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Chemical Stress Response of Wild Oat to 1, 2, 7, 8-Diepoxyoctane Treatment

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Abstract

This work aimed for positive genetic or physiological alteration in the quantitative traits of oat species specifically and other cereals in general in a way that may reflect human benefits in terms of increasing crop yield for both human and livestocks consumption. The results indicated that the treatment of both diploid oat species *(Avena clauda* and *A. eriantha*) with 100 μl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) as compared with the 200 µl and the untreated ones (control) had resulted in an increased final plant height (82-104 cm as compared with 70-9 cm for the treated with 200 µl and 12-48 cm for the untreated plants of *A.clauda* and *A. eriantha* respectively). Also, the former treatment had lead to an increase in ; leaf number (18-16 respectively as compared to 15-2 in the 200 µl treatment and 4-14 in the untreated). Furthermore, the 100 μl treatment had induced the germination of fertile node tillers (2-3) and apical tillers (6-9) which had increased the spikelets number between 16-26 within the node and apical tillers for *A. clauda* and between 4-5 for *A. eriantha* compared to 1-6 in the other treatments which is related to the complete absence of node tillers and to the presence of just single apical panicle in the other treatments. The presence of several fertile apical and node tillers in the diploid species as a result of treatment with 100 μl – DEO had reflected 4-5 folds increase in plant productivity in terms of increasing leaf number and seed density. These results may suggest that such tillers had arised as a result of physiological and/or chemical stress responses to DEO treatment rather than to genetic effects, Since the F2 progeny which is resulted from DEO-untreated F1 had shown no tillers as compared to the re-treated ones. These findings may suggest that the re-application of the 100 μl DEO treatment on cultivated oats as well as other cereals may be beneficial in terms of increasing productivity and this requires further studies on such plants.

الملخص

تهدف هذه الدراسه إلى إحداث تغيير أو تعديل وراثي أو فسيولوجي ايجابي في الصفات الكميه لمحصول الشوفان والمحاصيل ألحقليه عامة بطريقة تعكس منفعة بشريه ممثله بزيادة المحصول للاستهلاك البشري وتغذية الحيوانات الداجنة. تظهر التحاليل الإحصائية للنتائج بان معالجة أنواع الشوفان التي تحوي أربعة عشر كرموسوما (*Avena clauda* and *A. eriantha*) بمقدار 100 µl من المطفر الكيميائي DEO -Diepoxyoctane 1,2,7,8 أدت إلى زيادة كبيره في كل من طول النبات النهائي, عدد الأوراق (زياده بمقدار 3.5 ضعف), زياده في طول الشمراخ الزهري (بمقدار 2 - 15 ضعف) وزيادة في طول موسم النمو. مقارنة مع العينات المعالجة بمقدار 200 µl من نفس المركب.DEO حيث عكست المعالجه الثانيه 200 µl تأثيرات سلبيه على الصفات المذكوره أعلاه في جميع الأنواع الخاضعه للدراسة. إضافة إلى ذلك فأن المعالجة الأولى 100 µl للأنواع ذات الأربعة عشر كروموسوم أدت إلى ظهور تفرعات خصبه في كل من منطقه القمة النامية للنبات وعند العقد على طول الساق. نستطيع أن نستنتج بأن ظهور صفات كمية مرغوبه هو ناجم عن استجابة للتغيرات الفسيولوجية في النبات أو استجابة لتأثير المركب الكيميائي المستخدم DEO وليس لتأثيرات وراثيه , حيث أن أفراد الجيل الثاني F2 غير المعالجه بالمركب الكيميائي أظهرت نموا طبيعيا في حين أن المعالج منها أظهر زيادة إنتاجيه عن طريق زيادة إنتاجية الصفات الكمية من حيث زيادة طول النبات وعدد الأوراق والبذور وكذلك ظهور التفرعات الخصبه من القمم الناميه وعند العقد لبعض النباتات . وهذا يعد صفة ايجابيه مرغوبه في النبات كونها تؤدي إلى مضاعفة الانتاجيه على الرغم من أن مثل هذه الصفات لم تكن مورثه من جيل إلى آخر. تفيد هذه النتائج بان اعادة تطبيق هذه المعالجه 100 µl من المركب DEO على النباتات الاقتصاديه التي تزرع في البيوت البلاستيكيه أو الزجاجيه للاستهلاك البشري قد تؤدي الى زياده كبيره في كمية المحصول وهذا يتطلب اعادة الدراسه على مثل هذه النباتات مستقبلا.

Keywords: Avena species; 1,2,7,8-diepoxyoctane treatment; Apical and node Tillers;

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1. Introduction

Wild Oats (*Avena L.*) Species grow as weeds in Jordan and mostly found as contaminants in fields of cultivated crops, but as a crop in world production, it ranks fifth among all cereals and is distinctive among cereals due to their relatively high protein content. Seven species of Oats occur at three ploidy levels, diploids (5 species), tetraploids (1 species) and hexaploids (1 species) (Ladizinsky and Zohary, 1971; Kanan, 1987). Concerning the three oat species which were included in this particular work, The diploid species (*A. clauda Dur* and *A. eriantha Dur*) were found to be very restricted in their microenvironments when compared with the other species from different ploidy levels including the wild hexaploid species *Avena sterilis* which is characterized by having a wide range of distribution world wide and throughout the geographical regions of Jordan (Sampson, 1954; Ladizinsky and Zohary, 1971; Rajhathy and Thomas, 1974; Price and Kahler, 1983; Kanan, 1987; Jaradat, 1991; Kanan and Jaradat, 1996). The geographical differentiation between the species of the three ploidy levels may be related to specific genetic variability that provides each particular species with specific features to enhance its adaptation to the microenvironment in which it survives (Imam and Allard, 1965). The aim of this study is to evaluate the potential genotoxic or cytotoxic effects of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) treatment by means of in-vitro short-term mutagenicity tests on growth and survival of wild oat plants collected from Jordan. Also, to specify its significant effects on various (19) quantitative traits as compared with the control (DEO-untreated) or the natural populations, since no previous work had indicated the effect of DEO or other related compounds on oats or other cereals up to our knowledge. Several previous works had reported the genotoxic activity of several mutagens including DEO on the filamentous fungi including *Aspergillus nidullans* and *A. niger* were the mutagenic activity was tested by selecting chlorate and /or bromate resistant mutant strains that lie in genes responsible for nitrate assimilation (Kanan, 1996; Appleyard, *et al*., 1998). The obtained results had confirmed that DEO was serving as a strong mutagen (Kanan, 1996; Appleyard, *et al*., 1998; Kanan, *et al*., 2002; Kanan, 2002; Al-Najar, 2005 and Kinghorn *et al.*, 2005). In order to discriminate between genotoxic and cytotoxic mechanisms of DNA fragmentation, time-dependant dose response relationships for the induction of DNA double-strand breaks (DSB) was assessed by pulsed-field gel electrophoresis (PFGE) using cultured human lung epithelial cells treated with diepoxides where, the viability was evaluated by cytotoxicity tests (Cedervall, *et al*., 1995; Vamvakas, *et al*., 1997; Vock, *et al*., 1998; and Vock *et al*., 1999). Results obtained from such tests had confirmed that the chemical agent DEO and other diepoxides have induced DSB by a genotoxic mode with concentrations that did not affect cell survival (Blocher, *et al*., 1989; philips and Morgan, 1993; and Vock *et al*., 1999) . The diepoxybutane induced cross-links were of the unreparable types while, considerable repairs were observed for the DEO induced cross-links. However, DSB induction by formaldehyde and glutaraldehyde were found to be the consequence of extragenomic damage and viability loss (Saladino, *et al*., 1985 and Vock *et al*., 1999). Furthermore, the chemical mutagen 1,2,7,8-diepoxyoctane (DEO) whose deletogenic activity was also demonstrated in ad-3 system of the fungus *Neurospora crassa* and then in different fungal and bacterial species, has also been tested in *Samonella typhimurium* tester strain *(hisG428*) (de Serres, *et al*., 1995 and Picada, *et al*., 1999; Martinez, *et al*., 2000). The obtained results had confirmed that DEO is a cross-linking deletogenic agent and considered as a direct acting mutagen (Picada, *et al*., 1999). The sensitivity responses of two groups of *pso* mutants of *Saccharomyces cerevisiae* (The first; is involved in repairing damaged DNA or in RNA processing whereas the second group is related to metabolic steps but not responsible for DNA repair) towards three mutagens including DEO indicated that all mutants of the DNA repair group were sensitive to DEO while the other group of mutants had revealed wild-type or near wild-type resistance to all tested mutagens (Henriques, *et al*., 1997 and Pungartnik, *et al*., 2002). The relative DNA intra-strand cross-linking capabilities of several diepoxides (including DEO) with respect to chain length, molecular flexibility, carcinogenic potential, and DNA sequences targeted had been examined with a fragment of the 5S RNA gene of *Xenopus borealis*. Sites and efficiencies of intrastrand cross-linking were probed through denaturing polyacrylamide gel electrophoresis and quantitative phosphorimagery (Millard and Wilkes, 2001). Results obtained from such conducted mutagenicity and carcinogenicity tests using Mono-and Diepoxides had confirmed that the diepoxides (1,2,5,6-diepoxihexane and 1,2,7,8-diepoxyoctane) share the 5'-GNC target sequence and DEO also targeted 5'—GNNC sites where the efficiency of cross-linking this sequence may reflect carcinogenicity (Yunes, *et. al*., 1996 and Millard and Wilkes, 2001). The carcinogenicity of epoxide compounds has been attributed to covalent binding to DNA. Whereas, monoepoxides form only mono-adducts, diepoxides can form both mono-adducts and intra-strand cross-links (ICL) and show higher carcinogenicity and mutagenicity than their monoepoxide analogues (Yunes, *et al*., 1996 and Millard and Wilkes, 2001) where it is believed that ICLs are more significant cytotoxic lesions (Yunes, *et al*., 1996, Picada, *et al*., 1999, Vock, *et al*., 1999).

2. Materials and Methods

2.1. Collection of wild oat samples

The wild oat samples were collected from natural populations within the two restricted environmental sites for the included diploid species within the mountainous region of Jordan. The species *A. clauda Dur.* was found to be restricted to Mehna-Ajlun area (Lat: 32 22; Long: 35 46, Alt: l029) whereas, *A. eriantha Dur.* was found to be restricted to AL-ramaimeen-Al-Salt area (Lat: 32 07; Long 35 47; Alt: 671). In contrast, *A. sterilis* has been shown to be widely distributed throughout the mountainous region and Jordan valley. Fifty samples of mature (i.e. fruiting stage) whole plants and seeds from each species were

collected from the target regions. 19 quantitative traits were recorded for each plant and these are: plant height; date of seedling emergence; date of leaf emergance; leaf number; leaf length; leaf width; internode distance; date of panicle emergance; mature panicle length; number of flowering whorles; number of spikletes; distance between whorles; glume length; glume width; floret size; awn length; ground tillers. The collected seeds were subjected to a lobratory work where each treatment was repeated just once (i.e.Two independent experiments).

2.2. Germination of 1,2,7,8-diepoxoctane (DEO)-untreated seeds

Germination of seeds was induced by dehulling and piercing their coats with a 5 ml syringe needle (2-3 holes) followed by placing the seeds on three discs of filter paper (9 cm in diameter) in 9 cm petridishes. The seeds were considered germinated when the radical and the plumule become visible. Germinated seeds were transplanted at a depth of 3-4 cm in 12 x 12 cm plastic pots containing 3:1 claylaom: peatmoss. 20 pots with 4 plants/each Pot were used for each species. Pots were placed in lightened shelves (18.81KLx) inside the laboratory (10 x l0 m) under 16 hours light period and the following physical conditions were scored at least once /day throughout the growing season: light intensity, air temperature and %RH. Where the scored ranges of temperature and %RH were as follows: June 25.2C°-28.8C° and 26.1 – 80.3 %RH; July : 27.6C° – 30.3C° and 87.9 - 95.2 %RH; August, 26.4C° - 33.2C° and 77.3 – 95.4 % RH.

2.2.1. Germination of 1,2,7,8-diepoxoctane treated seeds

Germination of seeds was induced as described above except that seeds were soaked for 6 hours in tap water then treated with DEO. Where, a wide range (i.e. 5, 10, 20, 40, 80, 100, 200, 300, 400, and 500 μl per 25 ml water) of DEO concentrations for various incubation periods (i.e. 3, 5, 10, 15 and 30 min) was used. After treatment, seeds were washed three times every 5 min with sterile tap water to remove any traces of the mutagen. Seeds were placed on three wet discs of filter paper in 9 cm petridishes to germinate then transplanted in pots as mentioned above. However, 33%-57% of survivals was generated within 10 days with seeds treated for only 3 minutes with 100 µl and 200 µl DEO. Whereas, the other combinations of treatments were showing a range of 0.0%-83% over different incubation periods ranging from 3 to 30 minutes. Therefore, the decision was to treat seeds with 100 µl and 200 µl DEO where only one third to one half of seeds have survived.

2.2.2. Generation of F2-progeny from the studied species

In order to determine that the obtained results (i.e apical and node tillers) had arised as a result of genetical events and will be transmitted to the succeeding generations or due physiological or chemical stress responses as a result of DEO treatment. Fourty DEO–untreated fertile seeds of F1 generation from each of the three studied species were grown to maturity as mentioned above, another fourty seeds from each species were treated with 100 µl DEO (i.e. The treatment that had yielded apical and node tillers) until maturity.

2.3. Measurements of the studied traits

Every single plant was checked daily for scoring the date of emergence for every single leaf or panicle. Plant height was measured every 3 days starting from the date of implantation in soil until each plant has reached maturity (i.e. forming mature panicle) then the internode distance was measured using scaled ruller. Whereas, the remaining traits were scored at maturity.

2.4. Statistical analysis

From the experimental observation there were apparent difference between the treatments on the studied traits. Therefore, to test the statistical significance of these differences, SPSS programme (version 10) was used to carry out one way ANOVA for F1 progeny in order to determine the effect of different treatments on the studied variables while, two way ANOVA was used to study the effect of the applied treatment, the plant species and the interaction between them on the studied variables generated from F1 progeny this is followed by Post Hoc comparisons (Sheffe test). The results of the statstical analysis were used to study the significant differences of applied treatments on the studied quantitative traits within and between studied species. The results of different treatments on plant height, leaf number and percentage of survivals for tested plants from the three studied species were expressed as means ± SE and analyzed statistically by student's t-test using SPSS programe. *P* values of less than 0.05 was considered as the lowest limit of significance.

3. Results

All of the analyzed results presented here were obtained from the F1 (DEO-treated and untreated) generation. The F2 plants generated from the treated with 200 µl DEO or the untreated F1 were just like the natural plant population and no single plant had shown apical or node tillering capacity. Whereas, six *A. clauda* F2 plants (Re-treated with 100 µl DEO) out of fourty that were generated from the 100 µl DEO treated F1 had restored the tillering capacity.

3.1. Statistical analysis

Results presented in table (1) indicated that there is a highly significant effect of treatments on all of the studied quantitative traits (16 traits) from the three studied species except for the awn length in *A. sterilis,* the distance between whorles in *A. eriantha* and the date of emergence in *A. clauda*.

3.1.1. Multiple comparison by sheffe method – one way ANOVA

***A.clauda*/ Plant height**: Results presented in table (2) indicated that the applied treatments had significantly affect the plant height of *A. clauda*. Where, the highest plant height was obtained after 100 μl DEO treatment (104.0 cm) followed by the 200 μl DEO treatment (70.3571 cm) then the untreated (control) plants where the

plant height had reached only 12.6375 cm. However, plants from the natural population had shown a final height of 45.4231 cm.

Leaf number:- No significant difference in leaf number was noticed between plants from natural population and the DEO-untreated (control) ones, where the leaf number has reached 3.7375 and 5.3077 respectively. Also, no significant difference in leaf number was noticed between plants treated with 200 µl DEO or 100 µl where the leaf number has reached 15.5536 and 16.000 respectively. However, both DEO-treatments have shown 3 folds increase in leaf number as compared with the control (Table 2).

***A. eriantha*/Plant height:** The obtained results indicated that the 100 µl DEO treatment had significantly affected the plant height where it had shown a 9-folds increase in plant height (82.1642 cm) as compared to the ones treated with 200 µl DEO (9.2237 cm). In addition, the former treatment had shown a double-fold increase as compared with the control (48.2167 cm). However, no significant difference in plant height was noticed between natural population plants (50.688 cm) and the untreated - control (48.217 cm) (Table 2).

Leaf number: The applied treatments had significantly affect the leaf number of *A. eriantha* plants. Where, the highest number was obtained after 100 µl DEO treatment (17.97) and the least (1.338) was obtained after 200 µl DEO as compared to 14.62 leaves obtained by the untreated (control) plants (Table 2).

Panicle length: The applied treatments had significantly affect the panicle length of *A. eriantha* plants. Where, the longest panicle was obtained after 100 µl DEO treatment (14.81 cm) and the least (0.844 cm) was obtained after 200 µl DEO as compared to 7.82 cm long obtained by the untreated (control) plants. These findings indicated that the 100 µl DEO treatment had shown 15-folds increase as compared with the plants treated with 200 µl DEO. and just 2 folds increase when compared to the untreated ones (Table 2).

Number of whorles: The 100 µl DEO treatment had significantly affected the number of whorles where, it had shown 20-folds (6.48) increase in whorles number as compared with the 200 µl DEO treatment (0.33) and 1.5 folds compared to the control (Table 2).

Number of spikelets: The plants treated with 100 µl DEO have shown an approximate of 2-folds increase in number of spikelets per plant (7.045) compared to the untreated plants (4.1). However, the former traetment had shown an approximate of 25 folds increase as compared to 200 µl DEO treatment (0.3250). These findings were based on a single apical panicle but in fact the 100 µl DEO treatment has lead to the generation of several plants showing uncommonly occurring fertile tillers at the apical meristems and at each node and these will be discussed later (Table 2).

***A sterilis*/ Plant height**:- Results obtained indicated that both DEO treatments (i.e. 100 µl and 200 µl) had negatively influenced the plant hright where, such treatments had lead to the generation of plants with approximately half-length (i.e. in the range of 41.4-44.5 cm respectively) as compared with the untreated ones (73.143). However, no significant difference in plant height was noticed between untreated plants and the ones from the natural population. Furthermore, both DEO-treatments as compared with the control had negatively influenced (shown the least means) all of the remaining *A.sterilis* traits presented in table 2. However, no significant difference between both treatments was noticed (Table 2).

3.1.2. Two-way analysis of variance

Results presented in table (3) indicated that the applied treatment, the plant species and the interaction between them have shown a significant effect on all variables presented except for the internode distance and the distance between the flowering whorles. However, no interaction between the treatment and the species was noticed for these two variables.

3.1.3. Multiple comparison by Sheffe method for means of five quantitative traits that mostly reflect plant productivity (Two-Way ANOVA)

Final plant height. Results presented in table (4) indicated that the 100 µl DEO was the most significantly effective treatment in terms of generating the highest plant height in *A. clauda* plants whereas, the untreated *A. clauda* and the treated *A. eriantha* with 200 µl DEO had shown the least. However, depending on the level of significance presented in table 4 for the effect of applied treatments on species and their interactions, such treatments can be organized as groups in a descending order starting from the most effective treatment (group 1) to the least as presented in table 5. where, all combinations of the treatments within the same group are not significantly effective on the studied trait.

Leaf number. Both DEO treatments applied to *A. clauda* plants and the 100 µl DEO treatement applied to *A.eriantha* plants have shown the highest number of leaves. Whereas, the untreated *A. clauda* and the *A. eriantha* plants treated with 200 µl DEO have shown the least leaf number. However, the untreated *A. sterilis* plants had shown an approximate of double leaf number as compared with both DEO treatments. The desending order of groups as mentioned above is presented in table 5.

Leaf length. The untreated plants of *A. sterilis* have shown the longest leaves whereas, all treatments applied to *A. clauda* plants, natural population of *A. eriantha* and the *A. sterilis* plants treated with 200 µl DEO have shown the shortest leaves where, the reduction was in the range of 2.5-3.0 folds as compared to the former treatments. However,the 100 µl DEO treatments applied to both *A. sterilis* and *A. eriantha* also the untreated *A. eriantha* plants have shown a medium range in terms of leaf length as compared with other treatments. The desending order of groups as mentioned above is presented in table 5.

Panicle length. The 100 µl DEO treatment applied to *A. clauda* plants is not included in the comparison here since such treatment has shown uncommonly occuring several (6-9) apical tillers/plant and a single node tiller/node, and this will be discussed separately. However, the results presented in table 4 showed that both untreated (control) and natural population plants of *A. sterilis* have shown the tallest panicles followed by the 100 µl DEO treatment applied to *A. eriantha*. Whereas, both DEO treatments that were applied to *A. sterilis*, the 200 µl DEO treatment applied to *A. eriantha* and the untreated

plants of *A. clauda* have shown the shortest panicles where these were about $1/8^{th}$-$1/10^{th}$ of *A. sterilis* natural population plants or the untreated ones respectively. The desending order of groups as mentioned above is presented in table 5.

Number of spikelets. The results of the 100 µl DEO treatment applied to *A. clauda* plants is not included in the comparison here since such treatment has shown as mentioned above apical and node tillers each with its own spikelets and this will be discussed separately. However, the presented results in table 4 indicated that both DEO treatments that were applied to *A. sterilis* plants as well as the 200 µl DEO-treatment applied to *A. eriantha* plants and the untreated *A. clauda* plants had shown the least number of spikeletes as compared to other treatments. The desending order of groups as mentioned above is presented in table 5.

3.1.4. The generation of uncommon apical and node tillers with fertile panicles after 100 µl of 1,2,7,8-Diepoxioctane (DEO) treatment

Approximately one tenth of treated *A. eriantha* plants and one half of treated *A. clauda* plants had generated fertile apical and node tillers (Table 6) where instead of having one common panicle (Figure 3) at the apex, several (6-9) apical tillers each with leaves and fertile panicles (Fertile seeds) were generated at the apical meristem (Figure 1). In addition, each single node along the culm had shown one tiller with leaves and fertile panicle (Figure 2). Surprisingly, such uncommonly occuring beneficial traits had reflected several folds increase in total number of leaves per plant also, increased the number of seeds produced by a single plant which leads finally to an increased crop yield. However, *A. eriantha* tiller's type had shown a double leaf length as compared to *A. clauda* tillers leaves. On the contrary, *A. clauda* tiller's panicles have shown a range of 4-5 folds increase in the number of spikelets as compared to *A. eriantha* ones. In addition, these results had indicated that there is no significant difference in leaf length between node tillers (Figure 2) and apical tillers (Figure 1) within the same species. In contrast, the leaf length in both types of *A.eriantha* tillers have shown double length as compared to *A. clauda* leaves.

3.2. General- growth habit

3.2.1. Plant height

A. clauda The growth rate is shown in Figure 4. The results showed that the growth rate for untreated *A. clauda* plants was very slow where the plants have gained very short culms (≈ 10 cm) in the second week then the growth rate has persisted relativley constant until the end of the short growing season (≈ 9 weeks). However, the 100 µl DEO treatment has positively influenced the growth rate in terms of final plant height and the length of growing season where, the plant height has increased almost linearly up to 13, weeks after emergence and reached a final height of approximately, 90 cm. Also, the 200 µl DEO treatment had increased the growth rate of *A.clauda* plants almost linearly up to 15 weeks after emergence where, the plant height has reached ≈ 75 cm then the rate has stayed relatively constant until week 20. Furthermore, there was a high significant difference in plant height between the untreated plants and the treated with either 100 µl DEO ($p<0.05$) or 200 µl DEO ($p<0.05$).

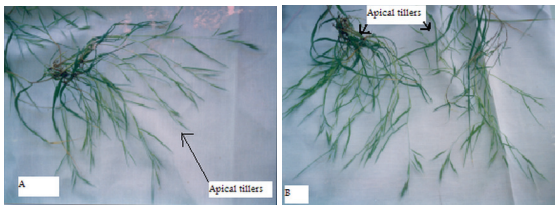

Figure 1. Fertile tillers generated at the apical meristems of plants from diploid oat species (*Avena clauda* (photo A) and *A. eriantha* (photo B)) treated with 100 µl 1,2,7,8-Diepoxyoctane (DEO), Photos were taken 60 days post treatment.

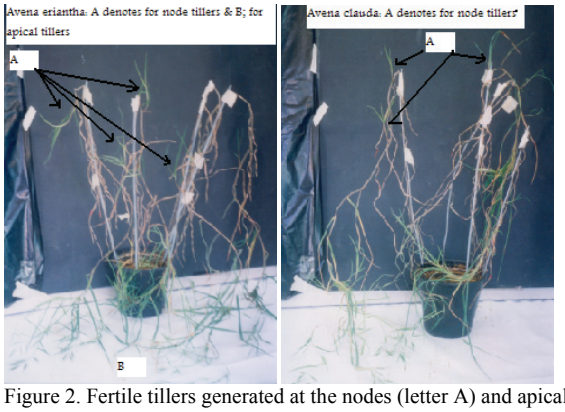

Figure 2. Fertile tillers generated at the nodes (letter A) and apical meristems (letter B) of plants from diploid oat species (*Avena clauda* and *A. eriantha*) treated with 100 µl 1,2,7,8-Diepoxyoctane (DEO). Photos were taken 60 days post treatment.

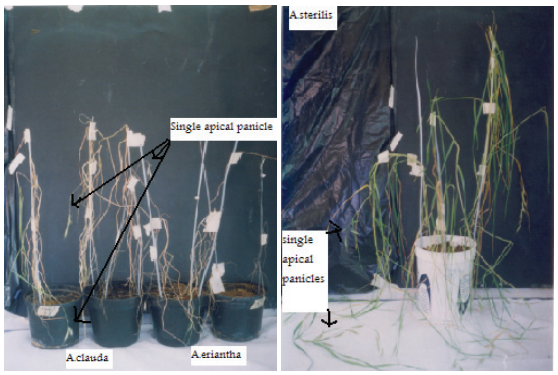

Figure 3. Representatives of 1,2,7,8-Diepoxyoctane (DEO)-untreated plants (control) from the three studied oat species (*Avena clauda*; *A. eriantha* and *A. sterilis*) indicating the formation of only the usual single apical panicle. Photos were taken 60 days post treatment.

A. eriantha The growth rate of untreated *A. eriantha* (Figure 5) plants has been accelarated rapidly during the first 6 weeks where the plant height has reached ≈ 35 cm then slight increase in height with increase in time has occurred until week 14 where, the plant has reached ≈ 50 cm in height. After that a steady state has occurred until week 23 . However, the 100 µl DEO treatment has positively influenced the growth rate in terms of final plant height and the length of growing season where, the plant height has increased almost linearly up to 21 weeks after

emergence and reached a final height of approximately 83 cm.

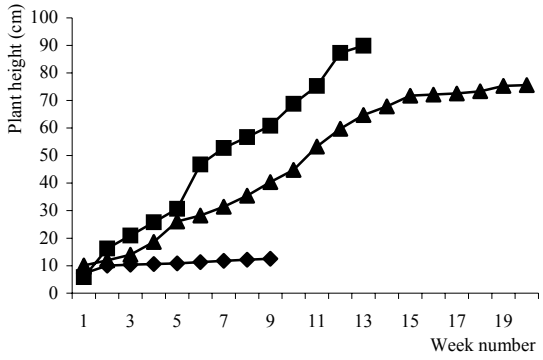

Figure 4. Time–course of growth expressed as means ± SE for *A. clauda* plants measured as plant height of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. clauda*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. The t-test results indicated that there is a high significant difference in plant height between the untreated plants and the treated with either 100 µl DEO (P<0.05) or 200 µl DEO (P<0.05). The significance was for all weeks of the study. As a result of reaching maturity (end of growing season) earlier than the treated plants there were no scored data for untreated *A. clauda* plants after week number 9. Also there was no increase in final plant height after week 13 for the plants treated with 100 µl DEO.

In contrast *A. eriantha* plants were extremly negatively influenced by the 200 µl DEO treatment where, the plant has shown a very stunted growth in which the height was ranging from ≈7 cm (week 1) to 9 cm (week 4; the end of the growing season). Moreover, there is no significant difference between the untreated plants and the treated with 100 µl DEO on plant height (P=0.186) while there is significant difference in plant height between the untreated and the treated with 200 µl DEO (P <0.05).

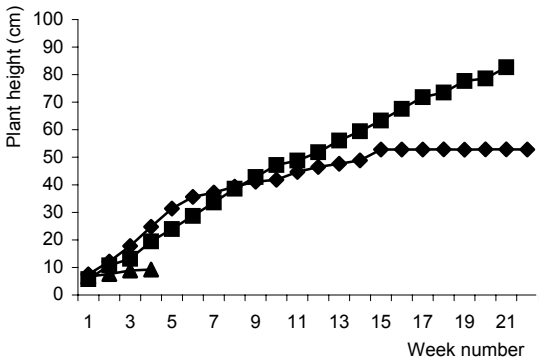

Figure 5. Time–course of growth expressed as means ± SE (for *A. eriantha* plants measured as plant height of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. eriantha*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. the t-test results indicated that there is no significant difference between the untreated plants and the treated with 100 µl DEO on plant height (P=0.186) while there is significant difference in plant height between the untreated and the treated with 200 µl DEO (P <0.05). The significance was for all weeks of the study. There were no scored data for *A. eriantha* plants treated with 200 µl DEO after week number 4 due to the end of the growing season.

Table 1. One – way analysis of variance for 16 quantitative traits of plants from hexaploid (*Avena sterilis*) and diploid (*A clauda* and *A eriantha)* oat species showing the effect of treatments on the presented quantitative characters. Results indicated that there is a high significant effect of the applied treatments (natural population; treated with 100 µl 1,2,7,8-Diepoxoctane (DEO); treated with 200 µl 1,2,7,8-Diepoxoctane (DEO) and DEO-untreated (control) plants on the studied traits. *NC: indicates not scored.

Character	*A. sterilis (2n = 42)*		*A. eriantha (2n = 14)*		*A. clauda (2n = 14)*	
	Observd F-value	Observed sig. level $\alpha = 0.05$	Observd F-value	Observed sig. level $\alpha = 0.05$	Observd F-value	Observed sig. level $\alpha = 0.05$
Date of emergence (days)	4.448	0.006	7.153	0.000	0.307	0.821
Plant height (cm)	48.955	0.000	202.638	0.000	162.665	0.000
Leaf number	10.594	0.000	389.968	0.000	180.521	0.000
Date of panicle emergence (days)	70.812	0.000	287.808	0.000	73.851	0.000
Panicle length (cm)	200.399	0.000	145.655	0.000	42.114	0.000
Whorle number	190.075	0.000	301.277	0.000	279.349	0.000
Spikelet number	236.872	0.000	251.816	0.000	300.883	0.000
Leaf length (cm)	30.163	0.000	60.407	0.000	24.84	0.000
Leaf width (cm)	161.694	0.000	23.518	0.000	4.95	0.003
Internode distance (cm)	0.000*	1.000	17.088	0.000	* NC	* NC
Distance between whorles (cm)	55.536	0.000	1.454	0.231	* NC	* NC
Ground tiller number	159.561	0.000	3.159	0.027	* NC	* NC
Upper glume length (cm)	4.675	0.004	97.719	0.000	2.719	0.051
Lower glume length (cm)	20.714	0.000	197.514	0.000	* NC	* NC
Floret size (cm)	*NC	1.000	8.582	0.000	9.896	0.000
Awn length (cm)	1.152	0.335	102.183	0.000	15.689	0.000

A. sterilis The growth rate of untreated *A. sterilis* plants (Figure 6) was accelarated rapidly during the growing season ($\approx$13 weeks) where, the plant has reached $\approx$75 cm in height. The 100 μl DEO treatment has positively influenced the growth rate in terms of final plant height and the length of growing season where, the plant height has increased almost linearly up to 14 weeks after emergence, where the final plant height has reached approximately, and 58 cm. The treated plants with 200 μl DEO have shown a rapid increase in height until week 5 where the mean height was $\approx$40 cm, then, the rate has stayed nearly constant until the end of the growing season (week 12) where, the plant height has reached $\approx$50 cm. There is a high siginificant difference in plant height between the untreated and the treated with 100 μl DEO (P=0.009) while there is no significant difference in plant height between the untreated plants and the treated with 200 μl DEO (P=0.074).

Table 2. Multiple comparison by Sheffe method for the means of various quantitative traits of plants from hexaploid (*Avena. sterilis*) and diploid (*A. clauda* and *A. eriantha)* oat species (One -Way ANOVA). Results indicated that the 100 μl DEO – treatment had lead to a significant increase in the quantitative traits that reflect plant productivity (plant height; leaf number; number of seeds) in both diploid species whereas, such treatment had lead to a significant reduction in the above mentioned traits in *A. sterilis*. *Treat (i) and (j) denote for treatment type where: (1): denotes for plants from natural population, (2): plants treated with 200 µl DEO, (3): plants treated with 100 µl DEO and (4): untreated plants with DEO (control).

Variable	Treat type (i)*	Treat type (j)*	Species								
			A.clauda			*A.eriantha*			*A.sterilis*		
			Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Mean difference (i-j)	Std.Er	Sig. level α= 0.05
Plant height	1	2	24.9341	3.7942	0	41.4638	3.2872	0	39.1854	1.0769	0
		3	58.5769	5.1839	0	31.4767	3.4047	0	42.316	4.2835	0
		4	32.7856	3.5095	0	2.4708	3.4866	0.918	10.5759	4.5623	0.152
	2	3	33.6429	5.1322	0	72.9404	2.9817	0	3.1306	3.9423	0.889
		4	57.7196	3.4327	0	38.9929	3.0749	0	28.6095	4.2437	0
	3	4	91.3625	4.9254	0	33.9475	3.2002	0	31.7401	4.4425	0
Leaf number	1	2	10.2459	0.6545	0	3.9542	0.5981	0			
		3	10.6923	0.8943	0	12.6785	0.6195	0			
		4	1.5702	0.6054	0.085	9.325	0.6344	0			
	2	3	0.4464	0.8853	0.968	16.6326	0.5425	0			
		4	11.8161	0.5922	0	13.2792	0.5595	0			
	3	4	12.2625	0.8497	0	3.3535	0.5823	0			
Panicle length	1	2				9.4688	0.7543	0	17.1806	0.9433	0
		3				4.4935	0.7812	0	19.625	0.9911	0
		4				2.4958	0.8	0.023	3.125	1.0556	0.036
	2	3				13.9622	0.6842	0	2.4444	0.9121	0.071
		4				6.9729	0.7056	0	14.0556	0.9819	0
	3	4				6.9893	0.7343	0	16.5	1.0279	0
Whorle number	1	2				6.3208	0.262	0	4.0028	0.252	0
		3				0.1682	0.2714	0.943	4.625	0.2648	0
		4				1.7958	0.2779	0	0.2679	0.2821	0.825
	2	3				6.1526	0.2377	0	0.6222	0.2437	0.094
		4				4.525	0.2451	0	4.2706	0.2624	0
	3	4				1.6276	0.2551	0	4.8929	0.2746	0
Spikelets number	1	2				12.7167	0.4765	0	14.3813	0.6062	0
		3				5.9969	0.4935	0	14.7813	0.6369	0
		4				8.9417	0.5054	0	9.5313	0.67884	0
	2	3				6.7198	0.4322	0	0.4	0.5862	0.926
		4				3.775	0.4457	0	4.85	0.631	0
	3	4				2.9448	0.4639	0	5.25	0.6606	0
Ground tillers number	1	2							9.8625	0.4833	0
		3							8.9514	0.5077	0
		4							7.7768	0.5408	0
	2	3							0.9111	0.4673	0.288
		4							2.087	0.503	0.001
	3	4							1.1746	0.5266	0.179

Table (3). Two-way analysis of variance for the treatment type * oat species. Results indicated that the studied quantitative traits were significantly affected by the applied treatment type (natural plant population; treated with 100 µl 1,2,7,8-Diepoxoctane (DEO); treated with 200 µl 1,2,7,8-Diepoxoctane (DEO) and DEO-untreated plants), the plant species (hexaploid (*Avena. sterilis*) and diploid (*A. clauda* and *A. eriantha*)) and the interaction between them. *: Significant (P ≤ 0.05).

Character	Treatment		Species		Treatment*Species	
	F-value	Sig.level α = 0.05	F-value	Sig.level α = 0.05	F-value	Sig.level α = 0.05
date of emergence (days)	3.668*	0.026	160.305*	0.000	5.672*	0.000
plant height (cm)	87.016*	0.000	27.723*	0.000	143.860*	0.000
internode distance (cm)	26.899*	0.000	70.025*	0.000		
leaf number	137.665*	0.000	62.236*	0.000	256.198*	0.000
leaf length (cm)	70.459*	0.000	26.630*	0.000	44.549*	0.000
leaf width (cm)	160.474*	0.000	24.556*	0.000	76.720*	0.000
date of panicle emergence (days)	26.089*	0.000	80.596*	0.000	344.794*	0.000
panicle length (cm)	112.993*	0.000	35.364*	0.000	173.043*	0.000
number of whorles	257.691*	0.000	107.567*	0.000	247.809*	0.000
distance between whorles (cm)	110.680*	0.000	188.478*	0.000		
number of spikletes	635.856*	0.000	9.455*	0.000	62.368*	0.000
glume length (cm)	14.099*	0.000	391.644*	0.000	12.389*	0.000
floret size (cm)	12.933*	0.000	186.598*	0.000	40.222*	0.000
awn length (cm)	81.157*	0.000	1115.594*	0.000	3.370*	0.036
ground tiller number	187.637*	0.000	9.638*	0.000	16.803*	0.000

*: Significant (P ≤ 0.05)

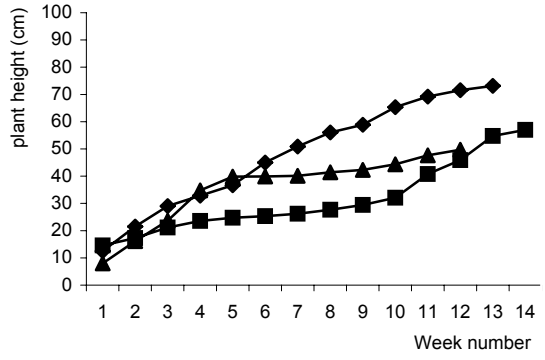

Figure 6. Time–course of growth expressed as means ± SE for *A. sterilis* plants measured as plant height of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. sterilis*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. The t-test results indicated that there is a high significant difference in plant height between the untreated and the treated with 100 µl DEO (P=0.009) while there is no significant difference in plant height between the untreated plants and the treated with 200 µl DEO (P=0.074). The significance was for all weeks of the study. There were no scored data for *A. sterilis* plants treated with 200 µl DEO after week number 12 due to the end of their growing season.

3.2.2. Leaf number

A. clauda The growth rate (leaf number vs . time in days) of untreated plants is shown in Figure 7. The obtained results indicated that the leaf number has almost increased linearly in all treatments where the leaf number has reached 8 leaves during 77 days, 23 leaves, over 130 days, and 18 leaves, over 98 days in the three applied treatments i.e. the untreated (control), the 100 µl DEO treatment and the 200 µl DEO treatment respectively. There is no significant difference in plant height between the untreated plants and both the treated with 100 µl DEO (P=0.176) or the treated with 200 µl DEO (P= 0.108).

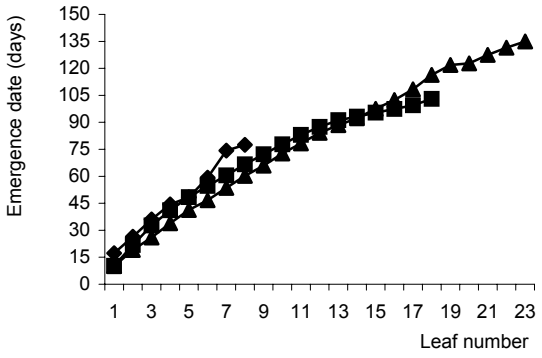

Figure 7. Time–course of growth expressed as means ± SE for *A. clauda* plants measured as as leaf number of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. clauda*; ▲- treated with 100 µl DEO and ■- treated with 200 µl DEO. The results of the t-test indicated that there is no significant difference in leaf number between the untreated plants and both the treated with 100 µl DEO (P=0.176) or the treated with 200 µl DEO (P = 0.108). The significance was for all weeks of the study. As a result of reaching maturity (end of growing season) earlier than the treated plants there was no emergence of more than 8 leaves for untreated *A. sterilis* plants as compared to 18 leaves and 23 leaves for the treated with 200 µl DEO and 100 µl DEO respectively.

A. eriantha The obtained results of untreated plants (Figure 8) indicated that the leaf number has almost increased linearly in all treatments where the leaf number has reached 21 leaves during 120 days, 24 leaves, over 140 days, and 4 leaves over 50 days in the three applied treatments i.e. the untreated (control), the 100 µl DEO

treatment and the 200 µl DEO treatment respectively. There is a high significant difference in plant height between the untreated plants and the treated with 100 µl DEO (P<0.05). Whereas, no siginificant difference was noticed in plant height between the untreated and the treated with 200 µl DEO (P = 0.0.392).

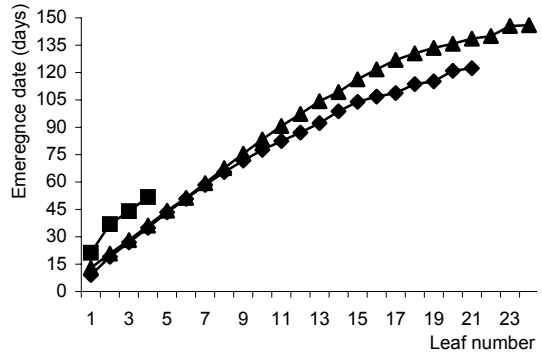

Figure 8. Time–course of growth expressed as means ± SE for *A. eriantha* plants measured as as leaf number of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. eriantha*; ▲- treated with 100 µl DEO and ■- treated with 200 µl DEO. The results of the t-test indicated that there is no significant difference in leaf number between the untreated plants and the treated with 100 µl DEO (P<0.05). Whereas, high significant difference was noticed between the untreated and the treated with 200 µl DEO (P = 0.0.392). The significance was for all weeks of the study. As a result of reaching the plants that were treated with 200 µl DEO maturity (end of growing season) earlier than other tested plants there was no emergence of more than 4 leaves for *A. sterilis* plants treated with 200 µl DEO as compared to 21 leaves and 24 leaves for the untreated and the treated with 100 µl DEO respectively.

A. sterilis The obtained results of untreated plants (Figure 9) indicated that the leaf number has almost increased linearly in all treatments where the leaf number has reached 12 leaves during 93 days, 12 leaves over 115 days and 9 leaves over 75 days, in the three applied treatments i.e. the untreated (control), the 100 µl DEO treatment and the 200 µl DEO treatment respectively. There is significant difference in plant height between the untreated plants and the treated with 100 µl DEO (P = 0.035). while there is no siginificant difference in plant height between the untreated and the treated with 200 µl DEO (P = 0.410).

3.2.3. Survival of plants up to maturity

A. clauda The untreated plants have shown the highest mortality rate and the shortest growing season where the percentage of survivals has decreased from 100% to ≈ 50% within 12 weeks the end of the growing season. Whereas, the percentage of survivals for the plants that were treated with 100 µl DEO has been kept constant (100%) during the first 10 weeks of the growing season (Figure 10) and then showed a slight decline (≈ 97% to ≈ 90%) starting from week 11 to the end of the season (week 21). However, the percentage of survivals in the treated plants with 200 µl DEO was nearly constant throughout the growing season (16 weeks) where, the percentage of survivals was in the range of 100% to 96%. There is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO (P < 0.05).

A. eriantha The untreated plants have also shown the highest mortality rate and the shortest growing season where the percentage of survivals has decreased from 100% to ≈ 50% within 20 week (Figure 11). The treated with 100 µl DEO have shown a gradual decline in percentage of survivals that range form 97% (week 2) to 75% (week 13) then the percentage has kept nearly constant until the end of the growing season (24 weeks). The 200 µl DEO treatment had shown negative influence on both the length of the growing season (9 weeks) and the percentage of survivals for *A. eriantha* plants where it has been droped down from 95% (week 3) to ≈13% (week 9). There is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO (P < 0.05).

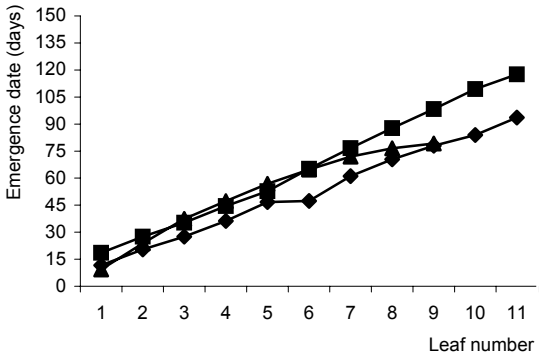

Figure 9. Time–course of growth expressed as means ± SE for *A. sterilis* plants measured as as leaf number of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. sterilis*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. The results of the t-test indicated that there is significant difference in leaf number between the untreated plants and the treated with 100 µl DEO (P = 0.035). while there is no significant difference in leaf number between the untreated and the treated with 200 µl DEO (P = 0.410). The significance was for all weeks of the study. As a result of reaching the plants that were treated with 200 µl DEO maturity (end of growing season) earlier than other tested plants there was no emergence of more than 9 leaves for *A. sterilis* plants treated with 200 µl DEO as compared to 12 leaves for the untreated and the treated with 100 µl DEO respectively.

A. sterilis The percentage of survivals for control plants (Figure 12) indicated that such percentage was nearly fixed (98%-100%) from the date of emergence until the end of week 15. However, the percentage survivals has decreased from ≈ 98% to ≈40% during the last four weeks (16-19) of the growing season. In contrast, the plants which were treated with 100 µl DEO treatment were the most negatively influenced ones where the percentage of survivals was kept nearly constant (≈98%) during the first 9 weeks then a sharp decline has occured where the percentage was ≈ 45% (week11). The plants which were treated with 200 µl DEO have shown a constant percentage (100%) of survivals during the first 6 weeks and then declined during the last 9 weeks where, the percentage of survivals has decreased to approximately 50% at the end of the season (16 weeks). There is no significant difference in the percentage of survivals between the untreated plants and either the treated with

100 µl DEO (p = 0.722) or the treated with 200 µl DEO ($P = 0.086$).

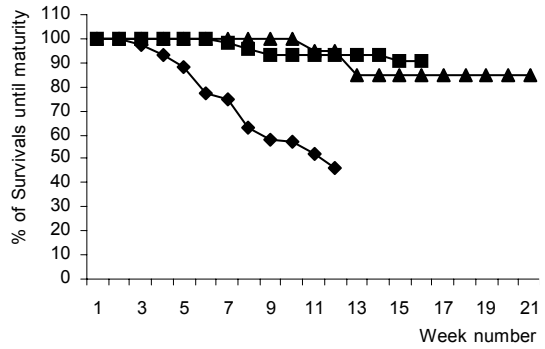

Figure 10. The pattern of change in the percentage of survivals from the date of emergence until reaching maturity for untreated (control), treated with 100 µl DEO and with 200 µl DEO plants of *A. clauda*. ♦-untreated *A. clauda*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO expressed as means ± SE. The results of the t-test indicated that there is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO ($P < 0.05$). As a result of having the highest mortality rate and the shortest growing season only 45% of the untreated plants have survived until week number 12 as compared to over 90% for the treated with 200 µl DEO (16 weeks) and the treated with 100 µl DEO (21 weeks).

4. Discussion and conclusions

The obtained results from the analysis of variance had indicated that the applied 100 µl DEO treatment to plants from both diploid species (*A. clauda* and *A. eriantha*) had positively influenced the growth rate of such plants.

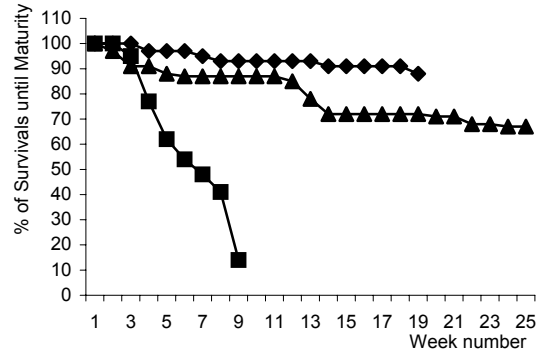

Figure 11. The pattern of change in the percentage of survivals from the date of emergence until reaching maturity for untreated (control), treated with 100 µl DEO and with 200 µl DEO plants of *A. eriantha*. ♦-untreated *A. eriantha*; ▲- treated with 100 µl DEO and ■- treated with 200 µl DEO expressed as means ± SE. The results of the t-test indicated that there is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO ($P < 0.05$). As a result of having the highest mortality rate and the shortest growing season only 13% of the treated plants with 200 µl DEO have survived until week number 9 as compared to over 90% for the untreated (20 weeks) and the treated with 100 µl DEO (75% until 24 weeks).

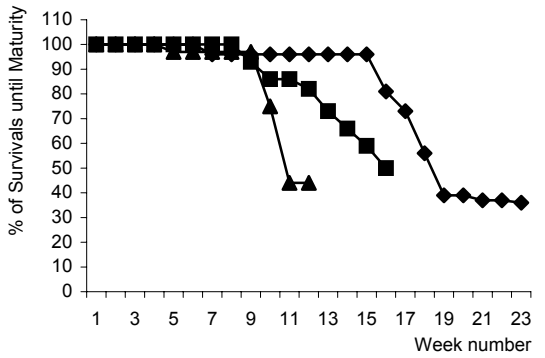

Figure 12. The pattern of change in the percentage of survivals from the date of emergence until reaching maturity for untreated (control), treated with 100 µl DEO and with 200 µl DEO plants of *A. sterilis*. ♦-untreated *A. sterilis*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO expressed as means ± SE. The results of the t-test indicated that there is no significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO ($P = 0.722$) or the treated with 200 µl DEO ($P = 0.086$). As a result of having the highest mortality rate and the shortest growing season only 40% of the treated plants with 200 µl DEO have survived until week number 11 as compared to over 40% for the untreated (23 weeks) and the treated with 100 µl DEO (50% until 16 weeks).

Such influence includes much extended (up to 24 weeks) growing season; several folds delay in panicle emergence; highest final plant height (2-folds increase); highest number of leaves (3.5 folds increase); longest panicles (2-5 folds as compared to untreated and 15-folds as compared to 200 µl treatment); highest number of flowering whorles reduced awn length to one half and the highest seed density. These findings may suggest that such plants are more resistant to intra-specific interferance between seedlings where by this would show a longer developmental period between the date of emergance and flowering or reaching maturity. In addition, such treated plants were subjected to approximately higher day-night temperatures, photoperiods and higher soil fertility than their natural environments. As a result of this more leaves will be produced in shorter periods between every two succession leaves. Also, much taller plants with an increases internode's distance, much longer, wider and healthy leaves and this suggestion would agree with the findings of (Bonaparte, 1975 and Sharma, *et al*., 1977). Where the gene action and its related secondary effects are highly influenced by the internal environmental factors in the external environment in which the plant was surviving. According to this wide range of modified phenotypic expressions will be generated and this may suggest that why several variable vegetative and floral characters could appear as a result of modified or altered gene(s) compared to normally expressed ones in the natural or the untreated plants. Furthermore, approximately one tenth of *A. eriantha* and one half of *A. clauda* plants that were treated with 100 µl DEO had shown several (6-9) fertile apical tillers/plant instead of one at the apical meristem and a single tiller at each stem node where each has its own small leaves and fertile seeds. Such induced traits would reflect human benefits in terms of increasing crop yield for human and farm animals consumption.

Table 4. Multiple comparison by Sheffe method (Two-way ANOVA) for means of five quantitative traits (Plant height; Leaf number; Leaf length; number of spikelets and panicle length) that generally reflect plant productivity. Plants of *A. clauda* and *A .eriantha* treated with 100 µl DEO had shown the highest means for traits reflecting productivity whereas, the same treatment had negatively influenced the same traits in *A. sterilis*. *Treat (i) and (j) denote for treatment type where: (1): denotes for *A. clauda* plants treated with 200 µl DEO, (2): *A. clauda* plants treated with 100 µl DEO, (3): untreated *A. clauda* plants, (4): *A. eriantha* plants treated with 200 µl DEO, (5): *A. eriantha* plants treated with 100 µl DEO, (6): untreated *A. eriantha* plants, (7): *A. sterilis* plants treated with 200 µl DEO, (8): *A. sterilis* plants treated with 100 µl DEO and (9): untreated *A. sterilis* plants.

Variable																									
Plant height					Leaf number					Leaf length					Spikelets number					Panicle length				
Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05
1	2	33.6429	4.8254	0	1	2	0.4464	0.8351	**1**	1	2	1.2443	2.3997	**1**	1	3	3.1339	0.4747	0	1	3	6.9518	0.7048	0
	3	57.7196	3.2275	0		3	11.8161	0.5586	0		3	16.2055	1.6679	0		4	3.7464	0.4747	0		4	8.183	0.7048	0
	4	61.1334	3.2275	0		4	14.2161	0.5586	0		4	8.294	1.7117	0.003		5	2.9733	0.4933	0		5	5.7792	0.7324	0
	5	11.807	3.3539	**0.337**		5	2.4166	0.5804	**0.07**		5	2.0615	1.8442	**0.996**		6	2.86E-02	0.5063	**1**		6	1.2101	0.7516	**0.989**
	6	22.1405	3.4419	0		6	0.9369	0.5957	**0.991**		6	13.3829	1.9679	0		7	3.6714	0.5455	0		7	6.5823	0.8099	0
	7	25.8238	3.7085	0		7	8.9091	0.6418	0		7	29.7321	2.1322	0		8	4.0714	0.5821	0		8	9.0268	0.8642	0
	8	28.9544	3.9572	0		8	9.4147	0.6848	0	7	8	15.4444	2.0599	0		9	1.1786	0.6306	**0.967**		9	7.4732	0.9363	0
	9	2.7857	4.2875	**1**		9	5.9464	0.742	0		9	31.7937	2.2174	0	4	5	6.7198	0.4512	0	4	5	13.9622	0.6699	0
4	5	72.9404	3.0677	0	4	5	16.6326	0.5309	0	2	5	17.4498	2.3473	0		6	3.775	0.4653	0		6	6.9729	0.6909	0
	6	38.9929	3.1636	0		6	13.2792	0.5475	0		6	9.5383	2.3786	0.044		7	7.50E-02	0.5077	**1**		7	1.6007	0.7538	**0.921**
	7	35.3096	3.4517	0		7	5.3069	0.5974	0		7	0.8172	2.4757	**1**		8	0.325	0.5468	**1**		8	0.8438	0.8119	**1**
	8	32.179	3.7176	0		8	4.8014	0.6434	0		8	14.6272	2.5691	0		9	4.925	0.5983	0		9	15.6562	0.8883	0
	9	63.9191	4.0674	0		9	8.2696	0.7039	0		9	30.9764	2.697	0	7	8	0.4	0.6093	**1**	7	8	2.4444	0.9046	**0.696**
7	8	3.1306	4.1421	**1**	7	8	0.5056	0.7168	**1**	5	6	7.9114	1.6374	0.004		9	4.85	0.6558	0		9	14.0556	0.9737	0
	9	28.6095	4.4587	0		9	2.9627	0.7716	**0.145**		7	18.267	1.7755	0	5	6	2.9448	0.4843	0	5	6	6.9893	0.719	0
2	3	91.3625	4.631	0	2	3	12.2625	0.8015	0		8	2.8226	1.9037	**0.974**		7	6.6448	0.5252	0		7	12.3615	0.7797	0
	4	94.7763	4.631	0		4	14.6625	0.8015	0		9	13.5267	2.073	0		8	7.0448	0.5631	0		9	1.694	0.9103	**0.968**
	5	21.8358	4.72	0.032		5	1.9701	0.8169	**0.829**	8	9	16.3492	2.3212	0		9	1.7948	0.6131	**0.574**	8	9	16.5	1.0193	0
	6	55.7833	4.7829	0		6	1.3833	0.8277	**0.986**	6	7	10.3556	1.8166	0	8	9	5.25	0.6866	0	3	4	1.2313	0.6396	**0.959**
	7	59.4667	4.9782	0		7	9.3556	0.8615	0		8	5.0889	1.9421	**0.552**	3	4	0.6125	0.4308	**0.996**		5	12.731	0.6699	0
	8	62.5972	5.1661	0		8	9.8611	0.8941	0		9	21.4381	2.1084	0		5	6.1073	0.4512	0		6	5.7417	0.6909	0
	9	30.8571	5.4233	0.001		9	6.3929	0.9386	0							6	3.1625	0.4653	0		7	0.3694	0.7538	**1**
5	6	33.9475	3.2925	0	5	6	3.3535	0.5698	0							7	0.5375	0.5077	**1**		8	2.075	0.8119	**0.768**
	7	40.7614	3.8279	0		7	11.8313	0.6625	0							8	0.9375	0.5468	**0.983**		9	14.425	0.8883	0
	8	9.0213	4.1685	**0.945**		8	8.363	0.7214	0							9	4.3125	0.5983	0	6	7	5.3722	0.7977	0
8	9	31.7401	4.6676	0	8	9	3.4683	0.8078	**0.051**						6	7	3.7	0.5373	0		8	7.8167	0.8528	0
3	4	3.4138	2.9289	**1**	3	4	2.4	0.5069	0.014							8	4.1	0.5744	0		9	8.6833	0.9258	0
	5	69.5267	3.0677	0		5	14.2326	0.5309	0							9	1.15	0.6236	**0.97**					
	6	35.5792	3.1636	0		6	10.8792	0.5475	0															
	7	31.8958	3.4517	0		7	2.9069	0.5974	0.009															
	8	28.7653	3.7176	0		8	2.4014	0.6434	**0.18**															
	9	60.5054	4.0674	0		9	5.8696	0.7039	0															
6	7	3.6833	3.653	**1**	6	7	7.9722	0.6322	0															
8	8	6.8139	3.9052	**0.99**		8	8.4778	0.6759	0															
	9	24.9262	4.2396	0		9	5.0095	0.7337	0															

Table 5. Multiple comparison by Sheffe method (Two-way ANOVA) for means of five quantitative traits. Treatments were arranged as groups in a descending order starting from the most effective treatment (group 1) to the least, depending on the level of significant effect at ά=0.05 presented in table 4 for the effect of applied treatments on species and their interactions. where, combinations of treatments within the same group reflect non- significant effect on the studied trait. The figure in brackets denotes for the treatment type where: 1, 5, and 9 denote for plants from natural populations of *A. clauda*, *A. eriantha* and *A. sterilis* respectively. 4, 8, and 12 denote for DEO-untreated (control) plants from the above mentioned species respectively. 3, 7, and 11 denote for for 100 µl DEO treratment of plants from the above mentioned species respectively. 2, 6, and 10 denote for 200 µl DEO treratment of plants from the above mentioned species respectively.

character	Group 1	Group 2	Group 3	Group 4	Group 5
Plant height (cm)	(3)104.0000	(2)70.3571 (12)73.1429 (7)82.1642 (9)83.7188	(11)41.4028 (10)44.5333 (1)45.4231 (8)48.2167 (5)50.6875	(6)9.2238 (4)12.6375	
Leaf number	(2)15.5536 (3)16.0000 (7)17.9701	(8)14.6167 (2)15.5536 (3)16.0000	(12)9.6071	(4)3.7375 (5)5.2917 (1)5.3077 (11)6.1389 (10)6.6444	(6)1.3375 (4)3.7375
Leaf length (cm)	(12)50.5714	(8)29.1333 (11)34.2222 (7)37.0448	(1)14.3462 (5)16.0417 (10)18.7778 (3)19.5950 (2)20.8393		
Panicle length (cm)	(12)16.5000 (9)19.6250	*(7)14.8060* *(12)16.5000*	(8)7.8167 (1)7.9423 (2)9.0268 (5)10.3125	(11)0.000 (6)0.8438 (4)2.0750 (10)2.4444	
No. of spikelets	(9)14.7812 (1)16.3846	(5)13.0417 (9)14.7812	(12)5.2500 (7)7.0448	(2)4.0714 (8)4.1000 (12)5.2500	(11)0.000 (6)0.3250 (10)0.400 (4)0.9375

Table 6. Mean ± standard deviation (SD) for quantitative traits measured on fertile tillers generated at the apical meristems and each node along the stem of plants from both diploid species (*A. clauda* and *A. eriantha*) after treatment with 100 µl of 1, 2,7, 8-diepoxyoctane (DEO). Approximately one half of the tested *A. clauda* and one tenth of *A. eriantha* plants had generated several (6-9) apical tiller/plant and a single node tiller per node after DEO treatment. Nc* : denotes for not scored.

Quantitative trait	*A. eriantha*			*A. clauda*		
	Ground tillers	Node tillers	Apical tillers	Ground tillers	Node tillers	Apical tillers
Number of tillers	5.00±4.05	3.06±1.51	5.73±3.64	Nc	3.020±1.67	8.93± 4.11
Length of tillers (cm)	33.22±11.81	34.79±8.39	31.84±12.22	Nc	22.40±8.96	27.03±7.88
Number of leaves/tiller	Nc*	5.70±1.06	5.70±1.06	Nc	8.30±2.367	7.04±1.53
Leaf length (cm)	11.14±6.21	10.28±7.71	10.44±4.33	Nc	4.78±3.91	4.57±2.11
Leaf width (cm)	0.16±0.05	0.17±0.05	0.44±0.14	Nc	0.229±0.05	0.24±0.05
Panicle length (cm)	9.28±3.70	9.59±4.15	10.76±4.36	Nc	9.50±7.54	9.71±3.83
Number of whorles	4.88±1.76	3.75±1.29	5.28±2.06	Nc	5.78±2.22	5.00±1.95
Number of spikelets	5.26±2.31	4.25±2.51	5.56±2.40	Nc	16.22±9.26	26.27±15.38
Internode distance (cm)	7.37±2.33	Nc	7.64±3.16	Nc	Nc	Nc
distance between whorles (cm)	1.21±0.26	Nc	1.39±0.41	Nc	Nc	Nc

However, the obtained results from the F2 progeny had indicated that such traits are not inherited but can be restored after the re-application of the treatment. The possible explanation for such effects that came as a result of DEO toxicity may be traced to alterations that cause low DEO sensitivity in some plants indicating that there is either a very low DNA damage (in number or in lesions) in silent and non-transcriptional regions of the genome (Verhage *et al*.; 1994) or that the still existing residual mismatch, excision, and the post replication repair mechanisms i.e. the error-free mechanisms (Reed *et al*., 1998) are sufficient enough to remove most of DEO induced DNA lesions. Such resistance might be due to genes encoding proteins with known or apparent functions in either the SOS-repair (i.e. Tolerance of the damage that allows the DNA polymerase to replicate past a lesion making replication faster but promote error-prone replication, where it introduces mutations while doing so) or error-free DNA repair. This effect demonstrates the importance of error-free and error-prone repair in handling the DNA lesions induced by the action of DEO mutagen. However, the generation of such apical and node tillers may indicate that the lack of ergosterol does not lead to a larger number of DNA lesions but such lesions might induce physiological changes or chemical stress responses that alter the metabolism or the production of balanced amounts of plant growth regulators such as cytokinines, auxins and gibberellines-GA3 (Devlin and Witham, 1983). where, each hormone has a multiplicity of effects depending on its concentration, site of action, developmental stage of the plant, and the balanced amounts with other hormones rather than on the absolute amount of each individual hormone. Moreover, the induced tillering capacity may arise as a result of altered rapid mitotic cell division, cell proliferation and cell elongation in the meristematic tissues. Where it seems to

be that the 1,2,7,8-Diepoxyoctane treatment has alters the hormonal balance between auxins and cytokinines because these are antagonistic hormones in controlling the apical dominance and the ability of the terminal buds to suppress the development of axillary buds. Auxins from apical bud inhibits the growth of axillary buds this leads to elongation of shoots main axis over lateral branches. Auxins in some way derepress a repressed gene, thereby releasing DNA template for RNA synthesis. The new RNA-presumably mRNA would then induce the formation of one or more new enzymes thus increasing wall plasticity and extension (Cohen and Bandurski, 1982). In addition, cytokinines which are produced in meristematic regions and areas of continued growth potential to induce cell division and enlargment are transported upwards from roots counter auxins and stimulating the growth of axillary buds, this explains why axillary buds near the shoot tips are likely to grow. Because a cytokinin intercalated into DNA causes template modifications that are important to the mechanisms of transcription and translocation and hence important to numerous physiological and morphogenetic processes (Burrows, 1975). Moreover, all gibberellines are able to stimulate cell division as well as elongation in the subapical meristem. Growth retardants retard growth by blocking the biosynthesis of gibberellines and by this inhibiting subapical cell division and induce lateral expansion of the apex (Jacobsen, 1977). In contrast, both DEO-treatments that were applied to *A. sterilis* plants and the 200 µl DEO treatment that was applied to all species have generated adverse antagonistic effects on almost all the studied traits. The altered responses in negatively influenced plants had shown reduced developmental processes that may result from reduced rate of cell division in meristematic tissues, such reduction will lead to reduced cell proliferation and elongation and these in turn will lead to dwarf plants with stunted growth, short growing season, low percentage of survivals, short internodes, reduced leaf number, reduced leaf length and width and mostly be lethal. One of the possible causes for such sensitivity may be traced to altered mutagen metabolism induced by an electron flow impairment (pungartmik *et al*., 1999). However, the extreme sensitivity of DNA repair—proficient altered plants suggest that DEO might be metabolized and this would generate highly genotoxic plants (Schimdt *et al*., 1999). Therefore, we might speculate that DEO metabolism in altered plants could lead to altered derivatives with significantly higher DNA-damaging potential. However, the genes that encode proteins apparently not involved in a DNA repair mechanisms but most probably confer sensitivity to certain mutagens as a result of loss from protection to reactive oxygen species (Schimdt *et al*., 1999). Furthermore, from a genetical point of view since DEO is considered as a DNA cross-linking genotoxic agent so it is suggested that all altered plants in the repair-deficient group were highly sensitive to DEO. More specifically, the altered plants are sensitive to intra-strand cross-links (ICL) producing mutagens including DEO and such suggestion agreed with that of Ruhland *et al*; 1981b. In addition, ICL repair in transcribed region of the genome might be more severily inhibited or lead to lethal RNA processing in the other repair-deficient plants and hence enhances their sensitivity over other plants. In summary, it can be suggsted that the DNA repair genes are rather important to elucidate genotoxicity in terms of survival, repair, mutagenesis (Henriques *et al*., 1997 and Pungartnik *et al*., 2002) of photo-induced DNA damage, thereby, controlling events that when not properly coordinated might eventually lead to cell death.

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