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EFFECT OF DIETHYL SULPHATE ON M1 ATTRIBUTES AND SDS-PAGE ANALYSIS IN GROUNDNUT (*Arachis hypogaea* L.)

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ABSTRACT

The present study was carried out to know the effect of diethyl sulphate on groundnut cultivar TMV-7 particularly in M₁ generation. The healthy and viable seeds of groundnut cultivar TMV-7 were exposed to different concentrations of diethyl sulphate (5 to 25 mM) and sown in the field. The various growth and morphological parameters like percentage of seed germination, survival percentage plant height, days to flowering, total height at time of harvest, root length and number of lateral roots, number of pods per plant and pod weight were decreased with increasing concentrations of diethyl sulphate. All the characteristics studied were inhibited when compared to control. The lower concentrations such as 5, 10 and 15 mM concentrations enhanced all the parameters. But there was a sudden decrease in 20 and 25 mM. The various concentrations of DES played a different role in the field condition itself. The shoots developed from different concentrations of DES showed variation in their number and height. The protein content also showed decreasing trend with increasing concentrations of DES. There was a steady increase of protein content up to 15 mM DES. After that, there was a drastic reduction in the protein content in 20 and 25 mM. From the diethyl sulphate treated seeds, the embryo axes were collected and subjected to SDS-PAGE analysis. The protein banding pattern also differentiated among various concentrations of DES. The protein band with 77.5 kDa was observed in 5 and 10mM treatments. Like that the 13.5 kDa was present in 10 and 25mM treatments. The 48.25 kDa recorded in 15 and 20 mM treatments.

Keywords: *Arachis hypogaea* L., Diethyl Sulphate and SDS-PAGE.

Introduction

The peanut or groundnut (*Arachis hypogaea* L.), a species in the legume family is an important oilseed legume grown worldwide and is known by many other local names such as earthnut, peanut, goober pea, monkey nut and pignut (McDonald, 1968). It is grown both for domestic market and export. The world groundnut production was estimated to be 35.367 million metric tons in 2011-2012. The world leading producers are China, India, and USA followed by Nigeria.

Groundnut is a nutritive crop with approximately 25% protein and about 45–50% oil. The skin of groundnut is contain rich in vitamin B and it is used as a base ingredient for cosmetics. It also provides important ingredients in numerous industries for confectionery and bakery products. Groundnut proteins content is of high biological value than other proteins. The residue of the extraction process is used as commercial groundnut cake which is a concentrate feed for livestock and poultry. The nuts are eaten raw or after roasting as snacks. The green leaves or shoot makes excellent fodder and hay for animals (National Peanut Council 1990; Anon, 1977).

Mutation breeding has been wide used for the development of plant characters in numerous crops. it's a

robust and effective tool within the hands of plant breeders particularly for autogamic crops having slender genetic base (Micke, 1988).

Crop improvement by mutation breeding has been applied in a very variety of crops for yield improvement, creation of recent varieties, stress and drought tolerance, sickness resistance and for farming or gardening functions (Ali *et al.*, 2007; Skoric, *et al.*, 2008). Iatrogenic mutations are wont to improve major crops that ar principally propagated by seeds (Adamu and Aliyu, 2007; Mostafa, 2011) and to introduce novel genetic variability in ornamental crops.

Materials and Methods

Seed material

Mature, healthy and uniform seeds of groundnut variety TMV-7 was obtained from Tamil Nadu Agricultural University, Coimbatore and used as an experimental material to carry out the mutagenic studies using Diethyl sulphate as a chemical mutagen.

Determination of imbibition period

For the purpose of chemical mutagen treatment the total imbibitions period was calculated before Diethyl sulphate

treatment, the imbibition period was divided in to(1) pre-soaking period- 4hrs and mutagen treatment period-2hrs.

Treatment with Diethyl sulphate

For chemical mutagen treatment, (DES) the groundnut seeds were placed in perforated polyethylene bags (with 3replica each containing 100 seeds in separate polyethylene bags) and presoaked in distilled water for 4 hrs. The different concentrations of Diethyl sulphate (5.0 to 25.0 mM) was prepared using phosphate buffer. Then the presoaked seeds were immersed in various concentrations DES for 2 hrs. After mutagenic treatment the seeds were carefully washed with distilled water to remove the traces of Diethyl sulphate present on the surface of the seeds. After treatment with Diethyl sulphate the seeds were immediately sown in the field.

M₁ characteristics

The following M₁ characteristics were studied in the field. Percentage of seed germination, Survival percentage, Plant height, Number branches, Days to flowering, Total height of the plant at the time of harvest, Total number of branches at the time harvest, Root length, Number of lateral roots, Number of pods per plant, Pod weight, Weight of the 100 seeds, All the above M₁ parameters were carefully taken in field it self in all the 3 replica (R1, R2 and R3). To know the effect of various concentrations of Diethyl sulphate in mature embryos after mutagen treatment, the SDS-PAGE analysis was done using mutagen treated embryos from mature seed. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method of Laemmli, 1976)

Results

Effect of Diethyl sulphate on groundnut cultivar TMV-7

The healthy uniform seeds of groundnut cultured TMV-7 were pre-soaked in distilled water for 4 hr and treated with different concentrations of Diethyl sulphate using phosphate buffer with a pH of 6.0. The diethyl sulphate concentration was ranged from 5 mM to 25 mM. In mutation breeding programme the breeders are more interested in the extent of variability, which is more reflected by mean, range and standard deviation. The variation in mean of yield and its contributing characters of groundnut TMV-7 in M₁ generation is given Table - 1. During M₁ generation, most of the characters *viz.*, seed germination percentage seedling height, shoot length, root length, number of branches, number of lateral root, number of lateral pod, its range was affected by various treatments of DES. The standard deviation was found decreased in all the treatments as compared to control.

The spectrophometric analysis of protein content was increased in all the treatments when compared to control. The maximum protein content was observed in 25mM treatment (3.350 mg/100g/fw). Whereas it was 3.432, 3.382, 3.368 and 3.329 mg/100g/fw present in 5, 10, 15 and 20mM respectively. There was a increasing trend protein content due to diethyl sulphate treatment (Table-2).

Electrophoretic analysis of proteins

The different concentrations of diethyl sulphate were subjected to SDS-PAGE analysis to know the protein profiling/banding pattern. The electrophoretic showed differences in number of bands per sample and molecular

weight of the band (kDa). In control, 10 mM, 20 mM and 25 mM diethyl sulphate treatments only five bands were observed. Like that in 5mM and 15mM concentrations the total number of bands were four. The protein band with kDa of 35.5 were present in all the six treatments. The kDa of 105.4 and 67.6 were present in all the six treatments. The kDa of 23.5 was present in control, 20 and 25mM. In a similar way 77.2 kDa was observed only in 5 and, 10mM. At the same time 13.2 kDa band was noticed in 10 and 25 mM treatments. Whereas The kDa of 27.6 was observed in all the six treatment. In 15 and 20mM the kDa of 48.2 were observed (Fig. 1).

Discussion

In the present study different concentrations of diethyl sulphate were used in groundnut cultivar TMV-7 as an experimental material to know the morphological and biochemical characteristics. The results obtained were discussed with earlier reports related with induced mutagenic studies of several chemical mutagens.

In the present experimental work the seeds of groundnut cultivar TMV-7 were exposed to different concentrations of diethyl sulphate and shown in the field. The various concentrations of diethyl sulphate reduced all the morphological and yield characteristics. The different treatments were found to affect percentage of germination, survival percentage, plant height, number of branches/ per plant and pod yield.

In our study the percentage of seed germination was decreased with increasing concentrations of DES. According to Constantin *et al.* (1976), reported that the reduction in survival and growth in soybean due to chemical mutagen treatment. The effect of mutagens was measured quantitatively by reduction in germination survival (lethality) (Ramasamy, 1973).

The present observation showed there was a decrease in plant height, number of branches with increasing concentrations of DES. This type of inhibitory effects of various mutagens were reported in several other crop plants (Reddy *et al.*, 1992). The found that the gamma irradiation significantly reduced some polygenic characters like length of pods in M₁ generations. Pavadai and Dhanavel (2004), reported the mutagenic effect was decreasing in quantitative characters in soybean. Like this Ojiewo *et al.*, 2006a suggested the M₁ generation plants developed from mutagens treated seeds suffered from growth inhibition, may be partly sterile.

In the study decreasing trend of growth and yield parameters with increasing concentrations of diethyl sulphate was in conformity with other chemical mutagens. The findings of similar nature were also reported by Badigannavar and Murty (2007) indicating root length, hypocotyl length and seedling height decreased with increased in doses of mutagens. Mutagenic sensitivity can be attributed to the level of differentiation of rudimentary plant parts at the time of treatment on one hand and extent of damage to the growth components like rate of the cell division, cell elongation and various hormones and biosynthetic pathways related to growth and development on the other.

Like that of present research communication Aliyu and Adamu (2007), reported the effect of diethyl sulphate on

some quantitative traits in tomato. The various concentration of DES affected seed germination, root length, height at maturity, number of branches per plants, and number of fruits per plant with increase in mutagen concentration.

Like this type research work, Sonone *et al.* (2010) achieved induced variability in quantitative characters of groundnut (*Arachis hypogaea* L.) whereas induction of mutations for plant height and inheritance of dwarf of mutant in groundnut (*Arachis hypogaea* L.) through gamma ray irradiation was reported by Anand *et al.* (2010).

Conclusion

The mutation methodology has been used to produce many cultivars with improved economic value and useful to studied the genetics, plant developmental phenomena. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops. Magnitude of induced variation was found to depend up on the mutagen used, character under study and the genotypic background of the mutant. These promising mutant lines need to be further utilized in next generation to derive distinct lines with improved agronomic traits. It is evident from our study, the diethyl sulphate is an useful chemical mutagen to obtain mutants in groundnut.

Table 1 : Effect of Diethyl sulphate on growth and yield characteristics in M₁ generation of groundnut cultivar TMV-7

Parameters	Control	5 mM	10 mM	15 mM	20 mM	25 mM
Percentage of seed germination	86.66± 4.72	80.55±1.57	76.33 ± 4.50	51.00 ± 5.29	29.33 ± 2.51	10.3 ± 3.05
Seedling height (on 30 th day)	7.92 ± 0.16	7.05±0.66	6.86 ± 0.30	6.65 ± 0.04	5.91 ± 0.31	4.70 ± 0.16
Shoot length (cm)	23.54 ± 2.25	17.4±1.68	16.18 ± 1.68	14.68 ± 1.60	12.12 ± 2.46	8.12 ± 1.97
Root length (cm)	12.17 ± 0.51	11.42±1.72	10.56 ± 1.35	9.02 ± 1.00	8.01 ± 2.24	7.40 ± 1.72
Number of branches	5.00 ± 0.89	4.60±0.89	4.10 ± 0.70	3.50 ± 1.58	2.80 ± 0.44	1.4 ± 0.54
Number of lateral roots	32.0 ± 6.20	30.22±1.88	28.25 ± 3.76	20.44 ± 2.07	16.12 ± 3.96	12.6 ± 3.04
Number Of pods/plant	16.6 ± 1.67	12.00±1.50	10.00 ± 1.64	6.00 ± 1.14	4.12 ± 1.58	2.4 ± 0.89

Table 2. Effect of diethyl sulphate on protein content in shoots (mg/100g/fw).

S.No	Treatment with DES	Protein content mg/100g/fw
1	Control	2.115
2	5mM	3.432
3	10mM	3.382
4	15mM	3.368
5	20mM	3.329
6	25mM	3.550

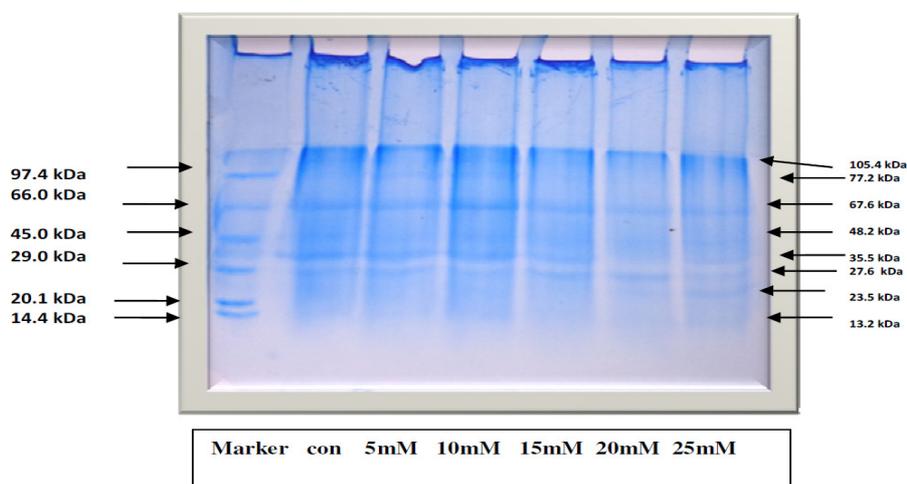


Fig. 1 : SDS-PAGE analysis of DES treated embryo in groundnut (*Arachis hypogaea* L.)

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