Available online at www.ilns.pl

International Letters of Natural Sciences

8 (2015) 25-35 ISSN 2300-9675

Studies on induced physical and chemical mutagenesis in groundnut (*Arachis hypogia*)

A. Gunasekaran[1], P. Pavadai[2]*

[1]Research and Development Centre, Bharathiyar University, Coimbatore, India

[2]P.G. & Research Department of Botany, Periyar Arts College, Cuddalore 607 001, TN, India

*E-mail address: indsankar@rediffmail.com

ABSTRACT

Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders. In any mutation breeding program, selection of an effective and efficient mutagen is very essential to produce high frequency of desirable mutation. Groundnut (*Arachis hypogia*) var. VRI-2. was treated with different concentration of physical and chemical mutagen namely gamma rays 10, 20, 30, 40, 50 and 60 KR and Ethyl methane sulphonate (EMS). For inducing mutation various concentration of EMS such as 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 % for six hours were applied to 200 seed sample of each concentration and one respective control. The LD_{50} value was observed in 50% of gamma rays and 0.5 % of EMS. The morphological and yield characters were significantly reduced seed germination, seedling survival, days to first flower, plant height, number of leaves per plant, number of grains per plant, grain length and breath, 100 grains weight, grain yield per plant, fresh and dry weight per plant. The increasing doses/concentration of gamma rays and EMS decreased in phenotypic and yield characters in M1generation. The mutagenized populations showed significantly higher variability in the M2 generation. Mutant lines showing higher yield per plant than the respective parents and checks were isolated in M2 and subsequent generation were significantly more pod yield and yield components than the untreated plants.

***Keywords**:* physical; chemical; mutagenesis; *Arachis hypogia*

1. INTRODUCTION

Groundnut (L.) is popularly known as peanut. It is one of the world`s most popular oil seed plants, cultivated in more than 100 countries of six continents. Major groundnut producers in the world are China, India, Nigeria, USA, Indonesia and Sudan. It is the single largest source of edible oils in India and constitutes roughly about 50 percent of the total oilseeds production. Among the major Groundnut growing states there has been consistent increase in area under cultivation in Andhra Pradesh. In recent times, groundnut is gaining importance as a food crop, due to its high content of digestible proteins, vitamins, minerals, phytosterols and due to increased consumer preference after value addition.The groundnut seed mainly comprised of protein, fat, carbohydrate which make it sensitive to radiation

induced stress. Among the environmental stresses, the radiation is the most important factor, which limits production of groundnut. This would result in drastic reduction in crop yield and magnitude of reduction would depend on groundnut varieties. Not only the yield of Groundnut but also the quality of products decreases under radiation stress. The seed stage is a convenient phase in the plant's life cycle for use in radiological studies to determine relative radio sensitivity of species and the effects of various factors on radio sensitivity. Earlier experiments in this field have indicated that ionizing radiation could cause permanent genetical effects, lethal or beneficial mutations, morphological modifications and other effects in plants. Several factors may be involved in the inhibition of germination and the growth of the plants from seeds following their exposure to high irradiation doses. A number of radiobiological parameters are commonly used in early assessment of effectiveness of radiation. Methods based on physiological changes such as inhibition of seed germination and shoot and root elongation have been reported for detection of irradiated legumes. Therefore, in present study the response of groundnut seed (cv. Narayani) to gamma radiation stress on germination and seedling parameters of groundnut was investigated compared to non irradiated seed.

Though the groundnut crop has morphological, biochemical, physiological variability, it has narrow genetic base because of its monophyletic origin, lack of gene flow due to ploidy barrier and self- pollination. The most popular method employed for creating genetic variability, is induced mutagenesis through gamma irradiation.

Commercially, groundnut is the world's fourth most important source of edible oil and third most important source of vegetable protein. Currently, groundnut is grown on nearly 25.20 million ha around the world with an annual production of 36.90 million tons of nuts-in-shell. The major producers are China, India and the USA which together account for two-thirds of the world output. Developing countries, account for 82 per cent of total groundnut area and 79 per cent of production of the world. Among the developing countries, production is mainly concentrated in Asia and Africa with Asia accounting for 51 per cent of global area and 60 per cent of production. India occupies 30 per cent of global area (6.6 million ha) and contributes 22 per cent (5.9 million tons) of total groundnut production (FAO, 2007).

Groundnut oil generally contains 45-50 per cent monounsaturated fatty acids, 30-35 per cent polyunsaturated fatty acids and 17-18 per cent of saturated fatty acids (Ory *et al*. 1992). Polyunsaturated fatty acids have high potential of developing off-flavors due to oxidation. Oxidative rancidity of groundnut oil is a major cause of spoilage of roasted groundnuts and groundnut based confections during storage. Oxidation leads to the formation of tasteless and odorless hydroperoxides as primary products. Decomposition ofhydroperoxides results in a wide variety of compounds, such as aldehydes and ketones, which result in off-flavors and odors. There exists a strong negative correlation between linoleic acid content and oil stability in groundnut (Holley and Hammons, 1968). Oxidation of linoleic and linolenic acid is approximately 10 and 25 times higher, respectively, than that of oleic acid (Frankel, 1991; Lands, 1997). Oil with higher ratios of oleic to linoleic acid retains its quality longer in the seed or as oil. Thus the content of the polyunsaturated fatty acid (linoleic acid) or the ratio of oleic to linoleic acid (O/L) in oils is the most important determinant of oil quality.

2. MATERIALS AND METHODS

2.1. Biological materials

The dry and dormant seeds of the Groundnut (*Arachis hypogia*) var. VRI-2 were obtained from Pulses Research Station, Tamilnadu Agricultural University, Virudhachalam. Tamil Nadu.

2.2. Mutagens

Physical and chemical mutagens like Gamma rays and Ethyl methane sulphonate(EMS) were used at various concentrations to induce mutagenesis.

2.3. Mutagen treatments

Seeds of peanut cultivar VRI-2 were treated with γ-radiation and ethyl methane sulphonate (EMS). Uniform size seeds of each cultivar were used for treatment. Treatments (200 seeds per treatment) consisted of six different doses of γ-radiation (10,20,30,40,50 and 60KR) and EMS (0.1,0.2,0.3,0.4,0.5 and 0.6%). Untreated seed stock of the respective cultivars was used as a control. Seeds were irradiated with γ-radiation at Sugar cane Breeding Institute, Coimbatore, Tamil Nadu India. EMS solution was prepared in 0.1 M phosphate buffer (pH = 7.0). Seeds were presoaked in distilled water for six hours to allow uptake of EMS. Presoaked seeds were than treated with EMS for two hours at room temperature in cloth bags. Treated seeds were then rinsed in running tap water for four hours and sown in the field plots along with untreated control. The seeds were sown in a randomized complete block design in three replications with spacing of 30 cm between the rows and between plants. The recommended package of practice for the crop was followed. The M_1 plants were harvested on a single plant basis. M_2 generation seeds were raised from M_1 generation, the seeds were collected from different individual mutagenic treatment. Segregating mutant lines based on visual observations and low performing were discarded in the initial stages of evaluation and progenies were advanced on the basis of superiority of their yield performance over the respective controls, finally ending up with 10 superior mutant lines in M_2 generation. The selected 10 mutant progenies were evaluated during the summer of 2013 (M_2) in a replicated trial to assess their performance and identify high-yielding mutants. The 10 mutants, untreated controls (parents) and two checks were grown in randomized complete block design with three replications in a plot of 4.0 m x 2.4 m with spacing of 30 cm x 10 cm over three successive generations. From each entry, 10 plants were randomly selected for recording observations.

2.4. Laboratory studies

Germination studies

Germinated seeds will count from 3rd to 7th day emergence of cotyledonary leaf will be taken as the indication of germination. Germination percentage will be worked out for the treatment in each genotype separately and lethality will be found out based on the mean value of 10 replicates.

Shoot and root length

The shoot and root length (cm) will be measured ten randomly selected seedlings with ten replications on the 15th day with the effect of physical mutagen along with control.

2.5. Lethal dosage (LD_{50} Value)

The LD_{50} value for Groundnut (*Arachis hypogia*) var. VRI-2 was observed at 50KR of gamma rays and 0.5% of EMS treatment.

Field studies

- Treated and control seeds were sown in the field (3 replication) in a randomized Block design (RBD) in order to raises the R_1 and R_2 generations.
- Each treatment of doses consists of hundred seeds including control.
- The seed to seed and row to row distance was maintained at15×60cm respectively.
- Cultural operations were carried out *viz.*, irrigation and weeding.
- The following characters were observed in all the treatments along with the control from R_1- R_2 generations.

2.6. Field Observations (Based on the LD_{50} value)

Germination %, Seedling survival %, days to first flowers, plant height, Number of leaves per plant, number of kernal per plant, 100seed weight, kernal yield per plant, oil content, protein content fresh weight and dry weight.

3. BIOCHEMICAL ANALYSIS

3.1. Protein content

Two seeds from the same plant of each M1,M2 plants were separately de hulled and ground in a mortar and the extracts were defatted by washing with three changes of cold acetone for 4 to 6 hrs. The acetone was removed by filtration and the extracts were air-dried at room temperature. The proteins from the defatted meal were precipitated with 10% trichloroacetic acidand recovered by centrifugation at 5000 rpm for 30 minutes at 40°C. The protein content was then determined calorimetrically according to the method of Lowry *et al*. (1951) using bovine serum albumin as standard.

3.2. Oil content

The oil content of the seed was estimated with petroleum ether in Soxhlet extraction apparatus (Cox and Pearson, 1962).About 50 g of seed was dried in adrying dish at 130°C for 20 min. in a forced draft oven. Then they were cooled to room temperature and passed through the nut slicer to slice the nuts. The sliced samples were mixed well and accurately 2 g of the sample was taken in to a filter paper fold. The filter paper was folded in such a way to hold the seed meal. A second filter paper was used to wrap around the seed which was left open at the top like a thimble. The sample packet was placed in the butt tubes of the Soxhlet extraction apparatus.Extraction was done with petroleum ether (150 drops min-1) for 6 hrs without interruption by gentle heating.Then the extraction flask was dismantled after cooling and then ether was evaporated on a water bath until no odor of ether remained. The dirt or moisture outside the flask was carefully removed and the flask was weighed. The heating was repeated to get constant weight.

4. RESULTS AND DISCUSSION

Mutagenic sensitivity, in general, is known to be influenced by a variety of factors which include moisture content, temperature, pH, concentration of catalytic ions, various pre and post-treatment conditions, genetic constitution of the material, strength of the mutagen used, duration of the treatment etc. (Konzak *et al*., 1965).The mutagens were effective in reducing the survival of M1 plants. The genotypic response varied with respect to mutagenic treatments. High dose/ concentration of mutagenic treatment were recorded highest reduction in survival and lethality percentage. The present findings in respect of dose dependent reduction in survival, mutagen differences and differential response of genotypes to mutagens are in conformity with the earlier reports of Wang *et al.* (2006). The decrease in survival percentage has been attributed to the physiological disturbance or chromosomal damage caused to the cells of the plant by the mutagen. The level of biological damage expressed in terms of percentage reduction in seedling growth parameters induced by different mutagens in both the genotypes. According to Branch *et al*., (1990), gamma rays revealed that seedling height and pollen fertility were very sensitive indicators of biological injury level. Among the two doses of gamma irradiation, the highest reduction in traits was observed at 300 Gy in both the genotypes. Similar effects in groundnut were observed for gamma rays and NaN3 (Mandal *et al.,* 2007).

M_1 generation

The M_1 generation results were observed and recorded. The results all parameters such as days to first flowers, plant height, number of branches per plant, number of leaves per plant, number of cluster per plant, number of pod per plant, number of seed per plant, seed yield per plant, decrease with increase dose of mutagenic treatment.

The effect of gamma rays 10, 20, 30, 40, 50 and 60 KR and Ethyl methane sulphonate treated with various concentrations of EMS (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %) and DES (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %). The morphological and yield characters were significantly reduced seed germination, seedling survival, days to first flower, plant height, number of leaves per plant, number of grains per plant, grain length and breath, 100 grains weight, grain yield per plant, fresh and dry weight per plant. The increasing concentration of EMS and DES decreased in phenotypic and yield characters have been attributed to the physiological difference M_1 generation were carried out. The present results confirm these earlier reports in soybean (Pepol and Pepo, 1989 and Pavadai et al., 2009); mung bean (Khan and Wani 2005) and sesame (Prabhakar 1985).

The effect of Ethyl methane sulphonate treated was survival percentage, mutation frequency and mutagenic effectiveness. The survival percentage and mean value of M1 generation were decreased with increase the dose of treatments. The present results confirm these earlier reports in soybean (Pepol and Pepo, 1989 Cheng, and Chandlee, 1999 and Pavadai et al., 2009); mung bean (Khan and Wani 2005) and sesame (Prabhakar 1985).

Plant height, number of branches, number of pods per plant and seed weight, but rather low for yield per plant were decreased with increase the dose/concentration of treatments. Previous studies have reported the hereditary changes in desirable characters of crop plants using gamma irradiation, which contributed to 64% of radiation-induced mutant varieties

(Ahloowalia et al., 2004, Arulbalachandran, 2006 Amarnath et al.,1991, Balakrishnan, 1991 and Biradar et al.,2006).

M_2 generation

The data on mean performance to the following quantitative characters, such as days to first flowers, plant height, number of leaves per plant, number of kernal per plant, 100seed weight, kernal yield per plant, oil content, protein content fresh weight and dry weight in table 1.

Days to first flower

The maximum days to first flower (37.65 days) was observed at 0.1 per cent EMS treatment while the minimum days to first flower (33.88 days) was observed at 60KR gammarays treatments when compared to control (36.28 days). Most of the treatment decrease the days to first flower were observed .

Plant height

The plant height was positive shift for most of the treatment. The maximum height of the plant was recorded at 50 KR gamma rays treatment (52.89 cm), while the minimum height of the plant was recorded at 0.6% EMS treatment (42.58 cm) when compared to untreated plant(43.87 cm).

Number of leaves per plant

The number of leaves per plant were recorded in positive shift at all treatment. Higher number of leaves were observed in 50KR gammarays (55.35) while the lower number of leaves were observed in 0.1% of EMS concentration (43.26) when compared to control (48.63).

Number of kernal per plant

The number of kernal per plant recorded the positive and negative shift. The positive shift recorded at 30,40, 50 KR of gamma rays treatment and 0.4 , 0.5 per cent EMS treatments. The negative shift were recorded at 10,20, 60 KR of gamma rays treatment and 0.1 , 0.2, 0.3 and 0.6 per cent EMS treatments compared to untreated plant (28.76).

100 seed weight(g)

The 100 seed weight was positive shift for most of the treatment. The maximum 100 seed weight was recorded at 50 KR gamma rays treatment (33.69 g), while the minimum 100 seed weight was recorded at 0.2% EMS treatment (26.55 g) when compared to untreated plant (30.27 g).

Kernal yield/plant(g)

The Kernal yield/plant was positive shift for most of the treatment. The maximum Kernal yield/plant was recorded at 50 KR gamma rays treatment (30.15 g), while the minimum Kernal yield/plant was recorded at 0.1% EMS treatment (23.54 g) when compared to untreated plant (25.64 g).

Protein content

The protein content range between (32.48%-35.86%). The maximum protein content (35.86%) was recorded at 50 KR gamma rays treatment. The minimum protein content (32.48 %) were observed at 0.2% EMS treatment. When compared to control (34.58%).

Oil content

The oil content recorded at positive values for all the mutagenic treatments. The maximum oil content (50.88%) was observed at 50 KR gamma rays treatment. The minimum oil content (45.25%) was observed at 0.2% EMS treatment. When compared to control (49.12 %).

Fresh and Dry weight (g)

TheFresh and Dry weight recorded at positive values for all the mutagenic treatments. The maximum Fresh and Dry weight was observed at 40 KR gamma rays treatment. The minimum Fresh and Dry weight was observed at 0.1% EMS treatment. When compared to control.

5. CONCLUSION

The M_1 generation results were observed and recorded. The results all parameters such as days to first flowers, plant height, number of branches per plant, number of leaves per plant, number of cluster per plant, number of pod per plant, number of seed per plant, seed yield per plant, decrease with increase dose of mutagenic treatment.

In M_2 generation all parameters were recorded in moderate and high value. The highest mean value for all parameters was recorded in 50 KR gamma rays and 0.5 % EMS treatment than the other treatments. The maximum fresh and dry weight were recorded in 40 KR gamma rays treatment.

Table 2. Effect of physical and chemical mutagens on quantitative parameters of groundnut in M_2 generation.

Treatment	Days to first flower	Plant height	No. of leaves/plant (30days)	No. of kernal/ plant	100 Seed weight (g)	Kernal yield/plant (g)	Oil contend	Protein contend	Fresh weight (g)	Dry weight (g)
Control	36.28	43.87	48.63	28.76	30.27	25.64	49.12	34.58	85.36	34.28
GAMMA RAYS 10KR	35.14	44.15	45.24	25.38	28.65	26.41	47.69	33.21	82.40	32.46
20KR	35.07	47.65	46.32	26.54	28.14	28.16	46.88	33.84	88.59	35.10
30KR	35.98	50.11	50.14	29.22	30.45	27.44	50.13	34.66	84.51	35.74
40KR	34.88	50.48	52.87	30.14	31.25	28.98	50.58	35.21	90.04	36.41
50KR	34.32	52.89	55.35	32.87	33.69	30.15	50.88	35.86	90.63	34.78
60KR	33.88	51.25	50.47	27.45	30.14	25.54	48.10	34.26	82.51	31.28
EMS 0.1%	37.65	45.26	43.26	26.31	28.47	23.88	47.36	33.25	80.20	31.55
0.2%	37.01	48.74	45.78	27.54	26.55	23.54	45.25	32.48	85.46	33.02
0.3%	36.44	48.99	51.45	28.58	29.10	26.58	46.36	34.67	87.08	34.55
0.4%	35.25	49.35	52.87	30.11	32.04	28.30	49.65	35.13	86.46	35.54
0.5%	35.10	51.21	53.68	31.88	33.20	28.51	50.41	35.38	88.29	34.18
0.6%	34.98	42.58	51.01	25.36	30.54	25.13	50.84	34.18	80.40	32.90
DES 0.1%	35.21	42.44	46.88	23.01	25.24	23.14	45.51	33.25	75.72	31.25
0.2%	35.18	43.69	47.12	22.57	27.35	25.36	47.12	32.17	74.18	31.01
0.3%	36.58	45.58	48.55	28.45	27.99	25.88	46.39	34.88	76.88	31.89
0.4%	37.31	47.10	48.94	27.63	28.47	26.47	47.17	34.44	80.54	33.26
0.5%	37.19	48.22	46.32	26.50	27.69	24.25	48.59	32.15	86.31	35.69
0.6%	38.55	45.35	45.12	24.25	25.15	23.44	44.05	30.25	81.20	32.78

Table 1. Effect of chemical mutagens on quantitative parameters of groundnut in m_1 generation.

Treatment	Germination %	Seedling survival %	Days to first flower (Days)	Plant height (cm)	No. of leaves/ plant (30days)	No. of kernal/ plant	100 Seed weight (g)	Kernal yield/ plant (g)	Fresh weight (g)	Dry weight (g)
Control	93.00	90.25	35.38	42.58	50.12	29.50	35.30	19.25	95.53	35.28
GAMMA RAYS 10KR	86.59	84.26	36.11	40.05	47.40	25.55	35.10	18.46	90.13	33.88
20KR	77.92	75.50	36.20	40.88	46.25	22.41	35.00	17.55	87.25	31.20
30KR	71.58	69.65	36.95	41.30	45.85	20.13	34.85	17.11	83.44	30.05
40KR	61.45	58.20	37.55	41.80	45.01	18.65	34.44	16.33	80.26	28.55
50KR	52.36	49.45	37.94	42.05	40.22	16.66	32.88	15.42	75.10	26.65
60KR	40.25	37.24	37.98	42.55	35.85	12.54	32.11	14.46	70.45	24.82
EMS 0.1%	88.13	86.56	35.99	40.10	48.36	25.39	34.12	18.00	90.20	33.55
0.2%	81.61	79.24	36.14	38.95	45.25	22.42	33.98	16.25	85.46	31.02
0.3%	73.48	71.49	36.50	37.45	43.58	20.13	33.15	14.36	80.08	29.55
0.4%	60.58	56.28	37.02	36.25	40.10	16.58	31.57	12.80	71.46	26.54
0.5%	51.25	47.10	37.45	32.85	36.26	14.46	31.22	12.14	65.29	24.18
0.6%	41.46	35.85	38.50	30.75	33.75	13.52	30.35	10.55	60.40	22.90
DES 0.1%	86.45	83.40	36.05	41.95	47.15	26.95	34.80	18.42	88.66	32.44
0.2%	75.14	74.59	36.88	37.45	46.59	25.38	34.21	17.56	82.15	30.64
0.3%	63.87	60.20	37.20	35.29	44.51	22.75	33.76	16.58	78.50	28.77
0.4%	50.48	47.89	38.00	33.85	40.30	20.15	33.52	15.50	69.47	25.19
0.5%	44.03	42.05	38.75	31.75	37.18	18.56	31.85	13.36	65.28	23.53
0.6%	32.04	30.21	39.56	29.65	35.42	15.57	31.02	11.55	60.07	21.75

References

[1] Arulbalachandran (2006). Effect of physical and chemical mutagenesis in lack gram (Vigna mungo (L.) Hepper.). Ph.D.,Thesis, Faculty of Science, nnamalai University, Annamalai Nagar, India.

[2] Amarnath, K.C.N., S.R. Viswanatha and G. Shivashankar, 1991. Genotypic and phenotypic variability and heritability of some quantitative characters in soybean (Glycine max (L.) Merill). *Mysore J. Agric. Sci*., 25: 26-31.

[3] Balakrishnan, P.C., 1991. Induced mutagenesis in soybean (Glycine max (L.) Merill). Ph.D. Thesis, Tamil Nadu Agrl. Univ., Coimbatore.

[4] Biradar, K.S., P.M. Salimath and R.L. Ravikumar, 2006. Genetic variability for seedling vigour, yield and yield components in local germplasm ollections of Green gram (Vigna radiata (L.)Wilckzek). *Kar. J. Agr. Sci*., 20 (3): 608-609.

[5] Cheng, T.S. and J.M. Chandlee, 1999. The structural, biochemical and genetic characterization of a new radiation-induced, variegated leaf mutant of soybean (Glycine max (L.) Merr.). *Proc. Natl. Sci. Counc*., 23(1): 27-37.

[6] Khan, S. and M.R. Wani, 2005. Genetic variability and correlations studies in chickpea mutants. *J. Cytol. Genet*., 6(2): 155-160.

[7] Pavadai, P., Girija, M. and Dhanavel, D., M. Effectiveness and efficiency and biochemical content of physical and chemical mutagens in soybean (Glycine max (L.) Merr.). *Journal of Phytology*, 1 (6): 444-447, 2009.

[8] Pepol, P. and P. Pepo, 1989. Preliminary experiment on inducing soybean mutants by fast neutron seed irradiation. Soybean Abstracts,12(5): 4-7.

[9] Prabhakar, L.V., 1985. Studies on induced mutagenesis in Sesamum indicum L. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

[10] FAO, 2007, Food and Agricultural Organization of the United Nations, FAOSTAT database, http://www.FAO.ORG

[11] Branch, W. D., Nakayama, T. and Chinnan, A., 1990, Fatty acid variation among U. S. runnertypepeanut cultivars. *J. Am. Oilseed Chem.*, 67: 591-593

[12] Frankel, E. N., 1991, Recent advances in lipid oxidation. *J. Sci. Food Agri.*, 54:495-511

[13] Konzak, C. F., Nkan, R. A., Wagner, J. and Foster, R. J., 1965, Efficient chemical mutagenesis-the use of induced mutations in plant breeding. *Rad. Bot.*, 5: 49-70

[14] Lands, W. E. M, 1997, The two faces of essential fatty acids. *INFORM*, 8: 1141- 1147.

[15] Mandal, S., Badigannavar, A. M., Kale, D. M. and Murty, G. S. S., 2007, Induction of genetic variability in a disease-resistant groundnut breeding line. *BARC News Lett.*, 285: 237-246

[16] Ory, R. L., Crippen, K. L. and Lovegren, N. V., 1992, Off flavors in foods and beverages, Elsevier Science Publishers, New York.

[17] Wang, C. T., Yang, X. D., Tang, Y. Y., Zhang, T. C., Xu, T. Z. and Lin, G. Z., 2006, EMSinduced variations in pod characters of peanut. *Electronic J. Env. Agri. Food Chem.*, 2427-2433.

(Received 26 February 2015; accepted 03 March 2015)