Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 2, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON CYTOGENETIC EFFECTS INDUCED BY TREATMENTS WITH DIFFERENT MUTAGENIC SUBSTANCES IN *Arachis hypogaea* L. (ARAHIDS)

Oana MÎRZAN[1], Margareta NAIE[1], Diana BATÎR RUSU[2], Mihai STAVARACHE[3], Elena STAVARACHE[3], Constantin LUNGOCI[4]

[1]Agricultural Research - Development Station Secuieni, 377 Principala Street, Secuieni, 617415, Neamţ County, Romania
[2]Plant Genetic Resources Bank "Mihai Cristea", 17, 1 Mai Avenue, 720224, Suceava County, Romania
[3]Development Station - Meadows Development Vaslui, 256 Stefan cel Mare Street, Vaslui, 730006, Vaslui County, Romania
[4]"Ion Ionescu la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Street, 700490, Iasi County, Romania

Corresponding author email: dia_sv@yahoo.com

Abstract

The mitotic division, from a genetic point of view, ensures the copying of the genetic message in duplicate, transferring it to the descending cells and transmitting the genetic message from one cell to another within an individual (organism), maintaining the constants, number, shape and size of the chromosomes. In mitosis, the processes that take place are irreversible. As a result of mitosis, each of the two daughter cells has a diploid number of chromosomes (2n) in which DNA is included which gives the identity of the genetic information with the mother cell Mitosis interests in a special way, due to the wide possibilities offered by the karyotype study, a basic component in the identification of species. The mitotic index is one of the parameters successfully used in cytogenetic studies of mitosis. The main purpose of the research was to capture the cytogenetic effects induced by the mutagenic treatments in arahids species (Arachis hypogaea L.). For cytogenetic investigations, radicular meristems obtained from the germination of peanut seeds were used, which were later used to obtain microscopic preparations by the method developed by Feulgen. Following the research carried out it was established that the most pronounced mutagenic effect of mitotic indecision reduction was obtained after sodium azide treatments. In the case of ethyl metalsulfonate and dimethyl sulfate, there were noted increases in mitotic indecin at concentrations of 0.2% and 0.4%.

Key words*: peanuts seeds, ethyl metansulfonate (EMS), dimethyl sulfate (DMS), sodium azide (SA), mitosis.*

INTRODUCTION

The number of somatic chromosomes in *Arachis hypogaea* L. is equal to 40 (2n = 40) (Gregory et al, 1973; Resslar, 1980; Stalker, 1991; Dhesi and Stalker, 1994; Krapovickas and Gregory, 1994; Lavia Graciela Inés, 1996; Lavia Graciela Inés and Fernandez, 2004, Neiva et al, 2001; Peñaloza and Valls, 2005; Valls and Simpson, 2005; Silvestri et al, 2015; Ortiz et al, 2017, Dima et al, 2023).

One of the most important early indicators of mutagenic action of any physical, chemical or biological mutagenic agent is the frequency of chromosomal aberrations in mitosis (Kumar and Sing, 2003; Bhat et al, 2007; Kumar and Yadav, 2010; Jafri et al., 2013).

Important mutant lines have been developed in production after treatment with ethyl metansulfonate on peanut seeds. Previous attempts to induce mutations using chemicals have reported alternations on morphological characteristics, but rarely mentioned whether there were changes on the quality characteristics of peanuts (Jung et al., 2000; Wang et al., 2006). Mutant plants obtained by treatment with sodium azide are able to survive in different environmental conditions and have improved harvests, increased tolerance to stress, and longer life compared to untreated plants (Quarainy and Khan, 2009).

The successful use of sodium azide to generate variability in plant breeding has been discussed in barley (Kleinhofs and Sander, 1975) and other

culture species (Avila and Murty, 1983; Micke, 1988; Routaray et al., 1995). The dose with the most pronounced effect for inducing morphological mutations was established at 0.03% sodium azide. The main advantage of improvement using mutations is the ability to improve one or two characteristics without altering the rest of the genotype (Mensah and Obadony, 2007; Seijo et al., 2017).

Dimethyl sulfate is a colourless or slightly yellowish liquid with a slight onion odour. The substance is toxic, corrosive and mutagenic (Rippey and Stallwood, 2005; Pohanish, 2008). Dimethyl sulfate may affect specific cleavage in DNA by breaking the imidazole rings present in guanine (Cartwright and Kelly, 1991; Rippey and Stallwood, 2005).

Sodium azide is considered to be a very potent chemical mutagen in culture plants. Mutant plants resulting from sodium azide treatment are able to survive in different environmental conditions, they have a better yield and are stress tolerant compared to normal plants (Fahad and Salim, 2009; Alka Ansari et al, 2012; Kumar and Srivastava, 2013).

The mutagenic effect results from the production of an inorganic sodium azide metabolite. This metabolite enters the nucleus, interacts with DNA and creates point mutations in the plant genome (Owais and Kleinhofs, 1988).

Ethyl metansulfonate causes the introduction into the nucleotide structure of some alkyl groups: at the atom level of the 7th position of the guanidine structure (most often), at the 3rd position of adenine, the, very rare in 1st position of adenine (Kumar and Rai, 2005; Srivastava and Kapoor, 2008; Srivastava et al., 2011; Girjesh Kumar and Kshama Dwivedi, 2013). Also, the presence of alkyl group in guanidine weakens the ß-glucoside binding and causes the elimination of guanine (depuration), with the formation of breaches in the DNA structure (Kumar and Dwivedi, 2013).

Pyrimidine nitrogen bases are more resistant to alkylating agents, although ethyl metansulfonate can sometimes cause cytosine alkylation. Alkylation is accompanied by DNA distortion or cross-linking or transverse nucleotides, which prevents normal mating. Ethyl metansulfonate is highly effective in modifying genetic material of both prokaryotic and eukaryotic cells (Roychowdhury et al., 2012).

The main purpose of the research carried out during 2013-2015 period was to capture the cytogenetic effects induced by the mutagenic treatments in two peanut genotypes.

MATERIALS AND METHODS

The biological material used for research was represented by *Arachis hypogaea* L. seeds, varieties Tâmbureşti and Jelud, varieties created at the University of Craiova, Romania.

The mutagenic substances used to induce variability were ethyl metansulfonate (EMS), dimethyl sulfate (DMS) and sodium azide (SA). Ethyl metansulfonate and dimethyl sulfate were in concentrations of 0.2%, 0.4%, 0.6% and 0.8%, and sodium azide was in concentrations of 0.02%, 0.04%, 0.06% and 0.08%, with each concentration having an action time of 6 hours. The control variant was represented by each variety, to which no mutagenic treatments were applied.

For the identification of chromosomal aberrations and their frequencies, the classical method was used which involves the following stages (Ţîrdea and Leonte, 2003): fixing, hydrolysis, coloring, performing microscopic preparations, examining preparations under a microscope.

Fixation was done when the roots reached the length of 10-15 mm. These were placed in the Carnoy I fixator (absolute ethyl alcohol and glacial acetic acid at 3:1 ratio) at room temperature for 24 hours (Figure 1).

Figure 1. Fixing, hydrolysis and coloring of peanuts roots

Fixation is intended to kill the cells in the state they are in at that time, a process that takes place very quickly, almost instantly. By fixation, the fine structure of all cellular organelles must be preserved. In addition, the biocoloids of the cell coagulate and as a result, the cellular constituents can be colored with different dyes. Until the roots were processed for coloring, they were stored in the refrigerator, in ethyl alcohol 70%.

Hydrolysis aimed the maceration of tissues by partial dissolution of peptic substances between cells, which facilitates the process of staining and then display of cells between the blade and the slide.
The fixed roots were washed with distilled water, then introduced into HCl 1 N solution and left for 14 minutes at 60°C (Figure 1).
Coloring was done by keeping the roots in Carr solution in the refrigerator, until the tip of them became intensely colored (about 24 hours).
The area of the root apex has been colored faster and more intense because here most of the cells are in division, while the rest of the root remained visibly uncolored because the frequency of divisions was lower (the cells being large and elongated).
This dye caused the nuclei and chromosomes to color in red-violaceus, the cytoplasm being pale pink (Figure 1).

Making microscopic preparations, after the squash method was done on a clean, dry and degreased microscope blade, in a drop of Carr dye with the help of tweezers put the roots of a peanut grain.
Using a spatula needle or a shaving blade, 1-2 mm of the colored tip of the roots (of a single grain) were cut over which a clean and degreased. With the help of a filter paper, the blade was fixed so that excess Carr dye was absorbed and at the same time the slide of the blade onto the blade was avoided. With the help of a match stick hit the lemon (in the area where the root is located) at first weaker, then stronger, then stronger, to ensure perfect display of metaphasic cells and chromosomes.
After the display, between the blade and the slide, a very fine, barely perceptible layer of red-violaceus coloured material was observed with the naked eye. After the display was finished, a piece of filter paper was placed over the blade, gently pressing to absorb excess acetic acid, to complete the cell display, and, dispersion of chromosomes and so that the cells to cling to the blade, but especially to the slide and not fall when performing permanent preparations. And this operation was carried out with great care to avoid sliding the blade on the blade, from the initial site, which leads to cell rolling and partial or total compromise of the preparation.
The examination of the preparations under the microscope was carried out in bright light, at first with the 10x lens, with which the preparation is examined in the sea, and the reading of the preparation was done at the 40x objective. The data obtained were used to calculate the mitotic index and the frequency of cell types in mitotic division.
The photos were taken using the Cannon digital camera, adapted to the Hund - Wetzlar optical microscope at the 100x immersion lens.
Photographs were taken of the interphase, prophase, metaphase, anaphase and various types of chromosomal aberrations encountered.
Following the analysis of mitosis phases, the mitotic index was determined. This represents the percentage of cells in division, compared to the total number of cells analyzed (Tirdea and Leonte, 2003). The mitotic index will be calculated using the following relation:

$$I_m\,(\%) = (N_m \times 100)\cdot N_t^{-1}, \text{ where:}$$

I_m = mitotic index;
N_m = number of cells in division;
N_t = total number of cells analyzed.

RESULTS AND DISCUSSIONS

In the Tâmbureşti variety, the mitotic index in the case of the control variant had the value of 18.04%. Table 1 shows that the mitotic index decreased as the concentration of the mutagenic chemical increased.
In the case of ethyl metansulfonate at a concentration of 0.2%, the mitotic index was higher, with a distinctly significant difference (5.12%). In the 0.4% concentration of ethyl metansulfonate, the mean value of the mitotic index was 13.17% and the concentration of 0.8% was 13.55%, the differences from the control being negative, distinctly significant (4.87% and 7.44%, respectively).

Table 1. Effect of treatments with mutagenic agents on mitotic index (%) of the Tâmbureşti variety

Variant		Average (%)	Diff. (%)	Signif.
Control		18.04	Control	Control
EMS 6 h	0.2%	23.16	5.12	**
	0.4%	13.17	-4.87	oo
	0.6%	13.55	-4.49	o
	0.8%	10.60	-7.44	oo
	LSD 0.05 = 3.1; LSD 0.01 = 4.7; LSD 0.001 = 7.5			
DMS 6 h	0.2%	27.95	9.91	***
	0.4%	23.78	5.74	***
	0.6%	14.00	-4.04	oo
	0.8%	9.82	-8.22	ooo
	LSD 0.05 = 2.2; LSD 0.01 = 3.4; LSD 0.001 = 5.4			
SA 6 h	0.02%	16.60	-1.44	
	0.04%	13.07	-4.97	oo
	0.06%	10.35	-7.69	ooo
	0.08%	9.59	-8.45	ooo
	LSD 0.05 = 2.3; LSD 0.01 = 3.4; LSD 0.001 = 5.5			

The decrease in the percentage of cells in the division was also found in the treatment with dimethyl sulfate at a concentration of 0.6% (14.0%) and 0.8% (9.82%), the differences with the witness are distinctly significant and very significant.

All dimethyl sulfate in the concentration of 0.2% and 0.4% had a strong stimulating effect, the mitotic index being higher than that of the control variant.

Sodium azide treatments had a more pronounced effect, negatively influencing the mitotic index at all concentrations of the chemical agent mutagen. In the 0.02% concentration, the mean mitotic index was 16.6%, the difference from the control variant being insignificant (Table 1). At 0.04% sodium azide treatment the difference from the control was distinctly significant (4.97%). Concentrations of 0.06% (7.69%) and 0.08% (8.45%) showed a sharp decrease in the percentage of cells in the division, the differences being very significant compared to the untreated control variant (Table 1).

From the analysis of the mitotic index, at the Tâmbureşti variety, it can be seen that the most pronounced mutagenic effect of reducing this value was recorded at the sodium azide treatments followed by those with ethyl metansulfonate and finally those with dimethyl sulfate. An increase in the mitotic index was observed in the concentration of 0.2% and 0.4% of dimethyl sulfate and 0.2% of ethyl metansulfonate. These concentrations have a slight stimulating effect on cell division (Table 1).

For the Jelud variety the mean value of the untreated control variant was 17.68% cells in the division (Table 2).

Table 2. Effect of treatments with mutagenic agents on mitotic index (%) of the Jelud variety

Variant		Average (%)	Diff. (%)	Signif.
Control		17.68	Control	Control
EMS 6 h	0.2%	17.68		
	0.4%	23.00	5.32	***
	0.6%	20.50	2.82	**
	0.8%	13.17	-4.51	ooo
	LSD 0.05 = 1.4; LSD 0.01 = 2.2; LSD 0.001 = 3.5			
DMS 6 h	0.2%	24.50	6.82	***
	0.4%	16.12	-1.56	oo
	0.6%	12.26	-5.42	ooo
	0.8%	8.42	-9.26	ooo
	LSD 0.05 = 0.9; LSD 0.01 = 1.4; LSD 0.001 = 2.3			
SA 6 h	0.02%	15.63	-2.05	o
	0.04%	12.27	-5.41	ooo
	0.06%	10.09	-7.59	ooo
	0.08%	8.40	-9.28	ooo
	LSD 0.05 = 1.5; LSD 0.01 = 2.3; LSD 0.001 = 3.6			

In the Jelud variety, treatment of peanut seeds with ethyl metansulfonate influenced the number of dividing cells compared to the control sample as the concentration of the mutagen increases. At the concentration of 0.2% an increase in the mitotic index was observed, the difference from the control being very significant (5.32%). Conversely, the 0.6% and 0.8% following ethyl metansulfonate treatment have very significant negative differences (4.51% and 8.30%, respectively) compared to the untreated control sample (Table 2).

Dimethyl sulfate at a concentration of 0.2% had a strong stimulating effect, with the mitotic index being higher than that of the control variant. With the increase in concentration, the percentage of cells in the division of dimethyl sulfate treatments appears to be reduced at a concentration of 0.4% (1.56%), 0.6% (5.42%) and 0.8% (9.26%), the differences from the control variant being distinctly significant and very significant (Table 2).

All dimethyl sulfate in the concentration of 0.2% and 0.4% had a strong stimulating effect, the mitotic index being higher. In the case of sodium azide, the mitotic index decreased as the concentration of the mutagenic chemical increased (Table 2).

Thus, the mitotic index recorded lower values, the differences being negative, significant at the concentration of 0.02% (2.05%) and very significant at the concentrations of 0.04%

(5.41%), 0.06% (7.59%) and 0,08% (9.29%) of the untreated control variant (Table 2).

In the control variant of the Tâmburești variety, chromosomal aberrations were identified in a proportion of 0.84%. Treatments with mutagenic substances in different concentrations caused chromosomal aberrations to occur in a higher percentage, the differences from the control variant were very significant. Exception to this rule were treatments with dimethyl sulfate at a dose of 0.2% (5.31%), the difference being significant compared to the untreated control variant (Table 3).

Table 3. Effect of treatments on frequency of chromosome aberrations (%) in mitosis of Tâmburești variety

Variant		Average (%)	Diff. (%)	Signif.
Control		0.84	Control	Control
EMS 6 h	0.2%	6.12	5.28	***
	0.4%	10.14	9.30	***
	0.6%	11.27	10.43	***
	0.8%	14.53	13.69	***
	LSD 0.05 = 1.8; LSD 0.01 = 2.7; LSD 0.001 = 4.4			
DMS 6 h	0.2%	6.15	5.31	*
	0.4%	13.11	12.27	***
	0.6%	14.69	13.85	***
	0.8%	20.32	19.48	***
	LSD 0.05 = 4.3; LSD 0.01 = 6.6; LSD 0.001 = 10.5			
SA 6 h	0.02%	5.41	4.57	***
	0.04%	6.57	5.73	***
	0.06%	11.29	10.45	***
	0.08%	15.36	14.52	***
	LSD 0.05 = 1.6; LSD 0.01 = 2.4; LSD 0.001 = 3.9			

Treatment with sodium azide at 0.02% resulted in a distinctly significant difference from the untreated control variant (5.47%).

Chromosomal aberrations identified in metaphase, anaphase and telophase of mitosis were obtained and photographed in microscopic preparations, resulting in the total frequency of chromosomal aberrations (Figures 2-5).

Like the Tâmburești variety, in the Jelud variety, most of the treatments made at peanut seeds have determined positive differences, very significant, compared to the untreated control variant (Table 4).

Ethyl metansulfonate at a concentration of 0.2% resulted in an increase in the number of chromosomal aberrations, with significant differences from the control variant (7.04%). Dimethyl sulfate at a concentration of 0.4% (4.01) produced an increase in the number of chromosomal aberrations, with distinctly significant differences from the control variant (Table 4).

Table 4. Effect of treatments on frequency of chromosome aberrations (%) in mitosis of Jelud variety

Variant		Average (%)	Diff. (%)	Signif.
Control		0.82	Control	Control
EMS 6 h	0.2%	7.86	7.04	*
	0.4%	13.19	12.37	**
	0.6%	19.85	19.03	***
	0.8%	20.70	19.88	***
	LSD 0.05 = 6.0; LSD 0.01 = 9.1; LSD 0.001 = 14.6			
DMS 6 h	0.2%	2.41	1.59	
	0.4%	4.83	4.01	**
	0.6%	14.05	13.23	***
	0.8%	16.36	15.54	***
	LSD 0.05 = 2.4; LSD 0.01 = 3.7; LSD 0.001 = 5.9			
SA 6 h	0.02%	6.29	5.47	**
	0.04%	13.04	12.22	***
	0.06%	19.82	19.00	***
	0.08%	22.83	22.01	***
	LSD 0.05 = 3.5; LSD 0.01 = 5.3; LSD 0.001 = 8.5			

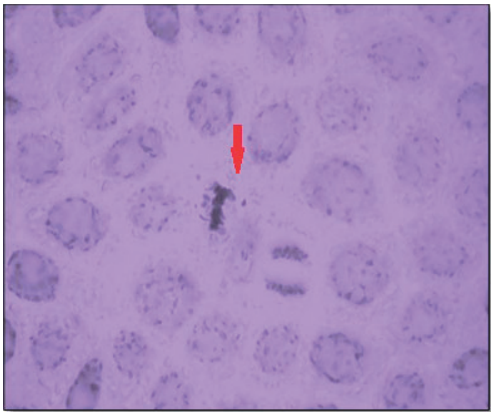

Figure 2. Metaphase, with the remaining chromosome and fragment

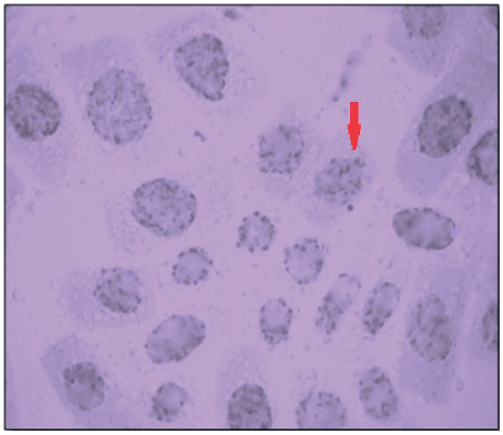

Figure 3. Telophase with micronucleus

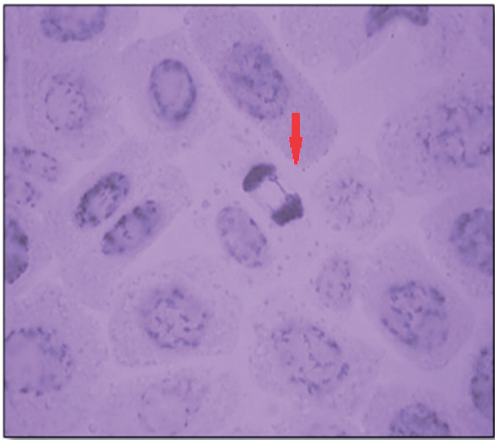

Figure 4. Anaphase with decks

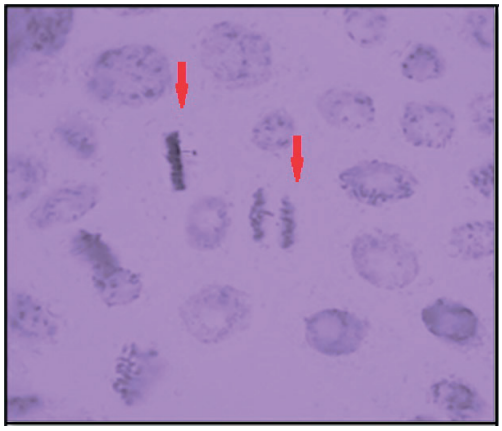

Figure 5. Metaphase with fragment and anaphase with bridge

CONCLUSIONS

As a result of the research carried out, the strongest mutagenic effect of mitotic index value reduction was recorded for sodium azide treatments followed by those with ethyl metansulfonate and dimethyl sulfate.

As the time of action of the mutagenic agent increases, a reduction in the mitotic index occurs. This is demonstrated by the presence of a smaller number of cell divisions, inversely proportional to the exposure time.

REFERENCES

Alka, Ansari M.Y.K., Towseef, M.B., Sana, C., Rumana, A. (2012). Genotoxic effect of EMS and Sodium Azide in Linum usitatissimum L. *International Journal of Plant, Animal and Environmental Sciences, 2.*

Avila, R., Murty, B.R. (1983). Cowpea and mungbean improvement by mutation induction. *Mutation Breeding Newsletter, 21:9-14.*

Bhat, T.A, Sharma, M., Anis, M. (2007). Comparative analysis of meiotic aberrations induced by diethylsulphonate and sodium azide in broad bean (*Vicia faba* L.) .*Asian Journal of Plant Sciences, 6:1051-1057.*

Cartwright, I.L., Kelly, S.E., 1991. *Probing the nature of chromosomal DNA protein content by in vivo footprinting.* Biotechniques, 11:188-190.

Dhesi, J.S., Stalker, H.T. (1994). Enhancing tehniques for studying mitotic peanut chromosomes. *Peanut Science, 21:92-96.*

Dima, M., Paraschivu, M., Partal, E., Diaconu, A., Drăghici, R., Titirica, I. (2023). *The impact of the sowing time on peanuts yield`s components in marginal sandy soils in Southern Oltenia, Romania. Romanian Agricultural Research, 40:307-316.*

Fahad A.Q., Salim K. (2009). Mutagenic effects of sodium azide and its application in crop improvement. *World Applied Sciences Journal, 6:1589-1601.*

Girjesh, K., Nitisha, S. (2013). *Efficiency and Effectiveness of Gamma Rays and Sodium Azide in Sesbania cannabina Poir. The Japan Mendel Society Cytologia ,78(1):1-10.*

Gregory, W.C., Gregory, M.P., Krapovickas, B., Smith, W. (1973). Structures and genetic resources of peanuts. *In peanuts- Culture and uses, American Peanut Research and Education Association, 47-133.*

Jafri, I.F., Khan, A.H., Gulfishan, M. (2013). Genomic damage induced by individual and combination treatment of gamma rays and ethyl methane sulphonate in *Coriandrum sativum* L. var. karishma. *International Journal of Botany, 2(3):pag. 79-86.*

Jung, S., Swift, D., Sengoku, E., Patel, M., Teule, F., Powel, G., Abbott, A. (2000). The high oleate trait in the cultivated peanut (*Arachis hypogaea* L.). Isolation and chraracterization of two genes encording microsomal oleoyl - PC desaturases. *Mol. Gen. Genet., 263:796-805.*

Kleinhofs, W., Sander, C. (1975). Azide mutagenesis in Barley. *Thrid Barley Genetics Simp. Garching. Proceedings of Symp, 1:113-122.*

Krapovickas, A., Gregory, P.C. (1994). Taxonomia del genero Arachus (*Leguminosae*). *Bonplandia, 8:1-186.*

Kumar, G., Singh, V. (2003). Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in barley. *JIBS, 82:19-22.*

Kumar, G., Rai, P. (2005). EMS induced genetic vriability in soybean (Glycine max). *The Nucleus, 48:46-51.*

Kumar, G., Yadav, R.S. (2010). Ems induced genomic disorders in sesame (*Sesamum indicum* L.). *Rom. J. Biol. - Plant Biol.,55(2):97-104.*

Kumar, G., Srivastava, N. (2013). Efficiency and Effectiveness of Gamma Rays and Sodium Azide in Sesbania cannabina Poir. *The Japan Mendel Society Cytologia, 78(1):1-10.*

Kumar, G., Kshama, D. (2013). Sodium Azide Induced Complementary Effect of Chromosomal Stickiness in Brassica campestris L. *Jordan Journal of Biological Sciences, 6:85-90.*

Lavia, G.I., Fernandéz, A., 2004. *Karyotypic studies in Arachis hypogaea* L. *varieties.* Caryiologia, 57(4):353-359.

Mensah, J.K., Obadony, B. (2007). Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.). *African Journal of Biotechnology, 6 (6): 668-671.*

Micke, A. (1988). Improvement of grain legume production using induced mutations IAEA, *Viena.*

Neiva, P.I., Leticia, G.M., Catalina, L.R. (2001). Application of C-banding in two Arachis wild species, Arachis Pintoi Krapov. And W.C. Gregory and A. villosulicarpa Hoehne to mitotic chromosome analyses. *Caryologia, 54(4):377-384.*

Ortiz, A.M., Robledo, G.A., Seijo, J.G., Valls, J.F.M., Lavia G.I. (2017). Cytogenetic evidences on the evolutionary relationships between the tetraploids of the section Rhizomatosae and related diploid species *(Arachis, Leguminosae). J Plant Res 130:791-807.*

Owais, W.M., Kleinhofs, A. (1988). Metabolic activation of the mutagen azide in biological systems*. Mutat. Res. 197: 313-323.*

Peneloza, A.P.S., Valls, J.F.M (2005). Chromosome number and satellite chromosome morphology of eleven species of Arachis. *Bonplandia, 14:65-72.*

Resslar, P.M. (1980). A review of the nomenclature of genus Arachis. *Euphytica, 29:813-817.*

Rippey, J.C.R., Stallwood, M.I. (2005). Nine cases of accidental exposure to dimethyl sulphate - a potential chemical weapon. *Emergency Medicine Journal, 22:878-879.*

Routaray, B.N., Mishra, R.G., Das, S.N. (1995). Genetic variability and effectiveness of some chemical mutagens of blackgram in relation to resistance source against Meloidogyne incognita. *Curr. Agric. Res., 8:3-4.*

Roychowdhury, R., Sudipta, D., Momezul, H., Tamoghno, K.M., Dipika, M., Parenneeta, G., Dipika, D., Jagatpati, T. (2012). Effect of EMS on genetic parameters and selection scope for yield attributes in M2 mungbean (*Vigna radiata* L.) Genotypes. *Romanian Journal of Biology Plant, 57: 88-99.*

Quarainy, F., Salim, K. (2009). Mutagenic effects of sodium azide and its application in crop improvement. *World Applied Sciences Journal. 6:1589-1601.*

Seijo, G, Samoluk, S.S., Ortiz, A.M., Silvestri, M.C., Chalup, L., Robledo, G., Lavia, G.I. (2017). Cytological features of peanut genome. In: Varshney R, Pandey M and Puppala N (Eds). *The peanut genome, compendium of plant genomes series, 2:37-52.*

Silvestri, M.C., Ortiz, A.M., Lavia, G.I. (2015). rDNA loci and heterochromatin positions support a distinct genome type for 'x = 9 species' of section Arachis (Arachis, Leguminosae). *Plant Syst. Evol., 301: 555-562.*

Stalker, H.T. (1991). A new species in section Arachis of peanuts with A D genome, *American Journal of Botany, 78:630-637.*

Srivastava, A., Kapoor, K. (2008). Seed yield is noimpared by chromosome stickiness in Sodiun Azide treated Trigonella foenum-graecum. *Cytologia, 73(2):115-121.*

Srivastava, P., Marker, S., Pandey, P., Tiwari, Z.D.K. (2011). Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (Triticum aestivum L. em. Thell)*. Asian J Plant Sci.,10(3):*190-201.

Ţîrdea, G., Leonte, C. (2003). *Citogenetică vegetală - metode de laborator.* Ed. Ion Ionescu de la Brad, Iaşi.

Valls, J.F.M., Simpson, C.E. (2005). New species of Arachis from Brazil, Raraguay. *Bonplandia,14:35-63.*