THE EFFECT OF MUTAGENIC FACTORS ON THE VIABILITY OF *IN VITRO* EXPLANTS

Köteles Nándor*, Pereş Ana Cornelia*

*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania, e-mail: kotelesnandor@yahoo.com

Abstract

This paper deals with the effects of mutagenic agents on the viability of in vitro explants. Six bird's foot trefoil species were included in the study: Alina, Nico, Suceava1, Suceava2, Suceava3 and Suceava4.

Looking at the effects of different concentrations added to the culture medium, it can be seen that a high concentration of the mutagenic agent causes the death of a significant number of plants, whereas a lower dose will decrease the incidence of mutations, but the effect on the viability of the biological material can be beneficial.

The results show that the decrease in the neo-plantlets' viability depends on the genotype and on the concentration of the mutagenic agent used. In the case of the genotypes included in the study, the lethal dose (LD50%) is represented by a concentration of 2.0 ppm when the DES (diethyl sulphate) mutagenic agent is used, the number of viable plantlets for the Alina and Nico species being 47% and 42% respectively.

Regarding the second mutagenic agent used, DMS (dimethyl sulphate), the LD50% values are close to those obtained with the DES (diethyl sulphate) mutagenic agent for a concentration of 2.0 ppm, that is, 48% viable neo-plantlets for the Alina species and 43% for the Nico one.

Key words: bird's foot trefoil, diethyl sulphate, dimethyl sulphate, mutagenic agent, neo-plantlets.

INTRODUCTION

The success of a breeding program depends directly on the availability of genetic variability sources. In this respect, an important step was made when the possibility of obtaining genetic variability by inducing gene mutations and recombinations was discovered at the beginning of the 20th century (Savatti M., et al., 2004).

The first *in vitro* mutants were induced and detected by Carlson P. S., (1975) and Binding (1970).

Using *Nicotiana tabacum,* Maliga et. al (1973) obtained cells from which they regenerated plants resistant to streptomycin, a characteristic passed down through the maternal line, a fact that shows a cytoplasmic mutation.

An important practical aspect is to obtain mutant lines with increased productions of amino acids. Thus, Hibberg (1978) obtained maize cell lines with a high methionine, lysine and isoleucine synthesis capacity.

Using amino-ethyl-cysteine, Charleff and Carlson (1975), obtained *Oryza sativa* mutant cell lines which have the capacity to accumulate a double quantity of lysine.

Despite all its advantages, *in vitro* mutagenesis also has some limitations. A number of agronomic characteristics cannot be manipulated during the genotype-medium as they would be at cell level. Several characteristics are expressed at the level of the entire plant, without being revealed at cell level (Tămaş Elena, 1998).

MATERIAL AND METHODS

In order to determine the viability of explants and the LD50% lethal dose for the six bird's foot trefoil species, Alina, Nico, Suceava1, Suceava2, Suceava3 and Suceava4, included in the study DES (diethyl sulphate) and DMS (dimethyl sulphate) were used as mutagenic factors, with concentrations between 0.001 and 3.00 ppm.

RESULTS AND DISCUSSION

Looking at the effects of different concentrations added to the culture medium, it can be seen that a high concentration of the mutagenic agent causes the death of a significant number of plants, whereas a lower dose will decrease the incidence of mutations, but the effect on the viability of the biological material can be beneficial. Therefore, an optimum of concentration is compulsory, but this can be rarely established precisely. In practice, prior experience is used to establish a dose and an appropriate treatment method which would not lead to a drastic lethality, while still producing a good percentage of mutations (Table 1, Table 2).

Table1

The effect of treatment with diethyl sulphate on the viability of explants and the determination of lethal dose DL50% for bird's foot trefoil

Genotype	Mutagenic factor	Concentration	No. of treated explants	Viable explants	% Percentage of viable explants	Unviable explants	% Percentage of unviable explants
Alina	DES Diethyl sulphate	v_0 – untreated	100	100	100	-	-
		$v_1 = 0.001$	100	96	96	4	4
		$v_2 = 0.01$	100	86	86	14	14
		$v_3 = 0.1$	100	82	82	18	18
		$v_4 = 0.02$	100	74	74	26	26
		$v_5 = 0.2$	100	63	63	37	37
		$v_6 = 2.0$	100	47	47	53	53
		$v_7 = 3.0$	100	28	28	62	62
Nico	DES Diethyl sulphate	v_0 – untreated	100	100	100	-	-
		$v_1 = 0.001$	100	92	92	8	8
		$v_2 = 0.01$	100	83	83	17	17
		$v_3 = 0.1$	100	76	76	24	24
		$v_4 = 0.02$	100	70	70	30	30
		$v_5 = 0.2$	100	61	61	39	39
		$v_6 = 2.0$	100	42	42	58	58
		$v_7 = 3.0$	100	30	30	70	70

continuation Table 1

Suceava 1	DES Diethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	94	94	6	6
		v_2 = 0.01	100	85	85	15	15
		v_3 = 0.1	100	80	80	20	20
		v_4 = 0.02	100	73	73	27	27
		v_5 = 0.2	100	64	64	36	36
		v_6 = 2.0	100	39	39	61	61
		v_7 = 3.0	100	25	25	75	75
Suceava 2	DES Diethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	84	84	16	16
		v_2 = 0.01	100	84	84	16	16
		v_3 = 0.1	100	72	72	28	28
		v_4 = 0.02	100	70	70	30	30
		v_5 = 0.2	100	61	61	39	39
		v_6 = 2.0	100	42	42	58	58
		v_7 = 3.0	100	24	24	76	76
Suceava 3	DES Diethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	82	82	18	18
		v_2 = 0.01	100	82	82	18	18
		v_3 = 0.1	100	73	73	27	27
		v_4 = 0.02	100	68	68	32	32
		v_5 = 0.2	100	63	63	37	37
		v_6 = 2.0	100	43	43	57	57
		v_7 = 3.0	100	25	25	75	75
Suceava 4	DES Diethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	90	90	10	10
		v_2 = 0.01	100	88	88	12	12
		v_3 = 0.1	100	70	70	30	30
		v_4 = 0.02	100	68	68	32	32
		v_5 = 0.2	100	61	61	39	39
		v_6 = 2.0	100	40	40	60	60
		v_7 = 3.0	100	23	23	77	77

Table2

The effect of treatment with dimethyl sulphate on the viability of explants and the determination of lethal dose DL50% for bird's foot trefoil

Genotype	Mutagen factor	Concentration	No. of treated explants	Viable explants	% Percentage of viable explants	Unviable explants	% Percentage of unviable explants
Alina	DMS Dimethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	93	93	7	7
		v_2 = 0.01	100	88	88	12	12
		v_3 = 0.1	100	77	77	23	23
		v_4 = 0.02	100	68	68	32	32
		v_5 = 0.2	100	60	60	40	40
		v_6 = 2.0	100	48	48	52	52
		v_7 = 3.0	100	28	28	72	72
Nico	DMS Dimethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	95	95	5	5
		v_2 = 0.01	100	83	83	17	17
		v_3 = 0.1	100	78	78	22	22
		v_4 = 0.02	100	69	69	31	31
		v_5 = 0.2	100	48	48	52	52
		v_6 = 2.0	100	43	43	57	57
		v_7 = 3.0	100	23	23	77	77

continuation Table 2

Suceava 1	DMS Dimethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	91	91	9	9
		v_2 = 0.01	100	83	83	17	17
		v_3 = 0.1	100	76	76	24	24
		v_4 = 0.02	100	69	69	31	31
		v_5 = 0.2	100	53	53	47	47
		v_6 = 2.0	100	48	48	52	52
		v_7 = 3.0	100	21	21	79	79
Suceava 2	DMS Dimethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	92	92	8	8
		v_2 = 0.01	100	86	86	14	14
		v_3 = 0.1	100	72	72	28	28
		v_4 = 0.02	100	64	64	36	36
		v_5 = 0.2	100	52	52	48	48
		v_6 = 2.0	100	46	46	54	54
		v_7 = 3.0	100	24	24	76	76
Suceava 3	DMS Dimethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	96	96	4	4
		v_2 = 0.01	100	82	82	18	18
		v_3 = 0.1	100	76	76	24	24
		v_4 = 0.02	100	68	68	32	32
		v_5 = 0.2	100	55	55	45	45
		v_6 = 2.0	100	48	48	52	52
		v_7 = 3.0	100	25	25	75	75
Suceava 4	DMS Dimethyl sulphate	v_0 – untreated	100	100	100	-	-
		v_1 = 0.001	100	91	91	9	9
		v_2 = 0.01	100	83	83	17	17
		v_3 = 0.1	100	78	78	22	22
		v_4 = 0.02	100	66	66	34	34
		v_5 = 0.2	100	53	53	47	47
		v_6 = 2.0	100	45	45	55	55
		v_7 = 3.0	100	21	21	79	79

The results show how the viability of neo-plantlets decreases depending on the genotype and on the concentration of the mutagenic agent.

CONCLUSIONS

In the case of the genotypes included in the study, the lethal dose (LD50%) is represented by a concentration of 2.0 ppm when the DES (diethyl sulphate) mutagenic agent is used, for the Alina and Nico species the number of plantlets viable is 47% and 42% respectively. There is a slight difference between the percentage of viable explants and genotypes, with higher values for Alina. The local bird's foot trefoil populations are also within the above parameters.

When the DMS (dimethyl sulphate) mutagenic agent is used, for the genotypes included in the study the values of DL50% are close to those obtained with DES (diethyl sulphate) for a concentration of 2.0 ppm, that is, for the Alina and Nico species 48% and 43% viable neo-plantlets respectively.

In this case too the two species have similar behaviour. Regarding the Suceava populations, the results do not show significant differences, the number of viable explants varies between 39 and 45%.

From the data in the two tables it can be seen that in the case of bird's foot trefoil DL50% is at the level of v_5 and v_6 variants (0.2 ppm and 2.0 ppm), and these are the concentrations that will be used for our experiments.

REFERENCES

1. Bandici G. E., 2001, Fiziologia plantelor, Ed. Dacia, Cluj-Napoca
2. Carlson P. S., 1975, Crop improvement through techniques of plant cell and tissue cultures, Bioscience 25
3. Dragomir N., 1981, Cercetări privind biologia, genetica şi ameliorarea ghizdeiului (*Lotus corniculatus L.*), Teză de doctorat, I.A. Cluj-Napoca
4. Dragomir N., 1998, Ghizdeiul, În: Ameliorarea plantelor furajere şi producerea seminţelor, Ed. Lumina, România
5. Evans J. R.,1983, Nitrogen and photosynyhesis in the flag leaf of wheat (Triticum aestivum L.), Plant Physiology, 72: 297-302
6. Halmagyi A., Anca Butiuc-Keul, 2007, Conservarea resurselor genetice vegetale, Ed. Todesco Cluj-Napoca
7. Köteles N., 2011, Regeneration and in vitro multiplication of Lotus Corniculatus L. species. Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Vol XVII, Anul 16, Editura Universităţii din Oradea, 2011, ISSN 1224-6255, pag. 677-684
8. Köteles N., Ana Cornelia Pereş, 2011, Capacity of germination *in vitro* of bird's foot trefoil seed (*Lotus Corniculatus L*), donor material of explants for culture and i propagation of the species in vitro. Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XVI A, Anul 16, Editura Universităţii din Oradea, 2011, ISSN 1224-6255, pag. 415-421
9. Köteles N., Ana Cornelia Pereş, 2012, The influence of cytoquinine in the *in vitro* morphogenesis at the Bird's foot trefoil (*Lotus Corniculatus L.*). Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XVIII, Anul 17, Editura Universităţii din Oradea 2012, ISSN 1224-6255, pag. 339-344
10. Köteles N., Ana Cornelia Pereş, 2013, Induction and acceleration of the *in vitro* proliferation of the axillary buds (the multiple axillary sprouting) of eight genotypes of bird's foot trefoil (*Lotus Corniculatus L.*), Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XXI, Anul 18, Editura Universităţii din Oradea 2013, ISSN 1224-6255, pag. 605-608
11. Köteles N., Ana Cornelia Pereş, 2013, The *in vitro* differential reaction of eight genotypes of bird's foot trefoil (*Lotus Corniculatus L.*) under the aspect of germination capacity, Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XX, Anul 18, Editura Universităţii din Oradea 2013, ISSN 1224-6255, pag. 33-38
12. Köteles N., Ana Cornelia Pereş, 2013, The reaction of the *Lotus Corniculatus L.* meristem cultivated in vitro on Linsmaier-Skoog (LS) basal medium Corniculatus L.), Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XXI, Anul 18, Editura Universităţii din Oradea 2013, ISSN 1224-6255, pag. 609-616
13. Köteles N., Ana Cornelia Pereş, M. Savatie jr, 2014, Ghizdeiul (*Lotus corniculatus L.*), actualitate şi perspectivă în culturile *in vitro*, Editura Universităţii din Oradea, pg. 166, ISBN 978-606-10-1268-8

14. Köteles N., Ana Cornelia Pereş, 2014, Evolution of I*n Vitr*o Multiplication of Bird's Foot Trefoil Subcultures, Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XXII, Anul 19, Editura Universităţii din Oradea 2014, ISSN 1224-6255, pag. 147-150
15. Köteles N., Ana Cornelia Pereş, 2014, Regenerative Capacity *In Vitro* of some Explant Types of Different Ages in Bird's Foot Trefoil, Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XXIII, Anul 19, Editura Universităţii din Oradea 2014, ISSN 1224-6255, pag. 667-670
16. Köteles N., Ana Cornelia Pereş, 2014, The Influence of β-Indolyl-Butyric Acid (IBA) and of β-Indolyl-Acetic Acid (AIA) on the Formation and Development of Bird's Foot Trefoil Plants, Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XXIII, Anul 19, Editura Universităţii din Oradea 2014, ISSN 1224-6255, pag. 671-674
17. Maliga P., A. Sz. Breznovits, L. Marton, 1973, Streptomycin-resistant plants from callus culture of haploid tobacco. Nature (New Biol.) 244, 29-30
18. McCay P. B., E. K. Lai, J. L. Poyer, C. M. DuBose, E. G. Janzen, 1984, Oxygen – and carbon – centred free radical formation during carbon tetrachloride metabolism, Observations of lipid radicals in vivo and in vitro, J. Biol. Chem. 259: 2135 – 2143
19. Murashige T., F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures, Plant. Physiol., 15
20. Pamfil D., 1980, Cultura de meristeme in vitro, Horticultura 3:20-25
21. Savatti M., Maria Zăpărţan, 1991, Capacitatea de regenerare in vitro a unor tipuri de explante de vârste diferite la sparcetă (Onobrychis viciifolia Scop.), Bul. IACN, A-H 45/1, Cluj-Napoca
22. Savatti M., M. Savatti jr., L. Muntean jr., 2003, Ameliorarea plantelor – teorie şi practică, Ed. AcademicPres, Cluj-Napoca
23. Savatti M., G. Nedelea, M. Ardelean, 2004, Tratat de ameliorarea plantelor , Ed. Marineasa, Timişoara
24. Seaney B. B., 1975, Bird's-foot trefoil, Third edition, The Iowa State University, Press/Ames., SUA
25. Tămaş Elena, 1998, Cercetări privind influenţa mutagenă a unor factori fizici şi chimici în vederea obţinerii de mutaţii utile pentru procesul de ameliorare a bobului (*Vicia faba* L.), Teză de doctorat, USAMV Cluj-Napoca