Neutron Irradiation in Plant Breeding

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ABSTRACT:

Neutron irradiation offer opportunities to improve plant genetic resources where the conventional breeding methods are limited. When a neutron is absorbed by a nucleus that conform the biological molecules, considerable amounts of energy, neutrons and ionizing radiations are released. These nuclear reactions in the genetic material produce chemical changes and free radicals. Genetic alters at different levels, especially in the chromosomes and chromatids. The results obtained in crops exposed at different physic mutagens have established that the effect for neutrons is greater due to the Linear Energy Transference (LET) and the Relative Biological Effectiveness (RBE). They are more effective in the mutant plants production than chemical mutagens. In plant breeding, neutrons can yield repeated and predictable results. Neutron radiosensitivity differences of genetic origin have been found in varieties and species. Diploid genotypes are more sensitive than polyploids. After neutron irradiation new cultivars carrying economic and scientific characteristics was founded. The advantages of in vitro system use (environmental changes independence and handling of large number of genotypes in a reduced space) are an important tool in the stabilization and selection of neutron mutant genotypes. From the mutant material, genes can be used in the molecular markers construction. Plants regenerated by cells and protoplasts previously irradiated with neutrons open the possibility to produce diseases resistance.

Keywords: Fast Neutrons, Thermal Neutrons, Irradiation, Mutants, Mutations, Protoplasts.

1. - NEUTRON PHYSICAL AND BIOLOGICAL INTERACTIONS:

The neutron is an electrically neutral particle insensitive to any electrostatic repulsive force having advantages over protons and deuterons. When a neutron is absorbed by a nucleus, it enters in an excitation state forming a compound nucleus with an excess of energy. It decay electromagnetically from this state to another emitting radiation in form of γ-rays or by transferring its energy through the electromagnetic field to one of the surrounding atomic electrons [1]. Fast neutrons dissipate their energies by elastic collision with nuclei of atoms.

The energetic distribution of energy into the tissue has great influence on the biological effects due to the fact the N, H, O, C in the cell compose 92 to 99 %. Transmutation reactions principally by neutron capture of $N^{14}(n,p)C^{14}$, $H^{2}(n,\gamma)H^{3}$, $C^{12}(n,\alpha)^{13}C$, $O^{16}(n,\alpha)C^{13}$ produce the ionization of the DNA or other biological structure. The ionized products subsequently scatter and produce secondary ionizations. The result may lead to the break-up or chemical change in the biological molecules.

Ionization of water molecules within the cell creates very chemically active agents (free radicals) which can chemically attack other biological molecules, increasing the neutron biological effects. The oxygen presence act preventing the occurrence of any restoration process. Low *LET* radiation is more affected by this factor than high *LET* radiation. Neutrons induce a relatively large number of chromosome breaks and cromatid changes. In mutation breeding neutrons can be expected to give repeated and predictable results, regardless of the physiological conditions and environment of plant tissue [2]. Due at these advantages, they are more efficient for the induction of specific mutants.

2. - LET AND RBE:

The induction of molecular alterations of a detectable size might be expected by the treatment with high LET (Linear Energy Transference) corpuscular radiation [3]. Neutrons are usually considered more effective in the mutants production due at the high-*LET* because they eject protons during the nuclear collisions. Therefore, the dose required to high LET radiations to produce a given effect is usually much less that the dose required for the same effect produced by X-rays. High-*LET* radiation has a larger Relative Biological Effectiveness *(RBE) (Table # 1).* Variations between effects of equal doses of different ionizing radiations are generally assumed due to differences in the spatial distribution the ionizations [4]. These, in tissue irradiated by X and gamma rays are distributed at random and sparsely [2],[5]. Contrasting with the dense ionization produced by neutrons. These radiations deposit most of their energy in a small volume magnifying the cellular damage. While the electron tracks from X-rays cause high degree of physiological, no genetic damage, the proton tracks from neutron bombardment act largely through direct effect in the chromosomal material [2].

TABLE #1. - VALUES OF IONIZING PARTICLES

Particle	Energy (MeV)	LET (keV/*u*)	Quality factor
Electron	0.001	12.3	1
	0.01	2.3	1
	0.1	0.42	1
	1	0.25	1
	200 Kvp x rays	0.4 – 36	0.6 - 1
	$Co^{60}\gamma$ rays	0.2 - 2	0.6 - 1
Proton	Small	32	-
	2	16	2
	5	8	2
	10	4	2
Alfa	Small	260	-
	3.4	140	10 - 20
	5	95	10 - 20
Neutrons	2.5	15 – 80	
		peak at 20	2.5
	14.1	3 –30	
		peak at 7	10

3. - NEUTRON MUTANTS EFFICIENCY WITH RESPECT TO OTHER MUTAGENS:

Ionizing radiation and chemicals mutagens in the biological material have a different genetic action mode. Induced genetic instability is manifested in multiple ways [6]. Sodium aside and neutrons form two extremes mutagens forms [5]. Alkylating agents have several different mutagenic effects, ranging from transversions to chromosome breakage. However, these mutagens show unstable reactions and can loss their efficiency due at the culture medium reactions or by the temperature changes. Plants did not display linearity of response with increased doses, as did the ionizing radiation mutagens [3]. Vitamins in cells culture mediums have chemicals antimutagenic effect.

The relation between LET and RBE in the biological material is greater to neutrons with respect to the biological response to other physical mutagens. The comparison between *X*-rays and thermal neutrons, the latter were six to twelve times more effective in reducing seedling height obtained from seeds and maize pollen [4]. The efficiency was emphasized, due to the different ionization trajectories. From the irradiation of ***Arabidopsis thaliana*** seeds, in mutants carrying morphological alterations, the difference was calculated 16 times more elevated for fast neutrons than for *X*-rays. For clorofilic and morphological viable mutants the mutations average for *Gy*/cell was approximately of 6.4 times higher with fast neutrons than *X* rays [10]. Wheat varieties exposed at different mutagen types established that the effectivity for clorofilics mutations is ethyleneimine < diethylsulphate ≤ fast neutrons ≤ ethylmethanesulphonate ≤ thermal neutrons. For morphologic mutations the relative efficiency was ethylemeimine < *X* rays < diethylsulphate < ethylmethanesulphonate ≤ thermal neutrons ≤ fast neutrons [9].

Neutron efficiency in the mutants production carrying new scientific and economic characteristics has been demonstrated in several plant species and organisms [2][3][4] [11][12][13][14][15] [16][17][18][19] [20][21][22][23][24][25] [26].

Nuclear techniques can be used for specific purposes, such as increase the genetic variability [27], chromosome translocations yield, dominant genes elimination [2] or desirable characters transference from wild species into cultivated species [28][29][30]. More than 1700 mutant varieties involving 154 plant species have been officially released in the world [31]. Around of all this material 3 % was produced using neutron irradiations [Van Derberg] *Table # 2* (personal communication).

4. - RADIOSENSIVITY:

The analysis of radiosensitivity is one of the requirements for mutation experiments that are planned for use in plant breeding [32]. The effectiveness of responses of plant tissues to neutrons and other mutagenic agents is extremely variable [3]. For example, dormant seeds are less neutrons radiosensitive than seeds with developing embryos. However, dry seeds have high radiosensitive due at the post-irradiation free radicals action. Different gene loci have a different mutability, regardless of treatment with one or other mutagen [5].

The radiosensitivity is influenced by the cell division stage. Cells in mitosis are most sensitive than cells in G_1. Cells in the S stage are intermediate sensitive and cells in the G_2 stage are the most resistant. Radiosensitivity associated with the chromosome number and size (genetic constitution between species and varieties) were founded some experiments [11][32][33][34][35]. Neutron mutants are more frequent in diploids and less frequent in polyploids [2][36]. However, the polyploidy radioprotective effect is associated with reduced chromosome volume and not with the protective effect of the genetic redundancy.

5. - CONSIDERATIONS:

Neutron biological effects study present two basic problems: (1) The Relative Biological Effectiveness *(RBE)* of the different quality radiations and (2) The rate of genetic damage lethal to the cell. RBE can be modified by the dose fraction, configuration of the irradiation facility, neutron category, dosimetry instrumentation control of quality. Gamma rays are the most common contamination source in a neutron irradiation facility affecting the neutron biological effect. Gamma rays measure using Thermoluminescent Dosimetry (TL) is widely used [35]. However, response of TL material during the particle irradiation has been observed [35][36]. TL's dosimeters thermal treatment procedures may influence in the trapping parameter [6] affecting the gamma rays dose estimation. To correct some differences, computerized codes have been developed [36].

6. - PROTOPLASTS ISOLATION AND SOMACLONAL VARIATION:

The use of in vitro techniques such as protoplast fusion can overcome some of the limitations in the application of mutation techniques in both seed and vegetatively propagated crops [31]. Protoplast fusion is an efficient method for restoring the fertility of somatic hybrids generated from sterile parent clones, and is a powerful procedure for the complementation of multigenetic disease resistance traits [39]. The results obtained indicate high morphogenetic potential of protoplasts. Consequently, it should be possible to involve wild species for the potato improvement by means of protoplast fusion [40].

TABLE # 2. - NEUTRON MUTANTS VARIETIES RELASED IN THE WORLD:

	YEAR	LATIN NAME	MUTANT VARIETIE NAME	COUNTRY	PARTICLE	AGRONOMIC TRAIT
1.		Achimenes sp.	Compact Arnold	Netherlands	x-rays or FN	
2.		Achimenes sp.	Cupido	Netherlands	x-rays or FN	
3.		Achimenes sp.	Early Arnold	Netherlands	x-rays or FN	
4.	1977	Achimenes sp.	Lollipop	Netherlands	FN	
5.		Achimenes sp.	Orion	Netherlands	x-rays or FN	Compact
6.		Achimenes sp.	Springtime	Netherlands	x-rays or FN	
7.	1959	Avena sativa L.	Florad	USA	ThN	Rust resistance
8.	1976	Begonia sp.	Flambeau	USA	FN	Flower morphology
9.	1975	Begonia sp.	Heirloom	USA	FN	Flower color
10.		Begonia sp.	Mikkel Limelight	USA	FN	
11.	1984	Cajanus cajan Millsp.	TAT 5	India	FN	Seed size
12.	1976	Cajanus cajan Millsp.	Trombay Vishakha-1	India	FN	Seed size
13.	1984	Cicer arietinum L.	Kiran	India	Neut	Erectoid type
14.	1984	Citrus paradisi Mafc.	Rio Red	USA	ThN	Food color
15.	1970	Citrus paradisi Mafc.	Star Ruby	USA	ThN	Seedless
16.	1983	Corchorus olitorius L.	Mahadev TJ-40	India	ThN	Yield
17.	1987	Glycine max L.	Heilong 31	China	ThN	Yield
18.	1987	Glycine max L.	Heilong 32	China	ThN	Yield
19.	1986	Glycine max L.	Heinong 28	China	ThN	Earliness
20.	1962	Glycine max L.	Tainung No.1(R)	China	ThN	Vigorousness
21.	1978	Hordeum vulgare L.	Alf	Denmark	ThN	Shortness
22.	1969	Hordeum vulgare L.	Bonneville 70	USA	ThN	Thereshability
23.	1963	Hordeum vulgare L.	Pennrad	USA	ThN	Winter hardiness
24.	1974	Hordeum vulgare L.	Radiation	Korea	ThN	Earliness
25.	1972	Hordeum vulgare L.	RDB-1	India	Neut	Shortness
26.		Hordeum vulgare L.	Shua	Iraq	FN	
27.	1986	Ipomoea batatas (L.) Poir.	Yan-shu 759	China	FN	Starch content
28.	1986	Ipomoea batatas (L.) Poir.	Yan-shu 781	China	FN	Starch content
29.	1970	Lespedeza cuneata Dum.	Interstate	USA	ThN	Compact
30.	1971	Oryza sativa L.	Fushe 94	China	Neut	Earliness
31.	1978	Oryza sativa L.	Juangyebai	China	Neut	Roll leaf
32.	1980	Oryza sativa L.	Mutashali	Hungary	FN	Blast resistance
33.	1975	Oryza sativa L.	Nongshi No.4	China	FN	Earliness
34.	1972	Oryza sativa L.	Nucleoryza	Hungary	FN	Earliness
35.	1981	Oryza sativa L.	RD 10	Thailand	FN	Gloutinus endosperm
36.	1968	Oryza sativa L.	Vellayani	India	Neut	------
37.	1976	Oryza sativa L.	Zhongbao No.2	China	FN	Earliness
38.	1985	Oryza sativa L.	Zhongtie	China	FN	Yield
39.	1970	Prunus armeniaca L.	Early Blenheim	Canada	ThN	Earliness
40.	1969	Ricinus communis L.	Aruna	India	ThN	Earliness
41.		Syringa vulgaris L.	Prairie Petite	USA	ThN	
42.	1985	Triticum aestivum L.	62-10	China	FN	Rust resistance
43.	1985	Triticum aestivum L.	62-8	China	FN	Rust resistance
44.	1979	Triticum aestivum L.	Jauhar-78	Pakistan	FN	Yield
45.	1964	Triticum aestivum L.	Lewis	USA	ThN	Stiffness
46.	1984	Triticum aestivum L.	Longfumai No.1	China	ThN	Earliness
47.	1987	Triticum aestivum L.	Spinnaker	Italy	FN	Lodging resistance
48.	1964	Triticum aestivum L.	Stadler	USA	ThN	Earliness
49.		Triticum aestivum L.	Tammuz-2	Iraq	FN	
50.		Triticum aestivum L.	Tammuz-3	Iraq	FN	
51.	1969	Triticum turgidum ssp. durum Desf.	Castel del Monte	Italy	FN	Stiffness
52.	1968	Triticum turgidum ssp. durum Desf.	Castelfusano	Italy	ThN	Stiffness
53.	1968	Triticum turgidum ssp. durum Desf.	Castelporziano	Italy	ThN	Stiffness
54.	1970	Triticum turgidum ssp. durum Desf.	G-0367	Greece	ThN	Shortness
55.	1987	Triticum turgidum ssp. durum Desf.	Icaro	Italy	FN	Short culm
56.	1979	Zea mays L.	Yuan 79-418	China	FN	Earliness

- FN = Fast neutrons.
- ThN = Thermal neutrons
- Nuet = Neutrons

Variability in tissue culture has been described in all levels of tissue culture process. Instability has been discerned at the karyotypic, morphological, biochemical and molecular levels. Specific gene effects can result from chromosomal interchanges because of gene inactivation, position effects and altered developmental timing. Mutant phenotypes can result either from insertion of the element into the structural gene or into regulator gene sequences [41]. Somaclonal variation and unstable mutants has been reported among plants regenerated from cell and vegetative tissue culture [41] [42][43][44][45][46][47][48].

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