C17

Mutation Induction in Cytoplasmic Genomes

A.R.Prina*, M.G.Pacheco and A.M.Landau

Instituto de Genética "Ewald A. Favret", CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), CC25, B1712WAA, Castelar, Buenos Aires, Argentina.
*Corresponding author, E-MAIL: aprina@cnia.inta.gov.ar

1. Introduction

Cytoplasmic genes are located in semi-autonomous cytoplasmic organelles, *i.e.* plastids and mitochondria. They are semi-autonomous in the sense that only a portion of the proteins they need are encoded by their own DNA, most are encoded in the nucleus, synthesized in the cytosol and finally imported into the organelles. It is widely accepted that mitochondria and plastids evolved from free-living prokaryotes and that during a long process of endosymbiosis they suffered a drastic loss of genetic material (90-95%). The lost genes were transferred to the nucleus of the host eukaryotic cell in which they originally invaded. In agreement with that idea both the mitochondrial and the plastid genomes share several similarities with bacterial genomes. During evolution these organelles became an essential component of the plant cell. The co-evolution of the three genomes involves an intensive and tightly coordinated cross-talk system. Many of the genes that still remain in these organelles encode components of their own genetic machinery and it can be inferred from information in bacteria that variability in these widely conserved genes not only includes diverse antibiotic resistances, but also differential abiotic stress responses.

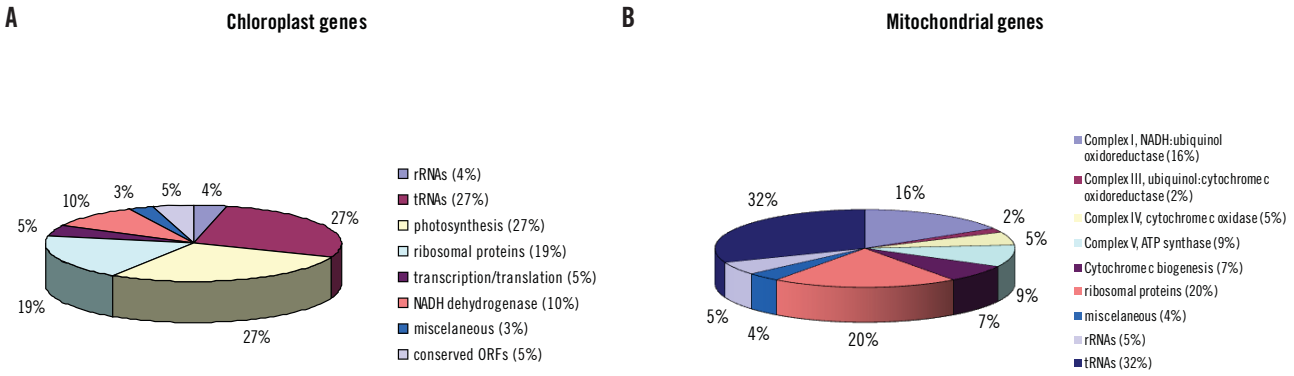
Natural genetic variability residing in the cytoplasmic organelles is extremely narrow in comparison with that of the nuclear genome. The generation of variability using conventional tools is very difficult in most crop species due to the particular mode of inheritance, mostly mono-parental, and reduced or null recombination by means of traditional hybridization. Over the last decades a huge amount of information on plastome genes was produced from experiments on site-directed mutagenesis using homologous recombination in *Chlamydomonas* and, more recently, in tobacco. However, in most crop plants species, plastid transformation is at present not easily performed and efficient procedures for plastome/chondriome mutagenesis are highly desirable.

Among the characters regulated by organelle genes, cytoplasmic male sterility (CMS) is by far the most commercially exploited, being a very useful tool for hybrid seed production in maize and several other crops. The CMS system has been determined in more than 150 species and it is usually associated with changes in mitochondrial genomes. Another cytoplasmic mutant commercially used in rapeseed varieties is a *psbA* gene allele that confers triazine tolerance. This plastid-encoded genotype has been found in several species as a spontaneous mutation.

2. Organelle Genomes and their Function

2.1. Plastid Genome (Plastome)

The plastid genome, or plastome, is a uni-circular DNA molecule of about 150 kbp encoding roughly 100-150 genes in the majority of higher plants. In addition to the components of the genetic machinery the plastome encodes most of the genes involved in photosynthesis (**Fig 17.1A**), including the reaction centre apoproteins, cytochromes and the large sub-unit of ribulose



The Joint FAO/IAEA Programme

Figure 17.1 The gene composition of organelle genomes. A: The barley plastome (NC_008590). B: The wheat chondriome (NC_007579).

1,5-biphosphate carboxylase/oxygenase (RuBisCO). RubisCO is the key enzyme in photosynthesis and photorespiration, and probably the most abundant protein on earth. Variability in these genes has been observed to affect responses to abiotic stress and photosynthetic efficiency, and even a slight improvement on these characters can have a huge impact in plant productivity.

2.2. Mitochondria Genome (Chondriome)

In contrast to the plastome, the mitochondrial genome or chondriome of higher plants consists of a complex population of circular and linear DNA molecules with different sizes and sequences. In the chondriome internal recombination events, which can originate new arrangements, are common. Consequently, the gene order is not as strict as in the plastome. The size of the chondriome, ranging from 200 to 2,000 kbp, is much more heterogeneous among different plant species than the plastome. However, the number of genes encoding proteins does not necessarily correlate with the genome size. In mitochondria the genetic machinery is even more incompletely encoded than in plastids and thus they need to import not only proteins, but also some tRNAs from the cytosol. Several hundreds of proteins are necessary for a functional mitochondrion, but the chondriome is responsible for encoding and synthesizing only a few of these, most of them involved in the electron transfer chain or belonging to the ATPase complex (**Fig 17.1 B**).

3. Mutation Induction of Cytoplasmic Genes

It is widely accepted that mutation induction of cytoplasmic genes by treatments with typical mutagens, like ionizing radiations and chemical mutagens is much more difficult than that of nuclear genes. In some reports, the obtainment of cytoplasmic mutants was only assumed from observations of non-Mendelian transmission, sorting-out patterns of chlorophyll deficiencies and/or the presence of mixed cells, but they were not actually proven. On the other hand, it has been sometimes discussed whether these mutagens directly induced cytoplasmic mutations or if they induced nuclear mutants, which in turn produced cytoplasmic mutants. Some peculiarities of cytoplasmic genomes that are relevant for mutation induction are highlighted in **Box 17.1**.

3.1. Physical and Chemical Mutagenesis

3.1.1. Plastome

Among the typical mutagens, nitrosourea compounds have been revealed as promising agents for the induction of plastome mutations in several higher plants species, including sunflower and tomato (Hagemann 1982).

Box 17.1: Cytoplasmic genomes and mutation induction

High number of copies of DNA molecules per cell: The cytoplasm of one cell contains numerous semi-autonomous organelles (plastids and mitochondria), each having multiple copies of DNA molecules. Thus, as mutations occur at the level of one of these copies, cytoplasmic chimeras cannot be avoided even when applying mutagenic treatments on uni-cellular organs (**see Chapter 15**).

Mixed cell: a cell carrying more than one type of allele in their plastids or mitochondria, also called heteroplastomic or heteroplastidic, when involving plastid DNA, and heteroplasmic, when involving any of these organelles. Cytoplasmic mutants always originate as mixed cells, they remain as mixed cells during several mitotic divisions and they can also persist as mixed cells after meiosis.

Somatic segregation or sorting out: a typical phenomenon of organelle genomes, which originates as a consequence of the so-called relaxed genome (see below). Thanks to somatic segregation an organelle mutant allele can differentially grow into some daughter organelles and daughter cells, segregating mutant vs. wild type homoplasmic cells without need of meiotic segregation.

Mode of inheritance and relaxed genome: in cultivated plants organelles have mostly a mono-parental mode of inheritance and they have the so-called relaxed genome, in contrast to the stringent nuclear one, which is copied only once per mitotic cell cycle. The more flexible mechanisms controlling replication and distribution of organelle-DNA molecules at cell division make the appearance of cytoplasmic mutants less predictable than that of nuclear ones (**see Chapter 15**). For nuclear genes, chimeras are usually limited to the soma of the M_1 plants, while segregation of solid mutants is expected to start in the M_2 generation. For cytoplasmic genes the appearance of chimeras is much more complex than for nuclear genes, they are not limited to the M_1 plants soma and the segregation of solid "homoplasmic" mutants is expected to occur in later generations.

Intracellular competition: as mentioned in **Chapter 15**, after mutagenic treatments applied on multi-cellular organs, competition between mutated vs. non-mutated cells can occur. This is true for both, nuclear and cytoplasmic mutations, but in the last case, as different organelle-DNA molecules can replicate more often than others into a single cell, intracellular competition can also occur.

Plastome-encoded antibiotics resistance was induced in *Solanaceae* species using N-nitroso-N-methylurea (NMU) (McCabe *et al.* 1989) and atrazine tolerance in *Nicotiana* after treatment of N-ethyl-N-nitrosourea (ENU) (Rey *et al.* 1990). Nitrosourea compounds seem to preferentially target the plastid DNA, but they also produce large amounts of nuclear mutations.

The availability of mutagenic treatments acting more specifically on the cytoplasmic DNA would be highly advantageous such that identification of cytoplasmic mutants can be easily performed in the absence of high amounts of nuclear gene mutants that occur when applying typical mutagens. In the uni-plastid *Chlamydomonas* alga, a model organism with several excellent attributes for plastid genetics studies, treatments with the antibiotic streptomycin induced plastome resistant mutants and also cytoplasmically inherited mutants carrying photosynthetic defects. Specific increase in chloroplast gene mutations was also reported after growth of *Chlamydomonas* in the thymidylate synthase inhibitor 5-fluorodeoxyuridine (FUdr), a drug that causes the plastid to become thymidine starved (Würtz *et al.* 1979). In this sense, some promising results came from experiments with the intercalating agent 9-aminoacridine hydrochloride in *Oenothera* (GuhaMajumdar *et al.* 2004).

3.1.2. Chondriome

In the case of mitochondrial genes, cytoplasmic male sterile (CMS) genotypes have been the main target of mutation induction. Results of EMS mutagenesis for CMS has been so far contradictory . There are two reports of successful obtainment of CMS from ionising radiation mutagenesis, one was in sugar beets with gamma-rays (Kinoshita *et al.* 1982) and the other in wheat with ion beam radiation (Wang and Li, 2005). Several chemicals known as effective inducers of cytoplasmic mutants in yeast have been successfully used in crop plants, *e.g.* acriflavine, streptomycin and ethidium bromide in sugar beets (Kinoshita *et al.* 1982), and the last two drugs along with mitomycin in pearl millet (Burton and Hanna, 1982). The method of CMS induction with streptomycin in maize was patented in the US in 1971. Examples of mutation induction in cytoplasmic genes by artificial mutagenesis are listed in **Table 17.1**.

3.2. Cytoplasmic Mutators

Cytoplasmic variability can also be generated using genetically unstable genotypes, which probably are

Table 17.1: Some artificially induced cytoplasmic mutations reported in literatures.

Plant species	Genome	Phenotype	Mutagen
Oenothera	Plastome	Chlorotic sectors	9-amynoacridine hydrochloride
Chlamydomonas reinhardtii	Plastome	Non-photosynthetic or antibiotic resistant	5-fluorodeoxyuridine
Sunflower, Tomato and *Antirrhinum majus*	Plastome	Clonally-variegated seedlings	N-ethyl-N-methylurea
Tomato	Plastome	Clonally-variegated seedlings	N-ethyl-N-methylurea
Solanaceae	Plastome	Antibiotic resistant	N-ethyl-N-methylurea
Nicotiana plumbaginifolia	Plastome	Atrazine tolerant (*psbA* gene)	N-ethyl-N-nitrosourea
Chlamydomonas reinhardtii	Chondriome	Minute mutants	Acriflavine or ethidium bromide
Maize	Chondriome	CMS	Streptomycin
Sorghum	Chondriome	CMS	Colchicine
Pearl millet	Chondriome	CMS	Ethidium bromide, streptomycin or mitomycin.
Sugar beet	Chondriome	CMS	Gamma rays, acriflavine, streptomycin or ethidium bromide
Wheat	Chondriome	CMS	Electron beam

defective in genes that control organelle-genome integrity and are hence referred to as cytoplasmic mutators. Mutator lines produce maternally-inherited changes and are therefore potential sources of organelle-genetic variability.

3.2.1. Genetic Control of Genetic Instability

The genetic instability of mutator lines can be encoded by cytoplasmic or by nuclear genes, however, the latter are more useful for inducing cytoplasmic variability because they allow easy separation of both components by means of hybridization and back-crossing. Mutator genotypes already exist in nature and they can also be induced by mutagenic treatments.

3.2.2. Spectrum of Cytoplasmic Mutants Induced by Mutator Genotypes

The width of the mutant spectrum generated by cytoplasmic mutators can be either wide or narrow. The *iojap* maize and *albostrians* barley are typical examples of the narrow spectrum group. Other mutators reported in several dicots (*Arabidopsis, Epilobium, Oenothera, Nepeta* and *Petunia*) and in barley are generating wide spectrum of mutations. The mutator genotype of *Oenothera* increases 200-1000 folds the spontaneous appearance of pigment deficient sectors. It was molecularly characterized as inducing deletions and duplications in the plastome through a replication slippage mechanism. Interestingly, when applying NMU on *Oenothera* plastome mutator genotypes a synergistic effect was observed suggesting an interaction between the mutator gene product and some repair systems in charge of correcting NMU-induced damage (GuhaMajumdar *et al.* 2004).

The barley chloroplast mutator genotype induces a wide spectrum of chlorophyll mutants, which includes several viable and normal-vigour types. Mutator effects are slightly manifested in the M_2 generation (approximately 3 *striata* over 1,000 F_2 seedlings) and only subtle mutational changes have so far been detected in plastid DNA of mutator-induced mutants. Remarkably, the mutator induced plastome variability is easily observed because it occurs on a homogeneous nuclear genetic background and without the simultaneous induction of nuclear gene mutants. Most of the mutational changes so far observed consisted of several T/A – C/G transitions and only one insertion. They were observed in the plastid genes *inf*A, *psb*A and the *ycf*3 locus (Prina *et al.* 2009). The *psb*A mutant was observed in families selected for atrazine tolerance (Rios *et al.* 2003). Both the wide spectrum of mutants and the subtle DNA changes induced; suggest that this chloroplast mutator genotype can be an exceptionally valuable tool to explore the potential functionality of the otherwise highly conserved plastid genome.

Several mutants with chlorophyll deficient sectors have been observed in association with mitochondrial anomalies. Thus, non-chromosomal stripe (NCS) maize mutants produce mitochondrial DNA rearrangements that cause deletion of essential genes; e.g. in the case of NCS 5 and NCS 6 mutants the mitochondrial *cox*2 (cytochrome oxigenase sub-unit 2) gene is affected and this finally impairs the development of normal chloroplasts showing yellow leaf stripes. Another chlorophyll-deficient mutant that alters the mitochondrial genome is the widely studied *iojap* maize, which has been observed to induce new cases of CMS (Lemke *et al.* 1988). Another example is the maize nuclear genotype denominated P2 line that was reported as a natural mutagenesis system for mtDNA. It highly destabilizes the mitochondrial genome by increasing low copy-number sub-genomes, amplifying aberrant recombination products and causing loss of normal components (Kuzmin *et al.* 2005). Finally, the *Arabidopsis chmchm* mutant, which confers maternally inherited leaf variegation and distortion and was previously classified as chloroplast mutator, has been observed to induce rearrangements in some mitochondrial genes for ribosomal proteins and to destabilize the mitochondrial genome. Furthermore, it causes accumulation of a rearranged subgenome, which was already present in the wild type at very low level, by specific copy number amplification (substochiometric shifting), *e.g.* in the maternal distorted leaf (MDL) mutant a defective ribosomal protein gene *rps3* replaces the wild type copy. This mutator gene, renamed AtMSH1, encodes a homologue to MutS gene in E *coli*, which is involved in DNA mismatch repair (Abdelnoor *et al.* 2003).

4. References

4.1. Cited References

Abdelnoor R.V., Yule, R., Elo, A. *et al.* **2003.** Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. *PNAS.* 100(10):5968-5973.

Burton, G.W. and Hanna, W.W. 1982. Stable cytoplasmic male-sterile mutants induced in Tift 23DB1 pearl millet with mitomycin and streptomycin. *Crop Sci.* 22:651-652.

GuhaMajumdar, M., Baldwin, S. and Sears, B.B. 2004. Chloroplast mutations induced by 9-aminoacridine hydrochloride are independent of the plastome mutator in Oenothera. *Theor. Appl. Genet.* 108:543-549.

Hagemann, R. 1982. Induction of plastome mutations by nitroso-urea-compounds. In: Methods in Chloroplast Molecular Biology, Edelman *et al.* eds. Elsevier Biochemical Press, pp. 119-127.

McCabe, P.F., Timmons, A.M. and Dix, P.J. 1989. A simple procedure for the isolation of streptomycin resistant plants in Solanaceae. *Mol. Gen. Genet.* 216:132-137.

Kinoshita, T., Takahashi, M. and Mikami, T. 1982. Cytoplasmic mutation of male sterility induced by chemical mutagens in sugar beets. *Proc. Japan Acad.* 58 Ser B:319-322.

Kuzmin, E.V., Duvick, D.N. and Newton, K.J. 2005. A mitochondrial mutator system in maize. *Plant Physiol.* 137:779-789.

Lemke, C.A., Gracen, V.E. and Everett, H.L. 1988. A second source of cytoplasmic male sterility in maize induced by the nuclear gene *iojap*. *J. Hered.* 79:459-464.

Prina, A.R., Landau, A.M., Colombo, N. *et al.* **2009.** Genetically unstable mutants as novel sources of genetic variability: The chloroplast mutator genotype in barley as a tool for exploring the plastid genome. *In*: Shu Q.Y. (ed) Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations. Rome. pp. 227-228.

Rey, P., Eymery, F. and Peltier, G. 1990. Atrazine- and diuron-resistant plants from photoautotrophic protoplast-derived cultures of *Nicotiana plumbaginifolia*. *Plant Cell Rep.* 9:241-244.

Rios, R.D., Saione, H., Robredo, C. *et al.* **2003.** Isolation and molecular characterization of atrazine tolerant barley mutants. *Theor. Appl. Genet.* 106:696-702.

Wang, L.Q. and Li, G.Y. 2005. Role of Mutation Induction for Wheat (T. aestivum L.) Improvement. In: Role of Classical Mutation Breeding in Crop Improvement, (S.K.Datta ed). Daya Publishing House, Delhi, pp. 156-185.

Würtz, E.A., Sears, B.B., Rabert, D.K. *et al.* **1979.** A specific increase in chloroplast gene mutations following growth of Chlamydomonas in 5-fluorodeoxyuridine. *Mol. Gen. Genet.* 170:235-242.

4.2. Further Reading

Birky, C.W. Jr. 2001. The inheritance of genes in mitochondria and chloroplasts: Laws, Mechanisms, and Models. *Ann. Rev. Genet.* 35:125-48.

Bock, R. and Khan, M.S. 2004. Taming plastids for a green future. *Trends in Biotechnology.* 22 (6):311-318.

Börner, T. and Sears, B. 1986. Plastome mutants. Review/ Genetics resources. *Plant Molecular Biology Reporter.* 4 (2):69-92.

Gustafsson, A. and Ekberg, I. 1995. Extranuclear mutations. In: Manual on Mutation Breeding, second Edition. Joint FAO/IAEA Div. of Atomic Energy in Food and Agriculture, Vienna. pp. 114-117.

Hagemann, R. 1976. Plastid distribution and plastid competition in higher plants and the induction pf plastid mutations by nitroso-urea–compounds. *In*: Genetics and Biogenesis of Chloroplasts and Mitochondria. Th Bücher *et al.* eds. Elsevier/NorthHolland Biomedical Press. Amsterdam. The Netherlands, pp. 331-338.

Hagemann, R. 1986. A special type of nucleus-plastid-interactions: nuclear gene induced plastome mutations. *In*: Regulation of Chloroplast Differentiation, G. Akoyunoglou and H. Senger eds. (A.R. Liss, Inc. New York), pp. 455-466.

Kirk, J.T.O. and Tilney-Bassett, R.A.E. 1978. The plastids. Elsevier, North Holland, Amsterdam. pp. 960.

Woodson J.D. and Chory, J. 2008. Coordination of gene expression between organellar and nuclear genomes. *Nature Reviews/Genetics*. 9:383-395.