Modulatory effect of the antioxidants turmeric and curcumin on oxidation-induced damage in Chinese hamster ovary cells

(Efeito modulador dos antioxidantes cúrcuma e curcumina sobre os danos oxidativos induzidos em ovário de hamster chinês)

Maria Cristina Paiva de Araújo*

Turmeric, the powder from the root of *Curcuma longa* Linn. (Zingiberaceae), has been widely used as a coloring and flavoring agent, and in the treatment of inflammatory conditions and other diseases. Curcumin, an important pigment of turmeric, is commonly used as a coloring agent in foods, drugs and cosmetics. This natural phenolic compound has been known since ancient times to possess a variety of pharmacological activities and therapeutic properties. Turmeric and curcumin are known to act as antioxidant, antimutagenic and anticarcinogenic agents. We investigated the role of both antioxidants, turmeric and curcumin, on the chromosomal damage induced by the oxidizing agents bleomycin and γ-radiation in Chinese hamster ovary (CHO) cells. The effect of curcumin on the chromosomal damage induced by thiourea, ferrous ions (Fe²⁺), ferric ions (Fe³⁺) and hydrogen peroxide (H₂O₂) was also evaluated.

Cultured cells received three doses of each drug, turmeric (100, 250 and 500 μg/ml) and curcumin (2.5, 5 and 10 μg/ml), one hour before they were submitted to bleomycin (10 μg/ml) or γ-radiation (2.5 Gy) treatment, which occurred during the G₁/S, S and G₂/S phases of the cell cycle. The bleomycin treatment lasted 30 min. Curcumin (15 μg/ml) was tested with three doses of Fe²⁺ (1.25, 2.5 and 5.0 μg/ml), Fe³⁺ (1.25, 2.5 and 5.0 μg/ml) or H₂O₂ (1.7, 3.4 and 6.8 μg/ml), by simultaneous treatment. Thiourea (10, 20 and 40 μg/ml) was added to cultures 15 min before the curcumin treatment. The combined treatment with curcumin plus Fe²⁺, Fe³⁺, H₂O₂ or thiourea lasted 13 h. Three hundred metaphases, one hundred in each experiment, were analyzed per treatment in order to determine the frequencies of chromosomal aberrations, and the mitotic index was obtained by counting the number of mitotic cells in 3000 cells per treatment.

Turmeric was not clastogenic by itself, whereas curcumin at 10-15 μg/ml increased the frequency of chromosomal damage. It mainly induced chromatid breaks and gaps. Neither turmeric nor curcumin had a protective effect against the clastogenicity of bleomycin and γ-radiation. Instead, a potentiating effect was observed with both natural antioxidants. Turmeric enhanced γ-radiation-induced chromosomal damage at G₅/S phase, and curcumin increased both bleomycin- and γ-radiation-induced chromosomal damage at the S and G₂/S phases of the cell cycle. These results clearly indicate that, beside their antioxidant behavior, turmeric and curcumin are also potentiating agents.

The clastogenicity of curcumin was significantly decreased in the presence of thiourea, a hydroxyl radical scavenger. This result strongly suggests that the clastogenicity of curcumin is at least partly mediated through reactive oxygen species. Ferrous and ferric ions also decreased the curcumin-induced chromosomal damage in a dose-dependent manner, suggesting that curcumin is a potent metal chelator. On the other hand, curcumin did not have any effect on H₂O₂-induced chromosomal damage.

It is known that many antioxidant natural compounds, such as phenols, also enhance oxidation under certain conditions, generating free radicals that damage DNA, lipids and proteins. It is suggested that the clastogenic and potentiating effects observed in this study depend on the oxidation-enhancing behavior of curcumin. However, the exact mechanism by which curcumin pronotes oxidation is not known.

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Development of a population genetics software for teaching and research

(Desenvolvimento de um programa de genética de populações com finalidades didáticas e de pesquisa)

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This software, developed in Visual Basic 3.0, allows the simulation of eight different population genetics models: panmixia, selection, genetic drift, gene flow, inbreeding, mutation and assortative matings (mixed or not with other mating systems).

The panmixia module allows the analysis of autosomal inheritance (with genotypic frequencies being represented in a triangular coordinate system) and X-linked inheritance (with representation of gene frequencies in a conventional Cartesian system).

The software also permits the study of a general selection model, where genotypic frequencies can be plotted using a triangular coordinate system or a Cartesian Δq graph. This program also uses a triangular coordinate system to present a model of frequency-dependent selection.

The phenomenon of genetic drift, in association or not with selection, can be simulated using the Cartesian system. The overall analysis of genetic drift is performed by Markov’s model, also contained in the software.
The inbreeding module can generate a series of curves formed with the possible values of genotypic frequencies in equilibrium, for any given value of the average inbreeding coefficient.

Gene flow is studied through a conventional Cartesian system, that shows the variation of gene frequencies under the influence of uni- or bidirectional migration. In a very similar manner to the latter module, the mutation module presents the variation of gene frequencies under the influence of uni- or bidirectional mutation.

Another module presents 14 different systems of assortative matings that can be studied either as inbred or sterile matings, mixed or not with panmixia. A model of admixture of positive and negative assortative matings with dominance was also included.

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**Genetic damage in exfoliated cells from the uterine cervix: association and/or interaction between cigarette smoking and the progression to malignant transformation**

(Danos genéticos em células esfoliadas do colo uterino: associação e/ou interação entre tabagismo e a progressão para transformação maligna)

Eneida de Moraes Marcílio Cerqueira*

Chromosomal damage in exfoliated cells from the uterine cervix, cervical cytological and histopathological modifications and associated risk factors for cervical intraepithelial neoplasia (CIN I, II and III) and cancer of the uterine cervix were evaluated in 200 women. They were divided into three groups: group I (N = 116), women periodically submitted to the Pap-nicolaou test, residents of the city of Salvador, Bahia; group II (N = 57), women resident in São Paulo, São Paulo, previously selected because of a cytopathologic test positive for conditions such as HPV infection, malignant or premalignant cervical lesions, and group III (N = 27), inmates of the Tatuapé Penal Institution (São Paulo).

Occurrence of risk factors for development of neoplastic lesions was statistically evaluated from information obtained by a questionnaire. Significant differences, particularly between groups I and II, as well as between I and II, were observed in relation to the number of sexual partners and age at first intercourse, when jointly evaluated by an index developed in this study. Groups I and II did not differ in frequency of smokers, but this habit was more frequent in group III. Smokers of group III differed from those of group I as in the daily number of smoked cigarettes and the cigarette load (daily number multiplied by years of smoking). There were no significant differences regarding age and multiparity. Analysis of the frequency of normal smears, inflammatory and koilocytic atypia and premalignant lesions by cyto- and/or histopathology revealed that premalignant changes were more frequent in groups II and III when compared with group I. Most of the premalignant lesions were associated with HPV infection. The results point to a relation between sexual variables, HPV infection and cigarette smoking in the development of cervical intraepithelial neoplasia.

Genetic damage, evaluated through the micronucleus test (MN), was significantly more frequent in groups II and III than in group I. Considering the 200-woman sample as a whole, the number of MN in women that smoked was significantly greater than in nonsmokers. It was also greater in the women who smoked more. No differences were observed in the occurrence of MN between the groups in relation to the sexual variables (age at first intercourse and number of sexual partners), use of hormonal contraceptives, multiparity, and presence of HPV. The occurrence of MN was significantly lower in the women with no cytopathological alterations, whether smokers or nonsmokers, than in those showing inflammatory atypia as well as low grade (CIN I) or high grade preneoplastic lesions (CIN II + CIN III). In nonsmoking women, occurrence of MN was similar in those with inflammatory atypia or CIN I and significantly lower among those with the two types of alterations and the ones presenting more severe lesions. Smokers with CIN I showed a larger number of MN than the nonsmokers with comparable diagnosis and smokers with inflammatory atypia. There was no significant difference in MN frequency between smokers with CIN I and smokers with CIN III. These results suggest that mutagenic effects of cigarette smoking occur in the uterine cervix cells and that the progression of cervical intraepithelial neoplasias is associated with an increase in the frequency of chromosomal damage. Moreover, they suggest that cigarette smoking introduces an additional risk to progression of low-grade intraepithelial neoplasia. We propose the inclusion of the micronucleus test in monitoring grade I cervical intraepithelial neoplasia in cigarette smoking women.

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**Characterization of the nanuzae group of lizards**

(Iguania: Tropiduridae): analysis of mitochondrial DNA restriction fragment patterns

(Caracterização de lagartos do grupo nanuzae do gênero Tropidurus (Iguania: Tropiduridae) através da análise de padrões de fragmentos de restrição do DNA mitocondrial)

José Carlos Passoni*

The nanuzae group of lizards includes three species, *Tropidurus nanuzae*, *T. divaricatus* and *T. amathites*. The first species is found along Serra do Espinhaço, in Minas Gerais...
(Brazil), and the other two in the northern region of Bahia, at the continental dunes of São Francisco River, inhabiting the two opposite margins of the river. T. divaricatus is located in the left margin, and presents different populations in two separated dune fields. Previous data from morphological, allozymic and chromosomal analyses were not conclusive about the phylogenetic relationships among these species. In the present study, we analyzed the restriction fragment patterns of the mtDNA in the three species and detected 53 restriction sites with the enzymes BclI, Drai, EcoRI, EcoRV, HindIII, PstI, PvuII and XhoI. Site and fragment length polymorphisms were characterized, and cases of heteroplasmy involving length variations of some of these fragments were observed. In T. divaricatus the length variations are of about 50-200 bp, suggesting the occurrence of changes in the number of small repeats in the control region of the molecule. In T. amathites the variation detected of about 400 bp is probably due to a duplication or deletion of a segment of this order of magnitude. The fragment length mutation rate also varied among the species: it is smaller in T. amathites when compared to T. divaricatus. In relation to site polymorphisms, we detected relatively low nucleotide diversity values in the populations analyzed, the smallest ones being found in the populations of T. nanuzae and T. divaricatus. In terms of haplotype diversity, on the other hand, the most polymorphic populations were those of T. divaricatus from the two different dune fields. The relatively high degrees of polymorphism occurring in these populations suggest recent population expansions after putative past reductions in population sizes. The differentiation levels found between conspecific populations of T. nanuzae and of T. divaricatus were compatible with those reported in literature for distinct species. The mtDNA data point to an evolutionary history for the nanuzae group that goes back to 5.5 million years ago and not 12.000 years ago, as originally proposed by Rodrigues in 1986, considering the time at which the São Francisco River found its way out to the Atlantic Ocean at the end of the last glaciation period. The nanuzae group phylogeny could not be determined unambiguously using the restriction fragment analysis of mtDNA. Considering the present data and the previous morphological, allozymic and chromosomal data, a hypothesis is proposed according to which the cladogram of this group would be, in fact, polytomous. In addition, the molecular data based on mtDNA analysis did not contest the monophyletic condition of the nanuzae group.

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The use of RAPD as a genetic marker in the study of DNA polymorphisms and genetic characterization of Drosophila species in the cluster buzzatii

(Juliana Polachini de Castro*)

The great differentiation found with the use of different genetic markers in the populations of the Drosophila species included in the buzzatii cluster and the intriguing aspects of its speciation process led to the present study, elaborated mainly with the purpose of evaluating the taxonomic potential of the RAPD assay for those species. The amplification of the genomic DNA with three different oligonucleotide primers was done in three strains of D. buzzatii, two of D. seriema, two of D. koepferae, one of D. serido type B and one of D. serido type D. As outgroups, one strain of D. mulleri and one of D. simulans were used. In total, 99 RAPD bands that varied from 290 to 5090 bp were produced. Strain-specific and species-specific DNA fragments generated by the different primers produced a characteristic pattern of RAPD bands that allowed to recognize the different strains and species. The largest coefficient of variation was obtained in the D. buzzatii strains followed by D. seriema strains. D. koepferae and D. serido types B and D strains were less polymorphic, except the strain A55 of D. serido type B and B25 of D. koepferae for the primer 69. The strain B31 of D. serido type D, unlike the others, presented a high number of monomorphic bands for all the primers used. The present RAPD data for the strains of D. buzzatii and D. seriema are the first evidence for the existence of a vast stock of unknown nuclear polymorphism segregating in those populations. The level of RAPD polymorphism found in the strains studied was remarkably higher than that observed in studies of esterases performed in the same strains. The similarity index and the cluster analysis confirmed the relationships obtained using other markers in the same strains, reinforcing the idea of a differentiation between the strains of D. serido types B and D and the other species in the cluster. However, the strain A55 (D. serido type B) from Brazilian cerrado showed to be closer to D. koepferae of the Argentinean Chaco. This finding was not observed with any other marker. The RAPD assay showed high resolution and efficiency to reveal new polymorphisms not detected by other genetic markers, to document the variability within and among populations and also for taxonomic purposes.

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Juliana Polachini de Castro*
Reproductive parameter and degree of assynapsis in polytene chromosomes of Drosophila species of buzzatii cluster

(Parâmetro reprodutivo e grau de assinapse em cromossomos políténios de espécies de Drosophila do cluster buzzatii)

Luciana Paes de Barros Machado*

The Drosophila buzzatii cluster of the repleta group (mulleri subgroup) is widely distributed across different vegetation formations in South America. Due to polymorphism and polytypism this cluster is an excellent subject for ecological adaptation, population differentiation and speciation studies. In this work geographical strains of the Drosophila buzzatii cluster (D. seriema, D. koepeferae and D. buzzatii) were intra- and intercrossed, aiming to evaluate the reproductive and cytogenetic relationships of these species. The strains and their hybrids were analyzed regarding fertility, fecundity and degree of pairing in polytene chromosomes.

Fertility of crosses was evaluated by observing the presence or absence of larvae and the fecundity through the average number of descendants yielded in every oviposition period and the total for every cross. For this study mass crosses were used. Male and female virgins were kept apart until the desired age, seven to nine days. In each bottle 20 couples were put, and for each type of cross four replicates and two transfers to new culture medium were realized at intervals of seven days (after this period, the parental fly was discarded). The number and sex of descendants were computed in two weekly census, in a total of four scores. The crosses were kept at constant temperature of 20 ± 1°C. For F1 hybrids yielded in intercrosses the fertility and fecundity were evaluated through mass crosses, as in the parental crosses, but with only one transference to new culture medium. The cytogenetical analysis was realized in plates of polytene chromosomes of salivary glands of late third-instar larvae stained with lacto-acetic orcein. The data were analyzed with the Minitab and Statistica programs.

The strains were highly fertile in intracrosses. Differences of fertility were observed in intra- and intercrosses of strains belonging to the same species. Intracross fertility of D63M (D. seriema, Mucugê, BA) was significantly lower than that of every other D. seriema strain and D63M was also the only strain of these species that was fertile in crosses with D69R2 (D. buzzatii, BA). Another case was related to geographical strains B50Q3 (Milagres, BA) and A55F11 (Belém, PA), both classified as D. kasumiae (Milagres, BA) and A55F11 (Belém, PA), and the cross was fertile. On the other hand, B50Q3 showed asymmetrical isolation when intercrossed with D69R2.

Strains D69R2 and D69R5 were sterile in intercrosses with strains of D. seriema. The analysis of courtship showed prezygotic reproductive isolation mechanisms operating in these cases.

Sterility of hybrid males from some F1 intercrosses was observed: D. seriema (A55F11) x D. seriema (D71C1BM, Morro do Chapêu, BA), D. seriema (A9S3, Serra do Cipó, MG) x D. koepferae (B25D7, Famatina, Argentina) and D. koepferae (B25D7) x D. seriema B (B50Q3). The spermatozoa of these hybrids showed no motility at any age tested.

The cytogenetic analysis of hybrid polytene chromosomes showed chromosome pairing to be analogous to intracrosses, that is, complete pairing in all regions, except in the microchromosomes (VI) and proximal region of chromosomes X.

Aedeagus morphology is a good character to define groups that correspond to biological species. However, the intraspecific divergence exhibited in some strains and other results obtained in this study as to reproductive compatibility and chromosome pairing showed that these and other markers have similar importance due to the complexity of the speciation process.

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Molecular characterization of ectomycorrhizal fungi by PCR-RFLP rDNA, sequencing of the ITS region and RFLP analysis of mitochondrial DNA

(Caracterização molecular de fungos ectomicorízicos por PCR-RFLP de DNA, sequenciamento de sua região ITS e análise por RFLP do DNA mitocondrial)

Eliane Aparecida Gomes*

Ribosomal and mitochondrial DNA polymorphisms are important sources of molecular markers for taxonomic, phylogenetic and genetic diversity analyses of ectomycorrhizal fungi. In this work, the internal transcribed spacer (ITS) was first amplified by polymerase chain reaction (PCR) with specific primers and then cleaved with different restriction enzymes to evaluate inter- and intraspecific variation among 28 isolates of ectomycorrhizal fungi belonging to 19 genera and eight species. Amplification products ranged from 560 to 750 bp. The range of polymorphism observed did not allow proper identification of most of the isolates; however, cleavage of the amplified fragments with restriction enzymes AluI, HaeIII, HinfI, HpaII, and Sau3AII revealed extensive polymorphism. Specific restriction patterns were observed for all eight genera and most of the species. The species which could not be identified by a specific pattern belonged to two genera: Rhizopogon (R. nigrescens, R. reaii, R. roseolus, R. rubescens and Rhizopogon sp.) and Laccaria (L. bicolor and L. amethystea).
Inheritance of traits related to bread-making quality of wheat

(Herança de caracteres do trigo relacionados à qualidade de panificação)

Andréa Mittelmann*

Bread-making quality of wheat is important to the industry and farmers. It improves market value of wheat. Breeding for quality has been difficult because it is a very complex trait affected by genetic and environmental factors. In addition, it requires specific tests for early-generation selection.

In this study 10 crosses between five cultivars with different bread-making quality were analyzed. The evaluated traits were sedimentation volume, sedimentation index, protein content and percent glutenins in total protein. Parent means for sedimentation volume and index and protein content differed from each other. Variability for all traits was detected by variances and genetic parameters and the occurrence of complementary genes may be suggested. An important part of this variability was not explained by differences among parents for high molecular weight glutenin subunits. The cultivar CEP 24 – Industrial contributed with the highest number of genes to quality improvement. It also had favorable genes for protein content. The trait sedimentation volume and sedimentation index were strongly associated, but the second seemed to be the most suitable for bread-making quality selection because of its larger heritability. Protein content and percent glutenin in total protein were independent and they affected quality estimates obtained from the sedimentation test.

Efficiency of induced mutations and artificial hybridization in enhancing genetic variability of some triticale agronomic characteristics

(Eficência de mutações induzidas e hibridação artificial para aumentar a variabilidade genética de algumas características agronômicas do trigo)

Fábio Pandini*

The restricted genetic variability caused by the narrow genetic background of triticale is one of the important factors that minimize the development and the progress of this crop in Southern Brazil. Techniques that increase genetic variability may help breeding for adaptability and production stability, allowing the use of more efficient productive systems. Induced mutations by gamma radiation (0, 5, 10, 20 and 40 kR doses) and reciprocal artificial crosses were tested as mechanisms of enhancing genetic variability for plant height and flowering date in two triticale cultivars, BR4 and EMB18. The treatments showed similar genetic variability amplitude for both traits but different means and variances, being suitable for increasing variability in breeding programs. There were superior and inferior mutants or recombinant genetic material for plant height and flowering date. Genotypes showed different responses as the gamma ray doses were increased, expressing short plant height and late flowering date. The decision of which of the treatments should be used depends on the material that is available and the selection method to be used.


Analysis of genetic variability in species of the family Anostomidae (Pices, Characiformes) from the Tibagi River basin

(Análise da variabilidade genética em espécies da família Anostomidae (Pices, Characiformes) da bacia do rio Tibagi)

Lucimara Chiari*

Genetic variability is an important feature of populations for both short term fitness of individuals and the long term survival of the population, allowing for adaptation to changing environmental conditions.

The RAPD technique was applied to evaluate the genetic variability of eight fish species of the family Anostomidae (Schizodon intermedius, S. nasutus, Leporinus friderici, L. octofasciatus, L. striatus, L. amblyrhynchus, L. elongatus and L. obtusidens) from the Tibagi River basin, in the State of Paraná, Brazil.

The samples were collected in three different regions of this basin (upper, central and lower Tibagi). RAPD data was obtained from the analysis of 10 previously selected primers. NTSYS-PC application software was employed to analyze the RAPD data; a similarity matrix was constructed using Jaccard’s coefficient of similarity and a dendrogram was obtained by the UPGMA method.

Based on the RAPD patterns, the genetic variability was estimated by the proportion of polymorphic loci (P) for the species collected in just one region and by the mean proportion of polymorphic loci (Pm) for the species collected in more than one region.

Leporinus elongatus was collected in two regions (central and lower Tibagi) and presented the greatest estimated genetic variability (Pm = 58.7%) and L. amblyrhynchus, collected in just one region, exhibited the least (P = 29.3%). The other species collected in just one region (S. intermedius, L. striatus and L. friderici) possessed P values between 40 and 50% and the species collected in more than one region (S. nasutus, L. elongatus, L. obtusidens and L. octofasciatus) presented Pm values > 50%.

The dendrogram analysis revealed that, with the exception of L. obtusidens, specimens of the same species collected in different regions were grouped, suggesting gene flow. This result reinforces the fact that these species are migratory as are the majority of Anostomidae species.

The dendrogram constructed by using the comparative analysis of the eight species clearly demonstrates the separation of the two genera (Schizodon and Leporinus) and also the separation of the two species of the genus Schizodon and four of the six species of Leporinus. There was no evident separation of L. elongatus and L. obtusidens, suggesting a strong genetic similarity between these two species.

Research supported by CAPES.

Apparently balanced chromosomal translocation associated to mental retardation and/or congenital malformations. Clinical-cytogenetic considerations and proposed mechanisms for the abnormal phenotype in six cases

(Translocação cromossômica aparentemente balanceada associada ao retardo mental e/ou malformações congênitas. Considerações clínico-citogenéticas e mecanismos propostos para o fenótipo anormal em seis casos)

Elenice Ferreira Bastos*

Structural chromosomal abnormalities have been used as an important tool in research towards genetic diseases, specially in the construction of the Morbid Anatomy Map of the Human Genome (OMIM, 1998). In this context, balanced chromosomal translocations associated to abnormal phenotypes may be relevant for the identification of specific chromosomal sites associated with a particular disease. This investigation comprises the study of six cases of apparently balanced chromosomal translocations associated with abnormal phenotypes through several cytogenetic techniques, including molecular cytogenetic (FISH). Referral for cytogenetic studies were: congenital malformations (cases I, II and V), mental retardation (cases III and IV) and primary amenorrhea (case VI). The six karyotypes were characterized according to ISCN (1995) as:

- Case I = 46,XX, t(3;8)(p23;q22.1);
- Case II = 46,XX t(4;16)(q21;q22);
- Case III = 46,XY, t(2;15)(q23;q22);
- Case IV = 46,XY, t(2;17)(q11.2;q25.3);
- Case V = 46,XX, t(11;13)(p13;p33);
- Case VI = 46,XX, t(6;18)(p23;q22).

A phenotype-karyotype correlation was attempted based on the smallest overlapping region and delineated in cases I, IV and V as: BPES syndrome and region 3p23, 2p deletion syndrome and region 2p11.2, and isolated aniridia and region 11p13, respectively. In cases II (Binder-like syndrome), III (unspecific mental retardation) and VI (primary amenorrhea) both chromosomal breakpoints involved in the translocation could be candidate regions. The parental origin of the anomalous chromosomes in two out of four informative de novo cases was paternal. Concerning the etiological mechanisms attributed to the abnormal phenotype, haplo-insufficiency was considered in cases I, II and V, and genomic imprinting, position effect, and microdeletion syndromes were considered in the remanescent cases.

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