APPLICATION OF COMET ASSAY TO ASSESS GENOTOXICITY OF AQUEOUS EXTRACTS FROM PERSIAN WALNUT (*JUGLANS REGIA* L.) HUSKS AND APPROPRIATE STATISTICAL EVALUATION

M. PETRICCIONE [1] C. CINIGLIA[1]

[1]Consiglio per la Ricerca e la Sperimentazione in Agricoltura – CRA – Unità di Ricerca per la Frutticoltura, Via Torrino 3, 81100 Caserta Italy. milena.petriccione@entecra.it; [2]Dipartimento di Scienze della Vita, Seconda Università di Napoli, Via Vivaldi 43, 81100 Caserta, Italy

The aim of this study was to confirm the usefulness of the Comet assay as a predictive genotoxicity screening test in evaluating the impact of walnut green husks. Aqueous extract of green husks from *Juglans regia* L. cv Sorrento was tested for its inhibitory activity and genotoxicity; radish has been choosen as a model, thanks to its quick germination and root growth (Einhellig and Rasmussen, 1978).

Seeds were germinated in Petri dishes (90 mm diameter) with 25 ml of autoclaved and agarized (1%) soil extract. After 4 days treatment with both undiluted and diluted (1:1, 1:2 and 1:4) walnut husk aqueous extract, seeds germination and radicles/hypocotiles lengths were expressed as seeds germination inhibition (%) and growth inhibitory activity (%). The DNA damage studies were carried out using comet assay according to Singh *et al.* (1988) with slight modifications. Radish seeds were germinated under sterile conditions on filter paper disks embedded with distilled water. At the stage of 2 true leaves, radicles of radish seedlings were immersed in plastic vials containing 2 ml of defined dilutions ofwalnut husk aqueous extract for 24 h at room temperature with a 16 h photoperiod. After this period, excised radicles of the treated plants were placed in a Petri dish kept on ice and spread out with cold 400mM Tris buffer pH 7.5. Using a fresh razor blade, the radicles were gently sliced, and the isolated nuclei collected in the buffer and embedded in a two-layered microgel, (Ciniglia *et al*., 2010). The slides were dipped into a lysis solution for 1 h and then in an electrophoresis buffer (300mM NaOH and 1mM Na2EDTA; pH>13) for 15 min at 4 ◦C, to allow unwinding of DNA. Electrophoresis was carried out using the same buffer at 4 ◦C for 20 min at 25V and 300 mA. Then, the gels were neutralized embedding the slides twice in 400mM Tris buffer (pH 7.5); DNA molecules were stained with 80μl ethidium bromide (20μg/ml). The nuclei images were observed using epifluorescent microscopy with an excitation filter of BP546/10 nm and a barrier filter of 590 nm (Nikon Eclipse E800) equipped with a digital camera. % tail DNA, tail length and tail moment were measured using Tritek CometScore software.

Phytotoxicity tests have revealed negative impact of walnut aqueous extract on both germination and early seedling growth of radish plantlets, after 96 h treatments. Comet assay has been proved to be ideal in the predictive detection of damage in plant nuclei affected by walnut extract, just after 24h, as well; high correlations between DNA damage in radish radicles and walnut husks water extract dilutions were observed with all parameters; since a high asymmetry in frequency distribution in % tail DNA data were observed, risk of an incorrect interpretation of experimental outcomes. By comparing different distribution types to the histograms of the data, Kolmogorov-Smirnov test is resulted to be the best statistical analysis to evaluate the goodness of the fit and Johnson SB distribution was the best distribution describing Comet assay data.

Acknowledgements. We thank the Ministero delle Politiche Agricole Alimentari e Forestali for financial support for the Project BIONUTS.

Einhellig F.A., Rasmussen J.A., 1978. Synergistic inhibitory effects of vanillic and p- hydroxybenzoic acids on radish and grain sorghum. J. Chem. Ecol. 4: 425-436.

Singh N.P., McCoy M.T., Tice R.R., Schneider E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell. Res. 175: 184-191.

Ciniglia C., Pinto G., Sansone C., Pollio A., 2010. Acridine orange/Ethidium bromide double staining test: A simple In-vitro assay to detect apoptosis induced by phenolic compounds in plant cells. Allelopathy J. 26: 301-308.

INDICE