Types of immunolabeling pattern in interphase nuclei of Pennisetum nervosum (Nees) Trin. (Poaceae) obtained with phosphorylation of histone H3 at serine 10

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In different species, H3S10ph is involved or with condensation or the cohesion of the chromatids and chromosomes at mitosis and meiosis. However, recently, the role of phosphorylation of histone H3 at serine 10 (H3S10ph), during interphase, has been associated with transcriptional regulation, DNA replication and apoptosis. In this study, intraspecific variation in interphase nuclei (IN) with different patterns of immunodetection signals of H3S10ph was investigated in four accessions of Pennisetum nervosum (Nees) Trin. (15736; 15737; 15742 and 15749) of the Germplasm Bank of Embrapa Gado de Leite. For immunodetection, roots were fixed in 4% paraformaldehyde, macerated in PBS 1X with 1% Triton X-100, blocked with 3% BSA and digested with pectinase:cellulase (2%:4%, ph 7.5) and cytohelicase (1%), for 4h. Primary antibody anti-H3S10ph (Rabbit polyclonal IgG, Santa Cruz Biotechnology, USA) was diluted 1:100 and detected with the secondary antibody Goat anti-rabbit IgG FITC conjugated (Santa Cruz Biotechnology, USA). We evaluated 200 IN per accessions and the comparison of the means were carried out by the Scott Knott test (p<0.05). Immunodetection of H3S10ph revealed six types of interphase nuclei. Type I, G1 stage of interphase, has low density and intensity of phosphorylation signals. Type Il shows diffuse signals, with high intensity in the nucleolus and / or with two small foci of the phosphorylation around the nuclear envelope. In some cases, it was also observed two regions intensely phosphorylated peripheral to the nuclear envelope. Type III presents two to six foci phosphorylation marked on the center of the nucleus. Types II and III characterize the transition from the S-G2 phases. Type III was found exclusively in accessions 15737. Type IV shows diffuse signals with high intensity in the nucleus and nucleolus and several foci distributed by the nucleus. Besides of statistically different among accessions, nucleus type IV is characteristic of the accessions 15742. Type V shows diffuse signal without intense labeling in the nucleolus. Type VI has polarized phosphorylation signal and was observed more frequently in the accessions 15749. The intraspecific variation of the pattern of distribution of immunodetected signs of H3S10ph in the interphase nuclei contributed to characterize four accessions of the P. nervosum. Similarly, as proposed for the tobacco and maize, it is suggested that this epigenetic modification is also related to gene regulation in interphase nuclei in this species.

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