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ALTERATIONS IN LEAF PROTEIN PROFILES OF ANNUAL RYEGRASS UNDER DIFFERENT LEVELS OF OSMOTIC STRESS

ALTERAÇÕES NO PERFIL PROTEICO DE FOLHAS DE AZEVÉM ANUAL SOB DIFERENTES NÍVEIS DE ESTRESSE OSMÓTICO

Passos, LP[1]; Freitas, JCE[1]; Santos, ASO[1]; Martins, MF[1]; Mittelmann, A[1]; Fogaça, GN[1]; Dias, DHS[1]

[1]Embrapa Gado de Leite - Rua Eugênio do Nascimento 610, Juiz de Fora, MG 36038-330 Brasil. leonidas.passos@embrapa.br; joberconde@gmail.com; alessa.siqueira@gmail.com; marta.martins@embrapa.br; andrea.mittelmann@embrapa.br; gisele_fogaca@hotmail.com; diegohsdias@gmail.com

The growing importance of annual ryegrass (*Lolium multiflorum*) for dairy cattle feeding has prompted a growing need for better understanding the physiological responses of the species to abiotic stress. Therefore, a study was carried out to evaluate the short-term responses of annual ryegrass cv. BRS Ponteio to sudden root exposure to increasing levels of PEG 6000 as estimated by protein profiles. The effects were determined after 30 days of continuous stress through the microfluidic *Lab-on-a-Chip* technique (MF) in comparison with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The analysis through MF considered a complete block design, with five treatments (0, 200, 250, 300, and 350g.L^{-1} PEG6000), 12 blocks (i.e. the number of microfluidic chips) and one observation per plot. SDS-PAGE evaluations considered a complete randomized design, with five treatments (0, 200, 250, 300, and 350g.L^{-1} PEG6000), six replications (i.e. the number of wells) and one observation per plot. The protein extraction was carried out with a commercial kit (Sigma-Aldrich) with minor alterations. The proteins expressed in each treatment were separated according to their respective molecular mass and retention times. SDS-PAGE results demonstrated that increased osmotic stress altered annual ryegrass leaf protein profiles, with band intensification becoming detectable from the level of 250 g.L^{-1} PEG6000. The most conspicuous modifications were observed in the molecular mass ranges of 20-30 and 50-60 kDa. Results obtained with MF, besides nearly reproducing those verified with SDS-PAGE, revealed additional and striking protein expression bands in the molecular mass range of 3.5-6.5 kDa, which also increased gradually in intensity as the level of osmotic stress was enhanced. It is concluded that annual ryegrass exhibits changes in protein profiles in response to varied levels of stress, suggesting there is room for advancements in knowledge through the use of proteomic strategies. MF is likely to be the procedure of choice as compared to SDS-PAGE, given its higher sensitivity and the ability of detecting minor differences in the lowest protein molecular mass ranges. The applicability of such results to breeding programs appears to be immediate.

Keywords: *Lolium multiflorum*; Proteomics; Forage grass, BRS Ponteio