Genetic confirmation of Johnsongrass accessions and their weedicide resistance per six geographical locations of Tennessee

The geographic profiling of invasive weeds like Johnsongrass (*Sorghum halepense*) is crucial for understanding their herbicide resistance patterns per regions. This notorious weed cause huge agricultural losses in different regions of Tennessee. By confirming the Johnsongrass samples collected from regional populations, using specific genetic markers associated with true type identification, subsequent diversity studies are possible. Johnsongrass leaf samples were collected from different counties across Tennessee during summers of years 2022-2024. To ensure comprehensive representation, three distinct samples of Johnsongrass were procured from separate locations within each selected Tennessee county. In total, 51 out of 92 counties, comprising nine from Northwestern Tennessee, 10 from Southwestern Tennessee, six from Northeastern Tennessee, seven from Southeastern Tennessee, 12 from the Northcentral Tennessee and seven from the Middle Tennessee regions were covered. Polymerase Chain Reaction is employed for species confirmation, and in this study five different primers were used to amplify DNA sequences unique to the Johnsongrass conserved genome towards true to type identifications of all samples gathered. EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) is a crucial enzyme in the shikimate pathway, responsible for synthesizing aromatic amino acids in plants. Weedicide glyphosate inhibits EPSPS, disrupting this pathway leading to plant death. The EnzChek Phosphate Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA) was utilized to measure the enzymatic activity of EPSPS, a key target in glyphosate resistance studies, in Johnsongrass (*S. halepense*) samples. Thereby, glyphosate-resistant Johnsongrass regional populations potentially exhibiting mutations in the EPSPS gene or amplified EPSPS expression, for reducing herbicide efficiency, remained major guest of this study; which has significant impact for Tennessee agricultural landscape.

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