REPRODUCTION AND GENETIC VARIATION IN JOHNSONGRASS *(SORGHUM HALEPENSE)* POPULATIONS

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Summary. Johnsongrass is a perennial weed that is capable of reproducing by means of seed and rhizomes. Polymerase chain reaction-random amplified polymorphic DNA (PCR-RAPD) technique was used to examine the reproduction and genetic variation within 10 johnsongrass populations in Kansas. Two twenty-base primers provided a suitable number of fragments to distinguish between individual johnsongrass genotypes. All populations had different degrees of genetic variability, with the occurrence of many genotypes per population and few clones, indicating the importance of reproduction by seed in population maintenance and dissemination.

INTRODUCTION

Johnsongrass has been listed by Holm et al. (2) as one of the 10 worst weeds of the world. It reproduces by seeds as well as by rhizomes, and thus, is extremely difficult to control (4). To date, little is known of the frequency or importance of seed and rhizome reproductions to the maintenance and dissemination of johnsongrass. However, considerable intraspecific variation has been described for this weed species (1). The aim of this research was to examine the mode of reproduction and genetic variation occurring within johnsongrass populations.

METHODS

Sites and plant collections. Ten populations of johnsongrass were collected in 1991. The collection sites were located in the state of Kansas, USA on an east-west transect extending approximately 520 km and a north-south transect extending approximately 325 km. At each site, plants were selected at random and at intervals along a transect. Each plant was unearthed, all soil was removed from the roots, and the shoots were clipped back to 10 cm. The plants were transplanted to styrofoam cups containing a mixture of peat:sand:vermiculite (1:1:1, v/v/v). The plants were placed in a mist chamber for a week to allow for the re-establishment of roots and shoots, then removed from the chamber and transplanted to 15 cm plastic pots containing a mixture of peat:vermiculite:silt loam soil (1:1:1, v/v/v). Pots were arranged and maintained in a greenhouse by populations until DNA extraction.

DNA extraction. One gram leaf samples were obtained from the plants growing in the greenhouse. The samples were placed in a mortar, frozen with liquid nitrogen, and crushed with a pestle. Five mL of CTAB buffer was added during crushing. The mixtures were transferred to plastic test tubes and placed in a 65°C oven for 30 min. The tubes were removed from the oven, 5 mL of chloroform was added to each tube, then were centrifuged at 13,000 x g (2,200 rpm) for 15 min. The supernatants were transferred to new tubes, and 5 mL of isopropanol added to each to precipitate the DNA. The tubes were then placed in a -20°C freezer for 8 h. The samples were centrifuged at 13,000 x g for 15 min, and all solutions removed, leaving the DNA attached to the bottom of each tube. Two mL of TE buffer (3) were added to the DNA to resuspend it. The above procedures were repeated once to further purify the DNA samples.

Amplification. One µL (approximately 100 ng) of the DNA extract was added to a well on a PCR-assay plate containing 200 µL/L each of dNTP's, 12 pg/30 µL of either HPH-1 or HPH-2

primer, 20X reaction buffer and 0.3 units/30 µL reaction of the thermostable DNA polymerase (Replitherm™). Fifty cycles of PCR amplification were performed in a thermocycler (MJ Research™) with the following temperature profile: 94°C (2 min), 92°C (30 sec), 28°C (30 sec), and 72°C (1 min) followed by a final incubation period of 5 min at 22°C.

Electrophoresis and analysis. Amplification products were analyzed by electrophoresis in a 1.25% gels in half-strength TBE buffer and detected by staining with 0.5 ug/mL of ethidium bromide. A 1 kb ladder (BRL™) DNA marker was run on each gel. Gels were placed on top of a UV light source to reveal DNA bands and photographed. Gels were scored by recording the presence or absence of bands. Data were analyzed using PAUP (Phylogenetic Analysis Using Parsimony) computer software.

RESULTS AND DISCUSSION

The PCR amplification of the DNA using two twenty-base primers (HPH-1 and HPH-2) resulted in the amplification of discrete fragment profiles among the johnsongrass individuals that were examined. Results of our study indicated that Marshall and Scott Co. populations were the most diverse having 25 and 23 genotypes (out of 30 individuals), respectively. Overall data also indicated that clonal reproduction was predominant in 8 populations, while reproduction from seeds was higher in the remaining 2 populations. Genetic diversity within populations varied by location.

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