



Introduction

Purple nutsedge (*Cyperus rotundus* L.) is an important agricultural weed in tropical and warm temperate regions of the world. It reproduces primarily by asexual means through the production of rhizomes and tubers. Therefore, one would predict that genetic diversity in purple nutsedge would be minimal, and if present, would manifest itself as several stable, easily differentiable lineages. Accessions of purple nutsedge from different geographically locations have been shown to exhibit quantifiable, morphological variations that include: shoot, bract, and culm length; inflorescence shape; number of leaves per shoot; leaf color and shape; and tuber size, texture, and shape. The objective was to determine whether genetic variation exists within a morphologically and geographically heterogeneous cross-section of purple nutsedge accessions using traditional genomic analytical techniques.

Materials and Methods

Purple nutsedge growth conditions: Purple nutsedge were grown in 15 cm. pots in a greenhouse maintained at 35 +/-2°C using a 1:1 mixture of soil [Bosket sandy loam] and Jiffy Mix growth mixture. Pots were irrigated as necessary and fertilized with a complete fertilizer. A 14 hour photoperiod was maintained with sodium vapor lamps.

Sample collection and genomic DNA extraction: Mature purple or yellow nutsedge leaves were cut into 1 inch sections and freeze-dried. Samples were ground into powder using a ball mill. DNA was extracted using a CTAB/phenol/chloroform extraction procedure. DNA was precipitated in isopropyl alcohol, washed with 75% ethanol, and air-dried before re-dissolving in Tris/EDTA buffer.

Randomly amplified polymorphic DNA (RAPD) analysis: 240 randomly designed 10bp oligonucleotide primers were screened using DNA samples from five geographically diverse locations. Primers that generated easily scorable bands, and differentiated between samples, were screened against our set of genomic DNAs. PCR amplification was performed using JumpStart RedTaq Ready Mix (Sigma-Aldrich) on a MJ Research PTC 225 (Biorad, Hercules, CA 94547) with conditions of 95°C for 120 s; 30 cycles of 95°C for 30 s, 46°C for 30 s, 72°C for 30 s; and one cycle of 72°C for 300 s followed by maintenance at 4°C until detection.

Agarose gel electrophoresis: Samples were loaded into 1.5% agarose gels and run at 24V for 16-18 hours, and then stained with ethidium bromide. Visualization and photography was conducted on an Alphamager 3300 transilluminator (Alpha Innotech Inc).

Data analysis: Dendrograms were drawn in Phylowidget (<http://www.phylowidget.org>) based on cluster analysis results in SAS using data from band patterns on agarose gels, and coded morphological traits from a study by Wills, 1998 (Weed Technol. 49:1-503).

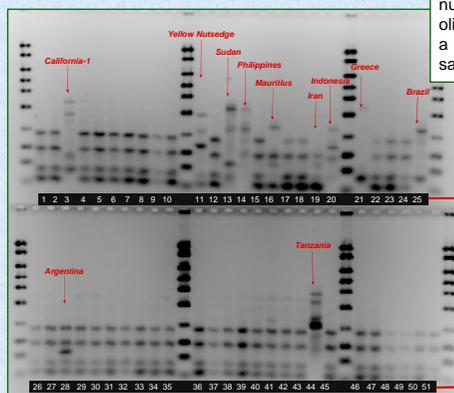
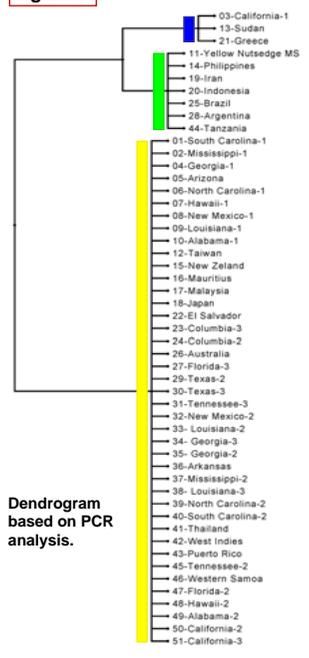


FIGURE 1. DNA extracted from 50 accessions of purple nutsedge and 1 sample of yellow nutsedge was subjected to Random Amplified Polymorphic DNA PCR analysis using an oligonucleotide designated Operon G-17. Lanes 1, 12, 23, and 29 (top) or 30 (bottom) contain a 1kb DNA ladder. All other lanes consist of nutsedge PCR products ordered sequentially by sample number. Multiple samples from same state or country are indicated by number.

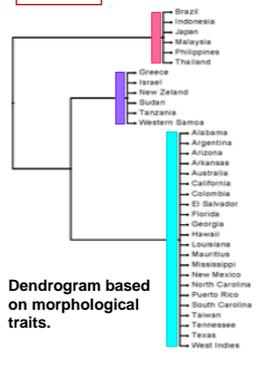
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|--------------------------|------------------|----------------------|
| 1. South Carolina-1 | 18. Japan | 35. Georgia-3 |
| 2. Mississippi-1 | 19. Iran | 36. Arkansas |
| 3. California-1 | 20. Indonesia | 37. Mississippi-2 |
| 4. Georgia-1 | 21. Greece | 38. Louisiana-2 |
| 5. Arizona-1 | 22. El Salvador | 39. North Carolina-2 |
| 6. North Carolina-1 | 23. Columbia-1 | 40. South Carolina-2 |
| 7. Hawaii-1 | 24. Columbia-2 | 41. Thailand |
| 8. New Mexico-1 | 25. Brazil | 42. West Indies |
| 9. Louisiana-1 | 26. Australia | 43. Puerto Rico |
| 10. Alabama-1 | 27. Florida-2 | 44. Tanzania |
| 11. Yellow Nutsedge (MS) | 28. Argentina | 45. Tennessee-2 |
| 12. Taiwan | 29. Texas-2 | 46. Western Samoa |
| 13. Sudan | 30. Texas-3 | 47. Florida-3 |
| 14. Philippines | 31. Tennessee-2 | 48. Hawaii-2 |
| 15. New Zealand | 32. New Mexico-2 | 49. Alabama-2 |
| 16. Mauritius | 33. Louisiana-2 | 50. California-2 |
| 17. Malaysia | 34. Georgia-2 | 51. California-3 |

Figure 3



Dendrogram based on PCR analysis.

Figure 2



Dendrogram based on morphological traits.

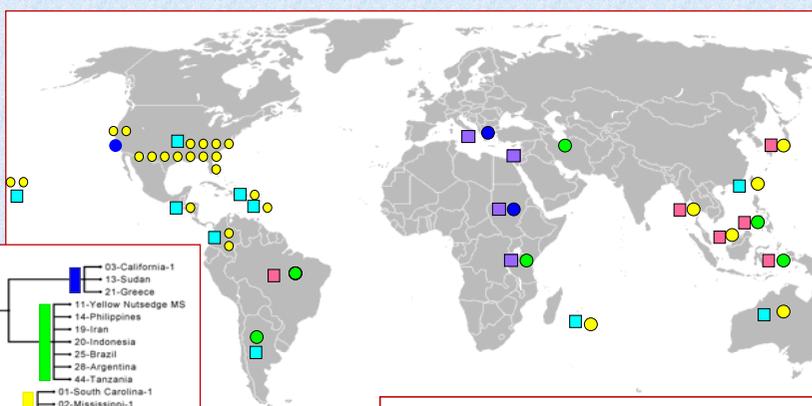


Figure 4. Global distribution of accessions based on PCR molecular markers (circle) and morphological traits (square). Like colors indicate similar groups.

Results

DNA from 50 accessions of purple nutsedge from 21 countries and 14 states in the United States, and one of yellow nutsedge from Mississippi, were subjected to RAPD PCR analysis using 14 random primers and 20 soybean SSRs. RAPD profiles revealed genetic variation in the following accessions: Argentina, Brazil, California-1, Greece, Indonesia, Iran, Mauritius, Philippines, Sudan, and Tanzania. All other samples displayed strikingly similar RAPD profiles (Figure 1).

Dendrograms based on cluster analysis in SAS using morphological characteristics (Figure 2) and molecular marker patterns (Figure 3) were similar, providing evidence for divergent groups of purple nutsedge (Figure 4). The two or three broad groups worldwide (i.e. Americas, Europe-Africa, Southeast Asia-Pacific) may indicate regional evolution of purple nutsedge, in which stable, nonlethal mutations have been maintained in subsequent generations through asexual reproduction. Evidence for hybridization among groups is currently lacking. Some accessions with distinct marker profiles may have been introduced into new locations (i.e. California-1 may have originated from Greece).

The regional groups may have differences in agronomically important weedy traits such as growth rates, fecundity, competitiveness, and allelopathic potential, or responses to herbicides. Understanding the genetic basis for differences among regional groups may improve our knowledge of controlling elements in weediness, survival in different habitats, and allow predictive mitigation in agricultural and non-agricultural settings.