

What do chloroplast sequences tell us about the identity of the commonly named Guinea grass, an invasive Poaceae in the southern United States?



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GUINEAGRASS: A TARGET FOR BIOCONTROL IN TEXAS

The commonly named Guinea grass of the Poaceae family is a perennial, tufted grass with a creeping rhizome and a large multi-branched, open panicle with loose, flexible branches (Fig 1). Native to Africa, the grass has been extensively and successfully introduced as a source of animal fodder to other tropical areas of both hemispheres. On a global scale but particularly in the southern United States (Fig 2), the Caribbean and Hawaii, Guinea grass is becoming a serious threat to biodiversity not only due to its invasiveness but also because it produces high fuel loads for fires (Fig 3). In Texas, it is particularly invasive in plantations of citrus (Fig 4) and sugar cane.

A biological control program is being attempted in Texas by the Subtropical Agricultural Research Center of USDA-ARS that is located at Weslaco in the U.S. and a preliminary survey of the natural enemies of Guinea grass was conducted in Cameroon, Central Africa in 2009 and 2010 (Mercadier et al., 2009). Source populations of the Texan invasion have to be identified in the native range in order to facilitate the search for potential biological control agents. However, investigating this first issue represents a challenge by itself to biocontrol practitioners as Guinea grass constitutes a polyploid agamic complex.



Fig1. multiple-branched panicle



Fig2. Distribution in the U.S.



Fig3. Ruderal invasion in Texas



Fig4. Invasion in a citrus orchard in Texas



Fig5. Site in Australia



Fig6. Site in Texas



Fig7. Site in Cameroon

GUINEAGRASS: A POLYPLOID AGAMIC COMPLEX

Polyploidy, defined as the condition of having more sets of homologous chromosomes instead of the two present in diploids, is an evolutionary phenomenon that is a widespread mode of evolution among invasive weeds. It has also long been known that polyploidy could be accompanied by agamospermy, or asexual seed production. When the two phenomena, polyploidy and agamospermy, occur together in a single plant species the result is the polyploid agamic complex.

Therefore, the most confounding problem when considering the taxonomy of the polyploid agamic complex in Guinea grass is the apparently limitless amount of morphological variation (Figs 5, 6, 7), compared to that found in the sexual diploid species, in addition to variation in ploidy levels and mode of reproduction. The agamic complex includes three botanical species, *Panicum maximum* Jacq (Fig 8), *P. infestum* Anders. (Fig 9), *P. trichocladium* K. Schum. (Fig 10), and several morphologically intermediate types and hybrids (Assenian & Noirot, 1991). The systematic position of the agamic complex remains uncertain and as illustrated by its periodic transfer from *Panicum* genus to *Urochloa* and more recently from *Urochloa* to *Megathysus* genus (Salariao et al., 2010).



Fig8. *P. maximum*



Fig9. *P. infestum*

Fig10. *P. trichocladium*

MOLECULAR TOOLS AND HISTORICAL/MODERN SPECIMENS TO IDENTIFY THE INVASIVE

To unravel the taxonomic identification of the invasive, we have begun to analyze the sequences of the two maternally inherited chloroplast regions i.e. NADH dehydrogenase subunit F (*ndhF*) and *trnL-trnF* intergenic spacer (*trnL-F*) that are considered as the most useful markers to resolve the phylogenetic relationships of taxa within the Guinea grass agamic complex.

The analyses were carried out firstly on modern leaf samples i.e. two locations in Texas (Los Indos and Weslaco) and five locations in Cameroon and two locations in Benin in Africa and lately in 14 African historical leaf specimens and one French Guiana specimen in the CIRAD collection, some dating back to 1944, prior to all extensive improvement programs in Africa. Total DNA was extracted from all dried specimens using a classical CTAB protocol that was modified in order to amplify the targeted Cp regions in recalcitrant historical specimens (Jeanneau et al., in preparation). Both *ndhF* and *trnL-F* regions were amplified using the Polymerase Chain Reaction conditions described by Salariao et al. (2010).

Under the experimental conditions developed within the framework of this study, amplifications of *ndhF* and *trnL-F* fragments were successful in historical specimens, 93% (14/15) and 86% (12/14) respectively and sequences of 490bp for *ndh* and of 300 bp for *trnL-F* were obtained (Fig 11). For both genes, the sequence similarities were found by Blast searches of all "*Panicum*" sequences deposited in Genbank database.



Fig11. Amplification of *trnL-F* in historical samples (1-3); historical specimens (4-12); positive control (13), negative extraction control (14), negative PCR control (15).

TWO *ndhF* MATERNAL LINEAGES

Of the 13 sequences analyzed, only two types of *ndhF* sequences were obtained. One has the highest similarity score with validated voucher *Megathysus infestum* (100%). There was no sequence in the database which matched exactly with the second type. Similarity ranged from 98.5% with *M. maximum* and 98.7% with *M. infestum* to 99.3%. It is important to note that the database is lacking any *ndhF* sequence of a validated voucher of *M. trichocladium*.

TWO *trnL-F* MATERNAL LINEAGES

Similar results were obtained with the sequencing of the second gene. Of the 5 sequences analyzed, only two types of *trnL-F* sequences were obtained. One has the highest similarity score with validated voucher *Megathysus infestum* (100%). There was no sequence in the database which matched exactly with the second type. Similarity ranged from 93.4% with both *M. maximum* and *M. infestum*. It is important to note that the database is lacking any *trnL-F* sequence of a validated voucher of *M. trichocladium*.

The distribution of the two lineages is presented in the map (Fig 12). The *M. infestum* lineage (blue in color) is present in Texas and mostly eastern-Southeastern parts of Africa and Cap Verde Island. The other lineage (red in color) is circumscribed to Western-Central Africa and French Guiana.

For the latter, it is likely that the latter corresponds to *M. trichocladium* in light of data obtained from historical records of the genetic improvement programs carried out in Africa and of release scenarios of accessions, the distribution of *M. trichocladium* (Fig 13) and isozyme data of the organization of the polyploid agamic complex obtained by Assenian and Noirot (1991; 1995).

Fig12. Distribution of the two maternal lineages obtained in both genes. *P. infestum* (blue color); putative *P. trichocladium* (red color)

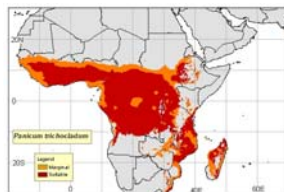


Fig13. African distribution of *P. trichocladium*



CONCLUSIONS

For the first time, our results provided evidence that the maternal lineage of the Texan invasive is *M. infestum* and is similar to the lineage found mainly in Eastern Africa. Then next step will be to analyze the same samples with a biparental-inherited nuclear marker. Future programs of exploration and collection of natural enemies are to be reviewed in light of this finding. Moreover, one should highlight the great benefit of using historical specimens at the onset of this program. The present study represents a new example of the importance of herbarium collections to research and biological control.