Identification of eight Panicum species in Riverina region of NSW using DNA sequence analysis

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Summary  Australia has over 30 Panicum spp. (panic grass) including several non-native species that cause crop and pasture loss due to competition. To develop appropriate management strategies for each species, it is critical to correctly identify panic grass species encountered. Currently, panic grass identification relies on microscopic examination of the inflorescence and spikelets, an approach that is only useful for flowering specimens and requires significant taxonomic expertise. To overcome this limitation, we applied both morphological and molecular techniques for identification of Panicum spp. in the Riverina region of New South Wales. We identified three molecular markers: one nuclear gene region (ITS) and two chloroplast gene regions (matK and trnL intron-trnF) capable of differentiating eight Panicum spp. Concatenation of sequences from ITS, matK and trnL intron-trnF gene regions provided clear separation of eight species collected regionally and identified a maximum intraspecific distance of 0.22% and minimum interspecific distance of 0.33%. Based on comparison with verified voucher specimens, P. hillmanii Chase was prevalent and constituted 78.9% of all samples collected and identified. DNA barcoding represents an accurate and potentially cost-effective tool for distinguishing Panicum spp. at the species level regardless of growth stage. Molecular markers may also be useful for accurate demographic analysis of Panicum grass invasion in Australia and abroad.

Keywords  Panicum spp., Panicum hillmanii Chase, DNA barcoding, matK, trnL intron-trnF, ITS, phylogenetic tree.

INTRODUCTION

The genus Panicum is one of the largest genera in the Poaceae, and is globally distributed (Aliscioni et al. 2003). Depending on the taxonomic concepts followed, up to 500 species are recognised (Byng 2014). Major photosynthetic types (C3, C4, and C3/C4 intermediate) reported in the Poaceae are represented in the genus (Zuloaga et al. 2010).

Panicum spp. are widely distributed in Australia (Figure 1) with 24 indigenous and 9 non-native species currently recognised (CHAH 2005 onwards). They are currently ranked as one of the most economically important weeds of summer fallows in Australia (Llewellyn et al. 2016). Their toxic profile in livestock is suggested to relate to the presence of saponins or sapogenins (Miles et al. 1992). However, variable steroidal saponin profiles may exist in different Panicum spp., and could be associated with their differential ability to cause hepatogenous or secondary photosensitisation in livestock (Quinn et al. 2014).

Panicum is a taxonomically challenging group due to the wide range of variation in both morphological and physiological features (Zuloaga et al. 2010). Subtle morphological differences required for species differentiation have not been well documented (Zuloaga et al. 2010). Existing identification keys to Panicum spp. in Australia are based primarily on reproductive features and therefore are only effective for plants in their reproductive phase (Coissac et al. 2016). Furthermore, keys for identification can be difficult to use with confidence and even experienced taxonomists find Panicum identification problematic at the species level. Therefore, establishment of robust genotyping methods are desirable for unequivocal plant species identification. Identification of Panicum at an individual plant level may also be critical for development of targeted management strategies for invasive and potentially toxic Panicum spp. (Quinn et al. 2014).

DNA barcoding, a simplified DNA-based species identification approach (Hebert et al. 2003), has been
utilised as an important and complementary method to traditional morphological identification (Zhu et al. 2014). DNA barcoding uses standardised DNA regions (‘barcodes’) to identify different species (CBOL Plant Working Group 2009). However, the optimal suite of barcoding loci for *Panicum* spp. identification in Australia has not yet been established. Following on from Hollingsworth et al. 2011, one nuclear locus (ITS) and two chloroplast loci (*matK* and *trnL* intron-*trnF*) were evaluated in this study for their ability to differentiate eight *Panicum* spp. occurring in the Riverina region of New South Wales.

**MATERIALS AND METHODS**

In early 2017, fresh leaf samples and accompanying herbarium voucher specimens were collected from 90 *Panicum* plants dispersed across multiple sites within a 200 km radius range of Wagga Wagga, NSW (Figure 2). Voucher specimens were lodged at the Australian National Herbarium (ANH). Fresh leaf samples were stored in silica gel prior to extraction.

To supplement the field-collected materials, an additional 19 dried leaf samples, representing seven *Panicum* spp., were sampled from specimens within the ANH collection. A total of 36 samples, representing eight species found in Riverina, were selected for further analysis in this study (Table 1).

DNA extraction, PCR and sequencing were performed as described (Zhu et al. 2014) using primer pairs ITS5A-ITSR, 390F-1326R and ucp_c-ucp_f for ITS, *matK* and *trnL* intron-*trnF* regions, respectively. Geneious version 11.0.5 (Kearse et al. 2012) was used
to read, edit and align the sequences. Sequence alignments were analysed using MEGA7.0.26 (Kumar et al. 2016) to calculate intra- and interspecific distances with the Kimura 2-parameter (K2P) model. Sequences of all loci for each Panicum specimen were concatenated and phylogenetic relationships among species were inferred by MrBayers 3.2.6 (Huelsenbeck and Ronquist 2001) using default settings with GTR+R substitution model as suggested by JModelTest 2.1.10 (Darriba et al. 2012).

Table 1. *Panicum* specimens used for DNA extraction and sequencing.

<table>
<thead>
<tr>
<th>Species identification</th>
<th>Fresh leaf</th>
<th>Dried leaf (ANH)</th>
</tr>
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<tbody>
<tr>
<td><em>P. capillare</em> L.</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>P. decompositum</em> R.Br.</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><em>P. effusum</em> R.Br.</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>P. glivum</em> Launert</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>P. hillmanii</em> Chase</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>P. laevinode</em> Lindl.</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>P. miliaceum</em> L.</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>P. queenslandicum</em> Domin</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
The majority of field-collected specimens were identified as *P. hillmanii* (78.9%, 71/90) by comparison with verified voucher specimens at the ANH (Figure 2). Other species identified included *P. glivum* (10.0%, 9/90), *P. decompositum* (5.6%, 5/90), *P. effusum* (4.4%, 4/90) and *P. capillare* (1.1%, 1/90). The predominance of *P. hillmanii*, a non-native species that was previously rarely reported in the Riverina region, suggests the species has spread considerably in recent years. Therefore, further investigation in regards to its invasion ecology should be conducted for the development of a more holistic management strategy.

Successful PCR amplification and sequencing was achieved in three selected gene loci (ITS, *matK* and *trnL* intron-*trnF*) for all specimens. Alignments were then truncated to 641, 730, and 750 bps for ITS, *matK* and *trnL* intron-*trnF*, respectively. In addition, concatenated data over all three gene loci were evaluated for phylogenetic analysis. Intraspecific and interspecific genetic distances were calculated for the eight species identified (Table 2).

The efficacy of DNA barcoding depends on the extent of intraspecific and interspecific divergence in a selected locus or combined loci, and the ideal situation...
is a lack of overlap between these two distance values (Aliabadian et al. 2009). For Panicum spp., ITS showed the highest minimum interspecific distance (0.71%), which was over 2-fold higher than the highest maximum intraspecific distance (P. effusum, 0.34%). This result confirmed that the ITS locus was used as an efficient barcode for separation of the eight Panicum spp. in this study. Conversely, the overlap between intraspecific and interspecific values of either the matK or the trnL intron-trnF regions suggests that use of these barcodes alone may prove problematic for differentiation of some Panicum spp.

In order to gain strong interspecies discriminatory power, the three loci were concatenated for phylogenetic analysis. The phylogenetic tree showed clear differentiation between the eight Panicum spp. evaluated (Figure 3). Investigation of the efficacy of this approach with other Panicum spp. will be undertaken in future studies using these loci.

When considering the phylogenetic tree generated, the primary division of interest with respect to the relationship between indigenous and non-native species is presented (Figure 3). Not surprisingly, all four indigenous species (P. decompositum, P. effusum, P. laevinode and P. queenslandicum), clustered together on clade 2, while the four non-native species (P. capillare, P. gilvum, P. hillmanii and P. miliaceum) clustered together on clade 1. This deviation suggests that indigenous Australian Panicum spp. maintain a unique genetic identity despite the opportunity for possible introgression or hybridisation events experienced during the naturalisation process by their non-native relatives (Hovick and Whitney 2014). However, a broader and geographically defined set of global Panicum spp. specimens are required to further explore this observation.

In conclusion, this study indicates that a genetic approach using three concatenated loci provided an accurate and potentially cost effective tool for distinguishing Panicum spp. at the species level, regardless of growth stage. Using this method, we confirmed that the majority of Panicum specimens collected across the Riverina in 2017 were P. hillmanii. This was somewhat unexpected since very few (6) herbarium collections had been made in NSW prior to our study, indicating that the species has been overlooked or its range has expanded relatively recently. Although voucher specimens keyed well to P. hillmanii based on morphology, further molecular analysis of samples from American herbaria will be undertaken to confirm whether the name is being correctly applied in Australia.

The genetic approach utilised in this study could have application in: 1) comprehensive surveillance studies of Panicum spp.; and 2) the development of more useful diagnostic tools, including loop-mediated isothermal amplification (LAMP), for rapid identification of Panicum spp. by stakeholders. Results may also provide improved options for invasive Panicum spp. control in Australian pastures and summer fallows.

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Figure 3. Bayesian phylogenetic relationships among eight Panicum spp inferred from the concatenation of three gene sequences. Clade posterior probability support >90% indicated at nodes.

REFERENCES


