

# Developing New *Cordylines* via Interspecific Hybridisation of the Australian Native *Cordyline stricta* with the New Zealand Natives *C. australis* and the Cultivar ‘Red Fountain’ (*C. hybrida*)

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## Abstract

*Cordyline* species from New Zealand, particularly *C. australis* and its hybrids are popular ornamental plants in temperate regions. ‘Red Fountain’ which is a hybrid involving three New Zealand species, *C. banksii*, *C. australis* and *C. pumilo*, is a very successful interspecific hybrid. However the use of *Cordyline* species and cultivars may be limited by factors such as plant form, disease resistance, cold and heat tolerance. An Australian native, *C. stricta* was chosen for its putative heat tolerance and genetic potential for creation of new forms of *Cordyline*. Two hybrid plants were produced from the cross *C. stricta* × *C. australis* and three hybrid plants were achieved from the cross ‘Red Fountain’ × *C. stricta*. Leaf descriptions for parents and their hybrids are provided. Inter-Primer Binding Site (iPBS) DNA molecular markers were applied to confirm the true hybrid nature of these five interspecific hybrids. This marker system was also chosen to test its efficacy for this purpose. Primer 2394 indicated that hybrid DNA bands came from both putative parents. This is the first report for interspecific hybrids achieved between New Zealand and Australian *Cordyline* species.

## INTRODUCTION

*Cordyline* is a small genus consisting of about 24 species according to the Royal Botanical Gardens, Kew, with eight species native to Australia (Wilson, 1994) and a further five native to New Zealand (Armitage and Clarkson, 2008; International *Cordyline* Society, 2015). *Cordyline australis*, the cabbage palm or Torbay palm, was first introduced from New Zealand to cultivation in Europe in about 1823 (Bean, 1988) as *Dracaena indivisa* or *Cordyline indivisa* (Heenan, 1991b). Traditionally cultivars from *C. australis* were mostly planted in coastal areas due to their sensitivity to frost and resistance to salty winds but were also used as a dot plants in bedding schemes (Heenan, 1991a; Armitage and Clarkson, 2008).

Among five of the *Cordyline* species from New Zealand, several hybrid combinations have been recorded and speculated upon at various times (Kirk, 1874; Carse, 1930; Cockayne and Allan, 1934; Esler, 1961). Selecting interspecific hybrids from *C. australis* with other species from the New Zealand region started in 1886 when ‘*C. rashleighana*’ was given in a list of plants growing at Menabilly in Cornwall for a plant from the cross of *C. australis* × *C. banksii* (Anon., 1886). Reginald Cory selected a plant named as ‘*C. ×austrachis*’ from the same parentage and received an Award of Merit from the RHS in 1923. The cultivar ‘Purple Tower’ was selected in 1972 by Duncan and Davies’ nursery from a cross between *C. australis* and *C. banksii* (Metcalf, 1987; Heenan, 1991b; Harris and Heenan, 1991; Cave and Paddison, 1999). *Cordyline kaspar* is endemic on the Three Knights Islands off the north coast of New Zealand and cultivar ‘Green Goddess’ resulted from the cross *C. kaspar* × *C. australis* (Metcalf, 1987). The Cultivar ‘Red Fountain’ was first developed by Felix M. Jury and Mark C. Jury in Taranaki, New Zealand in 1996 using

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‘Purple Tower’ and a pollen parent of *C. pumilo*. This cultivar later received a US Plant Patent using the name *Cordyline* ‘Jurred’ and was marketed as Festival Grass (Jury, 2003; US Plant Patent 14224, 2003). ‘Red Fountain’ can be planted from full sun (along the coast) to deep shade and proves to be hardy to around  $-7^{\circ}\text{C}$ .

Currently cultivars of *C. australis* and its interspecific hybrids such as ‘Red Fountain’ are popular pot, garden and landscape plants. Their use has even extended to annualized mixed container use as a vertical accent. Cultivars with red leaves and derived mutations, along with white and green variegated forms are particularly popular (Simpson, 2000; Harris, 2001). Estimated sales of protected cultivars are about 15 million plants and with unprotected cultivars such as ‘Red Star’, the total sales could be more than double this figure.

*Cordyline stricta* is native to Australia and is distributed in a band running from Sydney in New South Wales to southern Queensland. Its normal habitat is temperate rainforest and open forest (Wilson, 1994). Its green foliage and less striking form have limited its horticultural popularity despite a number of other characteristics which make it an attractive plant particularly for landscape use. Importantly for warmer and dryer climates, *C. stricta* appears to have superior heat and drought tolerance compared to the popular *Cordyline* species such as *C. australis* and *C. banksii* from maritime New Zealand. *C. stricta* also has relatively short leaves and the ability to grow well in shady conditions combined with the ability to withstand periods of full sun. With space, shade and water increasingly becoming limiting selection criteria for plant choice, particularly in city and urban development, *C. stricta* has the potential to be used as a source of useful genetic variations in *Cordyline* hybridisation. Hybrids which combine the attributes of *Cordylines* from both countries may increase the horticultural value of this crop.

There are very few publications studying genetic diversity in the genus *Cordyline*. The AFLP marker system has been developed for *C. australis* (Beever et al., 2013) and *C. fruticosa* (Hinkle, 2007) to reveal the geographical population structure of the species. Recently, the inter-Primer Binding Site (iPBS) DNA marker system was developed as an alternative method to explore genetic diversity and relationships in plants (Kalendar et al., 2010, 2011; Smykal et al., 2011; Kalendar and Schulman, 2014; Alzohairy et al., 2014). This marker system was recently also used for fingerprinting in apricot (Baránek et al., 2012), *Saussurea esthonica* (Gailite and Rungis, 2012), guava (*Psidium guajava*) (Mehmood et al., 2013), Cicer (Andeden et al., 2013), grape (*Vitis vinifera*) (Guo et al., 2014), *Myrica rubra* (Chen and Liu, 2014) and Lens (Baloch et al., 2015). The present research aimed to confirm the true hybrid nature of the interspecific hybrids using this new iPBS technique.

## MATERIALS AND METHODS

The plant materials used in this study are listed in Table 1. In September 2010, *C. stricta* ( $2n \approx 114$ ) with moderate branching was used as a female in crosses with a green selection of *C. australis* ( $2n = 38$ ) with a single stem plant architecture. Cultivar ‘Red Fountain’ which has a multi branched clumping habit was used as the female parent in crosses with *C. stricta*. One hundred flowers on each female parent were emasculated and bagged, then pollinated two days later. Pollinations were performed following Beever and Parkes’s (1996) method.

Two hundred mg of young fresh leaves collected from 8 plants listed in Table 1 were used for DNA isolation using the plant DNA isolation Mini Kit (Biolone, Australia) in accordance with the manufacturer’s protocols. Quality and quantity of DNA was checked following 2.0% agarose gel electrophoresis by comparison with known  $\lambda$  DNA concentrations. Portions of the isolated DNA were diluted in molecular grade water to 2 ng/ $\mu\text{L}$  concentration and used as templates for PCR.

The iPBS primers listed by Kalendar et al. (2010) used in this study were from Sigma Aldrich (Castle Hill, NSW, Australia). DNA amplification was carried out by a slightly modified protocol. PCR was performed in 15- $\mu\text{L}$  reaction mixtures containing 10 ng genomic DNA, 1 time GoTaq buffer (Promega), 0.5  $\mu\text{M}$  primer (single primer), 0.2 mM

dNTPs, 0.5 U Taq DNA polymerase (GoTaq, Promega) and 2.0 mM MgCl<sub>2</sub>. The PCR program had an initial hot start at 95°C for 3 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 36-56°C for 30 s, and an extension at 72°C for 2 min. There was a final extension at 72°C for 5 min, and the program was terminated by holding at 10°C. The reaction was performed in a Bio-Rad T100™ Thermal Cycler with 0.2-mL tubes or 96-well plates. A 5-μL sample of each PCR product was electrophoresed at 70 V for 3.5 h in a 1.5% (w/v) thin agarose gel with 1× TAE buffer (0.04 M Tris·acetate, 0.001 M EDTA). A Thermo Scientific GeneRuler 1 kb ladder (Fermentas, Australia) was used to estimate fragment lengths. Gels were post-stained with GelRed (Biotium) for 15-30 min and photographed using the Gel Doc-It Imaging System (UVP) at an exposure of 1 to 2 s.

Seventeen primers were initially screened for PCR using ‘Red Fountain’ DNA, and 7 primers generated a varied number of bands. These 7 primers (2083, 2087, 2271, 2375, 2377, 2386 and 2394) were further screened with *C. stricta* and ‘Red Fountain’ DNA samples, and all of these primers generated polymorphic bands using their specific annealing temperature (Table 2). Primer 2271 and 2394 were selected to amplify 8 accessions of DNA because of the large number of polymorphic PCR bands produced.

For each primer, PCR was performed 3 times to confirm band pattern consistency. DNA bands were sized and scored by LabWorks software (v. 4.5, UVP) and carefully checked manually; only clear bands were scored and faint bands were ignored. Bands with the same size were assumed to represent a single locus. For each locus, data were recorded using ‘1’ for presence of a band and ‘0’ for absence. For each cross, all the bands in the hybrid plants were checked with their alignments to the original parents. Bands from the male parent expressed in the hybrids were especially highlighted for demonstrating the true hybrid nature for the hybrids. Some of the bands in the hybrids were coming from the ovule parent.

## RESULTS AND DISCUSSION

Two viable seeds were germinated from the first combination (*C. stricta* × *C. australis*) and numbered hybrids 1 and 2. Three seeds from the second combination (‘Red Fountain’ × *C. stricta*) germinated and were numbered hybrid 3, 4 and 5. The putative hybrids were grown at the Plant Breeding Institute, Cobbitty in pots. After 5 years, hybrids 1 and 2 have grown as single stem plants with limy green leaves and a yellow midrib, whilst hybrids 3, 4 and 5 have light reddish brown leaves and are multi stemmed. Some of the leaf and stem characteristics for these two interspecific populations are also presented in Table 1.

The mature leaf structure of *C. stricta* and *C. australis* and their F<sub>1</sub> progeny 1 and 2 are presented in Figure 1. Leaf morphology of this interspecific F<sub>1</sub> population shows that leaf length, width and color are varied beyond the two parents. The mature leaf of ‘Red Fountain’ and *C. stricta* and their F<sub>1</sub> progeny 3, 4 and 5 are shown in Figure 2. Leaf length, width and color of this interspecific population are also varied beyond their two parents. These results demonstrate that there is heritable leaf variation at the interspecific level between the Australian species *C. stricta* and the New Zealand species *C. australis* and cultivar ‘Red Fountain’. Traditionally some of the morphological traits from the pollen parent expressed in their hybrids were used to prove the hybrid nature for the achieved hybrids (Kirk, 1874; Carse, 1930; Cockayne and Allan, 1934; Esler, 1961). These hybrids form part of our “4D landscape breeding” program where parents are chosen for traits that may endow the resulting hybrids a particular function for landscape use rather than primarily for other aesthetic reasons.

A single primer will often amplify several different regions of the genome and the result is a set of differently sized amplified fragments of DNA. Two interspecific F<sub>1</sub> plants were characterized together with their parents by primer 2394 (Fig. 3). Four DNA bands from the pollen parent were present in the two F<sub>1</sub> plants (hybrids 1 and 2) generated from the cross *C. stricta* × *C. australis*. Primer 2394 was also used to characterize the interspecific population ‘Red Fountain’ × *C. stricta* in Figure 4. There were bands from the pollen parent present in hybrids 3, 4 and 5. The presence of the maternal bands in these five hybrids proved their hybrid nature.

## CONCLUSIONS

Investigation of the genetic diversity and relationships in *Cordyline* germplasm is important for breeding, conservation, management and utilisation of the various species. Accurate identification of accessions in a germplasm collection is an important challenge faced by plant improvement projects. For germplasm identification, molecular marker systems such as the iPBS can add to the assessment of genetic diversity and relationships in plant phylogenetic analysis as well as selective plant breeding because they are objective and offer reproducible means of identification, independent of environmental influences. The use of iPBS markers has already provided valuable information on grapes (*Vitis vinifera* L.) (Guo et al., 2014) and guava (*Psidium guajava*) (Mehmood et al., 2013). In our study, polymorphic iPBS markers also enabled identification of a range of *Cordyline* accessions including the Australian species *C. stricta*, *C. australis* and cultivar 'Red Fountain' involving three New Zealand species. The level of information generated by iPBS and morphological analysis (Mehmood et al., 2014) suggest that both methods can be important for diversity studies in *Cordyline*.

The general consensus traits for a good molecular marker system are that it is polymorphic and evenly distributed across the entire genome; it should distinguish between genetic differences; it should be cheap, quick, and easy to use, requiring minimal amounts of DNA and should require no previous knowledge of the genome (Odong et al., 2011). The results from our study confirm that the iPBS method used on genus *Cordyline* exhibit all these characteristics.

## FURTHER INVESTIGATION

The iPBS marker system has been found to show informative variation among genetically distant species and their hybrids in the genus *Cordyline*. Further attempts could test all 60 primers designed by Kalendar et al. (2010) on a wider range of species to establish a robust and stable nomenclature for *Cordyline* in cultivation. This project would involve screening all the primers using the PCR protocols in this study and then selecting some of the most polymorphic primers to amplify all the collected accessions of *Cordyline*.

This program only used one Australian species (*C. stricta*) for hybridization with one green biotype of *C. australis* and the complex hybrid 'Red Fountain' from New Zealand. To expand the program there are other *Cordyline* species from New Guinea and Australia which could be explored for their interspecific compatibility with their New Zealand counterparts.

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## **Tables**

Table 1. Leaf and stem characteristics of the eight *Cordyline* accessions used for iPBS analysis.

| Accessions          | Leaf length | Leaf width | Leaf color          | Midrib color | Stem   |
|---------------------|-------------|------------|---------------------|--------------|--------|
| Red Fountain        | 79.5        | 1.6        | Red                 | Red          | Multi  |
| Hybrid 3            | 52          | 2.2        | Light reddish brown | Brown        | Multi  |
| Hybrid 4            | 79          | 2.2        | Light reddish brown | Brown        | Multi  |
| Hybrid 5            | 84.5        | 2.1        | Light reddish brown | Brown        | Multi  |
| <i>C. stricta</i>   | 51          | 2.7        | Green               | Green        | Multi  |
| Hybrid 1            | 77          | 1.5        | Limy green          | Yellow       | Single |
| Hybrid 2            | 56          | 1.7        | Limy green          | Yellow       | Single |
| <i>C. australis</i> | 62          | 2.0        | Green               | Light green  | Single |

Table 2. Primers (Kalendar et al., 2010) used in PCR amplification.

| Primer | Ta °C | Sequence      |
|--------|-------|---------------|
| 2083   | 41    | CTTCTAGCGCCA  |
| 2087   | 41    | GCAATGGAACCA  |
| 2271   | 56    | GGCTCGGATGCCA |
| 2375   | 43    | TCGCATCAACCA  |
| 2377   | 43    | ACGAAGGGACCA  |
| 2386   | 36    | CTGATCAACCCA  |
| 2394   | 44    | GAGCCTAGGCCA  |

## Figures



Fig. 1. Mature leaf shape for *C. stricta*, *C. australis* and two of their hybrids. From top to bottom: *C. stricta*, hybrids 1 and 2, *C. australis*.



Fig. 2. Mature leaf shape for Red Fountain, *C. stricta* and three of their hybrids. From top to bottom: *C. stricta*, hybrids 3, 4, 5, Red Fountain.

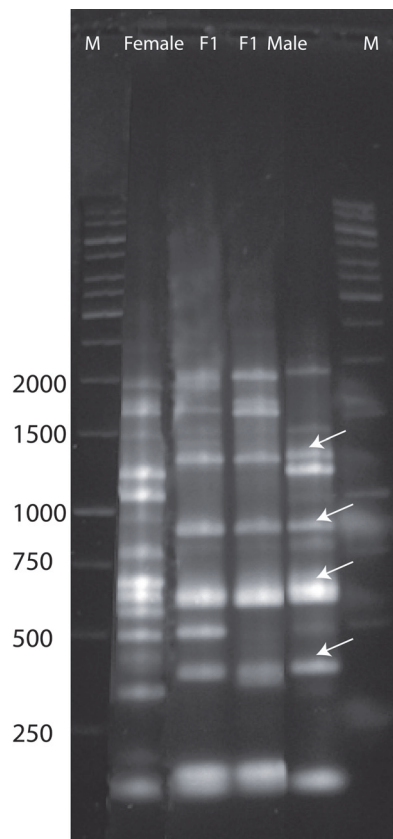


Fig. 3. The interspecific cross *C. stricta* × *C. australis* F<sub>1</sub> population amplified with primer 2394.

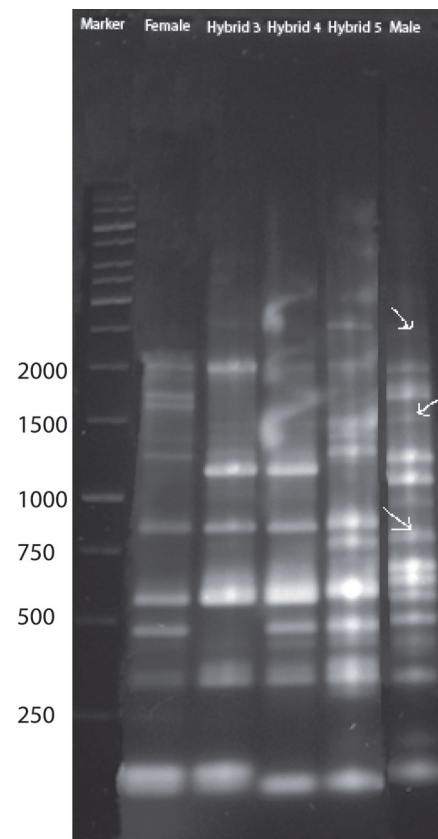


Fig. 4. The interspecific cross 'Red Fountain' × *C. stricta* F<sub>1</sub> population amplified with primer 2394.