

final report

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Leuceana DNA profiling

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Abstract

In beef producing areas of northern Australia with average rainfall > 800 mm the production of commercial leucaena (*Leucaena leucocephala* ssp *glabrata*) can be significantly affected by the leucaena psyllid (*Heteropsylla cubana*). Consequently, in 2002 an MLA-supported breeding program (B.NBP.307 and B.NBP.0610, Shelton, Dalzell, Lambrides) was initiated to develop psyllid-resistant leucaena from genetic material derived from a psyllid-resistant wild relative *L. pallida*. A psyllid resistant variety has now been developed and seed has been distributed to two commercial partners, (Leucseeds Pty Ltd and Bandana Station, Carnarvon pastoral) for commercialisation and distribution. The purpose of the project reported here was to provide a DNA profile of the new psyllid variety named 'Redlands' (BL12) and compare it to other psyllid resistant leucaenas (BL24, BL34, BL39), and two commercial cultivars Wondergraze and Cunningham. The DNA profile will assist the seed companies, MLA and UQ secure a level of protection over the new psyllid resistant leucaena. Using 426 DNA markers scored among the different leucaena lines, a dendrogram (family tree) showed that Redlands had a unique DNA profile that was distinct from other psyllid resistant breeding lines BL24, 34, 39 and from commercial leucaena cultivars Wondergraze and Cunningham.

Executive Summary

In beef producing areas of northern Australia with average rainfall > 800 mm the production of commercial leucaena (*Leucaena leucocephala* ssp *glabrata*) can be significantly affected by the leucaena psyllid (*Heteropsylla cubana*). Consequently, in 2002 an MLA-supported breeding program (B.NBP.307 and B.NBP.0610, Shelton, Dalzell, Lambrides) was initiated to develop psyllid-resistant leucaena from genetic material derived from a psyllid-resistant wild relative *L. pallida*. Commercial cultivar Wondergraze was used as the recurrent parent in the breeding program to recover a leucocephala-type leucaena for fattening cattle. Forty breeding lines with psyllid resistance and other desirable attributes were developed from this breeding initiative. After several years of testing, 4/40 breeding lines BL12, BL24, BL34 and BL39 were selected for precommercial testing. In 2013-14, one breeding line BL12 was placed in a trial at Redlands Research Station to gain protection via PBR (Plant Breeders Rights) through IP Australia. IP Australia have granted temporary protection of BL12 with a final decision pending early in 2016. Psyllid resistant BL12 has been given the commercial name 'Redlands' and is to be commercialised and distributed by two companies (Leucseeds Pty Ltd and Bandana Station, Carnarvon pastoral). The aim of this current study was to obtain a DNA profile of BL12 (Redlands) and determine its relationship to Wondergraze the recurrent parent used in the breeding program. A secondary aim was to study the relationship between Redlands and other psyllid resistant leucaenas developed in the breeding program. Using 426 DNA markers scored among the different leucaena lines, a dendrogram (family tree) showed that Redlands had a unique DNA profile that was distinct from other psyllid resistant breeding lines BL24, 34, 39 and from commercial leucaena cultivars Wondergraze and Cunningham. The DNA profile of Redlands will assist Leucseeds, Bundoora Station, MLA and UQ secure a level of exclusivity in the market place. Importantly, it will mean that the large investment into breeding psyllid resistant leucaena made by MLA and UQ and the royalty base generated from commercial releases will be protected.

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1 Background

In beef producing areas of northern Australia with average rainfall > 800 mm the production of commercial leucaena (*Leucaena leucocephala* ssp *glabrata*) can be significantly affected by the leucaena psyllid (*Heteropsylla cubana*). Consequently, in 2002 an MLA-supported breeding program (B.NBP.307 and B.NBP.0610, Shelton, Dalzell, Lambrides) was initiated to develop psyllid-resistant leucaena from genetic material derived from a psyllid-resistant wild relative *L. pallida*. The initial goal of the breeding program was to develop a shy seeding, psyllid resistant leucaena by random mating resistant types. However, this strategy was unsuccessful because of the possible genetic linkage of psyllid resistance and low forage yield in the donor parent *L. pallida*. Consequently, it was decided to adopt a backcrossing program where commercial cultivar Wondergraze was used as the recurrent parent in the breeding program to recover a leucocephala-type psyllid resistant leucaena for fattening cattle. Forty breeding lines with psyllid resistance and other desirable attributes were developed from this breeding initiative. After several years of testing, 4/40 breeding lines BL12, BL24, BL34 and BL39 were selected for precommercial testing. In 2013-14, one breeding line BL12 was placed in a trial at Redlands Research Station to gain protection via PBR (Plant Breeders Rights) through IP Australia. IP Australia have granted temporary protection of BL12 with a final decision pending early in 2016. Psyllid resistant BL12 has been given the commercial name 'Redlands'. A psyllid resistant variety has now been developed and seed has been distributed to two commercial partners, (Leucseeds Pty Ltd and Bandana Station, Carnarvon pastoral) for commercialisation and distribution. The aim of this current study was to obtain a DNA profile of BL12 (Redlands) and determine the genetic distance between BL12 and Wondergraze the recurrent parent used in the breeding program. A secondary aim was to study the relationship between Redlands and other psyllid resistant leucaenas developed in the breeding program. The DNA profile of Redlands will assist the seed companies, MLA and UQ secure a level of protection over the new psyllid resistant leucaena in the market place.

2 Project objectives

UQ to provide DNA profiling between two sources of the new psyllid resistant leucaena variety (BL12), alongside a panel of commercially available leucaena cultivars (including Wondergraze, Peru, Cunningham and Tarramba). The objectives were modified slightly to include additional versions of BL12 and the other psyllid resistant leucaenas developed. Consequently to save on resources, two less important cultivars Peru and Tarramba were excluded from the study.

3 Methodology

3.1 Genetic materials

Leaf/seed material of the the leucaena genotypes tested in this study are listed in Table 1.

Table 1. A list of leucaena genotypes used for DNA profiling

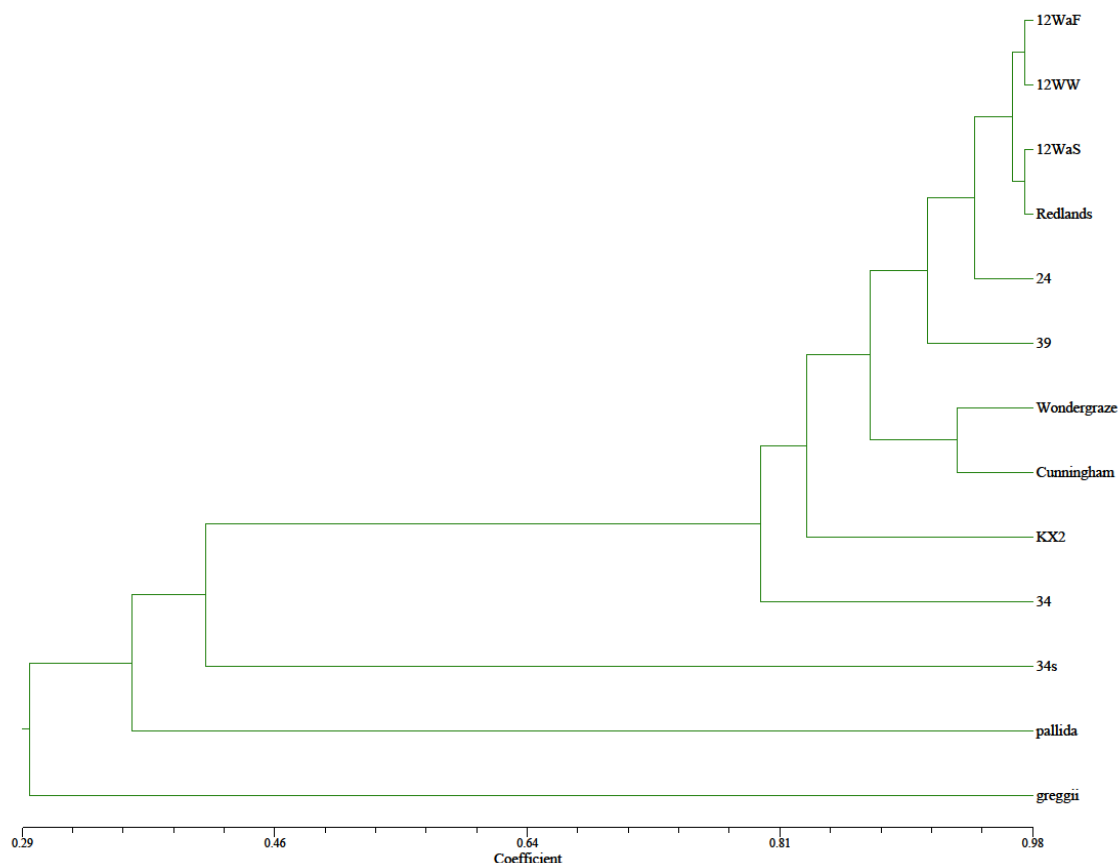
| Code | Genotype/Name | Taxon | Origin |
|-------------|---------------|-------------------------------|---|
| 12WaF | BL12 | <i>leucecephala x pallida</i> | BL12 Walkamin Research Station |
| 12WW | BL12 | <i>leucecephala x pallida</i> | BL12 Whitewater, Mt Surprise |
| 12WaS | BL12 | <i>leucecephala x pallida</i> | BL12 Walkamin seed (April) |
| Redlands | BL12 | <i>leucecephala x pallida</i> | BL12 Redlands Research Station |
| 24 | BL24 | <i>leucecephala x pallida</i> | BL24 Whitewater, Mt Surprise |
| 39 | BL39 | <i>leucecephala x pallida</i> | BL39 Whitewater, Mt Surprise |
| Wondergraze | Wondergraze | <i>leucecephala</i> | Wondergraze Whitewater, Mt Surprise |
| Cunningham | Cunningham | <i>leucecephala</i> | Cunningham Whitewater, Mt Surprise |
| KX2 | KX2 | <i>leucecephala x pallida</i> | Germplasm orchard, Redlands Res Station |
| 34 | BL34 | <i>leucecephala x pallida</i> | BL34 Whitewater, Mt Surprise |
| 34s | BL34 | <i>leucecephala x pallida</i> | BL34 seedless plant Whitewater, Mt Surprise |
| pallida | pallida | <i>pallida</i> | Germplasm orchard, Redlands Res Station |
| greggii | greggii | <i>greggii</i> | Germplasm orchard, Redlands Res Station |

3.2 DNA isolation, PCR and dendrogram construction

Under sterile lab practices to prevent the chance of contamination, DNA was isolated from each genotype using a standard CTAB protocol (see Jewell et al, 2010). The PCR (polymerase chain reaction) marker system used for DNA fingerprinting was ISSR (Inter-simple-sequence-repeat). Full details of these procedures are provided in the study by Loch et al (2009). Three ISSR marker primers, 5'-VIC-GA9(C)-3', 5'-Ned-GA9(T)-3', 5'-6FAM-AG9(C)-3' were used to generate DNA marker fragments among the panel of genotypes. The fragments were run through an ABI3730 Capillary electrophoresis genotyper and visualised using GeneMapper software. Each genotype was given a score 1/0 for presence/absence of a DNA marker band. Based on these data a similarity matrix of pairwise relationships between the genotypes was created using the DICE coefficient to determine relatedness. The genotypes were clustered using an UPGMA (unweighted pair group method with arithmetic mean) algorithm and a dendrogram (family tree) produced showing the inter-relationships within the test panel. DICE coefficient and UPMGA clustering were options of the NTSYS pc vers 2.21f software package.

4 Results

A total of 426 ISSR markers were generated across the panel of leucaena genotypes tested, enabling a dendrogram of relationships to be constructed (see figure below). The figure shows that the four versions of BL12 i.e 12WaF, 12WW, 12WaS and Redlands were highly related with a degree of relatedness (coefficient) > 95%. The BL12 genotypes were genetically distinct from all other genotypes especially the commercial cultivars Wondergraze and Cunningham. The four psyllid resistance genotypes BL12, 24, 34 and 39 were also genetically distinct from one another and BL34 was more closely related to the KX2 hybrid than it was to BL12, 24, and 39. The psyllid resistant genotypes were more closely related to the leucocephala genotypes (Wondergraze and Cunningham) than they were to *L. pallida* the psyllid resistant parent used in their breeding. Genotype 34s was a single sterile plant identified in a plot of BL34 at Whitewater, Mt Surprise. 34s was genetically different from BL34 suggesting that this plant may have resulted from an outcross with an unrelated line in the seed increase block at Redlands Research Station.



5 Discussion

The newly bred psyllid resistant leucaena Redlands developed by MLA/UQ has a unique genotype that is genetically distinct from other psyllid resistant breeding lines BL24, 34 and 39. In addition, Redlands is genetically distinct from Wondergraze the recurrent parent used in the breeding program and the check variety Cunningham. A recent PBR trial conducted for IP Australia also showed that in terms of DUS (distinctness, uniformity, stability) criteria, Redlands is phenotypically distinct from Wondergraze, Cunningham, Peru and Tarramba. The DNA profile of Redlands will assist Leucseeds, Bundoora Station, MLA and UQ secure a level of exclusivity in the market place. Importantly, it will mean that the large investment into breeding psyllid resistant leucaena made by MLA and UQ and the royalty base generated from commercial releases will be protected.

6 Conclusions/recommendation

Redlands has a unique DNA fingerprint that is genetically distinct from other psyllid resistant breeding lines BL24, 34 and 39. Importantly, Redlands is genetically distinct from Wondergraze the recurrent parent used in the breeding program.

7 Key message

The newly bred psyllid resistant cultivar Redlands has a unique DNA profile that will allow the commercialising seed companies, MLA and UQ to gain exclusivity in the market place. Importantly, it will mean that the large investment into breeding psyllid resistant leucaena made by MLA and UQ and the royalty base generated from commercial releases will be protected.

8 Bibliography

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