References


Brassica oleracea and atrazine-resistant Brassica campestris. Theoretical and Applied Genetics, 83, 201-208.


Acknowledgments

My sincerest gratitude to my supervisors, Dr Mary Christey and Professor Tony Conner whose guidance, constant encouragement, and support on all frontiers has made this project a learning curve in my career. I am greatly privileged to have worked under your guidance.

I thank my associate-supervisor Dr Hayley Ridgway for supporting and encouraging me at crucial junctures during this project.

I would like to thank Pastoral Genomics, Auckland, New Zealand, and New Zealand Institute for Crop & Food Research Ltd., New Zealand for financially supporting me. I am thankful to Foundation for Research Science and Technology, New Zealand for awarding me the Enterprise Scholarship. Special thanks to Pastoral Genomics, for extending the financial support for an extra year, without which it would have been very difficult to complete this project.

I would like to thank Robert H. Braun for his immense support in the laboratory, for having long discussions and giving me tips which have contributed greatly to the completion of this work. THANK YOU Robert.

Thanks to Ross Bicknell, Sylvia Erasmuson, and Andrew Catanach for helping me with the AFLPs and flow cytometry. My grateful appreciation to Ruth Butler for helping me with the statistical softwares. Thank you Ruth.

My sincere thanks to the Graphics team at Crop & Food, Robert Lamberts for taking attractive pictures of my specimens, Astrid Erasmuson & Carolyn Barry for helping design posters at various stages of this project. Thanks also to Sam Wakelin for designing the graphic pictures and posters.

I would like to thank all members of the Plant Genetic Technologies Team at Crop & Food, past and present. A big thanks to Annemarie Lokerse who has constantly supported me. Thanks to Dianne Hall for watering my plants in the glass house.

My thanks to all my past teachers whose teachings have made me qualify for this project.
Many thanks to my wife, Deepa, who has stood alongside me in the ups and downs of this project. I would also like to thank my family members who have encouraged me all throughout my career.

Lastly but most importantly, I would like to thank my parents who have constantly supported me and guided me in my endeavours.
Appendix 1

Species Description

a. *Lolium perenne* L. (Perennial ryegrass)

*Lolium perenne* (see inset) is a grass belonging to the graminace family Poaceae. It has a diploid chromosome number of $2n=2x=14$.

Leaves of perennial ryegrass are *folded* in the bud (in contrast to *annual ryegrass* (*Lolium multiflorum* L.), in which the leaves are *rolled*). Leaf blades are 2 to 6 mm wide, 5 to 15 cm long, sharply tapered, and keeled. Blades are bright green, prominently ridged on the *adaxial* (upper) surface, and smooth, glossy, and glabrous (hairless) on the *abaxial* (lower) surface. Leaf margins are slightly scabrous (rough to the touch, covered with minute serrations). Blades increase in size from the first to the seventh leaf on a tiller, although tillers rarely have more than three live leaves at one time (Balasko *et al.*, 1995). Leaf sheaths usually are not keeled, compressed but sometimes almost cylindrical, *glabrous*, pale green, and reddish at the base. *Sheaths* may be closed or split. The collar is narrow, distinct, glabrous, and yellowish to whitish-green. *Auricles* are small, soft, and claw-like. The ligule is a thin membrane, from 0.5 to 2.5 mm, obtuse (rounded at the apex), and may be toothed near the apex.

The shallow root system is highly branched and produces *adventitious roots* from the basal *nodes* of *tillers*. Perennial ryegrass has no *rhizomes* or *stolons*. It will, however, produce a dense and closely knit *sward* or turf with high plant densities and proper management (Spedding & Diekmahns, 1972).
b. *Lotus corniculatus* L. (Bird’s foot trefoil).

*Lotus corniculatus* (see inset) which belongs to the family Fabaceae, is a tetraploid with 2n=4x=24. It is a glabrous to sparsely pubescent perennial plant, though not a long-lived perennial with a life span of 2-4 years. Growth form ranges from prostrate to erect with numerous stems arising from a basal, well developed crown and branches arising from leaf axils. Primary growth comes from the crown, but regrowth comes from buds formed at nodes on the stubble left after defoliation. Leaves are pentafoliate, alternately on short stalks with the two leaflets at the petiole base resembling stipules. The asymmetrical pointed leaflets are mainly glabrous and are more slender and pale green.

Inflorescences with up to eight flowers are umbel-like cymes at the end of long axillary branches. The calyx is a dentate tube and the yellow five-petalled corolla is often tinged red. The flowering period is indeterminate and so seed is set over an extended period in summer. The self-sterile plants are cross-pollinated, mainly by honey bees. Seed pods, 2-5 cm long, contain 15-20 seeds attached to the ventral suture (Refer Fig 4.1); the seeds are released by a sudden split of the pod along both sutures after one to two weeks of ripening during which the pods change from green to brown. The seeds vary from round to oval in shape and from greenish yellow to dark brown in colour. The plant root system consists of a deep taproot with numerous secondary roots that have a good lateral spread. Some Moroccan genotypes produce rhizomes that arise from axillary buds on stem bases (Skerman et al., 1988).
Appendix 2: Flow cytometric analysis

Profiles generated from the flow cytometric analysis of the fusion parents (*L. perenne* and *L. corniculatus*) and the 14 putative fusion colonies obtained. The fusion colony profiles all resembled the *L. corniculatus* profile.

![Flow cytometric analysis profiles](image_url)
**L. corniculatus** callus

**FC2** callus
FC3 callus

FC4 callus
FC7 callus

FC8 callus
FC11 callus

![Graph of FC11 callus]

FC12 callus

![Graph of FC12 callus]
FC13 callus

FC14 callus
Appendix 3  Polyacrylamide Gel Preparation

1.1. Thoroughly clean the small and the large glass plates with Jiff, followed by generous acetone, then 95% ethanol and facial tissue.

1.2. In a chemical fume hood, prepare fresh binding solution by adding 6ul of Bind Silane (methacryloxypropyl-trimethoxy-silane, Sigma M-6514, store at 4°C) to 2 ml of 0.5% acetic acid in 95% ethanol in a 15 ml Falcon tube. Wipe large glass plate using facial tissue saturated with the freshly prepared binding solution. Be certain to wipe the entire plate surface with the saturated tissue.

1.3. Wait 5 minutes for the binding solution to dry. Wipe the glass plate with 95% ethanol and facial tissue to remove the excess binding solution.

Note: Failure to wipe excess binding solution from the glass plate will cause the gel to stick to both plates, and the gel will be destroyed upon separation of the glass plates after electrophoresis.

1.4. Apply 3 cm splodge of Sigmacote® (Chlorinated organopolysiloxane SIGMA) onto one side of the other glass plate. With a dry facial tissue tissue, spread the Sigmacote over the entire surface of the plate.

1.5. Wait 5 minutes for the Sigmacote to dry. Remove the excess Sigmacote with ethanol and facial tissue.

1.6. Place 0.4mm spacers on the large glass plate. Put the small glass plate on the large one. Assemble the glass plates by using clamps to hold them in place (2 clamps on each side). Place the gasket, bottom corner first, removing clamps when required.

Note: Take special care to prevent the treated surfaces from touching each other.

1.7. Pour 6% acrylamide solution (60ml for one gel) into a beaker. For each 60 ml solution, add, 60ul of TEMED (N,N,N',N'-Tetraethyl-ethylenediamine) and 600ul of 10% APS (ammonium persulfate) to the acrylamide solution, and mix gently.

1.8. Suck the acrylamide into a syringe. Take care to remove bubbles. Carefully inject the acrylamide solution into the space in the gasket.

1.9. Insert a multi-well sharks-tooth comb, straight side into the gel, between the glass plates (approximately 6-8 mm of the comb should be between the two glass plates). Secure the comb with four clamps.

1.10. Allow polymerisation to proceed for at least 1 hour to overnight.

Note: The gel may be stored overnight at room temperature if a paper towel saturated with dH2O and plastic wrap are placed around the well end of the gel to prevent the gel from
drying out. As an alternative, the gel can be stored overnight by keeping in the electrophoresis apparatus with 1xTBE buffer in the bottom and top chamber of the apparatus (see steps 2.1 and 2.5).

2. Polyacrylamide Gel Electrophoresis

For more efficient use of gel space, one may load one gel multiple times using samples from either the same or different loci. When loading samples from different loci, load the samples in order of smallest to largest size ranges, or load the mixed solution of two samples with equal quantity.

2.1. Remove the clamps from the polymerised acrylamide gel assembly and clean the outside of the glass plates with paper towel saturated with dH2O.

2.2. Shave any excess polyacrylamide away from the comb. Remove the comb, and shave and wash any excess polyacrylamide from the top of the glass plates. Wash surfaces of the gel setup.

2.3. Gently lower the gel (glass plates) into the lower slot with the large plate facing out and the well-side on top. Secure the glass plates, increasing the pressure a little at a time.

2.4. Ensure the tap between chambers of the electrophoresis apparatus is closed. Add 1xTBE (1 litre) to the top of the electrophoresis apparatus until the small plate is well covered. Pour the rest of the TBE into the bottom tank.

2.5. Using a disposable Pasteur pipette, remove the air bubbles and unpolymerized acrylamide on the top of the gel. Be certain the well area is devoid of air bubbles, small pieces of polyacrylamide and urea.

2.6. Pre-run the gel to achieve a gel surface temperature of approximately 45-50°C. Set the power constant at 70W and the voltage should be between 1400 and 1800V. Any higher and the plates may be getting too hot. Pre-run for about half an hour to an hour.

2.7. Prepare PCR samples by mixing 8ul of each sample with 5ul of STR 3x-loading solution, or add 10ul STR to the whole solution.

2.8. Prepare 500ng molecular weight marker (Marker DNA + 3x STR) for each marker lane.

2.9. Denature the samples and markers by heating at 95°C for 1.5-2 minutes and immediately chill on ice.
2.10. After the pre-run, use a disposable Pasteur pipette to flush the urea from the well area and carefully insert the sharks-tooth comb teeth into the gel approximately 1-2mm.

2.11. Load 6ul of each sample and marker into the respective wells. To prevent the gel from cooling, gel loading should not exceed 20 minutes.

2.12. Run the gel using the same conditions as the pre-run step (2.6), until Xylene cyanol is about half way (1.5 to 2 hours).

*In a 4% gel, Bromophenol blue migrates at approximately 40 bases, and Xylene Cyanol migrates at approximately 170 bases.

3. **Silver Staining**

3.1. After electrophoresis, empty the buffer chamber and carefully loosen the gel clamps. Remove the glass plates from the apparatus.

3.2. Place the gel (glass plates) on a flat surface. Remove the comb and the side spacers. Use a plastic wedge to carefully separate the two glass plates. The gel should be strongly affixed to the large glass plate.

3.3. Place the gel (attached to the small plate) in a shallow plastic tray.

3.4. For silver staining, follow the steps listed below:

<table>
<thead>
<tr>
<th>Step</th>
<th>Solution</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Fix/stop solution</td>
<td>30 minutes</td>
</tr>
<tr>
<td>b.</td>
<td>dH2O</td>
<td>2 minutes</td>
</tr>
<tr>
<td>c.</td>
<td>Repeat step b. twice</td>
<td>2x2 minutes</td>
</tr>
<tr>
<td>d.</td>
<td>Staining solution</td>
<td>30 minutes</td>
</tr>
<tr>
<td>e.</td>
<td>dH2O</td>
<td>10 seconds</td>
</tr>
<tr>
<td>f.</td>
<td>Developer solution (4-10°C)</td>
<td>2-5 minutes</td>
</tr>
<tr>
<td>g.</td>
<td>Fix/stop solution</td>
<td>5-6 minutes</td>
</tr>
<tr>
<td>h.</td>
<td>dH2O</td>
<td>2-3 minutes</td>
</tr>
</tbody>
</table>

Note: All steps should be conducted on a shaker and the plates should be well covered by the solutions.

3.5. Position the gel upright and allow it to dry overnight.
Composition of solutions

4% acrylamide solution (1000 ml)
   420 g urea,
   450 dH2O
   100 ml 10x TBE
   100 ml 40 % acrylamide/bis-acrylamide (19:1)

6% acrylamide solution (500mls)
   75mls 40% acrylamide:bis
   230g urea (=>7.67M)
   50mls 10X TBE
   Add acrylamide with some water and add half the urea, stir. Add rest of urea. Incubate at 37 degrees to aid dissolving in a shaking water-bath. Add the TBE and make up to volume.

0.5% acetic-acid in 95% ethanol
   Add 1 ml of glacial acetic acid to 199 of 95% ethanol

10% APS (ammonium persulfate)
   Add 1.0g of ammonium persulfate to 10 ml of dH2O

Developer solution

sodium carbonate 90g 120gm
37% formaldehyde (H2CO) 4.5ml 6ml
10mg/ml sodium thiosulfate (Na$_2$S$_2$O$_3$5HO) 600 µl 800µl
dH2O To 3L To 4l

Note: Prepare the solution and chill to 10'C before use. Prepare fresh before each use. The sodium carbonate can be dissolved and chilled overnight in the cold room. Add the formaldehyde and sodium thiosulfate before use.

Staining solution:

2 g silver nitrate (AgNO3) 3g 4g
3 ml 37% formaldehyde (H2CO) 4.5 6ml
2000 ml dH2O To 3L To 4 L

Fix/stop solution (10% acetic acid)

200 ml glacial acetic acid 300ml 400ml
1800 ml dH20 2.7L 3.6 L
Appendix 4  Publications and presentations

Publications


Oral Presentations


Poster Presentations

