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Molecular markers for classification of seed collections of *Lolium* cultivars

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Key words : *Lolium* ,taxonomy ,microsatellites ,SNPs

Introduction Molecular based methodologies have been successfully used by several workers to expose discriminating levels of diversity between ryegrass seed accessions but cannot be used for registering new varieties as they do not protect against plagiarism . They can , however , assist in assessing relatedness for Essential Derivation relationships (Roldan-Ruiz *et al* 2000) , and so identify varieties as highly similar pairs . If more widely related groupings could be identified , in which all varieties still conform to a shared seed sample profile , then discrimination of new varieties from all members of a group may only require one group representative to be sown in traditional field based identification trials .

Materials and methods Seed of twenty four ryegrass (*Lolium* spp) varieties representative of the diversity in commerce , were genetically screened by sequencing three chloroplast genome regions (*trnT-trnL* intergenic spacer , *trnL* intron and *trnL-trnF* intergenic spacer ; Taberlet *et al* .1991) , to identify microsatellite polymorphisms (method : Provan *et al* .2001) and single nucleotide polymorphisms (SNPs) , using the allele-specific PCR (AS-PCR) approach (Provan *et al* .2008) .

Results and discussion Sequencing of the *trnT-L-F* region identified both single nucleotide polymorphisms (SNPs) and microsatellite length polymorphism . Both of these clearly delineated *L. perenne* (10-24) , which have the shorter microsatellite motif lengths in the *trnL* intron and the longer microsatellite motif lengths in the *trnL-trnF* intergenic region , as well as a diagnostic SNP at position 377 in the *trnL* intron , from the *L multiflorum* complex (1-9) . This complex included one *Festulolium* (1) , one *Westerwold* (2) , four *L. perenne x L. multiflorum* hybrids (3-6) and three *L. multiflorum* (7-9) . A notable exception was Bealey (13) , which displayed a *L. multiflorum* type cytoplasm , suggesting that in the breeding of this *L. perenne* , the final polycross was constructed upon Italian or hybrid mothers . The hybrid Foyle (6a , 6b) contained both perennial and Italian cytoplasm , indicating that maternal plants from each species existed in the final multicross , whereas all the other hybrids were created only from Italian mothers . Interestingly , Foyle expresses a very strong perennial-like phenotype . The three New Zealand varieties (5 , 11 , 14) all expressed unique SNPs in contrast to the European or American material , possibly indicating a gene pool provenance .

Conclusions While diagnostic species patterns were identified , markers for unique variety types were also observed , indicating the potential of the combined SNP and chloroplast microsatellite approach to reveal detail on breeding lineages from seed samples . Examination of more similarly diverse regions should divide varieties into similar genotypes within species .

References

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Table 1 Alignment of three regions of the chloroplast genome sequenced in 24 cultivars . 1 BX421 , 2 Tewera , 3 Barsilo , 4 Brutus , 5 Glds Manawa , 6 Foyle , 7 Abercomo , 8 Lemtal , 9 Danergo , 10 Aberelf , 11 Loretta , 12 Numan , 13 Bealy , 14 Bronsyn , 15 Moy , 16 Aberdart , 17 Aberelan , 18 Aberstar , 19 Fennema , 20 Kent Indig^N , 21 Melle , 22 Tyrone , 23 Veritas , 24 Herbal . Sites in bold show SNPs .

	<i>trnT-trnL</i>	<i>trnL</i> intron	<i>trnL-trnF</i>
	2 2 5 6 3 3 3 3 3 3 3		1 1 1 1 1 1
	3 3 6 2 5 5 5 5 6 6 6 7 6 7 8 8 8 8 9 9 9 9 9 9		
	6 1 1 2 1 7 8 9 0 1 2 7 2 9 0 1 2 3 0 1 2 3 4 5		
1	C G C G T T T T T - - G A A A T A T C - - - -		
2	C G C G T T T T T - - G A A A T A T C - - - -		
3	C G C G T T T T T - - G A A A T A T C - - - -		
4	C G C G T T T T T - - G A A A T A T C - - - -		
5	A A C - T T T T T T - G G - - - - - - - - - -		
6a	C G C G T T T T T - - - G A A A T A T C - - - -		
6b	C G C G T T - - - - T A - - - - - C C C C - -		
7	C G C G T T T T T - - G A - - - - - C - - - -		
8	C G C G T T T T T - - G A - - - - - C - - - -		
9	C G C G T T T T T - - G A - - - - - C - - - -		
10	C G C G T - - - - - T A - - - - - C C C C C C		
11	C G C G T T - - - - - T A - - - - - C C C C - -		
12	C G A G T T - - - - - T A - - - - - C C C C C -		
13	C G C - A T T T T - - G A - - - - - - - - - -		
14	C G A G T T - - - - - T A - - - - - C C C C - -		
15	C G C G T T - - - - - T A - - - - - C C C C C -		
16	C G C G T - - - - - T A - - - - - C C C C C C		
17	C G C G T T - - - - - T A - - - - - C C C C C -		
18	C G C G T - - - - - T A - - - - - C C C C C C		
19	C G C G T - - - - - T A - - - - - C C C C C C		
20	C G C G T T - - - - - T A - - - - - C C C C C -		
21	C G C G T T - - - - - T A - - - - - C C C C C -		
22	C G C G T T - - - - - T A - - - - - C C C C C -		
23	C G C G T T - - - - - T A - - - - - C C C C C -		
24	C G C G T T - - - - - T A - - - - - C C C C C -		