Allelic Discrimination: A PCR Tool for Ryegrass Purity Determination

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ABSTRACT:

The ryegrasses are important worldwide for forage and turf use. Those of highest economic value are taxonomically classified as subspecies of perennial ryegrass (*Lolium perenne L.). The close taxonomic* relationships make it difficult to separate these grasses in seed labs. In areas such as Oregon where intensive seed production occurs, the "annual-types" often contaminate the more economically important "perennial-types" through seed mixture, pollen flow, or both. Seedling root fluorescence (SRF) has been used to distinguish "annual-types" from perennial ryegrass since the 1930s, but at times the test has been unreliable and overestimates the amount of "annual-type" contamination. The test is based on a loose genetic linkage among the gene(s) responsible for SRF and those responsible for other "annual-like" characteristics. We examined genes involved in flowering control and vernalization responses that are closely associated with growth type, and found two genes were effective in predicting growth type. DNA samples were extracted from leaf tissue of SRF-tested seedlings and were analyzed by real-time PCR using Allelic Discrimination (AD) as a purity test to differentiate between alleles (alternate forms of a gene). Twenty cultivars were examined to validate the AD protocol. Following the SRF test, all seedlings were transplanted to a growth chamber and grown under continuous, high intensity light for a grow-out test (GOT) that lasted for 84 days. Heading rate approached a plateau at about 70 days for those plants that had SRF as seedlings. These results supported the proposal that the GOT should be longer than the suggested 42 days if it is to be effective. Further, the rate of SRF was highest in the plants that headed earliest and was lower in later heading plants. The minimum SRF level was 30% over all the plants tested, demonstrating that the SRF test lacks accuracy in predicting contamination. In contrast, the AD test based on two flowering genes, *Vrn-1 and ID1 detected growth type differences equivalent to a 70day GOT, with less than 1% error rate*. Data presented demonstrates that the AD test is an effective and rapid method of marker assisted purity analysis in perennial ryegrass.

The Seedling Root Fluorescence Test and Grow-out Test simply MUST be fixed! The DNA A/D assay is a solution.

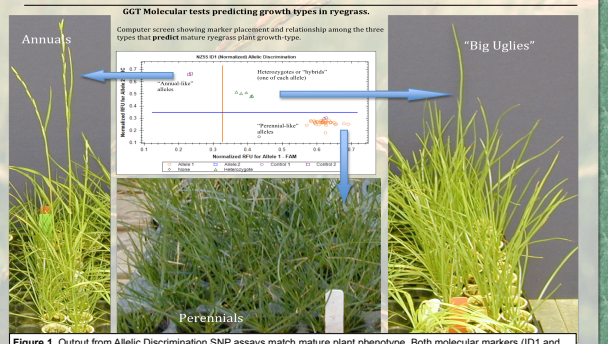


Figure 1. Output from Allelic Discrimination SNP assays match mature plant phenotype. Both molecular markers (ID1 and Vrn-1) are used in final genotype determinations.

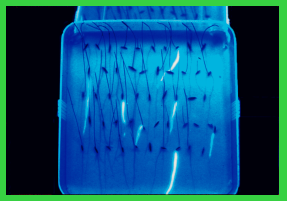


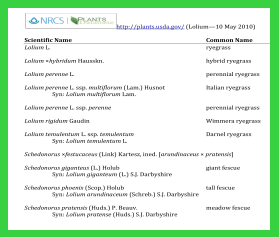
Figure 2. Germination box showing fluorescing seedling root traces. At one time, ryegrass seedlings that fluoresced under UV light were considered "annual" ryegrass. NOT ANY LONGER!

Figure 3. Contamination in ryegrass turf. "False-positives" from fluorescence and grow-out tests are still not being detected.

Table 1. Grow-out tests conducted on 20 seed lots from Agri Seed Testing in 2005 and 2006. Molecular markers (ID1 and Vrn-1) run on all seedlings in the USDA GOT that was done in intensity growth chamber.

	1st AST test					2nd AST test			Calc % 'A' from USDA growth chamber GOT				Calc % 'A' from USDA ID1& Vrn-1 DNA markers	
AST inc. TEST#	VFL	% Germ	TFL	Normal FI plants	Calc %"A" from AST GOT	% Germ	TFL	Normal FI plants	42 da	56da	70da	<84da	'A'-type plants only	Combined 'A' & 'H' plants
20741	n/d	96.00	10.68	41	4.08	95.00	8.68	33	6.95	7.29	7.99	7.99	2.08	4.17
21959	0.92	93.00	5.11	19	0.00	96.75	4.91	19	1.13	1.89	3.40	3.78	0.38	0.38
25264	0.72	90.00	5.00	18	0.29	95.00	4.74	18	2.03	3.05	4.06	4.40	0.00	2.03
21808	5.88	94.00	9.57	36	1.16	93.25	11.26	42	2.43	6.09	8.22	8.83	0.61	2.74
22395	n/d	94.50	7.94	30	2.06	95.25	8.92	34	3.25	4.60	5.41	6.49	1.89	3.25
22701	1.17	96.75	5.17	20	0.82	96.50	3.63	14	0.91	1.21	1.21	2.12	0.00	0.60
20736	1.22	96.00	5.44	21	0.00	96.75	4.39	17	0.34	0.68	1.01	1.35	0.00	0.34
19097	n/d	92.00	4.89	18	0.82	90.25	8.59	31	2.64	2.64	2.64	2.97	0.99	4.95
22036	0.53	93.75	3.20	11	0.59	92.25	2.98	11	2.09	2.38	2.68	2.68	1.19	1.79
18240	2.72	94.25	6.63	25	0.58	93.00	3.23	12	1.43	2.15	2.51	2.87	1.08	2.15
20604	0.88	91.00	8.52	31	0.00	87.00	10.63	37	2.58	4.19	5.15	6.77	0.64	1.93
19245	0.20	96.25	3.38	13	0.52	89.00	0.28	1	0.00	0.00	0.00	0.00	0.00	0.00
25867	0.15	93.50	9.63	36	0.30	95.75	8.62	33	3.19	6.38	7.02	7.02	0.64	3.19
19528	n/d	94.25	4.77	18	0.80	92.25	7.59	28	3.30	4.62	5.28	5.94	0.99	3.96
19660	n/d	97.25	4.63	18	0.00	95.75	3.66	14	2.13	2.44	2.74	3.35	1.22	2.13
19173	1.36	93.50	6.15	23	0.29	93.50	8.29	31	2.65	4.64	5.64	5.97	0.00	0.99
26065	0.83	87.75	1.23	13	0.57	91.00	0.82	3	0.41	0.82	0.82	0.82	0.00	0.41
21629	n/d	94.50	5.56	21	2.22	94.00	5.85	22	7.31	7.68	8.41	8.41	2.56	5.12
24737	n/d	93.75	3.47	13	0.80	91.25	1.92	7	1.28	1.28	1.28	1.28	0.32	0.96
25290	n/d	91.00	13.74	50	4.37	91.25	9.32	34	5.90	6.21	6.83	6.83	3.11	4.97
n/d = not determined														
Average	1.38	93.65	6.24	24	1.01	93.24	5.92	22	2.60	3.51	4.12	4.49	0.88	2.30
			Total FL+	475			Total FL+	441						

Current Ryegrass Taxonomy



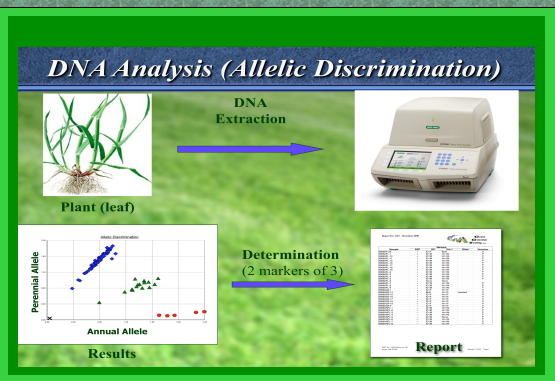


Figure 4. Concept of the overall process for the M*3 A/D assay.

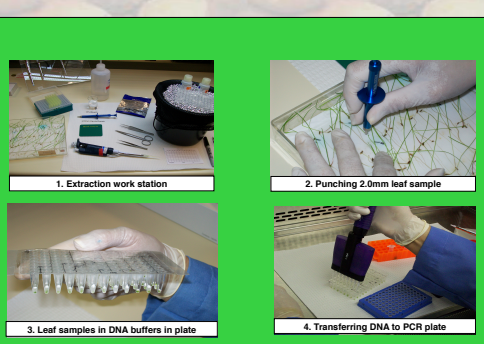


Figure 5. Pictorial sampling and preparation steps for the M*3 A/D assay.

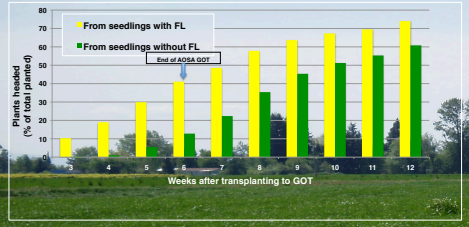


Figure 6. Heading rates of plants with and without fluorescent seedling roots.

Performance of Plants With and Without Fluorescence as Seedlings

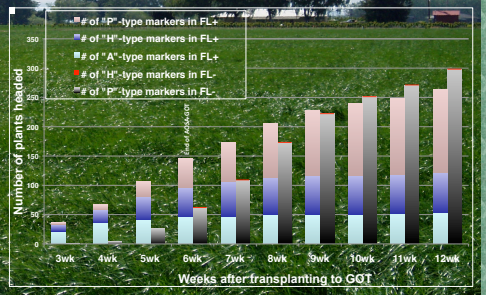


Figure 7. Heading rate of fluorescent (FL+) and non-fluorescent (FL-) seedlings with molecular markers for each, P=perennial, H=hybrid, A=Annual. Data from from seedlings of 20 cultivars.

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