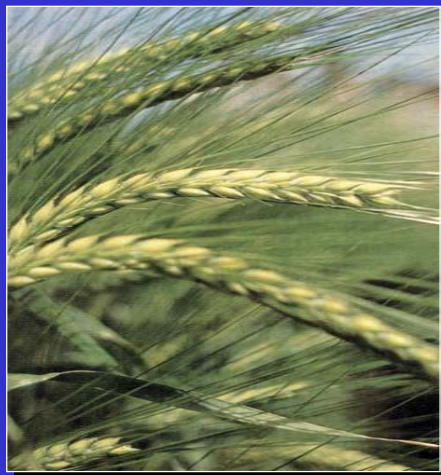


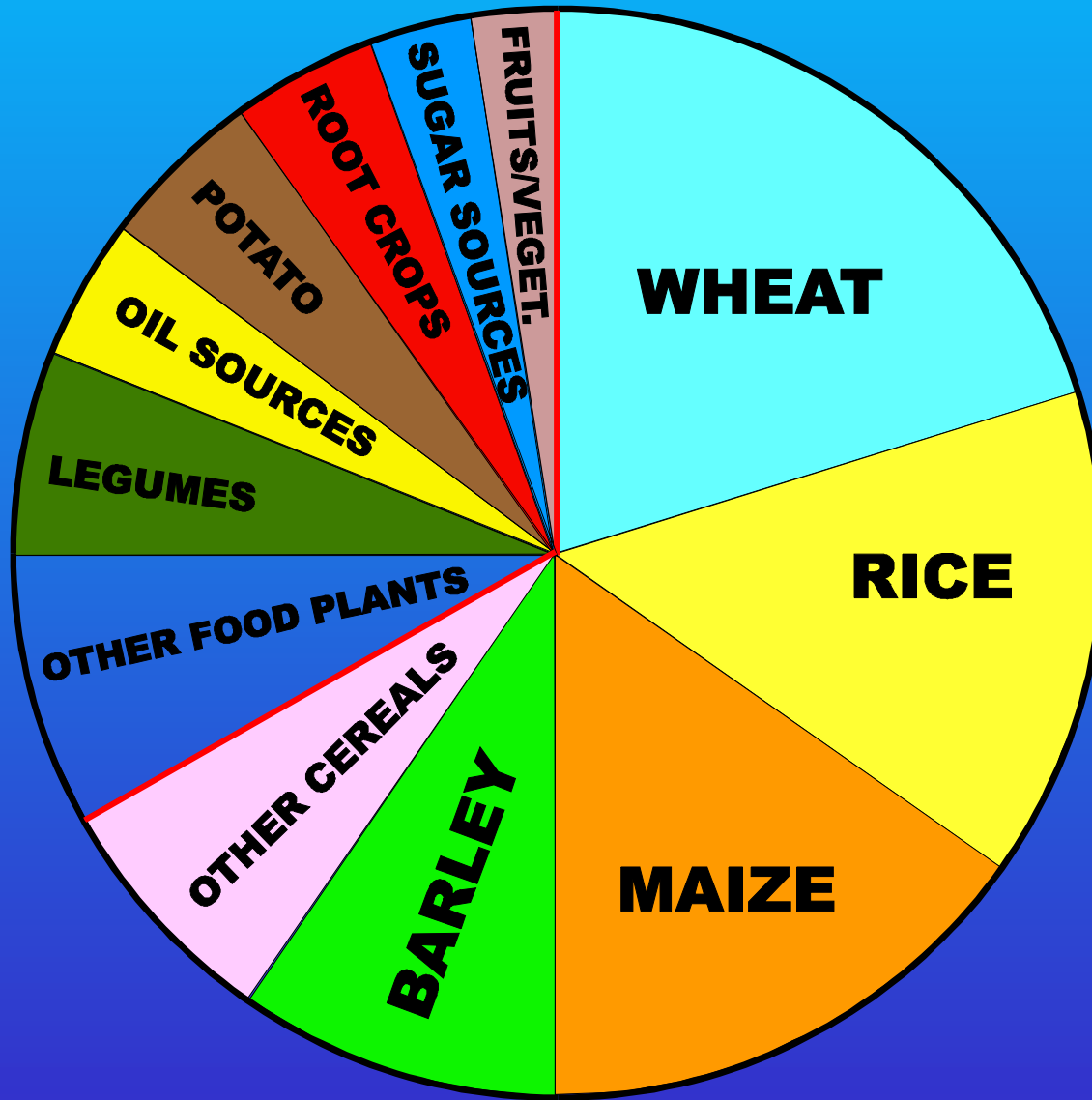
qPCR and Small grain Cereals: Species and Transgene Detection.



Valeria Terzi

Ist. Sperimentale per la Cerealicoltura
Sez. Fiorenzuola D' Arda, Italy

Triticeae represent worldwide the main food stuff



The cereal composition of foods
is always a key factor
for their quality and safety.

Wheat content? Bread wheat content?



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Development of analytical systems based on real-time PCR for *Triticum* species-specific detection and quantitation of bread wheat contamination in semolina and pasta

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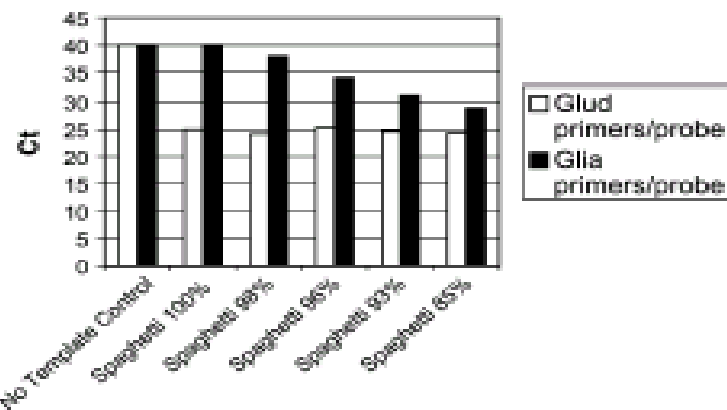


Fig. 4. Mean C_t s obtained from real-time PCR analysis using a Glud primers/probe combination and GliA primers/probe on 200 ng DNAs extracted from standard spaghetti made with 100, 98, 96, 93, and 85% of durum wheat and, respectively, 0, 2, 4, 7, and 15% bread wheat. Negative control is indicated as NTC (no template control).

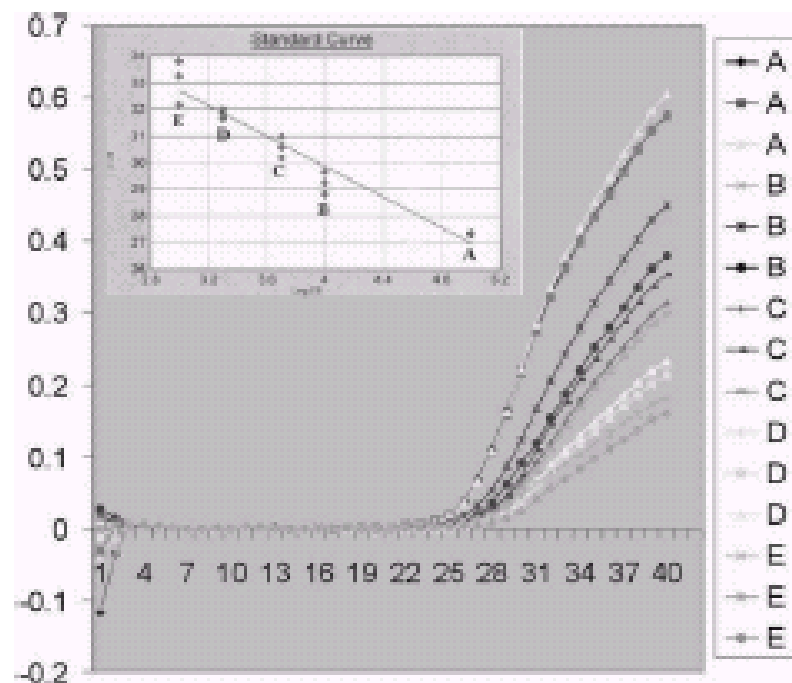


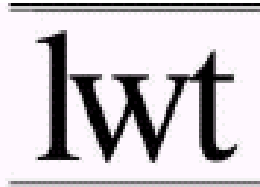
Fig. 5. Amplification plots (R_n versus C_t) and standard curve obtained from real-time PCR analysis using a GliA primers/probe combination on DNA samples (three replicates) extracted from mixtures of bread/durum wheat at the indicated percentages: A = 100% bread wheat (100 000 pg); B = 10% bread wheat (10 000 pg); C = 5% bread wheat (5000 pg); D = 2% bread wheat (2000 pg); E = 1% bread wheat (1000 pg). The standard curve slope is -2.85 , the intercept is 41.24 and the correlation is -0.97 .

Rye content?

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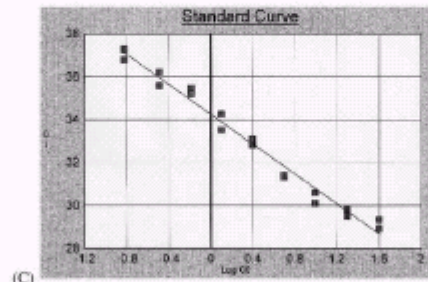
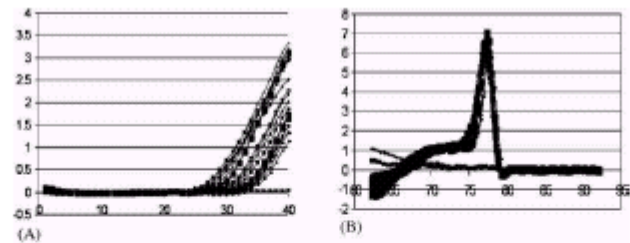
Quantitative detection of *Secale cereale* by real-time PCR amplification

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A. Michele Stanca^a, Primetta Faccioli^a

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Slope: -3.474503
 Intercept: 34.229931
 Correlation: -0.99062

Fig. 5. Real-time PCR with ESTSEC primers and SYBR green detection of the following rye DNA amounts: 40, 20, 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15ng and No Template Control (negative control without DNA). (A) The figure shows plots of ΔR_n vs. cycle number. NTC sample does not show any increase in fluorescence emission. (B) Dissociation curves of the amplicons. One single peak is observed, indicating the presence of a single product. (C) Standard curve obtained from samples containing different amounts of rye DNA, expressed as log of the concentrations.

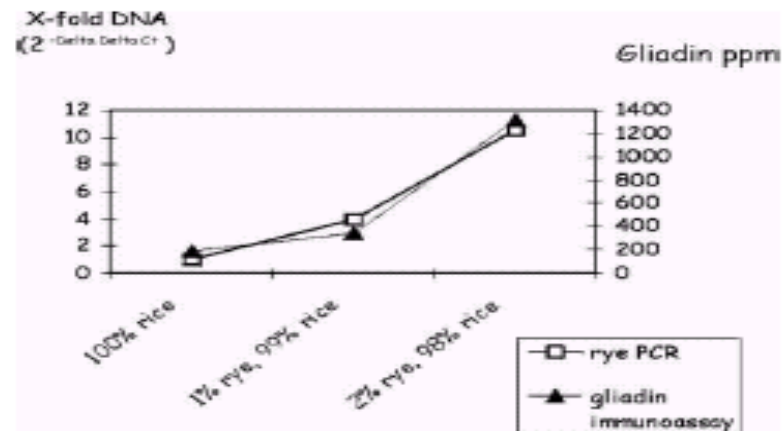


Fig. 6. Results obtained after evaluation of three samples (100% rice, 1% rye 99% rice and 2% rye 98% rice) with ESTSEC real-time PCR system to track rye DNA presence and with an immunoassay kit (RIDASCREEN Gliadin, R-Biopharm AG, Germany) to track gliadin content.

Some examples of transformed wheat genotypes to improve quality traits.

Species	Transformed traits	Reference
<i>Triticum aestivum</i>	1Ax1 and 1Dx5 HMW glutenin subunits	Barro et al, 1997
<i>Triticum aestivum</i>	1Ax1 HMW glutenin subunit	Altpeter et al., 1997
<i>Triticum aestivum</i>	HMW glutenin subunit composition	Alvarez et al., 2000
<i>Triticum aestivum</i>	Dy10:Dx5 HMW glutenin subunit	Blechl and Anderson, 1996
<i>Triticum durum</i>	HMW glutenin subunit composition	He et al., 1999
<i>Triticum aestivum</i>	LMWG1D1 promoter for seed specific expression	Stoger et al., 1999
<i>Triticum aestivum</i>	1Dx5 HMW glutenin subunits	Rooke et al., 1999
<i>Triticum aestivum</i>	Endosperm-specific storage protein promoter	Lamacchia et al., 2001
<i>Triticum durum</i>	Rab1 gene to up-regulate the transport of vesicles to the Golgi apparatus	Lamacchia et al., 2000
<i>Triticum aestivum</i>	<i>Aspergillus</i> phytase gene introduction to improved feed quality	Brinch-Peterson et al., 2000

GM wheat track

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doi:10.1006/jcrs.2002.0489



TaqMan PCR for Detection of Genetically Modified Durum Wheat

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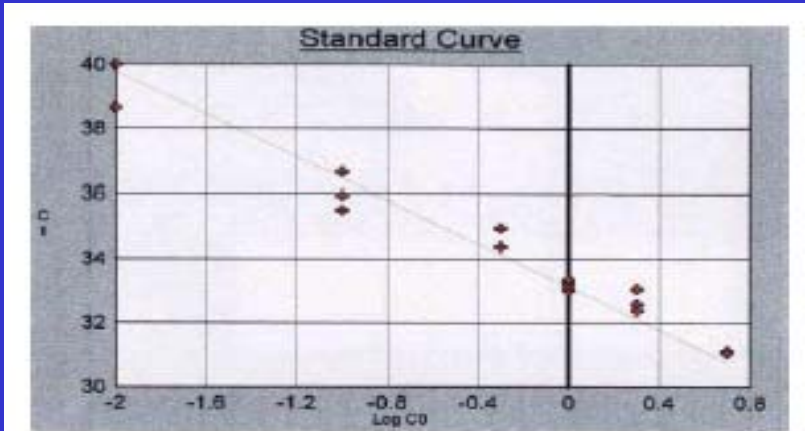
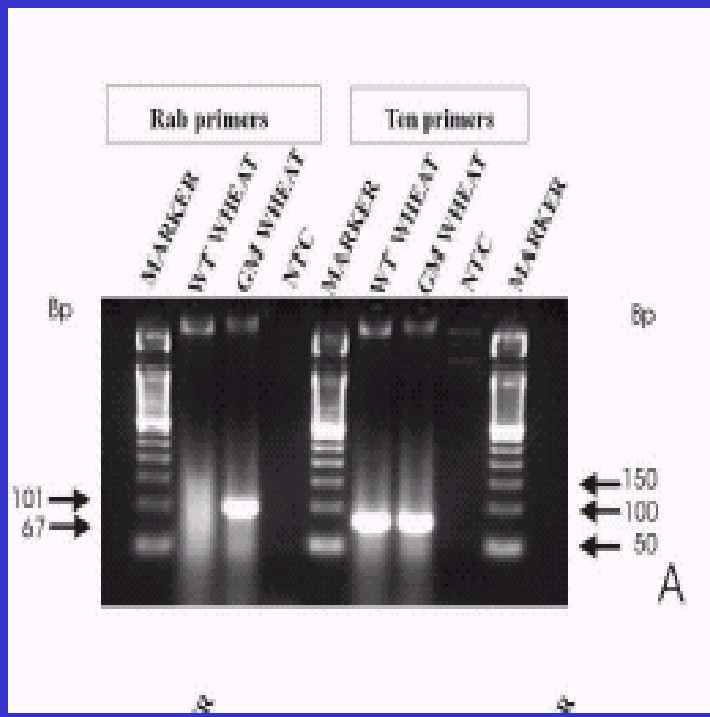
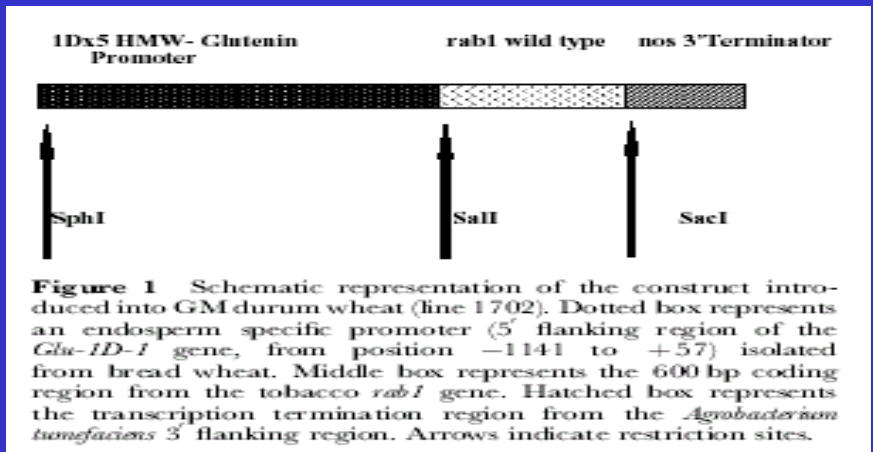
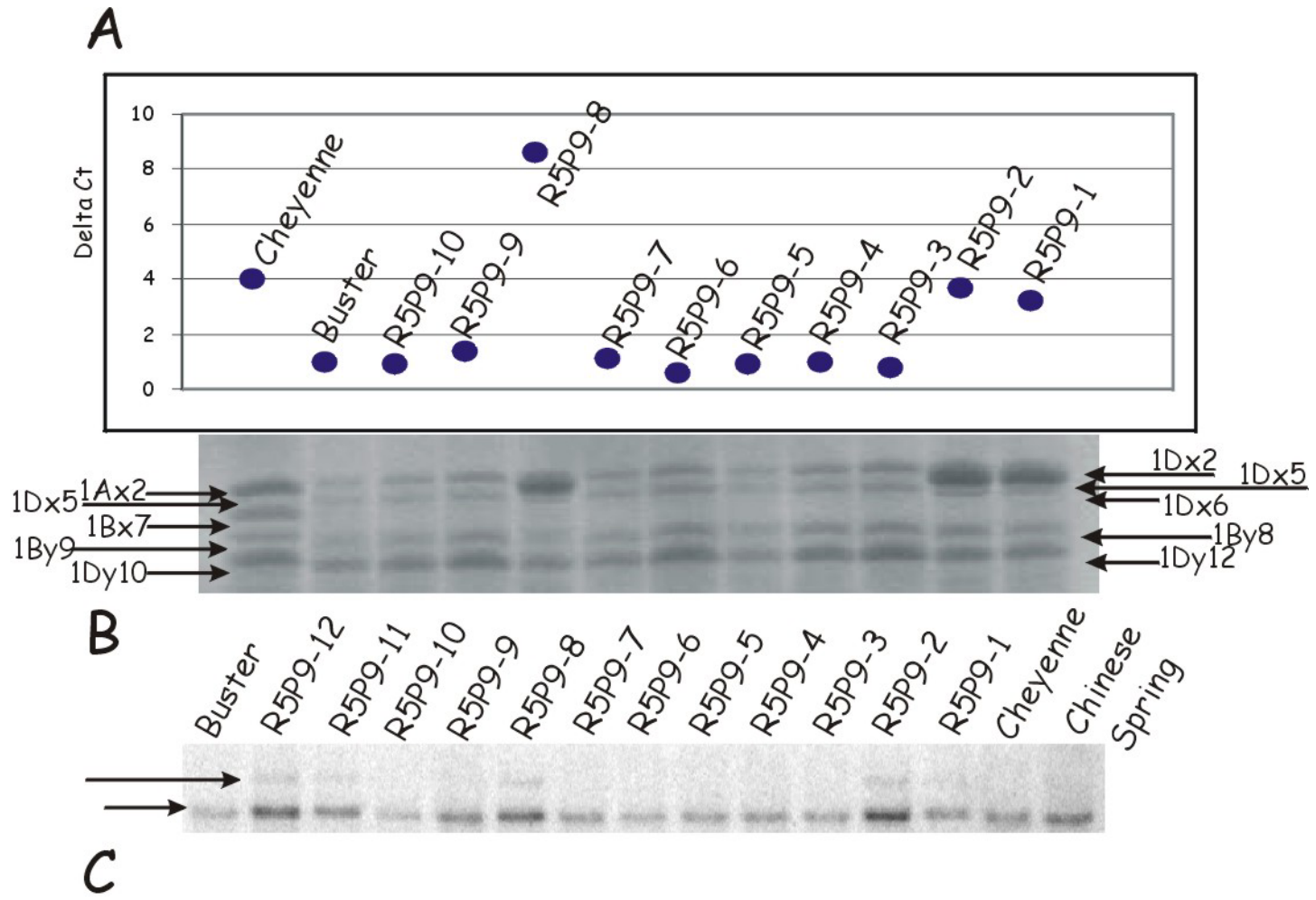


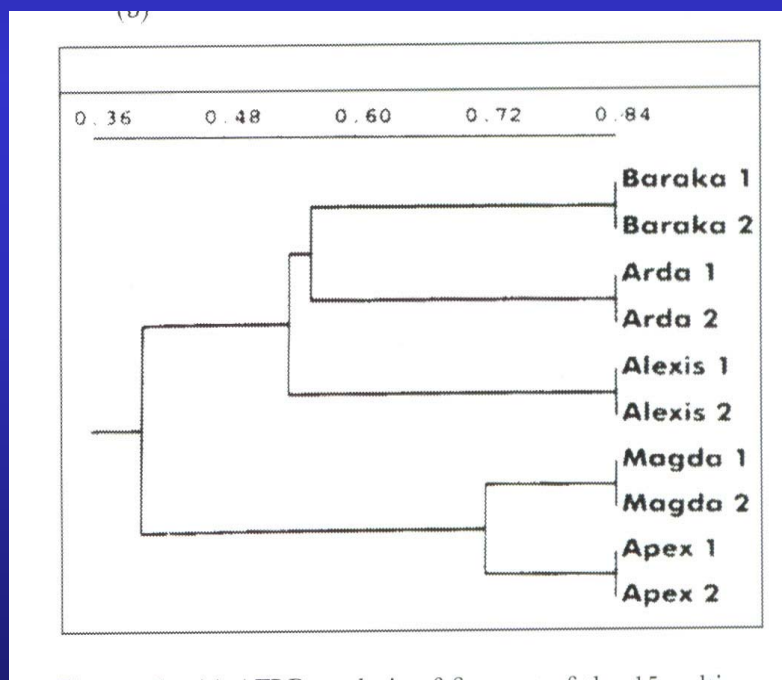
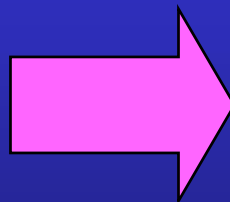
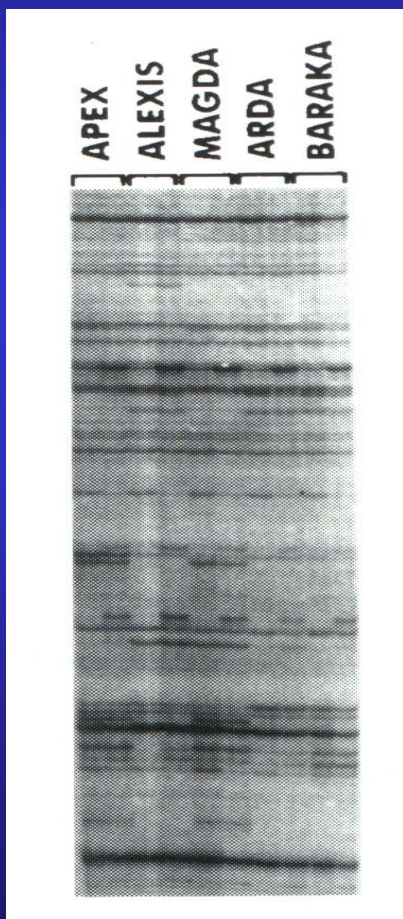
Figure 5 Standard curve (displaying the threshold cycle, Ct vs. Log₁₀ for each starting amount of standard) for determination of the *rab1* gene DNA by the TaqMan method, generated with DNA from blend of flours containing 0.01, 0.1, 0.5, 1, 2 and 5% (w/w) transgenic wheat. Equal amounts of blends containing 0.01 to 5% transgenic wheat were extracted and equal amounts of DNA (100 ng) were used in the analysis. The slope is -3.34, the intercept is 33.10 and R² is 0.99.

Real-time PCR assisted selection of wheat plants transformed with HMW gluten subunit genes.

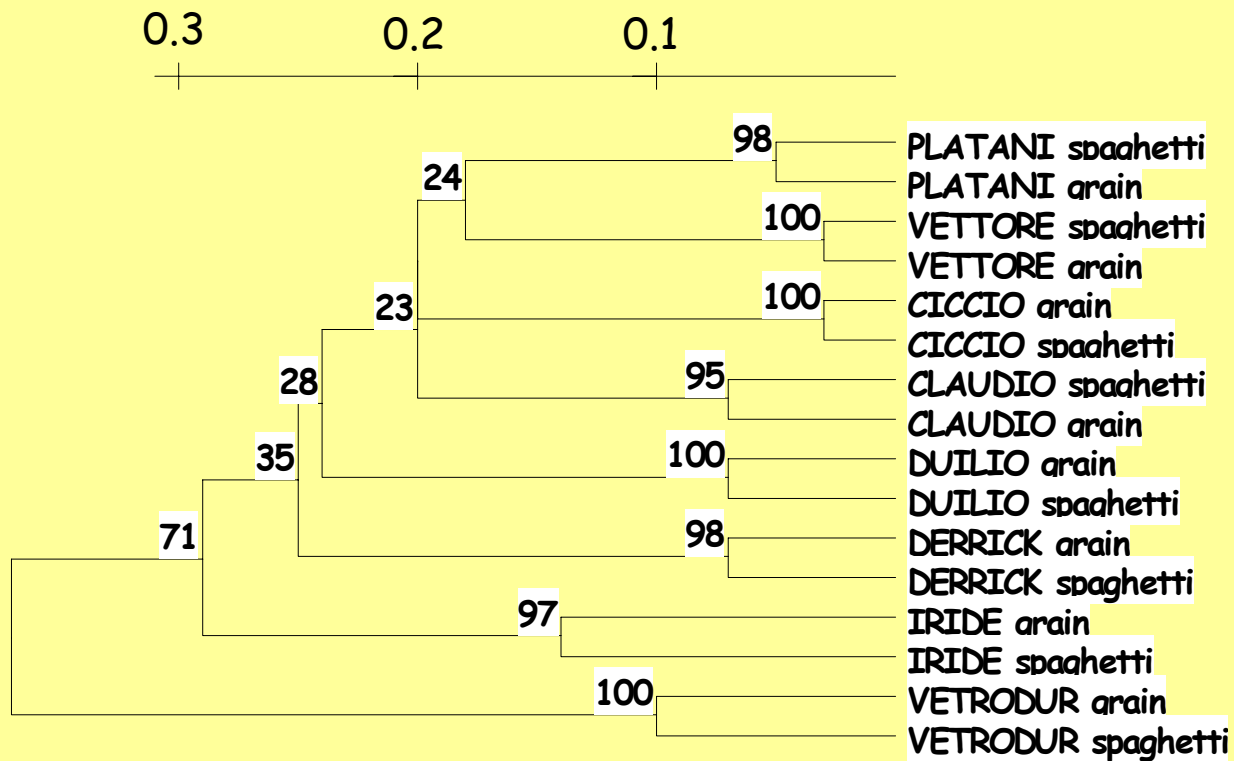
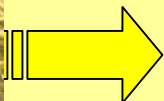
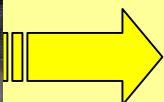


Amplified Fragment Length Polymorphism (AFLP) markers for barley malt fingerprinting

Faccioli P., Pecchioni, N., Stanca A.M., Terzi V.
J. Cereal Science, 1999, 29:257-260.



AFLP markers for monovarietal spaghetti fingerprinting



Thank you