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Development of a PCR-system for Detection of Rapeseed and Other Cruciferae

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Introduction

Today, a widespread method for surveillance of food ingredients and food products, especially of genetically modified organisms (GMO), is the polymerase chain reaction (PCR). Several simple or nested PCR systems were established for detection of even small amounts of DNA in raw or processed food products e.g. soy bean (1), maize (2), tomato (3) and potato (4). Specific PCRs have been developed for several GMOs currently authorised in the European Union and in the United States: Roundup Ready Soya (5), bt-maize (2), Maize Gard Corn (6) and FlavrSavr tomato (7). For all these systems an initial assessment of the quality and availability of DNA for PCR amplification is necessary. Therefore, specific PCR systems are necessary specifically amplifying the soya lectin gene, the maize zein gene or a tomato specific intergenic spacer (1–3). Beside quality assessment these systems allow, applied with an internal competitor, a quantification of the target DNA (8).

Results and Discussion

With respect to genetically modified rapeseed and other cruciferae available on the market two nested-PCR systems were developed, based on the sequence of two genes of *Brassica napus:* Cruciferin and napin. Both genes are encoding embryo-specific storage proteins; Cruciferin (12S storage protein) is the major storage protein of *Brassica napus* and is as well as the 1.7S seed storage protein napin encoded by a multigene family. Characteristic for the napin gene is the lack of introns. Primer sequences for both systems are shown in table 1.

DNA was extracted following methods described earlier (9) using Wizard DNA purification resin (Promega). PCR conditions for both systems were divergent from earlier reports as follows: First PCR (25 cycles) and nested PCR (2 µl of first PCR-

Table 1
Primersequences

Primer	*Amplicon*	*Sequence*	*$MgCl_2$-concentration*
CRU1		AGT GGA ACG CAA ACG CAA AC	1.5 mM
CRU2	372 bp	TCC TTG GCC CTC CGT AGC	
CRU3		AAC GAC AAC GGT GAC AGA	1.0 mM
CRU4	243 bp	TCC TTG GCC CTC CGT AGC	
NAP1		CTC GCC TTC TTC TTC CTT CTC ACC A	3.5 mM
NAP2	401 bp	ACC ATT TGC TGC TTT CCT TGC TGT T	
NAP3		GAA GAT GAT GCC ACA AAC TC	1 mM
NAP4	264 bp	TGG GCA AAC GCA AAG	

mix were used as template) (35 cycles) at 96 °C for 30 s and 60 °C for 30 s. $MgCl_2$ concentrations for each system were different and are shown in table 1. The primers CRU 1-4 generated a 243-bp-long PCR product after nested PCR, the NAP1-4 system a 264-bp-long amplicon (fig. 1). Sensitivity of the two nested PCRs was determined at 10 pg purified DNA from rapeseed (table 2). All rapeseed cultivars registered in Switzerland in 1998 (Capitol, Synergy, Jockey, Express and Libravo) yielded the expected PCR product with the same sensitivity. Performance with high concentrations of pure, high molecular DNA extracted from leafs yielded an additional, non-specific band (ca. 310 and 350 bp respectively) for both systems. Selectivity of both systems is not limited to rapeseed. But the control of availability for PCR of DNA extracted from rapeseed is usually performed with pure seeds and not with mixtures of different crops. Coamplification of other cruciferae means no limitation of applicability or reliability of the napin or cruciferin PCR-systems.

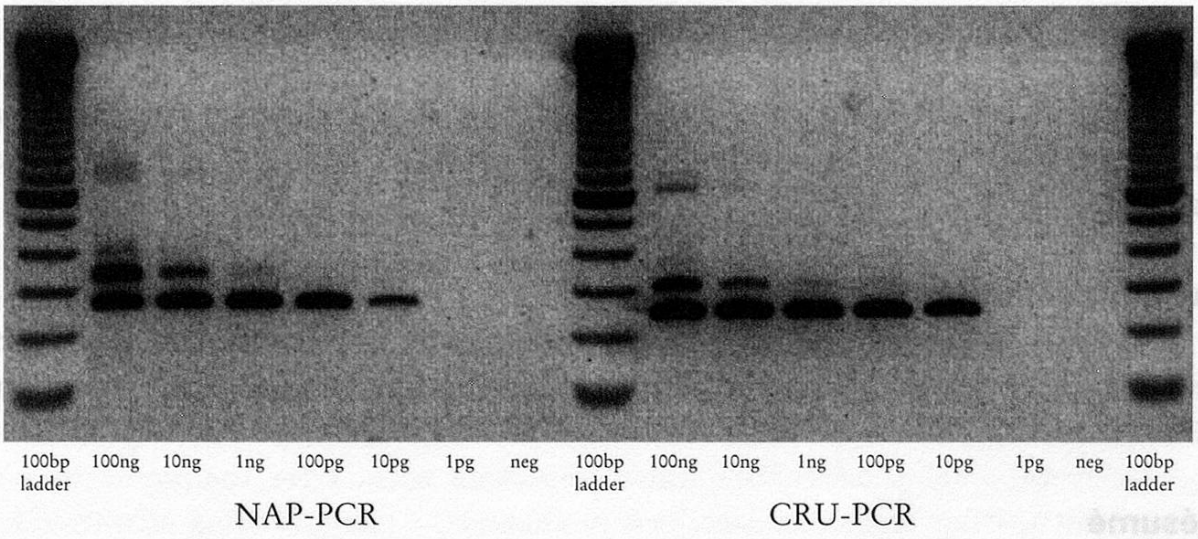

Figure 1 **Sensitivity of nested NAP- and CRU-PCR after 25/35 cycles of amplification. "neg" = negative control/PCR without DNA template**

Table 2
Sensitivity of cruciferin and napin PCRs

Primer pair	*Cycles*	*Sensitivity*
CRU 1-2	40	100 pg
CRU 3-4	40	1 ng
CRU 1-2	25	–[1]
nested CRU 3-4	35	10 pg
NAP 1-2	40	1 ng
NAP 3-4	40	1 ng
NAP 1-2	25	–[1]
nested NAP 3-4	35	10 pg

[1] not detected

Storage protein gene sequences differ not sufficiently to allow rapeseed specific systems due to affinity of *brassica napus* with other cruciferae. Further vegetables like broccoli or cauliflower (both cruciferae) yielded the same amplicons. Beside these two, other vegetables tested such as potatoes, tomatoes or cereals (wheat, barley, rye, oat), lentils, soya, sunflower, rice and animals (pig, chicken, and perch) gave negative results (data not shown).

With the above PCR a system for validation of availability of extracted DNA from various plants and plant products was developed. Especially for GMO analytics such systems are necessary for initial PCR.

Acknowledgements

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Summary

A sensitive PCR-system for detection of rape seed and other cruciferae was developed based on the two storage protein genes napin and cruciferin of brassica napus.

Zusammenfassung

Ein PCR-System für den sensitiven Nachweis von Raps sowie anderen Cruciferae wurde entwickelt, basierend auf den beiden Speicherproteingenen Napin und Cruciferin.

Résumé

Une méthode RCP sensible utilisant les gènes de deux protéines de réserve de *Brassica napus* (napine et cruciférine) a été développée pour mettre en évidence le colza et d'autres crucifères.

Key words

Rape seed, *Brassica napus*, DNA, cruciferin, napin

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