Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Control 17 (2006) 30–36
FOOD CONTROL
www.elsevier.com/locate/foodcont

Specific, semi-quantitative detection of the soybean allergen Gly m Bd 30K DNA by PCR

A.M. Torp [a,*], A. Olesen [a], E. Sten [b], P. Stahl Skov [c], U. Bindslev-Jensen [d], L.K. Poulsen [b], C. Bindslev-Jensen [d], S.B. Andersen [a]

[a] *Plant Breeding and Crop Science, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*
[b] *Allergy Clinic, National University Hospital, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark*
[c] *The Reference Laboratory, National University Hospital, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark*
[d] *Department of Dermatology, Allergy Centre, Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense C, Denmark*

Received 11 May 2003; received in revised form 10 August 2004; accepted 12 August 2004

Abstract

A semi-quantitative PCR-based system has been developed for detection of DNA sequences for the soybean allergen Gly m Bd 30K. The selected primers were highly specific for soybean and did not show amplification from a panel of legume relatives. Repeatability was assessed in a spiking experiment of soybean in wheat flour (0.0001–100% soybean), using known standards for comparison of the amount of output DNA from different PCR reactions. The frequency of PCR reactions with successful amplification of the soybean allergen sequence was highly dependent on initial target DNA concentration, and showed a rapid sigmoidal decrease, when target concentration approached the detection limit (0.01%). For samples with successful amplification there was a good correlation between the initial amount of soybean in the mixture and the output from PCR, when suitable block designs were used to control experimental errors. The simple approach used for quantification in this study proved efficient for assessment of homogeneity of self-prepared soybean bars used for provocation tests of food allergic patients in clinical practice. In addition the method was efficient in detecting soybean allergen sequences in a number of processed foods.

Keywords: Soybean; Allergen; Gly m Bd 30K; PCR

1. Introduction

At present, the only efficient treatment of patients suffering from food allergy or intolerance is avoidance of the offending food in the diet. However, hidden allergens in complex foods are a general problem for such patients due to lack of labelling or un-intended cross-contamination of commercial foods during processing. Thus for example, 5 of 17 commercial food products without declaration of peanut protein contained 2–18 ppm (micrograms/gram) of peanut protein when analysed with a sandwich type ELISA method (Holzhauser & Vieths, 1999a). Similarly, Holzhauser and Vieths (1999b) detected 2–48 ppm of hazelnut protein in 12 of 28 unlabelled commercial food products while Koppelman et al. (1999) found between 1 and 4000 ppm in 15 of 26 foods without label for hazelnut protein. Since low doses of the offending food have been found to elicit allergic reactions in some patients (Hourihane et al., 1997;

* Corresponding author. Tel.: +45 35 28 34 40; fax: +45 35 28 34 60.
E-mail address: amt@kvl.dk (A.M. Torp).

0956-7135/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2004.08.005

MalmhedenYman, Erikson, Everitt, Yman, & Karlsson, 1994) there is a need for sensitive detection methods to specifically detect such hidden allergens in order to assist patients suffering from food allergy in avoiding critical food contaminants.

Most available methods for detection and/or quantification of hidden allergens in food matrixes are based on immunological detection of protein components, and include analytical assays based on e.g. immunoblotting, Rocket immuno electrophoresis (RIE) and enzyme-linked immunosorbent assays (ELISA) (Holzhauser, Dehne, Hoffmann, Haustein, & Vieths, 1998; Holzhauser & Vieths, 1999a, 1999b; Koppelman et al., 1999; Meyer, Chardonnens, Hübner, & Lüthy, 1996; Stephan, Möller, Lehmann, Holzhauser, & Vieths, 2002). At present ELISA based methods are the most widely used and a number of commercial ELISA kits that allow detection and quantification of <0.1–5 ppm peanut protein, 1–10 ppm hazelnut protein, 1.5–10 ppm wheat protein and 1–<5000 ppm soybean protein in food samples are now available (Poms, Klein, & Anklam, 2004; Elisa Systems, Brisbane, Australia; R-Biopharm, Darmstadt, Germany; Pro-Lab diagnostics, Ontario, Canada; Tepnel Biosystems, Flintshire, UK; Neogen, Lansing USA).

Recently, polymerase chain reaction (PCR) based detection of specific DNA sequences has been suggested as an alternative to the immunological methods for detection of hidden allergens in complex foods. Several studies have shown a good correlation between presence of amplifiable DNA and protein as determined by ELISA based methods (Allmann, Candrian, Höfelein, & Lüthy, 1993; Dahinden, VonBüren, & Lüthy, 2001; Holzhauser, Stephan, & Vieths, 2002; Holzhauser, Wangorsch, & Vieths, 2000). Compared to immunological methods, PCR methods avoid the need for constant supply of high quality antisera. In addition, cross-reactivities and false-positive results may be minimized by careful selection of PCR-primers that can distinguish between sequences from biologically closely related species. A number of qualitative and quantitative tests kits for specific PCR based detection of below 10 ppm hazelnut, peanut and soybean, respectively, are now on the market. The test kits for hazelnut is based on detection of one of the major hazelnut allergens Cor a 1.04, while the kits for soybean and peanut are based on detection of the lectin gene and unspecified sequences, respectively (Poms et al., 2004; Tepnel BioSystems, Flintshire, UK; Congen GmbH, Berlin, Germany). In the present paper we describe a new set of primers for specific amplification of coding sequences of Gly m Bd 30K reported to be one of the major allergens in soybean (Ogawa et al., 1991; Ogawa et al., 1993). A semi-quantitative method was developed to evaluate the system with respect to detection level, specificity and stability.

2. Material and methods

2.1. Soybean and legume samples

Five cultivars of soybean supplied by the Nordic Gene Bank and Dr. J.R. Wilcox, Purdue University, USA were used as reference cultivars. Specificity of PCR with selected primers was tested with DNA isolated from groundnut (*Arachis hypogaea*), garden bean (*Phaseolus vulgaris var. vulgaris*), broad bean (*Vicia faba*), lima bean (*Phaseolus lunatus*), scarlet runner bean (*Phaseolus coccineus*), lentil (*Lens culinaris*), pea (*Pisum sativum* ssp *arvense* and *P. sativum* spp *vulgaris*), chick-pea (*Cicer arietinum*) and lupine (*Lupinus luteus*, *L. augustifolium*, *L. albus*). Seeds for these analyses were kindly provided by the Nordic Gene Bank (pea, soybean, garden bean, lentil), the ICRISAT gene bank (groundnut), the National Germplasm Resources Laboratory, Maryland, USA (chick-pea, lentil, broad bean and lima bean) and assistant professor Bjarne Jørnsgård, The Royal Veterinary and Agricultural University (lupine).

2.2. Extraction of DNA

Seed material (200–300 mg) from soybean and legume relatives was soaked in double distilled H_2O (dd H_2O) over night prior to DNA extraction. Individual seed samples were then transferred to 1.5 ml plastic tubes and ground using a small pistil. The homogenized samples were mixed with 860 μl extraction buffer (10 mM Tris–HCL, 150 mM NaCl, 2 mM EDTA, 1% (w/v) SDS, pH 8.0), 100 μl 5 M Guanidine hydrocloride, and 40 μl proteinase K (20 mg/ml) and subsequently incubated at 60 °C for 3 h with occasional mixing. After centrifugation for 10 min at 13.000 rpm, 500 μl of the supernatant was transferred to a new tube, mixed with 1 μl RNAse (10 mg/ml) and incubated for 30 min at 37 °C with shaking (250 rpm). DNA was purified using the Wizard DNA Clean-up system (Promega, Madison, WI., USA) and finally eluted with 2 × 50 μl of 70 °C dd H_2O. DNA concentrations were determined spectrophotometrically on a GeneQuant II RNA/DNA calculator (Pharmacia Biotech, Uppsala, Sweden) and adjusted to 10 ng/μl. To confirm the presence of amplifiable DNA, the plant-PCR system described by Taberlet, Gielly, Pautou, and Bouvet (1991) was initially applied to all DNA samples.

2.3. Polymerase chain reaction

PCR primers for amplification of Gly m Bd 30K DNA sequences (GenBank Accession JO5560) were designed using PRIMER3 software (Rozen & Skaletsky, 1997). One set of primers, Gly 30K-6F (5′-GCC ACG GGA GCC ATA GAA GC-3′, +492) and Gly