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Short communication

Development of triplex PCR for simultaneous detection of maize, wheat and soybean

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ABSTRACT

A reliable and fast detection of important food plants, such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max* L.) is of particular interest for food authenticity and safety assessment. In this study, the novel multiplex polymerase chain reaction (PCR) method was developed for the rapid qualitative detection of soybean, maize and wheat. To this purpose, new soybean-specific and maize-specific PCR primers were designed. Their specificity was assayed by uniplex PCRs with different plant species, namely maize, soybean, wheat, oats (*Avena sativa*), and barley (*Hordeum vulgare* L). Gel electrophoresis of the amplification products demonstrated high specificity of both primer pairs for identification of relevant species. Subsequently, based on the developed DNA markers, the species-specific triplex PCR targeting maize invertase gene, soybean lectin gene and wheat low-molecular-weight glutenin subunit was developed and optimized for simultaneous identification of these three plant species. The developed PCR method enables specific, effective and rapid detection of maize, wheat and soybean and may be used for food analysis.

1. Introduction

Maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max* L.) are important food crops worldwide. They are extensively used as raw materials and ingredients in modern food production. However, soy and wheat are recognized among the six top allergenic food materials, while maize belongs to the less common allergens (Terzi et al., 2004; Yamakawa et al., 2007). Food allergies are becoming increasingly more common worldwide. Currently, the only cure for a food allergy is to completely avoid the specific food. Exact information on the existence of the allergens in food constituents is important to prevent health problems. Therefore, reliable and fast detection of maize, soybean and wheat is urgently necessary for food authentication as well as food quality and safety monitoring. In addition, the current cereal transformation activities are focused on maize and wheat. Moreover, soybean and maize (corn) are the most distributed genetically modified (GM) plants worldwide (James, 2010). The precise identification of these species is necessary for GM food labeling and monitoring to meet the consumers' demand for freedom of choice.

DNA is the preferred analyte for plant materials, seeds, food ingredients and processed foodstuffs as it is a rather stable molecule. Because of its high specificity and sensitivity, the DNA-based polymerase chain reaction (PCR) is an effective and accurate technique for plant species and GMO detection (Anklam, Gadani, Heinze, Pijnenburg & Van den Eede, 2002; Marmiroli et al., 2008). PCR targeting a species-specific single copy gene is generally used to identify species' derived DNA. For this purpose, much effort has been put into the development of accurate PCR methods for detection of soybean, maize and wheat. Several studies (Germini et al., 2004; Meyer, Chardonnens, Hübner & Lüthy, 1996; Vodkin, Rhodes & Goldberg, 1983; Wang, Teng, Guan, Tian & Wang, 2013) demonstrated the PCR methods targeting soybean endogenous single copy lectin gene as effective tools for the specific identification of soybean DNA in all of the seeds, GM products and processed foods. In addition, β-actin gene-related DNA marker was successfully used in soybean-specific PCR (James, Schmidt, Wall, Green & Masri, 2003), while Glycine max intersperced repetitive element 1 (Yamakawa et al., 2007) was applied for the development of sensitive qualitative detection of soybean in food products. For specific detection of maize DNA, different amplicons, such as:

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