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SCAR marker: A potential tool to combat food adulteration

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Abstract

Nowadays viewing the present scenario of food adulteration Sequenced Characterized Amplified Regions (SCAR) markers had emerged out as authentic reliable tool for easy authenticity of food adulterants. Spices are most subjected to adulteration and even legal bodies are relying on the test conducted via DNA profiling by SCAR marker. Furthermore, research and studies needs to be conducted for other spices adulterants for care of health issues.

Keywords: scar markers, food adulterants, spices

1. Introduction

Food adulteration one of the mal practices adopted by tradesmen to increase monetary benefits. No doubt, it adds on to their pocket huge amount of money but on the same side they put the society to great risk of health hazards. With rapid increase in health issues nowadays, every country in the world is focusing on the authentication of food material they consume. For these purposes various food laboratories are established are set up. Though biochemical analysis is very common method of testing, some new technology such as SCAR marker had developed for quick, easy and reliable testing.

SCAR (Sequence characterized Amplified Region) marker is co-dominant in nature plays pivotal role in varietal identification and varietal purity. These markers were initially reported by Paran and Michelmore in 1993 [25]. These markers have reported to have developed using sequences derived from dominant markers i.e. RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism) (Negi *et al.*, 2000; Kalia *et al.*, 2017.) [22, 15]. Apart from co-dominance nature, other drawback features of dominant marker system which had given impetus to SCAR marker are mentioned below in the table 1.

Table 1: Comparison of ISSR, RAPD, AFLP and SCAR markers

Feature/Characteristics	ISSR	RAPD	AFLP	SCAR
Information content	High	High	High	Low
Specificity	Specific	Non-specific	Specific	Specific
Loci detection	Multiple or single	Multiple	Multiple	Single
PCR-based	Yes	Yes	Yes	Yes
Reproducibility	Moderate	Low	High	High
Genome Coverage	Whole	Whole	Whole	Partially
Reliability	High	Low	High	High
Nature	Dominant	Dominant	Dominant	Co-dominant

Adapted from: Rekha *et al.*, 2018 [29]

2. Steps for Development of SCAR marker

Steps for the development of SCAR markers is as follows:

- RAPD/ISSR/AFLP profiling is done
- Unique bands present are cut and eluted from the gel
- The eluted DNA is then cloned into *E. coli* cells
- Blue-white screening is performed for the selection of transformed cells

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e) Plasmid is extracted from the transformed colonies and is sent for sequencing

f) Sequences obtained are used for primer designing

g) Primer designed are then validated. The mentioned steps of SCAR marker has been depicted in the following Figure 1.

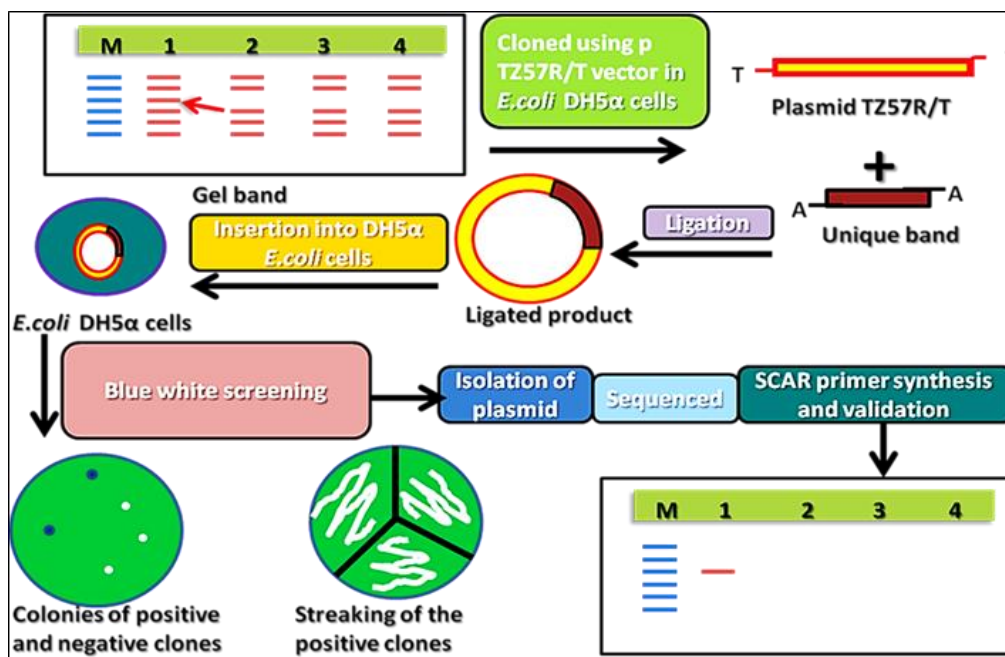


Fig 1: Flow chart of steps involved in SCAR development. Adapted from thesis of Rekha *et al.*, 2018 [29]

3. Food adulterants

When we talk about food adulterant the very first thing that comes to our mind is of spices. Spices are the key component of Indian cuisine which adds flavor and aroma to our taste buds. Some two-three decades ago, spices aroma were so strong that one could easily detect what is being cooked in their neighborhood by just its fragrance, but the rapid increase in the competition in the market every trader are losing their wisdom and following the path of fraudulence. The actual flavor of our food had eroded away from daily diet due to the adulterants added. Here is the list of some adulterants used commonly in our spices-

a) **Black pepper:** It is botanically known as *Piper nigrum* and is a flowering vine in the family Piperaceae, cultivated for its fruit, known as a peppercorn. This is common spice found in everyone's kitchen referred as 'King of Spices'.



Fig 2: Black paper seeds



Fig 3: Dried papaya seeds

In the Figure 3 is black pepper seeds and figure 4 depicts dried papaya seeds which are quite difficult to separate on visually.

Besides being used as a flavoring agent it is used as anti-inflammatory, anti-toxicity antimicrobial and antioxidant. It is essential ingredient in Ayurvedic and Unani Indian medicine system. It is marketed in the form of berries, grounded powder, pepper oil and oleoresin. Grounded pepper/Black pepper powder is most frequent form used by the consumers. Black pepper is commonly adulterated by dried papaya seeds which show similarity in shape and size with Black pepper. These adulterants increase the quantity of black pepper and trader make huge profits out of it. This adulterant apart from providing monetary benefits to traders is get setback for the common consumers of it. There are reports which states that due to papaya seeds infertility cases (Sareen *et al.*, 1961) [30] and harmful effects on genital organs (Das 1980) are quite detrimental for the health.

There are several reports which give various tests for distinction between black pepper and papaya seeds. Floatation test as reported by Pruthi and Kulkarni (1969) [28] which states that papaya seeds being lighter float while pepper seeds being

heavier sunk at the bottom. Others have reported for different levels of chromatography test conducted by Hartman *et al.*, (1973) ^[12], Curl *et al.*, (1983) ^[13], Paradkar *et al.*, (2001) ^[23], Paramita *et al.*, (2003) ^[24] and Jain *et al.*, (2007) ^[14]. The variation in starch concentrations have been used for authentication of black pepper seeds as per the reports of Smith *et al.*, 1926 ^[32].

The above methods of confirmatory test for black pepper are not reliable so DNA profiling of the sample are done using developed specific SCAR marker of easy identification and hastens the process of confirmation as reported by Dhanya (2009) ^[7]. In which papaya seed specific SCAR marker was developed which sharply distinguished between papaya seeds and various samples of black pepper seeds.

b) Chili: It is fruit of *Capsicum annuum* and belongs to family Solanaceae. Chilli is not chill but really hot when devoured. It is one of the spices which is eaten raw as well in dried form. It mostly used to season dal and kadis in Indian Dishes. It has additional medicinal properties and is used to cure asthma, cough, sore throat, rheumatic disorders (Pruthi, 2003) ^[27]. The natural anti-oxidant extract i.e. Capsaicin is known for its analgesic properties and is an essential component in various ointment and



Fig 4: Dried red chilli powder

In the above figure 4.0 depicts the picture of pure red chilli powder and in figure 5.0 depicts red fruit of choti ber which when gets riped is crushed in the form of powder and adulterated with the red chilli powder.

c) Turmeric: It is a perennial, rhizomatous, herbaceous plant native to Indian subcontinent and South East Asia. It's botanically known as *Curcuma longa* and belongs to family Zingiberaceae. Turmeric is basically the dried from its modified roots known as rhizome. It mostly used as spice in its powdered form for cooking purposes.

Apart from spice, it is used for cancer, diabetes, surgical pain treatment and important ingredient in mouthwash for reducing plaque (Chattopadhy *et al.*, 2004) ^[2]. The National Center for Complementary and Integrative Health (NCCIH) has studied curcumin for Alzheimer's disease, rheumatoid arthritis, and prostate and colon cancer (NIH reports, 2012; Daily *et al.*, 2016) ^[4].

lotion recommended various pharmaceutical companies (Srinivasan, 2005) ^[34].

It is marketed as whole dry fruit, crushed chili, chilli powder, chilli paste as per reports of Spices Statistics, 2004. Among these, chilli powder and chilli paste are mostly adulterated by using brick powder, talc powder, coal tar red, Sudan red etc. (Mitra *et al.*, 1961; PFA, 2003; Mazzetti *et al.*, 2004) ^[20, 26, 18]. There are reports available which states that some plant based adulterant like choti ber-is readily available cheap, red colored dried fruit obtained from *Ziziphus* species is powered and adulterated. (Dhanya *et al.*, 2008) ^[8]

Simple analytical tests are available which can help to separate brick powder and red color as per reports of Valencia *et al.*, 2000; Navarao *et al.*, 1965; Marshall 1977; Sun *et al.*, 2007; Mejia *et al.*, 2007; Ertas *et al.*, 2007; De la Cruz Yaguez *et al.*, 1986., Zhang *et al.*, 2005; Zhang *et al.*, 2006) ^[38, 21, 17, 35, 19, 10, 6] through various chromatography test.

But the plant adulterant i.e. Choti ber used in chilli are nearly impossible to detect so according to reports of Dhanya *et al.*, 2008 ^[8], RAPD derived SCAR markers were developed for choti ber in particular to have clear cut distinction between red chilli powder and choti ber. This tool provides authentic and reliable results.



Fig 5: Red colour choti ber
Obtained from *Ziziphus nummularia* shrub

Due to high usage of turmeric in above culinary purposes, it is high in demand which compels the tradesmen to adulterate with artificial colours like metanil yellow, lead chromate, chalk powder and yellow soapstone which can be easily detected by colorimetric, chromatographic and spectrophotometric methods (PFA, 2003; Tripathi *et al.*, 2004 and Tripathi *et al.*, 2007) ^[26, 36, 37]

Recently, there are reports available which states adulteration of *C. longa* with its closely wild related species *C. zedoaria* Rose. (Sasikumar *et al.*, 2005) ^[31]. Latif *et al.*, (1979) ^[16] in its studies had reported about the toxicity of *C. zedoaria* on consumption when experimented on rats. This report therefore, necessitates the reliable tool for authentication of turmeric. Dhanya (2009) ^[9] had reported the development of SCAR marker specific to *C. zedoaria* which helps in easy identification.



Fig 6: Inflorescence of *curcuma longa*



Fig 7: Inflorescence of *curcuma zedoaria* (white turmeric)

In the figure 6.0 depicts the white inflorescence of *Curcuma longa* taken from the field of Narendra Dev University of Agriculture and Technology, Ayodhya (U.P) while figure 7.0 depicts the pink inflorescence of wild species *Curcuma zedoaria* commonly known as white turmeric.

4. Conclusion

Adulteration though may add extra money to the account of traders, is an illegal practice. World Trade Organization at international level and Food Safety and Standards Authority of India (FSSAI) at national level are legal bodies which look into safety concern of food commodity. SCAR marker as we have cited in the above cases have successfully and reliable tools for food adulteration and efforts should be laid on the more development of SCAR markers for other spices and eatables.

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