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:

a) RAPD/ISSR/AFLP profiling is done
b) Unique bands present are cut and eluted from the gel
c) The eluted DNA is then cloned into E. coli cells
d) Blue-white screening is performed for the selection of transformed cells
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 Figure1.

**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’. 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.

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

**Fig 2:** Black paper seeds

**Fig 3:** Dried papaya seeds

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) [3], 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 [12].

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) Chilli: 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 lotion recommended various pharmaceutical companies (Srinivasan, 2005) [24]. 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, t alc 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.

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].

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.
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.

5. References
1. Calbiani F, Careri M, Elviri L, Mangia A, Pistara L, Zagnoni I. Development and in-house validation of a liquid chromatography-electrospray-tandem mass spectroscopy methods for the simultaneous detection determination of Sudan I, Sudan II, Sudan III and Sudan IV in hot chilli products. Journal of Chromatography A. 2004; 1042:123-130.
2. Chattopadhyay I, Kaushik B, Uday B, Ranajit KB. Turmeric and curcumin: Biological actions and medicinal applications. Current Science. 2004; 87(1):44-53.
3. Cull CL, Fenwick GR. On the determination of papaya seed adulteration of black pepper. Food Chemistry. 1983; 12:241-247.
4. Daily JW, Yang M, Park S. Efficacy of Turmeric Extracts and Curcumin for Alleviating the Symptoms of Joint Arthritis: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Journal of Medicinal Food. 2016; 19(8):717-29.
5. Das RP. Effect of papaya seed on the genital organs and fertility of male rats. Indian Journal of Experimental Biology. 1980; 18:408-409.
6. De La Cruz Yaguez LI, Pingarron Carrazon JM, Polo Diez LM. Polarographic study of the 1-(2, 4-dimethylphenylazo)-2-naphthol (Sudan II) in hydroalcoholic medium. Electrochimica Acta. 1986; 31:119-121.
7. Dhanya K, Syamkumar S, Sasikumar B. Development and application of SCAR marker for the detection of papaya seed adulteration in traded black pepper powder. Food Biotechnology. 2009; 23:97-106.
8. Dhanya K, Syamkumar S, Jaleel K, Sasikumar B. Random amplified polymorphic DNA technique for the detection of plant based adulterants in chilli powder (*Capsicum annuum*). Journal of Spices and Aromatic Crops. 2008; 17:75-81.
9. Dhanya K. Detection of probable plant based adulterants in selected powdered market samples of spices using molecular techniques. Ph.D. thesis, Mangalore University, Mangalore, India, 2009. 251.
10. Ertas E, Ozer H, Alasalvar C. A rapid HPLC method for determination of Sudan dyes and para red in red chilli pepper. Food Chemistry. 2007; 105:756-760.
11. Grieve M. "Turmeric". botanical.com. Retrieved on 05.01.2020.
12. Hartman CP, Divakar NG, Rao VNN. A study of identification of papaya seed in black pepper. Journal of Food Science and Technology. 1973; 10:43.
13. Herbs at a Glance: Turmeric, Science & Safety. National Center for Complementary and Integrative Health (NCCIH), National Institutes of Health, 2012.
14. Jain SC, Menghani E, Jain R. Fluorescence and HPLC-based standardization of Piper nigrum fruits. International Journal of Botany. 2007; 3:208-213.
15. Kalia P, Saha P, Roy S. Development of RAPD and ISSR derived SCAR markers linked to XcaIBo gene conferring resistance to black rot disease in cauliflower (Brassica oleracea var. botrytis L.). Euphytica. 2017; 213:232.
16. Latif MA, Moris TR, Miah AM, Hewitt D, Ford JE. Toxicity of shotti (Indian arrowroot: *Curcuma zedoaria*) for rats and chicks. British Journal of Nutrition. 1979; 41:57-63.
17. Marshall PN. Thin-layer chromatography of Sudan dyes. Journal of Chromatography A. 1977; 136:353-357.
18. Mazzetti M, Fascioli R, Mazzoncini I, Spinelli G, Morelli I, Bertoli A. Determination of 1-phenylazo-2-naphthol (sudan I) in chilli powder and in chilli containing food products by GPC cleanup and HPLC with LC/MS confirmation. Food Additives and Contaminants. 2004; 21:935-941.
19. Mejia E, Ding Y, Mora MF, Garcia CD. Determination of banned Sudan dyes in chili powder by capillary electrophoresis. Food Chemistry. 2007; 102:1027-1033.
20. Mitra SN, Sengupta PN, Roy BR. The detection of oil soluble coal tar dyes in chilli (*Capsicum*). Journal and Proceedings of the Institute of Chemistry. 1961; 33:69.
21. Navarao S, Ortuno A, Ooasta F. Thin-layer chromatographic determination of synthetic dyes in foods. 1. Fat soluble Azo dyes in paprika. Anales De Bromatologia. 1965; 17:269.
22. Negi MS, Devic M, Delseny M, Lakshmikumaran M. Identification of AFLP fragments linked to seed coat colour in Brassica juncea and conversion to a SCAR marker for rapid selection. Theoretical and Applied Genetics. 2000; 101(2):146-152.
23. Paradkar MM, Singhal RS, Kulkarni PR. A new TLC method to detect the presence of ground papaya seed in ground black pepper. Journal of the Science of Food and Agriculture. 2001; 81:1322-1325.
24. Paramita B, Singhal RS, Achyut SG. Supercritical carbon dioxide extraction for identification of adulteration of black pepper with papaya seeds. Journal of the Science of Food and Agriculture. 2003; 83:783-786.
25. Paran I, Michelmore RW. Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. Theoretical and Applied Genetics. 1993; 85:985-993.
26. PFA. Prevention of food adulteration act of India, 1954 and rules. Eastern Book Company, Lucknow, India, 2003, 436.
27. Pruthi JS. Advances in postharvest processing technologies of Capsicum. In: De AK (Ed.) Capsicum: the genus Capsicum, Taylor and Francis, London, 2003, 175-213.
28. Pruthi JS, Kulkarni BM. A simple technique for the rapid and easy detection of papaya seeds in black pepper berries. Indian Food Packer. 1969; 23:51-52.
29. Rekha K, Singh RS, Thakur D, Kishore C, Sinha S, Singh PK. Development of Scar Marker(S) For Aphid Tolerance in Brassica Juncea (L.) Czern. & Coss. M.Sc. Thesis of Bihar Agricultural University, Sabour (Bhagalpur, India), 2018.
30. Sareen K, Misra K, Verma DR. Oral contraceptives. V. antihelmintics as antifertility agents. Indian Journal of Physiology and Pharmacology. 1961; 65:125.
31. Sasikumar B, Syamkumar S, Renya R, John Zachariah T. PCR based detection of adulteration in the market samples of turmeric powder. Food Biotechnology. 2005; 18:299-306.
32. Smith ER, Samuel A, Mitchell LC. Detection of added papper-shells in pepper. Journal of the Association of Official Agricultural Chemists. 1926; 9:233.
33. Spices Statistics. Spices Board, Ministry of commerce and industry, Government of India, Cochin, Indiam, 2004, 281.
34. Srinivasan K. Role of spice beyond food flavouring: nutraceuticals with multiple health effects. Food Reviews International. 2005; 21:167-188.
35. Sun HW, Wang FC, Ai LF. Determination of banned 10azo-dyes in hot chili products by gel permeation chromatography-liquid chromatography electrospray ionization-tandem mass spectrometry. Journal of Chromatography A, 2007; 1164:120-128.
36. Tripathi M, Khanna SK, Das M. A novel method for the quantitative analysis of synthetic colours in ice cream samples. Journal of Association of Official Analytical Chemists International. 2004; 87:657-663.
37. Tripathi M, Khanna SK, Das M. Surveillance on use of synthetic colours in eatables Vis a Vis Prevention of Food Adulteration Act of India. Food Control. 2007; 18:211-219.
38. Valencia M, Uroz F, Tafersiti Y, Capitan-Vallvey LF. A flow through 1 sensor for the determination of the dyes sunset yellow and its subsidiary Sudan I in foods. Quimica Analitica. 2000; 3:129-134.
39. Zhang Y, Zhang Z, Sun Y. Development and optimization of an analytical method for the determination of Sudan dyes in hot chilli pepper by high performance liquid chromatography with online electro generated BrO-luminol chemiluminescence detection. Journal of Chromatography A. 2006; 1129:34-40.
40. Zhang Y, Zhang YJ, Gong WJ, Gopalan AI, Lee KP. Rapid separation of Sudan dyes by reverse-phase high performance liquid chromatography through statistically designed experiments. Journal of Chromatography A. 2005; 1098:183-187.