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Is Kasuri Methi Genetically Different from other Methi (Fenugreek) Varieties??

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ABSTRACT

Authors found that kasuri methi is genetically different from other methi varieties as an incidental finding. In gene expression experiments, annealing temperature of kasuri methi was found 4ºC lower than other methi varieties with the same primers. Further evidences supporting the hypothesis based on published studies are presented here.

Keywords: *Kasuri methi, fenugreek, HMG gene, genetically different, gene expression, melting point*

INTRODUCTION

Methi is hindi name of the plant Fenugreek, a Rabi season crop of India. Fenugreek is a dicot plant of Fabaceae (Leguminacae) family and genus Trigonella; its scientific name is Trigonella foenum-graecum. Globally, more than 75 varieties of fenugreek available out of which 18 have been reported by the researchers (Petropoulos 2002; Chaudhary et al. 2018).

Out of all available varieties of fenugreek (methi), kasuri methi is different in phenotypic characteristics, flavour, leaf sizes, fragrance and seed size also. Scientific name of Kasuri methi is Trigonella corniculata. It has sickle shaped pods, contains 10 to 20 small hard yellowish brown round seeds with about 3 mm size. Dry leaves of kasuri methi are rich in protein, iron and vitamin A and widely used as spice and herb in food preparation (Gurjar 2005).

METHODS

Kasuri methi is morphologically different from other methi varieties, an image is provided comparing the seeds of Kasuri and local methi available in India (figure1).We have hypothesized that kasuri methi is genetically different from other methi varieties based on our incidental finding.

Figure1. *Seeds of kasuri methi and local methi*

Originally, experiment was conducted to study the effect of various concentration of methyl jasmonate (a chemical elicitator) on the expression of genes related to the diosgenin biosynthesis pathway. The study included six different varieties of fenugreek namely Gujarat

Methi-2 (GM-2), Kasuri, Pusa early branching (PEB), Rajasthan Methi (RMT-1) and Maharashtra methi-5 (MMT-5) collected from India. Expression analysis of genes namely 3-hydroxy-3-methylglutaryl CoA reductase (HMG) and sterol-3-Beta Glucosyltransferase (STRL) were carried out in all six methi varieties (Chaudhary et al.2015).Authors have mentioned in the results section that the amplicons generated from first strand cDNA using housekeeping 18s gene, intensity of amplicons were found lower than other methi varieties. The reason for the difference in the amplicon concentration could be variability in GC content of the plant because all other factors like DNA template, primer concentrations and PCR conditions etc were constant.

Results and Discussion

In present study, we further investigated the possibilities of differential gene expression pattern of in different methi varieties by RT-PCR method. We used two genes namely the genes HMG and STRL as target gene and 18s gene as housekeeping gene. It was noticed that particularly in Kasuri methi variety, melting curve was different for HMG gene. For STRL and 18s gene, melting curve was same as found in other variety samples.

After PCR standardization we got the similar melting curve in kasuri methi at 56°C annealing temperature which was 4 °C lower than it was used for other varieties specifically for HMG gene. No much difference was observed in the expression profiling after changing the annealing temperature for HMG gene. Detailed PCR program used for RT-PCR assay for both Kasuri methi and other methi varieties is mentioned in table 1. Standard curve obtained for other varieties and Kasuri variety using mentioned PCR program is presented in figure 2.

Table1. *PCR conditions used for performing gene expression analysis on Kasuri and other methi varieties*

PCR steps	Local Methi Varietis (*HMG* gene)	Kasuri Methi Varietis (*HMG* gene)
Initial denaturation	94ºC for 5 minutes	94ºC for 5 minutes
De-naturation Annealing X 35 cycles Extension	94ºC for 30 seconds	94ºC for 30 seconds
	60ºC for 30 seconds	56ºC for 30 seconds
	72ºC for 30 seconds	72ºC for 30 seconds
Final extension	72ºC for 7 minutes	72ºC for 7 minutes

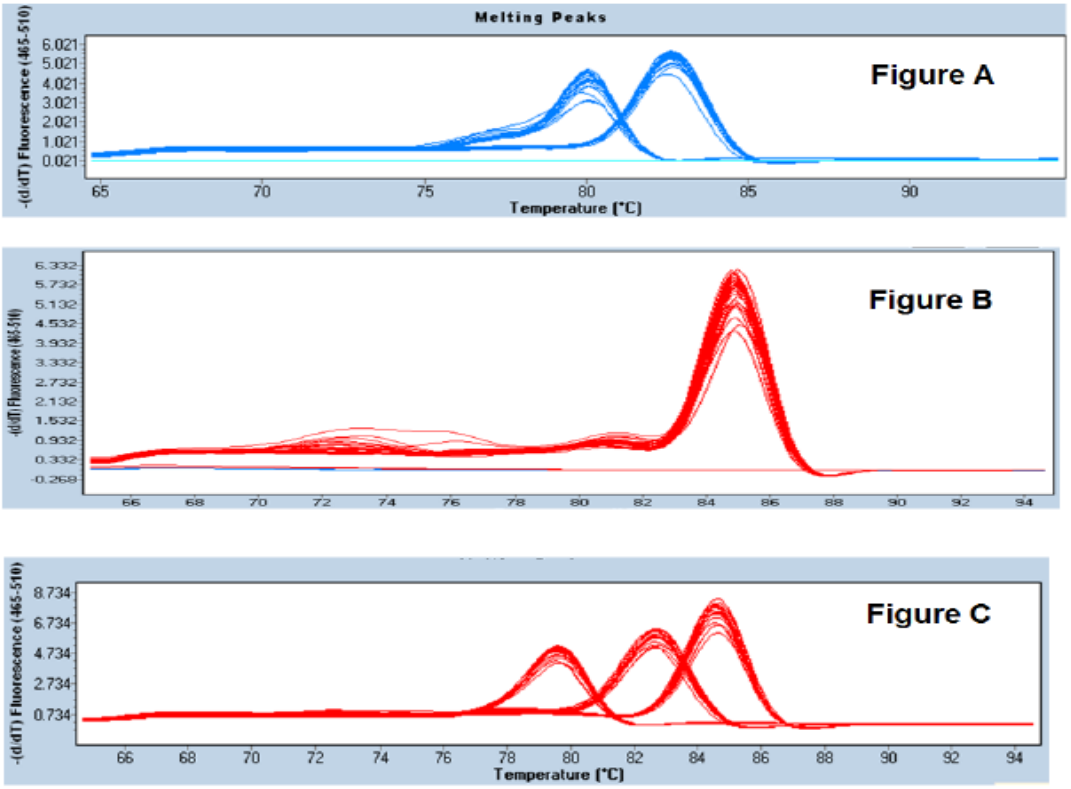

Figure2. *Melting curve profile for Kasuri methi and other local methi varieties. A represents melting curve profile of* 18s *and* STRL *gene in Kasuri methi, B represents melting curve profile of* HMG *gene in Kasuri methi and C represents* 18s, STRL *and* HMG *gene for all other local methi varieties.*

Annealing temperature is very important for a PCR reaction and it depends upon melting temperature of the primer- template hybrid. If the temperature is too high or too low, primer may not bind or will generate non specific product. Change in the annealing temperature of same primers among the species may be due to variation in the GC content of the genome. Reasons of GC content variation in the DNA are still unknown but few studies have reported some probable reasons.

Which includes UV resistance (Gause et al. 1967; Singer & Ames 1970), thermal adaptation (Kagawa etal.1984 Musto etal.2004), directional mutation pressure (Sueoka 1988), metabolism (Martin1995) environmental pressure (Foerstner et al. 2005) etc.

While few other researchers have postulated the hypothesis that genome size (Musto et al. 2006) and DNA polymerase III (Zhao et al. 2007) could also be reason for the GC content variability but these studies were conducted in prokaryotes. Positive correlation was reported between average chromosome size and GC content in dicot and monocot plant chromosomes (Li & Du 2014).

Two methi varieties namely Trigonella corniculata and Trigonella foenum-graecum have been reported to have same chromosome number 2n=16 (Tutin 1964) but differ in size of the chromosome. Chromosomal sizes of Trigonella corniculata (Kasuri methi) and Trigonella foenum-graecum (Local/Deshi methi) were reported to be 1.49–2.76 µM and 2.03–4.72 µM respectively (Martin et al. 2011) which proves that kasuri methi is genetically different from other varieties.

Sundaram et al., have conducted a study for assessment of genetic diversity among 61 accession of two species of Fenugreek, group 1 of Fenugreek (T. foenum-graecum) with 59 accessions and group 2 Kasuri methi (Trigonella corniculata) with 2 accessions from different location, they found that kasuri methi was classified as distinct variety (Sundaram & Purwar 2011).

Conclusion

Kasuri methi is phenotypically different from other local methi varieties but it is genetically also different. Based on published literature and study presented here, have provided some evidence for the same. However, comparative genome sequencing or other deep sequencing methods can provide some more insights.

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