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Research Article

AN OBJECTIVE METHOD OF PHENOTYPING POD FRAGRANCE IN DOLICHOS BEAN (*Lablab purpureus* L. SWEET) VAR. LIGNOSUS

UDAYKUMAR H.R.*[1], BYREGOWDA M.[2], RAMESH S.[1] and VASUNDHARA M.[3]

[1]Department of Genetics and Plant Breeding, University of Agricultural Sciences (UAS), Bengaluru, Karnataka, India
[2]All India Coordinated Research Project on Pigeon pea, UAS, Bengaluru, Karnataka, India.
[3]Department of Horticulture, UAS, Bengaluru, Karnataka, India
*Corresponding Author: Email-udayhr7096@gmail.com

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Abstract- In dolichos bean, pods with high fragrance fetch premium price in the market. Pod fragrance is routinely assessed through organoleptic means. Phenotyping pod fragrance by organoleptic (smelling) means is highly relative and subjective. Efficiency of breeding dolichos bean varieties with high pod fragrance hinges on an objective method of phenotyping fragrance in breeding populations. An attempt was made to devise an objective assessment of pod fragrance by quantifying the key fatty acids (FA) responsible for pod fragrance. Exudates were collected from the pods of HA-4 (released variety with high pod fragrance) and CPI-31113 (a germplasm accession with lack of pod fragrance). After esterification, the exudates were subjected for gas chromatographic mass spectrometry (GC-MS) analysis to identify the differences between HA 4 and CPI 31113 for key FA responsible for fragrance. The HA 4 pod exudates predominantly consisted of 24.58% trans-2-dodecenoic acid, 17.43% trans-tetradecanoic acid and 3.11% trans-2-tetradecenoic acid. Contrastingly, CPI 31113 pod exudates had 13.77% trans-2-dodecenoic acid and 12.92% of trans-2-tetradecenoic acid. The results indicated appreciable differences in the concentration of trans-2-dodecenoic acid and trans-2-tetradecenoic acids between fragrant variety HA-4 and non-fragrant germplasm accession CPI-31113. Quantifying these two FA in the pod exudates serve as a reliable and an objective method compared to organoleptic means for phenotyping pod fragrance in dolichos bean.

Keywords- Fatty acids, Pod fragrance, Trans-2-dodecenoic acid, Trans-2-tetradecenoic acid.

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Introduction

Dolichos bean is one of the important legumes grown in India. Karnataka contributes >90 per cent of total dolichos bean production in India. It is a multipurpose legume crop used as vegetable, pulse [1-7] and forage [6]. While fresh beans are used as a vegetable, dried seed as a split dhal is used in various food preparations. Fresh pods with immature beans are the harvestable economic products in dolichos bean. Pod fragrance is one of the 'farmers' and 'consumers' preferred traits in dolichos bean varieties [1-7]. The pods with high fragrance fetch a premium price in the market.

In dolichos bean breeding programmes, pod fragrance in germplasm accessions/ segregating populations/ advanced breeding lines is being routinely assessed by organoleptic (smelling) means using a panel of analysts. Phenotyping breeding populations for a pod fragrance by organoleptic means is highly relative and subjective. Also, sensitivity to smelling by the analysts will be reduced if there are subtle differences among the individuals of breeding populations. Besides, analysts differ in their ability to detect pod fragrance. Trans-2-Dodecenoic acid and trans 2-Tetradecenoic acids are the major fatty acids in the pod oil exudates of dolichos bean [5]. Based on similar kinds of chemical composition analysis, in apple [3] and in peach [4] attributed fruits aroma to various volatile organic compounds (VOC) and mapped quantitative traits (QTLs) controlling variation for respective VOCs. Taking cues from these studies, the limitation (relativity and subjectivity) associated with organoleptic means of phenotyping pod fragrance could be overcome by analyzing and quantifying trans 2-Dodecenoic acid and trans 2-Tetradecenoic acids, the key fatty acids (FA) in the oil exudates of dolichos bean pods. Hence, we undertook to quantify the key fatty acids for objective assessment of pod fragrance in dolichos bean.

Materials and Methods

The material for the study consists of HA 4 a high yielding photoperiod insensitive determinate pure-line variety with high pod fragrance and CPI 31113 a photoperiod sensitive indeterminate germplasm accession with lack of pod fragrance [8]. Phenotyping HA 4 and CPI 31113 genotypes for pod fragrance was carried out in three steps, (A) preparation of the pod exudates' samples, (B) esterification of pod exudates and (C) fatty acid profiling of esterified pod exudates using gas chromatographic mass spectrometry (GC-MS).

Pod exudates' sample preparation

The exudates of the pods borne on HA 4 and CPI 31113 genotypes were collected by wiping the surface of pods with the filter paper pieces (about 3 × 2 cm). Then, pieces of filter paper with the absorbed pod exudates were quickly transferred into a bottle containing 25 ml petroleum ether. The bottle containing the petroleum ether was shaken well and then decanted from the bottle. The filter paper pieces were washed using 50 ml methanol. Both the petroleum ether solution and methanol washings were combined and filtered. After that, the solvents were removed from a mixture under aspirator pressure at 40-50°c, leaving pleasant smelling oil extract. The oil extract was subjected to esterification.

Esterification of pod exudates using GC-MS

About 500 mg of oil extract was transferred to 250 ml flat-bottom flask and then 92 ml methanol and 8 ml Conc. H_2SO_4 was added to oil extract. After that, a mixture of 500 mg oil extract + 92 ml methanol + 8 ml Conc. H_2SO_4 was kept onheating mantle and connected to water condenser reflux for 90 min. Then oil extract + methanol + Conc. H_2SO_4 mixture was cooled to room temperature and transferred to separator funnel. To wash-out the acid content from the sample, 100 ml diethyl ether + 100 ml distilled water was added into the funnel and then diethyl ether layer was collected. This process was continued for 4-5 times. Subsequently, diethyl ether layer was passed through anhydrous sodium sulphate (Na_2SO_4) and collected in a 500 ml beaker. Finally, solvent was evaporated on water bath and concentrated to 1 ml. From the 1 ml concentrated solvent, 1-2 µl was used to inject in to GC column.

Fatty acid profiling of pod exudates using GC-MS analysis

An Rtx-5 column with a length of 30m, internal diameter 0.25 mm, and particle size of 0.25 micron meter was installed in the GC. One µL of esterified oils from the pod exudates of HA 4 and CPI 31113 genotypes were fractionated in GC-MS to estimate the composition of FA.

Results and Discussion

The GC-MS analysis indicated that pod exudates of HA 4 and CPI 31113 genotypes are fairly complex mixture of FA and their methyl esters [Fig-1 & 2]: [Table-1]. The HA 4 pod exudates consisted of 19 FA and their methyl esters. Among these, Dodecanoic acid (17.86%), trans-2-Dodecenoic acid (24.58%), Tetradecanoic acid (17.43%) and 2-Pentadecenoic acids (18.54%) dominated [Table-1]. On the other hand, CPI 31113 pod exudates consisted of 16 FA and their methyl esters among which, Dodecanoic acid (14.48%), trans-2-Dodecenoic acid 13.77%), Tetradecanoic acid (13.45%), trans-2-Tetradecenoic acid (12.92%), Hexadecanoic acid (11.10%) and 9, 12-Octadecadienoic acids (13.25%) predominated [Table-1]. In dolichos bean pod exudates, 42 compounds were reported, which mainly consisted of FA and their methyl esters [5]. In the ground raw and cooked soybeans, the presence of 62 and 71 compounds were reported respectively, which are a mixture of aromatics, furans, pyrazines, aldehydes, alcohols and ketones [2].

Table-1 *Proportion of fatty acids identified by GC-MS in HA 4 (fragrant) and CPI 31113 (non-fragrant) genotypes of dolichos bean.*

Sl. No.	Fatty acid	Composition (*Per cent*)		Difference (*per cent*)
		HA 4	CPI 31113	
1	Dodecanoic acid	17.86	14.48	3.38
2	Tridecanoic acid	2.14	2.23	0.09
3	2-Tridecenoic acid	-	2.55	2.55
4	**Trans- 2-Dodecenoic acid**	**24.58**	**13.77**	**10.81**
5	Tetradecanoic acid	17.43	13.45	3.98
6	**Trans- 2-Tetradecenoic acid**	**3.11**	**12.92**	**9.81**
7	Pentadecanoic acid	0.62	2.65	2.03
8	2-Pentadecenoic acid	18.54	-	18.54
9	Hexadecanoic acid	4.65	11.10	6.45
10	2-Hexadecenoic acid	0.25	0.64	0.39
11	Heptadecanoic acid	0.23	1.05	0.79
12	cis-10-Heptadecenoic acid	0.42	-	0.42
13	Octadecanoic acid,	1.20	3.07	1.87
14	9-Octadecenoic acid	1.20	-	1.20
15	10-Octadecenoic acid	-	3.63	3.63
16	Octadeca-9,12-dienoic acid	2.14	13.25	11.11
17	9,12,15-Octadecatrienoic acid	0.64	1.41	0.77
18	Eicosanoic acid	1.84	2.05	0.21
19	Heneicosanoic acid	0.43	-	0.43
20	Docosanoic acid	2.45	1.74	0.71
21	Tetracosanoic acid	0.29	-	0.29

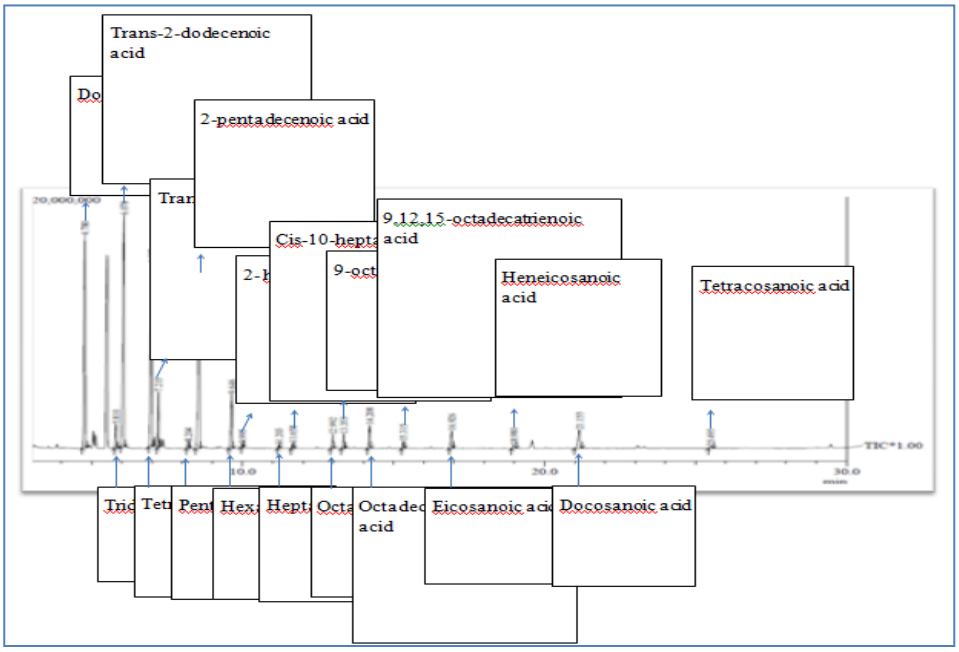

Fig-1 The GC-MS chromatogram of HA 4 (fragrant genotype) pod exudates in dolichos bean

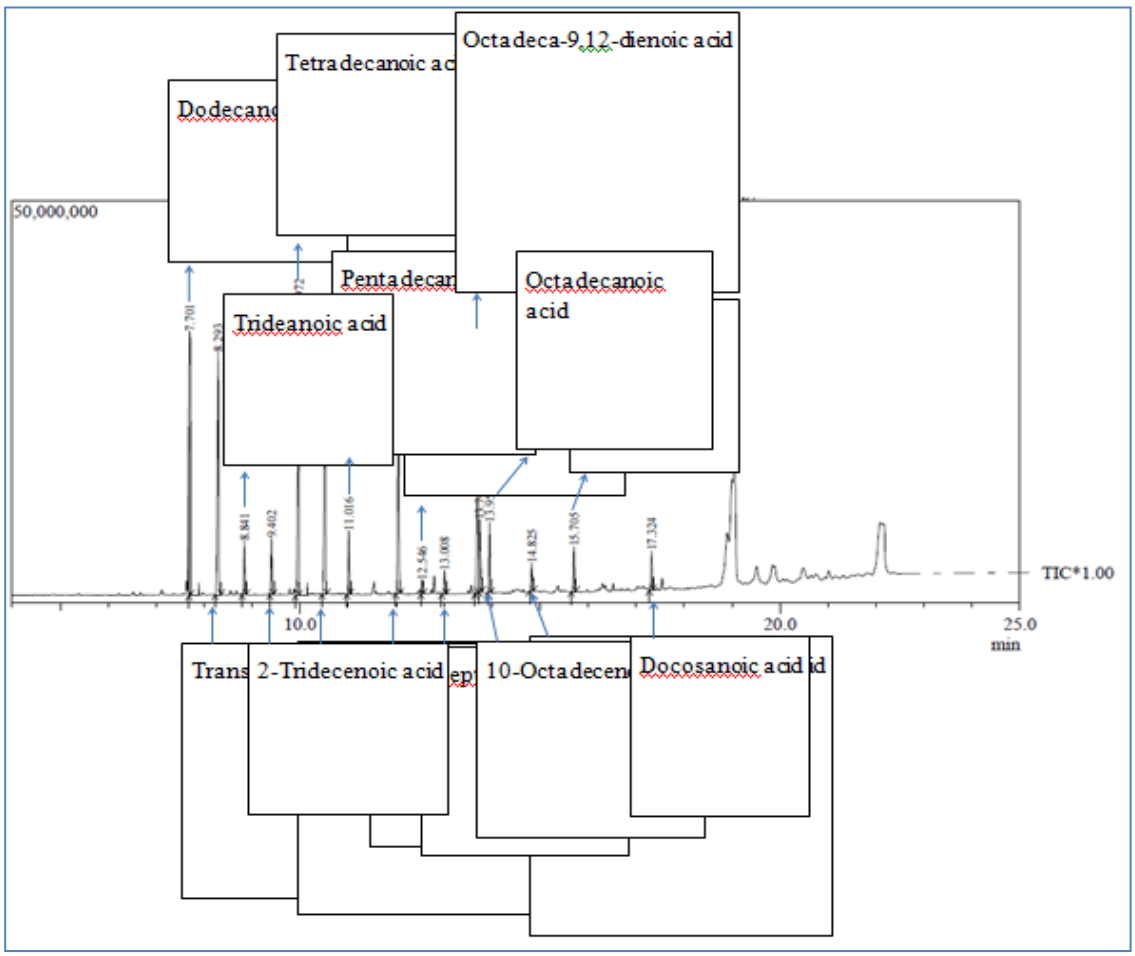

Fig-2 The GC-MS chromatogram of CPI 31113 (non-fragrant genotype) pod exudates in dolichos bean

The fragrant variety, HA 4 pod exudates predominantly consisted of 24.58% trans-2-dodecenoic acid and 17.43% trans-tetradecanoic acid. Contrastingly, the non-fragrant CPI 31113 pod exudates had 13.77% trans-2-dodecenoic acid, 13.45% trans-tetradecanoic acid and 12.92% trans-2-tetradecenoic acid [Table-1]. These results indicated appreciable differences in the FA composition between fragrant variety, HA 4 and non-fragrant germplasm accession, CPI 31113. The results and those reported by earlier scientists [5] confirmed the role of trans-2-dodecenoic acid and trans-2-tetradecenoic acids the key FA conferring pod fragrance in dolichos bean. Thus, quantifying these two FA in the pod exudates serve as a reliable and an objective method compared to organoleptic means for phenotyping pod fragrance in germplasm accessions/breeding populations/advanced breeding lines of dolichos bean. In the future, we can attribute different fatty acids for the pod fragrance and will be used to map QTLs for fatty acids and in turn will be useful to locate genomic regions governing pod fragrance.

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Conflict of Interest: None declared

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