Discrimination of Mungbean Cultivars/Varieties Based on Minor Saccharides Composition by HPLC Coupled with Multivariate Statistical Analysis

[1]Mudasir Majeed, [2]Abdullah Ijaz Hussain, [2]Shahzad Ali Shahid Chatha*, [3]Ghulam Mustafa Kamal and [4]Qasim Ali
[1]Department of Applied Chemistry, Government College University Faisalabad, Pakistan-38000.
[2]Department of Chemistry, Government College University, Faisalabad, Pakistan-38000.
[3]Department of Chemistry, Khwaja Fareed University of Engineering and information Technology Rahim Yar Khan, Pakistan-64200.
[4]Department of Botany, Government College University, Faisalabad, Pakistan-38000.
chatha222@gmail.com*

(Received on 11th March 2019, accepted in revised form 6th December 2019)

Summary: Present study reports the potential use of HPLC coupled with principle component analysis (PCA) and partial least squares discriminant analysis (PLSDA), for differentiation of approved mungbean variety from the promising lines based on minor saccharides profiles. A total of 48 mungbean samples from one approved variety and seven promising lines were analyzed for minor saccharides using HPLC and multivariate statistical analysis. PCA showed a clear separation among the classes. PLSDA was conducted to extract the variables that were responsible for the separation of mungbean approved variety from the lines. Maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole, betaine varied significantly while stachyose, raffinose, sucrose, lectitol, dulcitol, xylitol, galactose showed non-significant differences. Maltoheptaose, maltohexaose, maltotretraose, maltitol, mannitole and galactose were found as the most abundant compounds while stachyose, raffinose, sucrose, lectitol and betaine were found less abundant in all lines and approved variety of *V. radiata*. The study highlights metabolic variation among mungbean variety and lines for minor saccharides profiles and its usefulness for consumers to choose for their desired variety or line as well as for breeders to look into the genetic factors responsible for this variation.

Keywords: Maltoheptaose; Maltohexaose; HPLC; Partial least squares discriminant analysis; Principle component analysis; *Vigna radiata L.*

Introduction

Mungbean (*Vigna radiata* L.) belong to *Fabaceae* family and is considered as most important leguminous food crop native to Myanmar region of Asia. It is an important source of carbohydrates (46-65%) [1] and proteins (6.34-21.05%) [2] which are two folds higher than the levels found in other cereal grains [3]. *V. radiata* seeds provide high caloric energy and nutrition and are rich in minerals, fatty acids and bioactive compounds including phenolics and flavonoids [4]. *V. radiata* have antimicrobial, anti-inflammatory and anti-carcinogenic effects because of high phenolic contents [5]. It also contains sugars and proteins which are good source of energy and building blocks of human body respectively. Pulses are commonly used by vegetarians as a substitute of meat for energy and proteins and are referred to as 'the poor man's meat' [6].

Nowadays plant breading plays a key role in safety and increased food crops production in the world. For survival of cultivation, a compromised solution should be sought out to acquire maximum crop yield under varying atmospheric conditions and thus minimizing crop failure. This matter entails the best knowledge of impacts of plant breeding on crop genetic diversity [7]. To know about diversity of changes which occurred due to unique genetic makeup of each crop among its cultivars is very important for the development of a new variety and its maximum production with minimum loss occurring due to biotic and abiotic stresses [8]. Varied profile of sugar components in a crop is responsible for determining its quality which is important for both breeder as well as consumer. This enables breeder to earn more price premium and end user to ensure healthy growth of body.

Numerous chromatographic techniques have been used for the determination of sugars in fruits and vegetables [9] but due to the simplicity, exactness, and ease of sample preparation (involving just blending, extraction, dilution, demineralization and filtration steps) high performance liquid chromatography (HPLC-RI) has become invasive technique [10].

At present metabolomics offers the pledged solution to enhance the knowledge of high quality guidelines and provides information regarding variations depending on unique genetic makeup among cultivars as compared to its authenticated variety and among themselves [11, 12]. Metabolomics utilizes

***To whom all correspondence should be addressed.**

various techniques such as mass spectroscopy (MS), HPLC as well as nuclear magnetic resonance (NMR) or even coupled NMR spectroscopy along with LC-MS or MS for ample research associated with biomaterials [13, 14]. Actual aspects obtained from statistical multivariate model of metabolomics are promisingly helpful in discriminating the variances amongst the treatments very effectively due to which it has got popularity and is preferred among scientific community. Among a number of available multivariate statistical methods two are most suitable for recognition of variation patterns i.e. principal component analysis (PCA) and partial least square-differential analysis (PLS-DA). PCA and PLS-DA are more admired for the mapping associated with intrinsic variation within immense information models [15].

To judge the actual variations among mungbean approved and lined cultivars, HPLC data information combined with multivariate statistical analysis is very useful. Promising sugar contents in pulses make them beneficial for health of humans and animals being a vital part of their daily diet. Therefore, it is important to investigate the variations among different mungbean cultivars/varieties based on their sugar contents as almost all plants produce sugars like glucose and fructose in photosynthesis at early stages that are used in growth of plants as an important source of energy. This research was conducted with an aim to assess the variation in sugar content in mungbean cultivars and varieties under trial. Fructose, glucose, sucrose stachyose, verbascose and raffinose are the major sugars in some species of pulses and extensive research has been published on their content all over the world [16]. However, to the best of our knowledge, very few reports are available in the literature on the discrimination of different lines/varieties of *V. radiata* based on minor saccharides through HPLC coupled with multivariate analysis especially in Pakistan. So, the aim of this research work was estimation of essential minor saccharides of all newly modified lines of *V. radiata* and comparison of candidate lined cultivars with approved variety. It is anticipated that this work will provide useful information for crop breeders to finalize the candidate lines leading to its confirmation as a final approved variety with enhanced nutritional values as compared to existing lines/varieties.

Experimental

Materials and Methods

Collection of Sample

Dry samples of *V. radiata* seeds including one approved variety named as (NM-11) and seven lines of *V. radiata* named as (0708, E-1, E-2, E-12, E-14, E-15, E-18) as shown in (Table-1) were collected from Pulses Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan (latitude:31.398 & Longitude: 73.0553). The samples were further identified and authenticated from Dr. Qasim Ali, Department of Botany, Government College University Faisalabad.

Table-1: Sample information of selected samples of *V. radiata*.

Sr. No:	Cultivars Names	Status
1	NM-11	(Approved)
2	0708	Lined
3	E-1	Lined
4	E-2	Lined
5	E-12	Lined
6	E-14	Lined
7	E-15	Lined
8	E-18	Lined

Chemicals and reagents

Ultra-pure water obtained from Labconco system (Catalog Number: 9000521), was used in all the experiments. The standards of maltodextrin, maltose, glucose and fructose, all with purity exceeding 99.0% were obtained from Sigma and Fluka Chemicals Ltd., Dorset, UK, unless otherwise indicated.

Pretreatment and storage of samples

Samples of *V. radiata* were washed with water in order to remove dust particles, remaining water was removed using paper towel, then samples were air dried till constant weight was achieved. Dried samples than ground to semi powder using grinder (LG BL 999SP) and stored in air tight polythene bags.

Preparation of Extracts

The extracts of all seed parts were prepared by employing absolute ethanol as reported previously [17]. Extracts were concentrated under reduced pressure using rotary vacuum evaporator and preserved under refrigerator (-4 ^{o}C), until used for further analysis [18].

Sugar Analysis

Sugar analyses of selected samples were performed by HPLC using previously reported method by Johansen [19] with some modification. Briefly using water as a mobile phase which is inexpensive, nonhazardous and result in non-toxic waste instead of using acetonitrile, as it is expensive, hazardous and results in toxic waste in divergence to water.

Preparation of samples for HPLC

20 mg ethanolic extract blended with 5 mL double distilled water for 3 minutes and filtered through Whatman No. 1 filter paper [20]. The filtrate was de-mineralized by filtering through cation and anion resins. Before injecting to HPLC, the sample was filtered through syringe filter of 0.22 µm for the removal of microbes. All samples were prepared in triplicate and analyzed within 24 hours of preparation.

HPLC analysis

HPLC analysis of the sugars was performed on a Shimadzu HPLC LC-20A system (Singapore). The HPLC system consisted of a pump (model LC20AT Prominence), a solvent degasser (model G1322A), a column oven (model CT 020A/20AC) and refractive index detector (model RID10A) and was controlled by Shimadzu LC Solution software. The system was also assisted by CBM 20A/20A Light system controller. Carbohydrate separation was carried out on a Bio-Rad Aminex HPX-87K 300 × 7.8 mm column (Cat # 1250142) with Bio-Rad Guard column with ultra-pure H_2O as mobile phase at a flow rate of 0.50 mL/min. A 20 µL sample was injected. Refractive index detector maintained at 40 °C was used for detection purposes. Maltodextrin, maltitrose, maltose, glucose and fructose were identified and quantified on the basis of retention times, peak areas and comparison with calibration curve obtained by corresponding standards.

Developing an HPLC method that can resolve a mixture of mono-, di-, tri- and oligosaccharides in a short elution time is a challenge. Earlier described methods had several shortcomings and some of the methods have shown an unacceptable long retention time for raffinose and stachyose while some others had poor resolution or broad peaks.

Statistical Analysis

Multivariate Data Analysis

The HPLC data were imported to SIMCA-P+ 11 (Umetrics, Umea, Sweden) for multivariate statistical analysis. The UV (Unit variance) scaling was applied for all multivariate analyses. PCA, an un-supervised pattern recognition analysis was applied to reveal the intrinsic variations in the data set and to diagnose any possible outlier. The quality of the model was defined by total variance of the two components at a confidence level of 95%. The overall predictability of the model is assessed by cumulative Q^2 representing the fraction of the variation of the Y that can be predicted by the model, which was extracted according to the internal cross-validation default method of the SIMCA software. The PCA is a method requiring no prior knowledge of the data set and acts as a screening model to reduce the dimensionality of data while preserving most of the variance within it [21]. PCA was performed in order to find overall separation among all eight verities/lines. PCA score scatter plot shows the separation among different groups. To further maximize separation and to extract the variables responsible for the separation among different groups of mungbean PLSDA was also performed. PLSDA scatter plots give the separation among two groups whereas PLSDA loading plots give the variables responsible for the separation.

Results and Discussion

PCA score scatter plot was calculated from the data representing all the 8 cultivars of mungbean (Fig. 1). PCA score scatter plot efficiently separated all the eight classes' with R^2X cum. (61%) and Q^2 cum. (98%). This revealed that all classes are different from each other on the basis of various sugar metabolites with respect to their concentration as shown in (Table 2). Since, class 1 represents the approved variety NM-11 so all the other classes were compared one with class 1. First PLSDA (Fig. 2A & B) was calculated from the data representing sugar profile of class 1 and 2. The two classes were separated from each other by PLSDA scatter plot with PLS1 93% and Q^2 99%. Compounds responsible for this separation were extracted from the corresponding PLSDA loading plot. PLSDA loading plot revealed that maltohexaose, maltopentaose, maltitol, lectitol, betaine, galactose, maltoheptaose, maltotretraose and maltose are responsible for this separation. Maltohexaose, maltopentaose, maltitol, lectitol, betaine, galactose were present in higher concentrations in class 1 while maltoheptaose, maltotretraose and maltose were present in higher amounts in class 2.

Second PLSDA (Fig. 2C & D) was calculated on the basis of sugar profile calculated for class 1 and 3. The two classes were separated from each other by PLSDA scatter plot and sugar contents responsible for this separation were extracted from the corresponding PLSDA loading plot with PLS1 90% and Q^2 99%. PLSDA loading plot showed that maltohexaose, maltopentaose, maltotretraose, maltitol, betaine, galactose, maltoheptaose, maltose and mannitole are responsible for this separation. Maltohexaose, maltopentaose, maltotretraose, maltitol, betaine, galactose were present in higher concentrations in class 1 while maltoheptaose, maltose and mannitole were present in higher amounts in class 3.

Table-2: Average concentration of different sugar metabolites in *V. radiata* samples.

Compound Name	Retention Time	NM-11	0708	E-1	E-2	E-12	E-14	E-15	E-18
Maltoheptaose	7.2	1.121	29.189	39.337	44.481	33.108	44.556	34.981	23.318
Maltohexaose	7.4	40.342	12.111	14.903	14.472	17.902	14.472	14.677	17.827
Maltopentaose	7.8	17.974	0.000	0.000	0.000	14.615	0.000	0.000	14.162
Stachyose	7.9	0.000	3.683	0.000	0.000	0.000	0.000	0.000	3.956
Maltotretraose	8.4	9.788	13.231	9.087	16.290	0.000	16.126	12.076	10.983
Raffinose	8.7	0.000	0.000	0.000	0.000	6.597	0.000	0.000	0.000
Sucrose	10.1	0.000	15.326	0.000	0.000	0.000	0.000	0.000	0.000
Maltitol	10.2	15.872	0.000	14.252	7.782	0.000	7.847	12.075	13.645
Lectitol	10.6	0.000	0.000	0.000	0.000	5.879	0.000	0.000	0.000
Maltose	10.7	3.694	7.470	6.657	0.000	0.000	0.000	4.180	0.000
Mannitole	12.6	2.584	1.477	11.291	1.504	3.675	1.486	16.165	16.110
Dulcitol	12.9	0.000	12.951	0.000	7.914	0.000	7.239	0.000	0.000
Betaine	13	3.861	0.000	0.000	0.000	10.813	0.000	0.000	0.000
Xylitol	14.2	0.000	0.000	0.000	1.001	2.640	1.307	2.053	0.000
Galactose	15.1	4.765	4.563	4.474	2.915	0.000	3.170	3.794	0.000

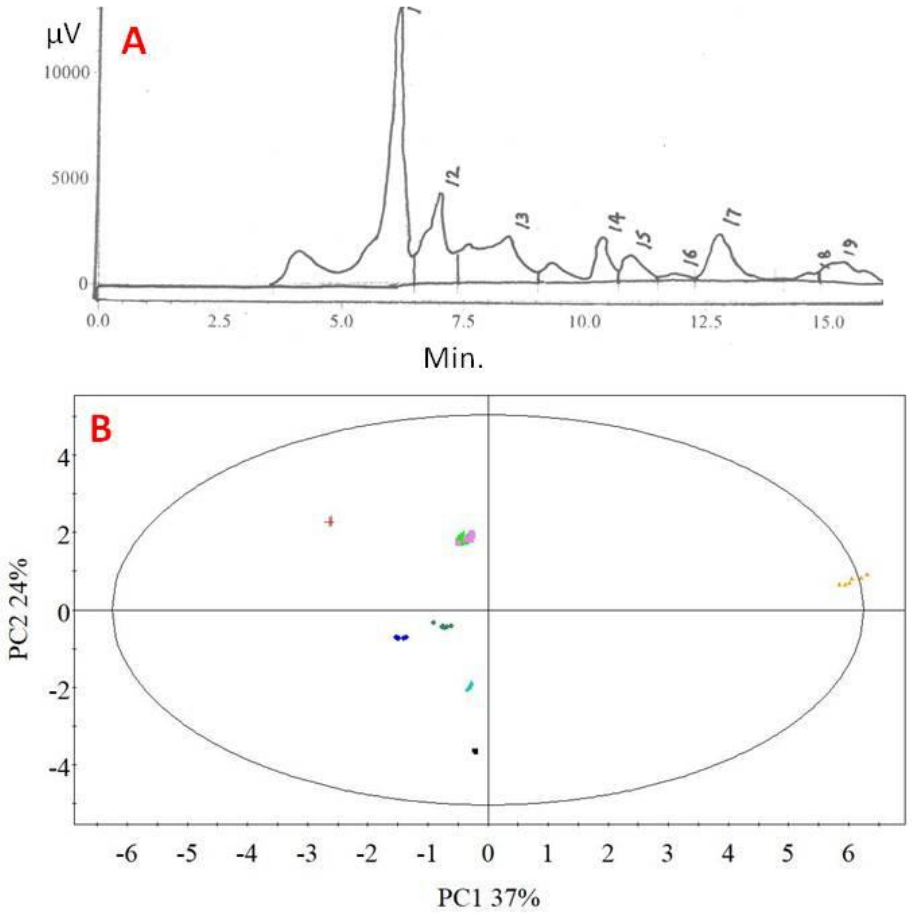
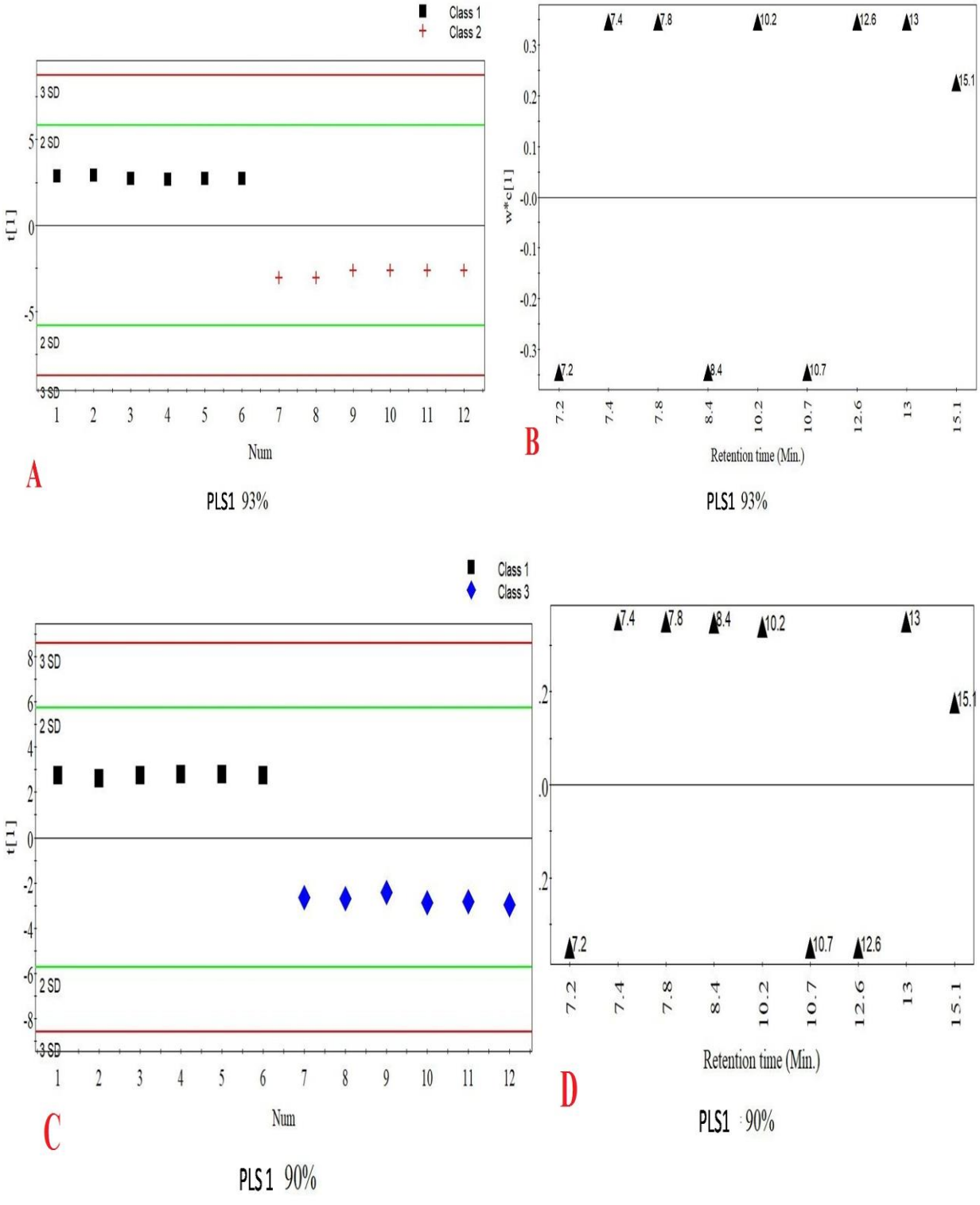
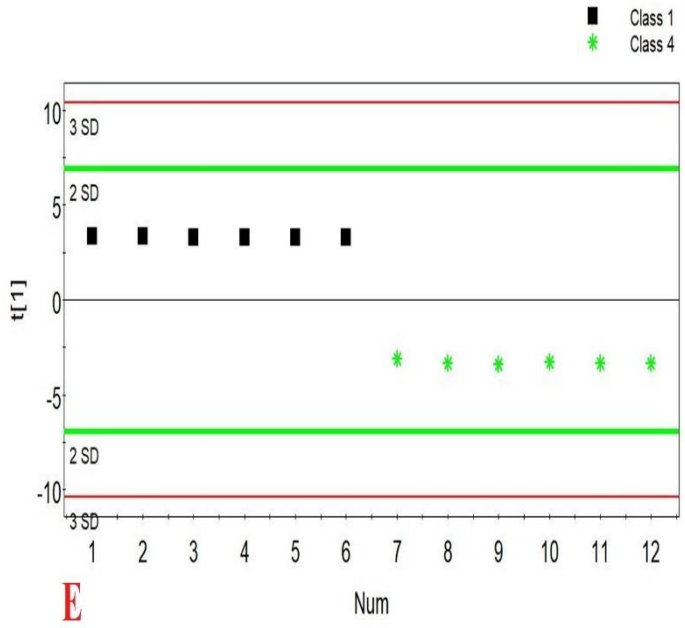


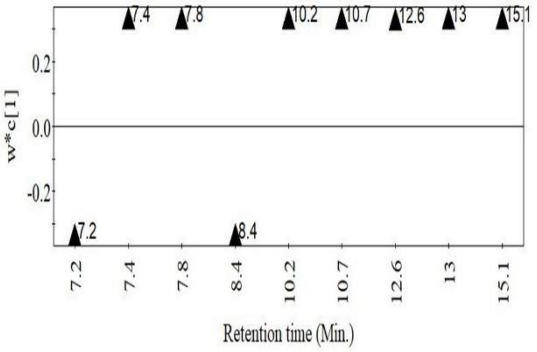
Fig. 1: (A) Chromatogram of a representative *V. radiata*. sample showing different sugar metabolites. Peaks: 1: Maltoheptaose, 2: Maltohexaose, 3: Maltotretraose, 4: Maltitol, 5: glactose, 6: Mannitole, 7: Dulcitole, 8: Xylitol, 9: Galactose.
(B) PCA scatter plot representing all cultivars of selected *V. radiata* samples.
Black Box; Class 1(NM-11), Red Cross; Class 2(0708), Blue Diamond; Class 3(E-1), Green Star; Class 4(E-2), Orange Triangle; Class 5(E-12), Violet Box; Class 6(E-14), Sea Green Dot; Class 7(E-15), Dark Turquoise; Class 8(E-18).


Class 1
Class 2
3 SD
2 SD
2 SD
3 SD
t[1]
Num
A
PLS1 93%
7.4
7.8
10.2
12.6
13
15.1
7.2
8.4
10.7
w*c[1]
Retention time (Min.)
B
PLS1 93%
Class 1
Class 3
3 SD
2 SD
2 SD
3 SD
t[1]
Num
C
PLS1 90%
7.4
7.8
8.4
10.2
13
15.1
7.2
10.7
12.6
Retention time (Min.)
D
PLS1 90%



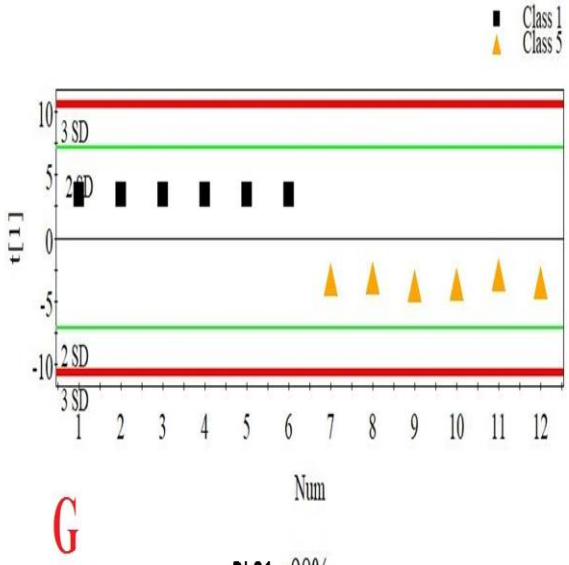
E

PLS1 99%



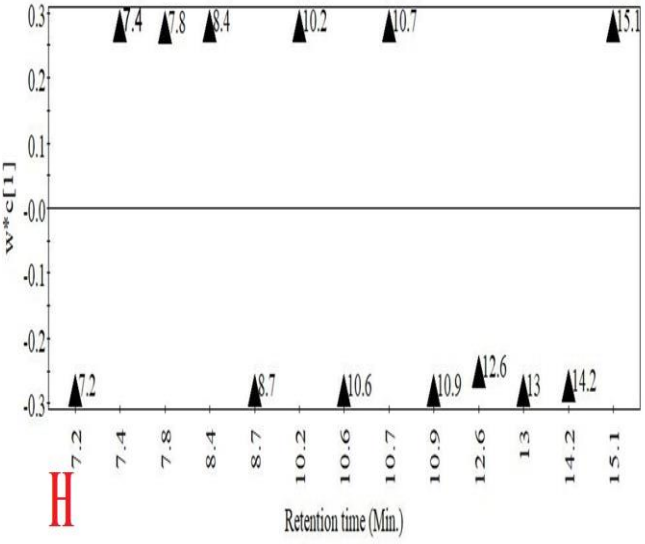
F

PLS1 99%



G

PLS1 98%



H

PLS1 97%

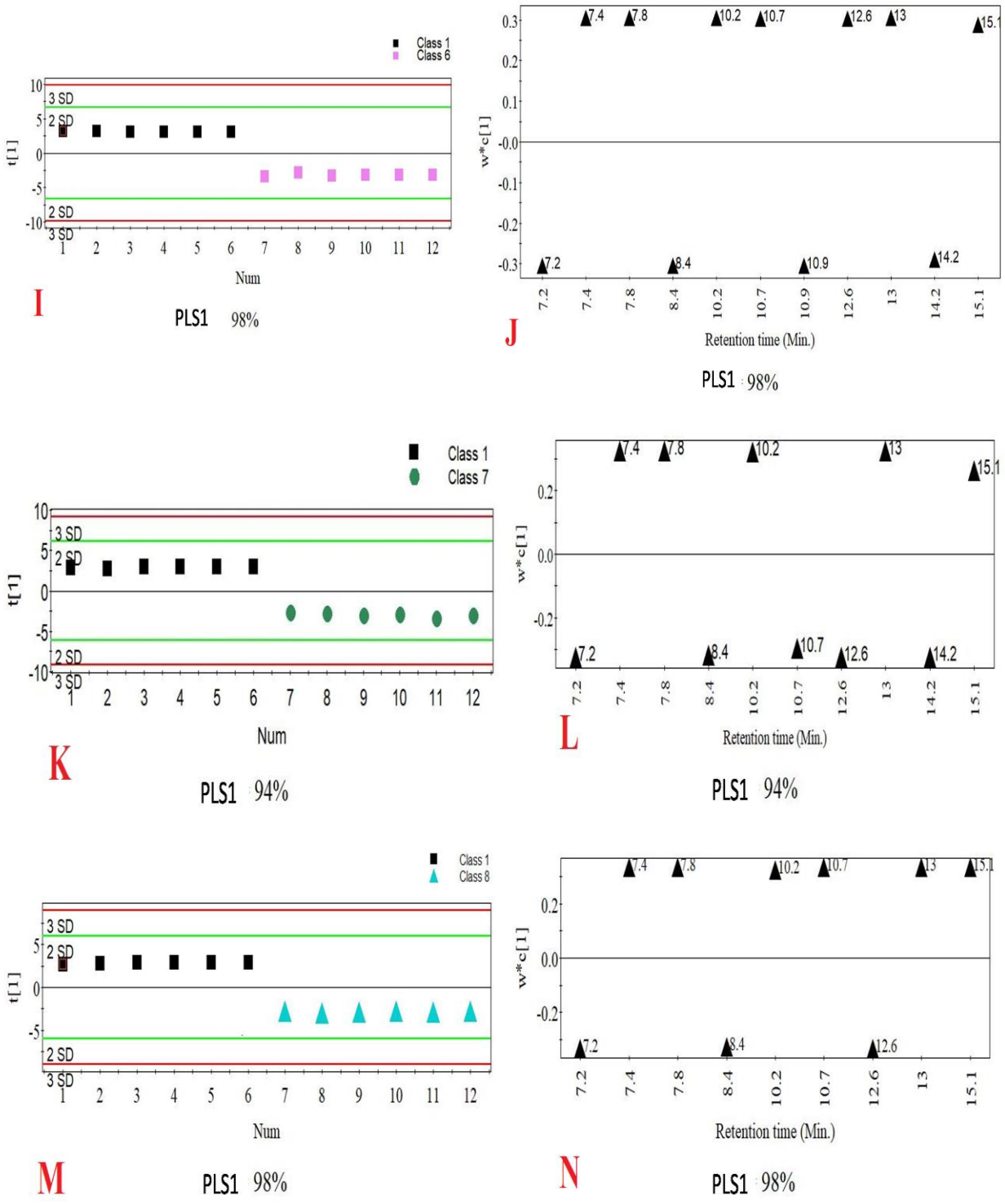


Fig.2: PLSDA scatter plots (A, C, E, G, I, K and M) and their crossponding loading plots (B, D, F, H, J, L and N) sowing the variables responsible for this separation derived from HPLC data for different concentration of sugar compounds among approved variety (NM-11) and lined cultivars (0708, E-1, E-2, E-12, E-14, E-15 and E-18) respectively.

Third PLSDA (Fig. 2E & F) was obtained on the basis of sugar contents calculated for class 1 and 4. These two classes were separated from each other by PLSDA scatter plot and sugar contents responsible for this separation were extracted from the corresponding PLSDA loading plot with PLS1 99% and Q^2 100%. PLSDA loading plot showed that maltohexaose, maltopentaose, maltitol, maltose, mannitole, betaine, galactose, maltoheptaose and maltotretraose are responsible for this separation. Maltohexaose, maltopentaose, maltitol, maltose, mannitole, betaine and galactose were present in higher concentrations in class 1 while maltoheptaose and maltotretraose were present in higher amounts in class 4.

Fourth PLSDA (Fig. 2G & H) was calculated on the basis of sugar profile obtained for class 1 and 5. These two classes were also separated from each other by PLSDA scatter plot and sugar contents responsible for this separation were extracted from the corresponding PLSDA loading plot with PLS1 97% and Q^2 99%. PLSDA loading plot showed that maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, galactose, maltoheptaose, raffinose, lectitol, mannitole, betaine and xylitol are responsible for this separation. Maltohexaose, maltopentaose, maltotretraose, maltitol, maltose and galactose were present in higher concentrations in class 1 while maltoheptaose, raffinose, lectitol, mannitole, betaine and xylitol were present in higher amounts in class 5.

Fifth PLSDA (Fig. 2I & J) was calculated on the basis of sugar profile obtained for class 1 and 6. These two classes were also separated from each other by PLSDA scatter plot and sugar contents responsible for this separation were extracted from the corresponding PLSDA loading plot with PLS1 98% and Q^2 99% PLSDA loading plot showed that maltohexaose, maltopentaose, maltitol, maltose, mannitole, betaine, galactose, maltoheptaose, maltotretraose and xylitol are responsible for this separation. Maltohexaose, maltopentaose, maltitol, maltose, mannitole, betaine and galactose were present in higher concentrations in class 1 while maltoheptaose, maltotretraose and xylitol were present in higher amounts in class 6.

Sixth PLSDA (Fig. 2K & L) was calculated on the basis of sugar profile obtained for class 1 and 7. These two classes were also separated from each other by PLSDA scatter plot and sugar contents responsible for this separation were extracted from the corresponding PLSDA loading plot with PLS1 94% and Q^2 99%. PLSDA loading plot showed that maltohexaose, maltopentaose, maltitol, betaine, galactose, maltoheptaose, maltotretraose, maltose, mannitole and xylitol are responsible for this separation. Maltohexaose, maltopentaose, maltitol, betaine and galactose were present in higher concentrations in class 1 while maltoheptaose, maltotretraose, maltose, mannitole and xylitol were present in higher amounts in class 7.

Seventh PLSDA (Fig. 2M & N) was calculated on the basis of sugar profile obtained for class 1 and 8. These two classes were also separated from each other by PLSDA scatter plot and sugar contents responsible for this separation were extracted from the corresponding PLSDA loading plot with PLS1 98% and Q^2 99%. PLSDA loading plot showed that maltohexaose, maltopentaose, maltitol, maltose, betaine, galactose, maltoheptaose, maltotretraose, maltose, mannitole and xylitol are responsible for this separation. Maltohexaose, maltopentaose, maltitol, betaine and galactose were present in higher concentrations in class 1 while maltoheptaose, maltotretraose and mannitole were present in higher amounts in class 8.

Results of LSD showing significant and non-significant differences in concentrations of sugar compounds among approved and candidate verities were summarized in (Table-3). While comparing NM-11 and candidate line 0708 maltoheptaose, maltohexaose, maltopentaose, stachyose, maltotretraose, sucrose, maltitol, maltose, mannitole, dulcitol, betaine was significantly responsible (p = 0.000) for the differentiation among NM-11 and 0708 while raffinose, lectitol, xylitol (p = 1.000) and galactose (p = .224) contributed non significantly towards differentiation among NM-11 and 0708. Concentrations of maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole and betaine varied significantly (p = 0.000) between of NM-11 and candidate line E1, while those of stachyose, raffinose, sucrose, lectitol, dulcitol and xylitol (p = 1.000) and galactose (p = 0.028) varied non-significantly. In another comparison between NM-11 and candidate line E2 numerous sugar compounds namely maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole, dulcitol, betaine, xylitol and galactose were found significantly varying with p = 0.000 whereas stachyose, raffinose, sucrose and lectitol varied non-significantly with p = 1.000. When NM-11 was compared with E-12 candidate line stachyose, sucrose and dulcitol were found to be non-significantly varying with p = 1.000. Maltoheptaose, maltohexaose, maltopentaose,

maltotretraose, raffinose, maltitol, lectitol, maltose, mannitole, betaine, xylitol and galactose contributed significantly (p = 0.000) towards the separation among NM-11 and E-12. In comparison between NM-11 and E-14 variety of sugar compounds that were maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole, dulcitol, betaine, xylitol and galactose varied significantly (p = 0.000) while stachyose, raffinose, sucrose and lectitol varied non significantly level (p = 1.000). Maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole, betaine, xylitol, and galactose contributed significantly (p = 0.000) while stachyose, raffinose, sucrose, lectitol and dulcitol varied non significantly (p = 1.000) for the separation among NM-11 and E-15. When separation among NM-11 and E-18 was authenticated using LSD test, maltoheptaose, maltohexaose, maltopentaose, stachyose, maltotretraose, maltitol, maltose, mannitole, betaine and galactose were found to be significantly varying with p = 0.000 while raffinose, sucrose, lectitol, dulcitol, and xylitol varied non significantly with p = 1.000.

Maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole and betaine are all sugar compounds that varied significantly with p = 0.000 while stachyose, raffinose, sucrose, lectitol, dulcitol, xylitol and galactose are varied non significantly at different levels among all studied *V. radiata* lines/cultivars. This discrimination provides important information for crop breeders to judge the extent of finalization of candidate lines as approved one variety with some modified nutritional levels as compared to existing lines/varieties.

Our results are in good agreement with the previously reported finding of [22] who studied the nutritional composition and antinutritional factors of mungbean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Bhardwaj and Hamama [23] and Bhardwaj and Hamama [16] also investigated the mungbean seed composition in Virginia. Banusha and Vasantharuba [24] found non-significant effect of malting on total sugars contents in two different lines of *V. radiata.*

Conclusions

It was observed that some compounds varied significantly including maltoheptaose, maltohexaose, maltopentaose, maltotretraose, maltitol, maltose, mannitole, betaine while other compounds (stachyose, raffinose, sucrose, lectitol, dulcitol, xylitol, galactose) varied non significantly. Maltoheptaose, maltohexaose, maltotretraose, maltitol, mannitole and galactose were found most abundant than other compounds while stachyose, raffinose, sucrose, lectitol and betaine were found less abundant in all cultivars of *V. radiata.* It is anticipated that this work will provide useful information for crop breeders to finalize the candidate lines leading to its confirmation as a final approved variety with enhanced nutritional values as compared to existing lines/varieties. At the same time the results would be useful for end users to choose for the appropriate mungbean variety for their food stuff. Results of present study also provide an opportunity for the future researchers to further verify composition of different lines and approved varieties by employing some advanced methodologies.

Table-3: Summary of least square discriminant analysis showing significant and non-significant sugar metabolites.

Approved Variety						**(NM-11)**	
Candidate lines	**0708**	**E-1**	**E-2**	**E-12**	**E-14**	**E-15**	**E-18**
Compound Names				***p*-Value**			
Maltoheptaose	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Maltohexaose	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Maltopentaose	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Stachyose	**.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**.000**
Maltotretraose	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Raffinose	**1.000**	**1.000**	**1.000**	**.000**	**1.000**	**1.000**	**1.000**
Sucrose	**.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
Maltitol	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Lectitol	**1.000**	**1.000**	**1.000**	**.000**	**1.000**	**1.000**	**1.000**
Maltose	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Mannitole	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Dulcitol	**.000**	**1.000**	**.000**	**1.000**	**.000**	**1.000**	**1.000**
Betaine	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Xylitol	**1.000**	**1.000**	**.000**	**.000**	**.000**	**.000**	**1.000**
Galactose	**.224**	**.082**	**.000**	**.000**	**.000**	**.000**	**.000**

Acknowledgements:

Acknowledgement is given to Dr. Nadeem Abbas Faisal for the HPLC facility.

References

1. N. Ahmad, M. Sarfraz, U. Farooq, M. Z. Mushtaq, M. A. Ali and A. Khan. Quality evaluation of some mungbean [*Vigna radiata* (L.) Wilczek] varieties/promising lines from Punjab. *Pakistan. J. Agric. Res.,* **54**, 85 (2016).
2. S. D. Chandrasiri, R. Liyanage, J. K. Vidanarachchi, P. Weththasinghe and B. C. Jayawardana. Does processing have a considerable effect on the nutritional and functional properties of Mung bean (*Vigna radiata*)? *Procedia. Food. Sci.,* **6**, 352 (2016).
3. X. Guo, T. Li, K. Tang and R. H. Liu. Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (*Vigna radiata* L). *J. Agric. Food. Chem.,* **60**, 11050 (2012).
4. A. Afshin, R. Micha, S. Khatibzadeh and D. Mozaffarian. Consumption of nuts and legumes and risk of incident ischemic heart disease, stroke and diabetes: a systematic review and meta-analysis. *Am. J. Clin. Nutr.,* **100**, 278 (2014).
5. O. Caliskan and A. A. Polat. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. *Sciente. Cult.,* **128**, 473 (2011).
6. M. S. Butt and R. Batool. Nutritional and functional properties of some promising legumes protein isolates. *Pak. J. Nutr.,* **9**, 373 (2010).
7. Y. B. Fu. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet.,* **128**, 2131 (2015).
8. G. Keneni, E. Bekele, M. Imtiaz, K. Dagne. Genetic vulnerability of modern crop cultivars: causes, mechanism and remedies. *Int. J. Plant Res.,* **2**, 69, (2012).
9. R. S. J. Rodriguez, V. Fernández-Ruiz, M. Camara, M. C. Sanchez-Mata Simultaneous determination of vitamin B1 and B2 in complex cereal foods, by reverse phase isocratic HPLC-UV. *J. Cereal Sci.,* **55**, 293, (2012).
10. L. D. A. Lima, A. Schuler, N. B. Guerra, G. E. Pereira, T. D. A. LIMA and H. Rocha. Otimizacao e validacao de metodo para determinacao de acidos organicos em vinhos por cromatografia líquida de alta eficiencia. *Embrapa Semiarido-Artigo em periodico indexado (ALICE)*. **33**, 1186 (2010).
11. W. Terouzi, M. De Luca *, A. Bolli, A. Oussama, M. Patumi, G. Ioele, G. Ragno. The methodology of the Moroccan olive varieties by using direct FT-IR analysis of the mesocarp section. *Spectr*. **56** 123-128 (2011).
12. K. S. Solanky, N. J. Bailey, B. M. Beckwith-Hall, S. Bingham, A. Davis, E. Holmes, L. K. Nicholson and A. Cassidy. Bio fluid ^{1}H NMR-based metabonomic techniques in nutrition research metabolic effects of dietary is flavones in humans. *J. Nutri. Biochem.,* **16**, 236 (2005).
13. D. J. Crockford, E. Holmes, J. C. Lindon, R. S. Plumb, S. Zirah, S. J. Bruce, P. Rainville, C. L. Stumpf, J. K. Nicholson. Statistical heterospectroscopy, an approach to the integrated analysis of NMR and UPLC-MS data sets: application in metabonomic toxicology studies. *Anal. Chem.,* **78**, 363 (2006).
14. M.D. Luca, D. Restuccia, M. L. Clodoveo, F. Puoci and G. Ragno. Chemometric analysis for discrimination of extra virgin olive oils from whole and stoned olive pastes. *Food Chem.,* **202**, 432 (2016).
15. S. Esslinger, J. Riedl, C. Fauhl-Hassek. Potential and limitations of non-targeted fingerprinting for authentication of food in official control. *Food Res. Int.,* **60**, 204 (2014).
16. H. L. Bhardwaj and A. A. Hamama. Cultivar, planting date, and row spacing effects on mungbean seed composition. *J. Agric. Sci.,* **8**, 26 (2016).
17. M. Majeed, S. A. S. Chatha, A. I. Hussain, M. R. Asi, S. Mahboob and M. Yousaf. Intervarietal variation in proximate composition and antioxidant potential of dry peas (*Pisum sativum* L). *J. Chem. Chem. Engi.,* **4**, 7 (2010).
18. H. E. Abdolrasoul, H. E. Ebrahim, D. Zahra, J. K. Fereshteh, M. Asma and B. Hossein. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of *Stachys inflata Benth* from Iran. *Food Chem.*,**119**, 452 (2010).
19. H. N. Johansen and V. Glitso, K. E. B. Knudsen. Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. *J. Agric. Food. Chem.,* **44**, 1470 (1996).
20. T. Mahmood. Investigations of valuable phytochemicals in native berries and cherries fruits (Doctoral dissertation, University of Agriculture, Faisalabad). (2011).
21. H. K. Choi, Y. H. Choi, M. Verberne, A. W. Lefeber, C. Erkelens and R. Verpoorte. Metabolic finger printing of wild type and trans genic tobacco plants by ^{1}H NMR and

multivariate analysis technique. *Phyto Chem.,* **65**, 857 (2004).

22. A. E. Mubarak. Nutritional composition and anti-nutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chem.,* **89**, 489 (2005).
23. H. L. Bhardwaj and A. A. Hamama. Cultivar, planting date, and row spacing effects on mungbean performance in Virginia. *Hort. Sci.,* **50**, 1309 (2015).
24. S. Banusha and S. Vasantharuba. Effect of malting on nutritional contents of fingermillet and mungbean. *Eurasian J. Agric. & Environ. Sci.,* **13**, 1642 (2013).