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Changes in the polypeptide/ protein banding pattern of guava (*Psidium guavajava* L.) fruits during ripening on-tree and in-storage

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Abstract
Changes in the polypeptide pattern of guava (*Psidium guavajava* L.) cvs. *viz.* Hisar Safeda and Hisar Surkha were examined during ripening on-tree as well as in-storage, at different stages of ripening. Observation of protein banding profile of guava fruits revealed that the profiles differed among cultivars as well as seasons. Both the hybrids revealed reproducible bands ranging from Rf values of 0.48 to 0.98 in rainy season and 0.36 to 0.93 in winter season. Polymorphism was exhibited clearly in fruits of rainy season crop than winter season crop. Also the profiles were affected differentially during ripening on-tree and in-storage. Differences were also observed with respect to maturity stages. There were few bands which were present only at green mature and half ripe stages but absent at full ripe stage while some bands were absent at green mature and half ripe stages but present at full ripe stage. In the present investigations, two unique bands were also observed. One band (Rf value 0.28) was present only in Hisar Surkha at full ripe stage whereas another band (Rf value 0.66) was present in Hisar Safeda at green mature stage only.

Keywords: Electrophoresis, guava, poly peptide/ protein banding pattern, polymorphism

Introduction
Guava (*Psidium guajava* L.) is one of the most important tropical and subtropical fruits, Fresh guava is rich source of Vitamin-C and also contains appreciable amounts of niacin, calcium, phosphorus and iron, seeds of guava are potential source of pectin and oil (Misra and Sheshdri; Sharma *et al*.,) [1, 2]. Guava is a perishable fruit and is susceptible to bruising and mechanical injuries. To reduce the percent losses in guava and to avoid glut, it becomes desirable to evolve technologies for prolonging its keeping quality through delaying softening process during ripening. Development of practical solution to the post harvest problems requires detailed understanding of biochemistry and molecular biology of fruit ripening. The various biochemical and molecular changes taking place in guava during ripening in isolation have been studied by many workers (Selvaraj *et al.,* Jain *et al.,*) [3, 4]. It has been observed that ripening behavior of fruit while attached to tree may not be the same as in the detached fruit during storage (Nunes *et al.,*) [5]. Softening during ripening involves structural as well as biochemical modifications. The study of seasonal variations in guava fruit characters and quality is required to evaluate commercial guava growing season and better performed cultivars (Neeraj *et al*., 2016) [6]. The use of storage proteins provides a more appropriate option to characterize and classify plant germplasm. Total protein is not sensitive to environmental fluctuations; its banding pattern is very stable which is advocated for cultivars identification purpose in crop plants. Analysis of SDS-PAGE is fairly simple and inexpensive, which are added advantages for use in practical plant breeding (Rahman and Hirata) [7]. However, the information on the SDSPAGE on different species of guava for genetic diversity is still limited and no literature is available on use of protein markers or electrophoretic banding pattern in guava in relation to ripening. Protein banding information in relation to changes during ripening can boost development of post harvest technologies to increase the shelf life of guava. Present study focused on changes in polypeptide pattern of guava fruits at various stages ripening.

Materials and Methods

The present investigation was carried out at Post Harvest Laboratory of Department of Horticulture CCS HAU, Hisar on rainy season and winter season crop, of guava fruit,. Fruits of guava cultivars Hisar Safeda and Hisar Surkha were harvested with scateur keeping a small intact pedicel with each fruit, from ten year old trees growing at Orchard of the department of Horticulture, CCS Haryana Agricultural University, Hisar. For studying ripening on-tree the fruits from tree were harvested on the basis of visual observation and firmness at three maturity stages *Viz.* Green mature stage (GMS): 100% green fruit ; Half ripe stage (HRS): 50% Yellow and 50% green fruit ;Full ripe stage (FRS): 80% yellow and 20% green fruit. For studying ripening, in-storage 10 kg uniform size fruits of both the cvs. Hisar Safeda and Hisar Surkha were harvested at green mature stage from the trees of uniform size and age. Fruits were divided into four replicates, each of 2.5 kg, packed separately in 2% perforated polythene bags (200 gauge) and stored at room temperature. Fruits were replicated four times and changes in polypeptide pattern of guava fruits at on respective stages for on-tree ripening and on alternate days for in-storage ripening were studied. Hanges in polypeptide pattern were studied by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Laemmili's method (1970) [8] was used for obtaining polypeptide pattern. Proteins were extracted and subjected to Denaturating discontinuous polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was done using the apparatus of M/S Atto Japan. The glass plates (13 x 0.1 x 15 cm) were used. Gel was removed from the glass plates and stained with the staining reagent. The excess stain was removed by diffusion using destaining solution. After complete distaining, the gel was transferred to 7% acetic acid solution and photographed.

Results and Discussion

Results regarding changes in polypeptide banding pattern during on-tree ripening of guava hybrids Hisar Safeda and Hisar Surkha in rainy and winter season have been presented in Plate 3 and 4. On scoring of reproducible bands in polypeptide pattern it was noticed that both the hybrids showed polypeptides ranging from Rf value 0.28 to 0.99 in both the season.

In rainy season, bands corresponding to Rf values 0.53, 0.58, 0.63, 0.86, 0.90 and 0.99 were very prominent in both the cultivars at all the three harvesting stages. There were 6 faint bands ranging from 0.33 to 0.46 and 0.64 to 0.71 Rf values, which were present in both the hybrids at all the stages of ripening except that a faint band of 0.28 Rf value was present only in cv. Hisar Surkha at full ripe stage. One more faint band of Rf value 0.83 was present in both the cvs. At green mature and half ripe stage but was found to be absent in full ripe stage (Plate 3).

In winter season, 6 very clear bands corresponding to Rf values from 0.52 to 0.58 and 0.80 to 0.95 were present in both the cultivars at all the stages of harvest. In this season, three clear bands of Rf value 0.69, 0.70 and 0.74 were found to be present in both the hybrids at all the stages of ripening. Six faint bands ranging in Rf value from 0.28 to 0.47 were also present in both hybrids at all the stages. Two faint bands corresponding to Rf values 0.62 and 0.64 were present only at green mature and half ripe stages and were absent at full ripe stage in both the hybrids. One faint band of Rf value 0.79 was present in green mature stage only and absent in half ripe and full ripe stages in both the hybrids (Plate 4).

Plate 5 & 6 present the results depicting changes in polypeptide pattern of guava hybrids Hisar Safeda and Hisar Surkha during ripening in storage in rainy and winter season. On scoring of bands both the hybrids revealed reproducible bands ranging from Rf values of 0.48 to 0.98 in rainy season and 0.36 to 0.93 in winter season.

In rainy season both the hybrids exhibited 9 very clear bands of Rf value ranging from 0.48 to 0.55, 0.70 to 0.80 and 0.84 to 0.98 at all the stages of harvest. Two clear bands of Rf value 0.58 and 0.60 and one faint band of Rf value 0.81 were also exhibited in both the hybrids at all the stages. No polymorphism was observed in bands with respect to cvs. as well as stages .

In winter season, seven very clear bands of Rf values 0.52 to 0.58 and 0.75 to 0.93 were present in both the hybrids at all the maturity stages (Plate 6). A band of Rf value 0.47 was exhibited faintly in both the cultivars, except at green mature stage in cv. Hisar Safeda where this band was exhibited clearly. One more band of Rf value 0.76 was also exhibited differentially in both the cultivars. In cv. Hisar Surkha this band was very clear whereas in Hisar Safeda its visibility was faintly clear. In cv. Hisar Surkha two bands of Rf value 0.63 and 0.64 were faintly visible at green mature and half ripe stages but became clear on full ripe. While in cv. Hisar Safeda these two bands were faintly visible at all the 3 stages. One faint band of Rf value 0.61 was present only green mature and half ripe stage and absent at full ripe stage in cv. Hisar Surkha while in cv. Hisar Safeda it was present only at full ripe stage and absent in green mature and half ripe stages. There was one more faint band of Rf value 0.66 present only in cv. Hisar Safeda at green mature stage.

On overall observation of banding profiles it was noticed that the profile differed among varieties as well as seasons. Differences were also observed with respect to maturity stages. Also the polypeptide banding pattern was affected differentially during ripening on-tree and in-storage.

The polymorphism was exhibited clearly during ripening on tree as well as in-storage in winter season whereas in rainy season polymorphism was expressed only during ripening on-tree in terms of stages and was not observed at all during ripening in storage.

On observing banding profile of proteins, in winter season during ripening on-tree it was observed that cv. Hisar Surkha and cv. Hisar Safeda had different protein profile at green mature stage. The band with Rf value 0.64 is missing in cv. Hisar Surkha at green mature stage while is present in cv. Hisar Safeda (Plate 2) but in half ripe and full ripe stage, both exhibited similar protein profiles. Two bands corresponding to Rf value 0.62 and 0.79 showed polymorphism with respect to stages. The band with Rf value 0.62 was present at green mature and half ripe stages but absent in full ripe stage in both the hybrids and band with Rf value 0.79 is present only in green mature stage and is absent in half ripe and full ripe stages in both the hybrids. This leads to conclusion that there are certain changes taking place in polypeptides of guava fruits, there are certain higher molecular weight polypeptides (Rf value 0.62) and some low molecular weight polypeptides (Rf value 0.79) which disappear during ripening. During on-tree ripening, in rainy season (Plate 1) also one band of Rf value 0.83 was observed at green mature and half ripe stages but disappeared at full ripe stage. However all these bands were visible faintly and were not discrete. Lelyveld *et al.* [9] have also demonstrated that at low temperatures polymorphism is clearer. They carried out polyacrylamide gel electrophoresis on proteins of pineapple cv. Queen and

observed that storage of fruit at two extreme temperatures of 2 ^{0}C and 16 ^{0}C resulted in a high intensity of high molecular mass proteins at 2 ^{0}C and an increase in intensity of low molecular mass proteins at 16 ^{0}C. There was a distinct "change over" of these protein bands intensities at 8 ^{0}C to 12 ^{0}C.

During ripening of guava fruits marked differences were observed in the protein profiles of cv. Hisar Surkha and cv. Hisar Safeda in-storage in winter season (Plate 4). One high molecular weight polypeptide band of Rf value 0.47 was exhibited clearly only at green mature stage in cv. Hisar Safeda only but was visible faintly in other stages in cv. Hisar Safeda and all stages in cv. Hisar Surkha. One band of Rf value 0.61 was present in cv. Hisar Surkha at early ripening stages (Green mature and half ripe stage) but absent at full ripe stage. However, the same band was observed in cv. Hisar Safeda at full ripe stages which indicates that there are some proteins which are present in cv. Hisar Surkha at initial ripe stages and are degraded at full ripe stage but are synthesized in cv. Hisar Safeda at full ripe stages which could be a character which is responsible for different ripening behaviour of both the hybrids.

Intensity of some bands also differed with respect to hybrids. Proteins of Rf value 0.63 and 0.64 clearly visible in cv. Hisar Surkha at full ripe stage but in cv. Hisar Safeda, it was faintly visible at all the stages. Similarly band of Rf value 0.76 was very clear in cv. Hisar Surkha but was faintly clear in cv. Hisar Safeda at all the stages. This difference in intensity can be related to corresponding higher protein content in cv. Hisar Surkha as compared to cv. Hisar Safeda observed during present investigation. This could be due to presence of coloured pigments in Hisar Surkha. Rouholamin and Saei (2016) [10] also showed that there is a relation between the color of pomegranate and the amount of protein because genotypes. Sharma (2002) [11] examined polyacrylamide gel electrophoresis of protein in fruits of two different Ber cvs. Umran and Kathaphal at different maturity stages, 5 clear bands A, B, C, D and E were present in both cvs. Studied at fruit set, before ripening and at ripening, which were dark (A & B) or medium (C, D and E) in their staining intensities. The bands A, C and D were present in cv. Umran, whereas, band B and E were present in cv. Kathaphal. However, band D was visible only in cv. Umran, whereas in cultivar Kathaphal this particular band (D) was missing. Their was no clear cut differentiation in both cvs. During different stages. Carter and Brock (1980) [12] also found sufficient differences in protein banding pattern of terminal shoots in 5 peach cultivars using polyacrylamide gel electrophoresis techniques and also observed the differences in the densities and Rf values of the bands.

Unique pattern/bands

In the present investigations, two unique bands were observed. One band (Rf value 0.28) was present only in Hisar Surkha at full ripe stage whereas another band (Rf value 0.66) was present in Hisar Safeda at green mature stage only. These unique bands, may be used to identify (fingerprint) the varieties based upon their presence/absence. These can even be used as one of the varietal identification document while registering with NBPGR.

Summary

Observation of protein banding profile of guava fruits, obtained after electrophoresis, revealed that the profiles differed among cultivars as well as seasons. Polymorphism was exhibited clearly in rainy season than winter season. Also the profiles were affected differentially during ripening on-tree and in-storage. Differences were also observed with respect to maturity stages. There were few bands which were present only at green mature and half ripe stages but absent at full ripe stage while some bands were absent at green mature and half ripe stages but present at full ripe stage. In the present investigations, two unique bands were also observed.

Plate 1: Fruits of guava cv. Hisar Safeda at different physiological stages of maturity.

Plate 2: Fruits of guava cv. Hisar Surkha at different physiological stages of maturity.

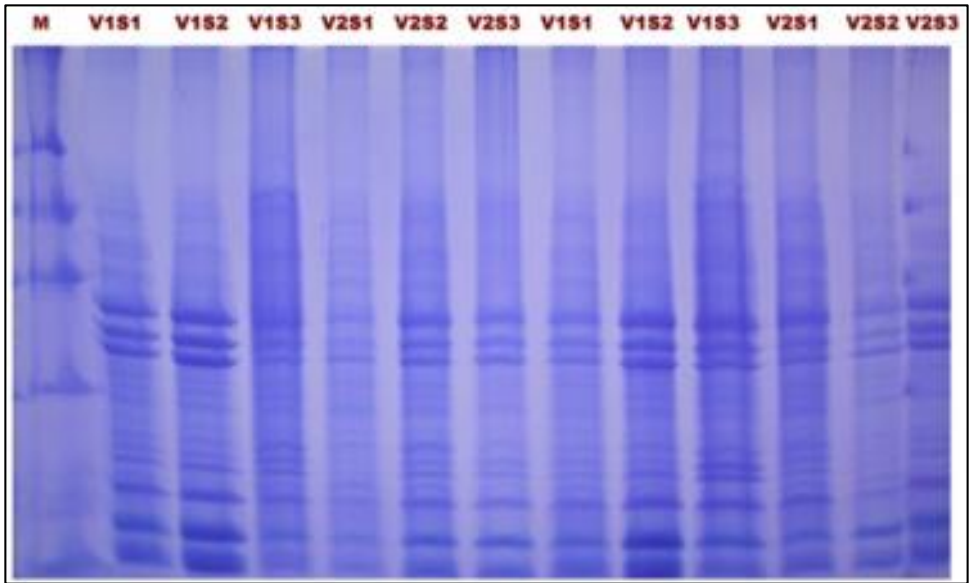
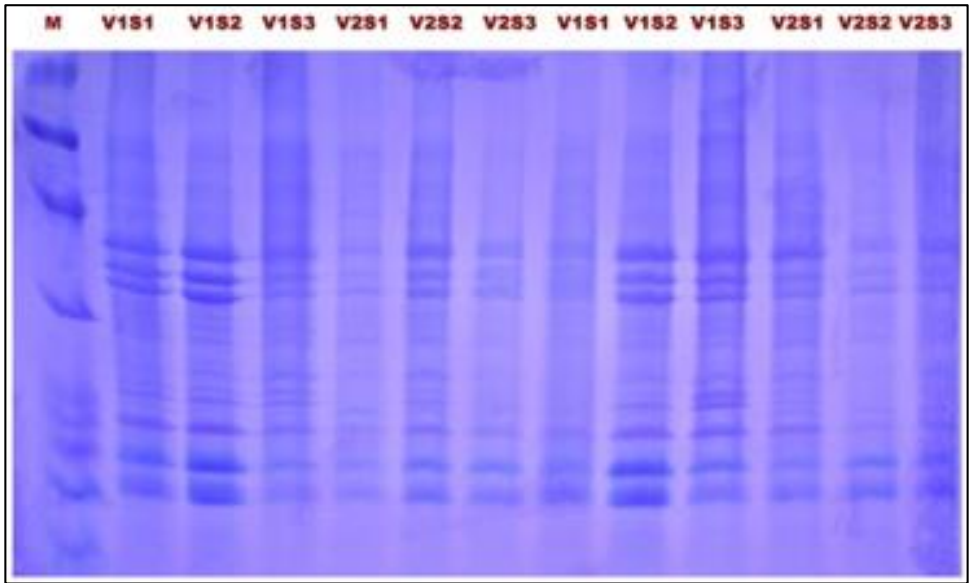


Plate 3: Polypeptide banding pattern of rainy season guave fruits at various maturity stages during ripening on – tree.



V1 - Hisar Surkha
V2 - Hisar Safeda

S1 – Green mature stage
S1 – Haif ripe stage
S1 – Full ripe stage

Plate 4: Polypeptide banding pattern of winter season guave fruits at various maturity stages during ripening on – tree.

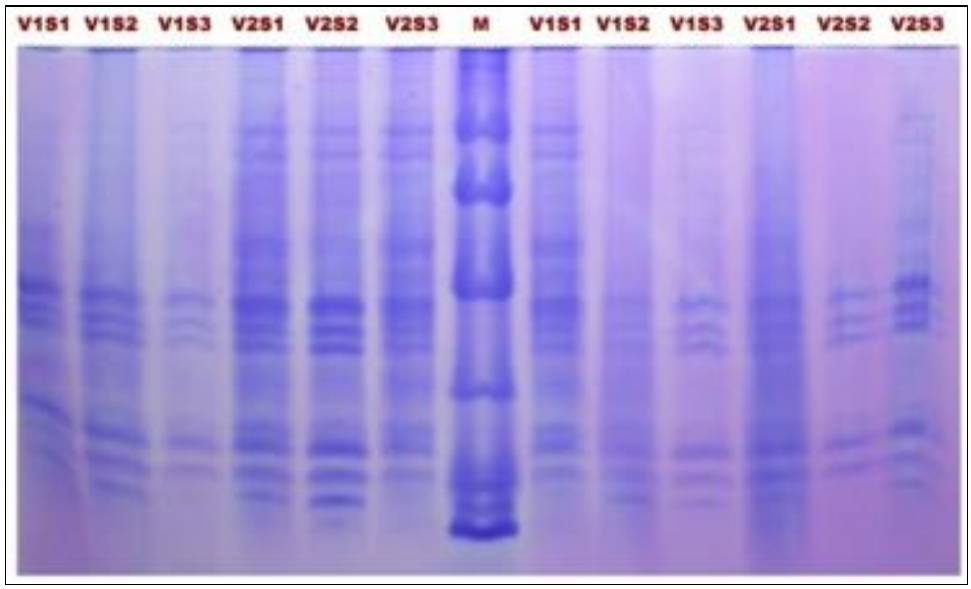
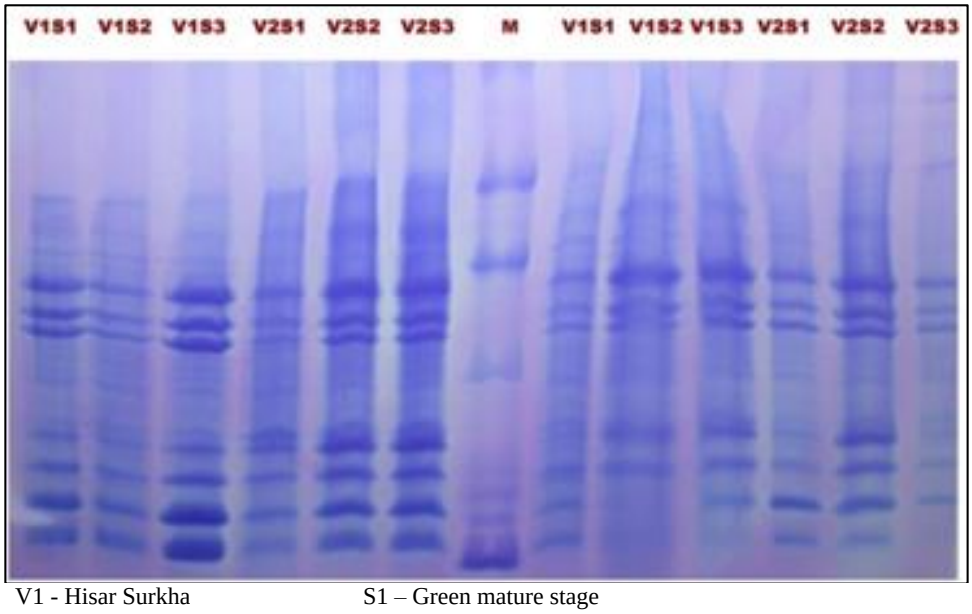


Plate 5: Polypeptide banding pattern of pattern of rainy season guave fruits at various maturity stages during ripening in – storage.



V1 - Hisar Surkha
V2 - Hisar Safeda

S1 – Green mature stage
S1 – Haif ripe stage
S1 – Full ripe stage

Plate 6: Polypeptide banding pattern of winter of season guave fruits at various maturity stages during ripening in – storage.

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