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Establishment of the relationship among the three morphotypes of Cape gooseberry (*Physalis peruviana* L.)

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Abstract
Cape gooseberry is a minor fruit crop and considerable variations have been recorded from the morphotypes collected from seven different districts of Uttar Pradesh, where Cape gooseberry is grown on a commercial level, based on the vegetative parameters and physico-chemical analysis of fruits of Cape gooseberry morphotypes collected from different districts of Uttar Pradesh. However, there was no any research available regarding the molecular level electrophoresis separation of macro molecule of Cape gooseberry leaves, it was the first time when research on Cape gooseberry at molecular level done. Hence, in order to analyse the variability among different morphotypes of Cape gooseberry at the molecular level the three morphotype of Cape gooseberry are grown together at different plots in the field and experiment conducted. After analysis of the photographed gel it was reported that most of the bands were similar and specific bands were rare. High molecular weight proteins were located at the upper portion of the gel; while low molecular weight proteins were located in lower side of the gel. The protein pattern of the three morphotypes (morphotype-1, morphotype-2, morphotype-3) when studied under electrophoresis it was observed that at different kDa the density of bands was found different, it indicates that at particular molecular weight the level of a particular protein of a morphotype may be increases or decreases. The variation was also recorded that at different doses of protein the level of protein among three morphotypes were found different.

Keywords: morphotypes, electrophoresis, molecular characterization, SDS-PAGE

Introduction
Cape gooseberry (*Physalis peruviana* L.) of family Solanaceae, with chromosome number 2n=24 is the only important annual herbaceous, minor tropical fruit crop of India, which has potential for use as nutraceutical (Ramadan and Morsel (2004)) [8]. It is a native of South America (Klinac (2012)) [3]. It is herbaceous in nature and reaches 2 to 3 feet height under favorable growing conditions (Ramadan and Morsel (2004)) [8]. The fruit is a berry with smooth waxy orange yellow skin. *Physalis peruviana* L. is rich source of bioactive compounds therefore, it is considered as functional food. The protein content of Rasbhari ranges from 0.05g/100g to 2.01g/100g (Ramadan and Morsel (2004)) [8]. SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis) has proved to be an effective technique for establishing variability at the molecular level among morphotypes of different crops like citrus (Zukas and Breksa (2005)) [12], kiwifruit Miraghaee *et al.* (2011) [6], olive Wei Wang *et al.* (2003)) [11] etc. SDS-PAGE is a high resolution method used universally for analyzing the mixture of proteins according to their respective size. SDS solubilized in soluble proteins makes possible the analysis of the other insoluble mixtures. Separation of the protein does not occur due to similar charge: mass ratio (Z/m). Therefore, such proteins are treated first with an ionic detergent called sodium dodecyl sulfate (SDS) before the start and during the course of electrophoresis (PAGE). Therefore, such electrophoresis is called SDS-PAGE. In analysis of a complex mixture of proteins, the resolution is improved by the initial movement through a stacking gel. The final bands in the separating gel are sharper and focused in a better way. After electrophoresis, mixture of protein is separated as discrete bands (Sengar *et al.* (2011)) [9]. A proteomics approach using two-dimensional electrophoresis analysis in combination with mass spectrometry has the potential to be a powerful tool in the selection and evaluation of new varieties. This approach permits simultaneous separation and identification of hundreds of proteins. (Zukas and Breksa (2005)) [12]. However, the electrophoretic separation of proteins from plant tissue extracts is often complicated by other nonprotein contaminants indigenous to the plant, such as organic acids, lipids, polyphenols, pigments, terpenes, etc. (Wei Wang *et al*, (2003)) [11]. These need to be processed for accurate estimation of the protein bands.

The realization of the full potential of two dimensional electrophoresis (2-DE) is dependent on good sample preparation, (Zukas and Breksa, (2005)) [12].

Materials and Methods

The present investigation entitled "Establishment of the relationship among the three morphotypes of Cape gooseberry (*Physalis peruviana* L.)*''* was carried out at Horticulture research laboratory of the Department of Horticulture, School of Agricultural Science and Technology, Babasaheb Bhimrao Ambedkar University Luck now,. Geographically Lucknow is situated at an elevation of 111 meter above the mean sea level in the sub-tropical climate of central Uttar Pradesh at 20.55^0 North latitude and 80.52^0 East longitude. The climate of the region is subtropical with maximum temperatures ranging from 22 ^{0}C to 45 ^{0}C in summer and minimum temperatures ranging from 3.5 ^{0}C to 15 ^{0}C in winter and relative humidity ranging from 60% to 80% in different seasons of the year. Lucknow is characterized by sub-tropical climate with hot dry summer and cold winter. Nearly 85% of the total annual rain fall (750mm) is received during the monsoon.

The leaves were collected at 5th node from the plants selected randomly at fruiting stage and kept in polyethylene bag, washed with water and wiped properly for removal of dirt particles then the sample was kept in aluminium foil and stored at -20 °C until extraction of protein. All the chemicals like Tris HCl, Bis-acrylamide solution, Ammonium acetate used were procured from HiMedia Laboratories Pvt. Ltd. The protein was extracted from the green leaves by liquid nitrogen method using Hurkman extraction buffer, PVPP (polyvinylpyrrolidone), Ammonium acetate etc. The SDS-PAGE was employed by using Cavoy Mini P-4 Vertical Electrophoresis System, MP-8003 offered by Br Biochem Life Sciences Pvt. Ltd. from New Delhi, India. The SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was followed by using the chemicals like acrylamide, sodium dodecyl sulfate etc. The glass sandwich was filled with the composition of two types of gel, the lower 2/3 part is filled with the separating gel and remaining portion was filled with the stacking gel. The composition of the separating and stacking gel is given in the table-1

7.5% of uniform concentration of SDS was prepared then gel electrophoresis apparatus was assembled and gel solution of 12.5% of the separating and 4% of stacking gels was prepared, APS and TEMED were added at the end just before pouring the gel solution into the glass sandwich, the glass sandwich was filled with micropipette up to mark with the prepared separating gel solution, thereafter for removing the bubbles from the gel the acrylamide solution is overlaid by the N-butanol solution, the N-butanol was removed by using the filter paper. Thereafter, freshly mixed requisite amount of 4% stacking gel solution was added (composition given in the table 1) and Comb was inserted carefully. With the polymerized gel, glass sandwich was placed into the electrophoretic chamber and running buffer is added to the level in both cathodic and anodic chamber. Denatured protein solution was loaded into the well; first one lane was filled with the 10µg standard molecular weight marker protein. Electrodes of the apparatus was tightly connected with the power supply and gel was run at 50 mA during stacking gel and 100 mA during separating gel, with the dye (bromophenol blue) mobility of the sample was tracked in the matrix. After completion, button was switched off and Gel was transferred to the staining tray containing the gel staining dye and stained overnight. At this stage the whole gel turned blue and shaked on a rocker shaker for 30 min, destaining solution was added and these steps were repeated until bands are clearly visible in gel and photograph was taken for analysis. Analysis of the gel was done by using the two applications *i.e.* Adobe photoshop and Image J software. The selected portion of the bands was cut and pasted by the application of Adobe photoshop and analysis was done by using image. The reading of the protein in quantitative densiometer at different KDa was recorded.

Table 1 Composition for casting gel-

Components	Separating gel	Stacking gel
	12.5%	**4%**
Distilled water	1.6ml	1.22ml
Acrylamide	2.1ml	260µl
Tris (1.5)	1.25ml	0.5ml (0.5 M)
Sodium dodecyl sulfate (10%)	50µl	20µl
Ammonium per sulfate	25µl	20µl
TEMED	7µl	10µl

Results and Discussion

Studying genetic variation has always been regarded essential to understanding gene pool, to guide in order to collect germplasm and to breed new fine cultivars. It is also necessary to identify affinities and similarity of plants for more comprehensive comparison of cultivars. Different methods such as protein and DNA molecular markers, or cytogenetical and biochemical, or morphological properties have been used for the analysis of genetic variations and cultivars identification (Collard and Mackill, (2008) [1]; Jaradat and Shahid, (2006) [2]; Malik *et al.,* (2009) [4]; Miernyk and Hajduch, (2011) [5]; Pettengill and Neel (2008) [7]; Tavaud-Pirra *et al*. (2009)) [10]. As the major purpose of the present study, it is tried to deeply observe the three morphotypes of *Physalis peruviana* L. (Cape gooseberry) for variability at the molecular level. This can assist the other researchers for obtaining a better viewpoint about correlation of variability at morphological and molecular level among the three morphotypes of Cape gooseberry. Similar study was also conducted in the wheat where SDS-PAGE was used to evaluate protein pattern after applying water stress. Thirty five protein bands appeared. Most of the bands were similar in the entries and specific bands were rare. Under drought stress, high molecular weight proteins were intensified, while low molecular weight proteins were faint. In results it is concluded that the effects of drought stress, proteins with low molecular weight which are located at the bottom of the gel have been intensified while proteins with high molecular weight which are located at the above of the gel have been weakened Moradpour *et al.* (2014) [13].

Variability among the different morphotypes of Cape gooseberry (*Physalis peruviana* L.) collected from different districts of Uttar Pradesh was characterized through SDS-PAGE of the proteins extracted from the leaf of the morphotypes. Separating gel of 12.5% and stacking gel of 4% composition was prepared and the protein samples in different concentrations were loaded on the gel. This was resolved at 50 mA during the stacking gel and 100 mA during the separating gel. It was apparent from the gel (fig-1) that the proteins of the three morphotypes at the different concentrations resolved into different bands which corresponded with the standard protein bands of the pre stained protein ladder loaded in the first lane. These bands showed varying densities among the three morphotypes. The first morphotype (Morphotype-1) at 20 µg protein sample showed 9 resolving bands, relative molecular weight mainly

ranging from 165-15 kDa, only 2 bands were heavily stained showed high density of protein. Similarly other morphotypes of different concentrations of protein sample also showed varying range of density and different number of bands. (Table-2).

Table 2: Number of bands and density of bands in different morphotypes of Cape gooseberry (*Physalis peruviana* L.) observed in SDS-PAGE at different concentrations of protein loaded on the gel.

		Morphotype-1		Morphotype-2		Morphotype-3	
		At 20 µg	At 40 µg	At 20 µg	At 40 µg	At 20 µg	At 40 µg
Number of bands		9	11	12	10	11	12
Density of bands							
•	High	4,7	3,5,8	5,7,8	3,5,7	3,4,6,7,8	3,4,5,6
•	Medium	3,9	1,2,10	3,9,12	1,2,8	1,2	1,2,8,9
•	Low	1,2,5,6,8	4,6,7,9,11	1,2,4,6,10,11	4,6,9,10	5,9,10,11	7,10,11,12

However, for better accuracy and clear observation, gradient gel analysis was also followed by using separating gel (gradient gel) 12.5%, stacking gel 4%, and in one lane pre stained protein ladder 10µg was employed, all three morphotypes were replicated, in first replication 20 µg while in second replication 40µg protein sample was loaded and it was recorded that the all three morphotypes were showing varying densities of protein content in the bands. The first morphotype (Morphotype-1) at 20 µg protein sample showed 8 resolving bands, from 165- 8 kDa, 4 bands were dark stained showed high density of protein. However, there may be a group of many proteins in heavily stained bands. Similarly other morphotypes at different doses of protein sample also showed varying range of density and different number of bands. (Table-3) The performance of the protein in bands was analyzed (fig-2). Analysis of the gel was done by using the two softwares *i.e.* Adobe photoshop and ImageJ. The selected portion of the bands was cut and pasted by the application of Adobe photoshop and analysis was done by using imageJ. The reading of the protein in quantitative densiometer at different kDa was recorded and represented in table-4 and figure-3

Table 3: Number of bands and density of bands in different morphotypes of Cape gooseberry (*Physalis peruviana* L.) observed in gradient gel in SDS-PAGE at different concentration of protein loaded on the gel.

		Morphotype1		Morphotype2		Morphotype3	
		At 20 µg	At 40 µg	At 20 µg	At 40 µg	At 20 µg	At 40 µg
Number of bands		8	9	10	8	9	11
Density of bands							
•	High	5,4,7	3,5,8	5,7,8	3,5,7	4,6,7	3,4,5,6
•	Medium	3,	1,2,6	1,3,9,12	1,2	1,2,3,8	1,2,7,9
•	Low	1,2,6,8	4,7,9	2,4,6,10	4,6,8	5,9	8,10,11

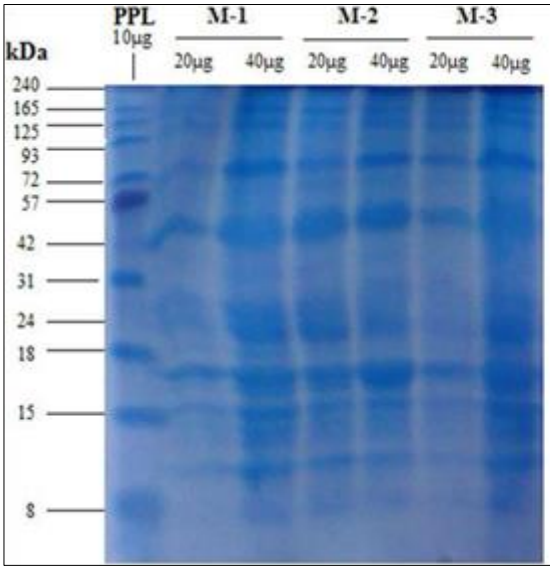

Fig-1 Gel using SDS-PAGE

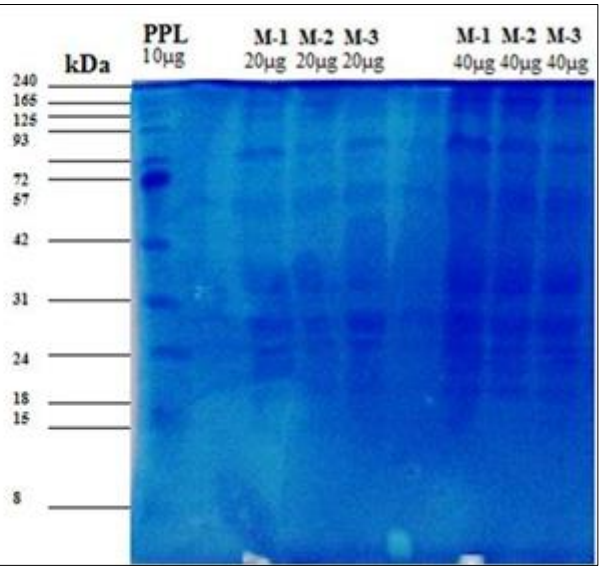

Fig-2 Gradient gel using SDS-PAGE

Where, PPL=Prestained Protein Ladder
M=Morphotype
kDa= Kilodalton

Table 4: Density of bands at 20 and 40µg of protein sample extracted from Cape gooseberry (*Physalis peruviana* L.) in quantitative densiometer recorded at different kDa (kilodalton).

		At 20 µg			At 40 µg	
Molecular	**M-1**	**M-2**	**M-3**	**M-1**	**M-2**	**M-3**
Weight						
72 kDa=	48046.16	43427.02	37106.67	41354.24	30560.39	23894.94
72000g/mole						
42kDa=	47458.66	40293.14	34391.38	39429.72	42465.67	51031.82
42000g/mole						
31kDa=	53533.007	23260.359	34852.593	42273.258	41414.451	41145.128
31000g/mole						
24kDa=	57374.421	38003.744	54465.401	51421.116	55418.338	49714.158
24000g/mole						

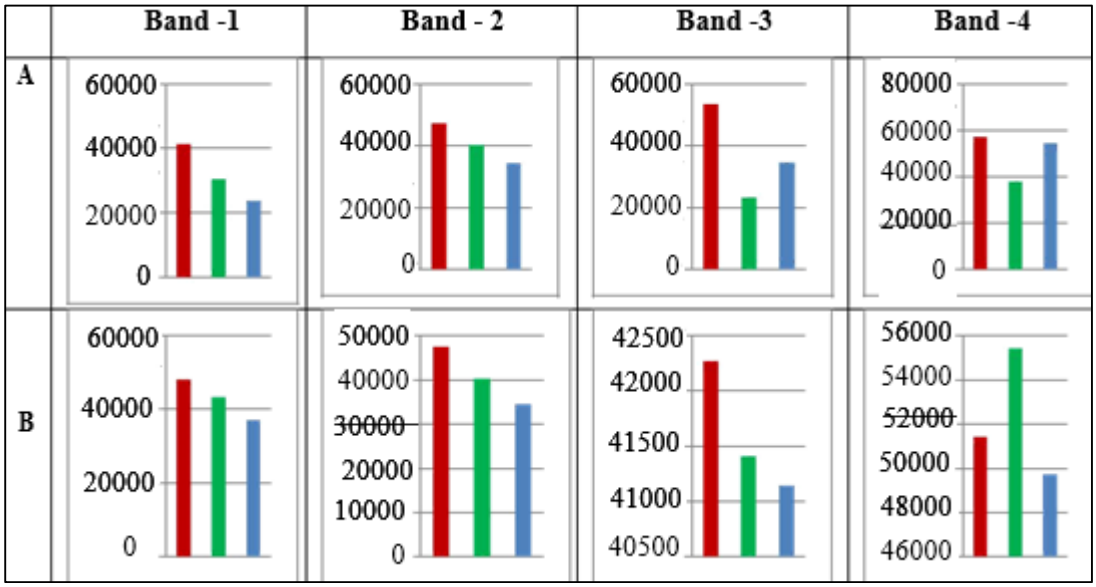

Fig 3: Graphic representation of relative protein content in different bands observed in SDS-PAGE in three morphotypes of Cape gooseberry (*Physalis peruviana* L.) at (A) 20 µg protein sample and (B) 40 µg protein sample for four bands 1, 2, 3 and 4

Morphotype-1
Morphotype-2
Morphotype-3

The protein pattern of the three morphotypes (Morphotype1, Morphotype 2, Morphotype 3) when studied under electrophoresis it was observed that at different kDa the density of bands was found different, it indicates that at particular molecular weight the level of a particular protein of a morphotype may be increases or decreases which is indirectly governed by the activity of m-RNA. The variation was also recorded that at different doses of protein the level of protein among three morphotypes were found different. However, in Cape gooseberry (*Physalis peruviana* L.) this type of research was not reported till date. It is the first research was done to study the variability among the three morphotypes of Cape gooseberry.

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