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Comparative protein profiling study of selected tomato varieties grown in polyhouse and shade house.

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ABSTRACT- *Tomato (Lycopersicum esculentum) is an edible citrus fruit and considered as an important vegetable in our day to day life. In this study three tomato hybrid varieties namely Anagha, Swaraksha, and Sakhtiman were selected for protein quantification and comparison. Aim of this study is to predict growth, yield and disease resistance of these three varieties according to their protein content estimated and separated. Leaves of these varieties were taken from both polyhouse and shade house grown plants for comparison. Folins-Lowry method was employed for protein quantification of them and SDS-PAGE for protein separation. Shade dried leaves and fresh leaves were taken as samples and two methods were adopted for sample preparation. Results showed that plants grown in polyhouse have high protein content than shade house grown plants. Among these three varieties Anagha and Sakhtiman shows more protein quantity than Swaraksha. Based on the results it was clear that the overall growth, yield and disease resistance is high for polyhouse plants than shade house plants; especially Anagha and Sakhtiman varieties are more potent for high yield, growth and disease resistance.*

Keywords; Anagha, Folins-Lowry method, Lycopersicum esculentum, Sakhtiman, SDS-PAGE, Swaraksha.

1. INTRODUCTION

Tomato is the edible citrus fruit of the plant *Lycopersicon esculentum.* Originally, tomato was named after the food family to which it belongs-the *Solanaceae* (sometimes called as 'Solanoid' or 'night shade') family. Regardless of its name, tomato is a wonderfully popular and versatile food that comes in over a thousand different varieties that vary in shape, size and colour. There are small cherry tomatoes, bright yellow tomatoes, Italian pear-shaped tomatoes and the green tomatoes (Anthon GE *et al.* [1]).

Only the fruits of tomato plant are eaten since the leaves often contain potentially problematic concentrations of certain alkaloids. Tomatoes have fleshy internal segments filled with slippery seeds surrounded by a watery matrix. They can be red, pink, yellow, orange/tangerine, green, purple, brown or black in colour (Aldrich HT *et al*. [2]).

Tomatoes provide a unique variety of polynutrients including carotenoids (beta caroteine, lutein and zeaxanthin), flavanoids (nanigenin, chalconaringenin, rutin, kaempferol and quercetin), hydroxycinnamic acids (caffeic,ferulic,coumaric acid), glycosides (esculeoside A) and fatty acid derivatives (9-oxo-octadecadienoic acid) (Slimestad R and Verheul M. [3]).

Tomatoes are also an excellent source of vitamin c and vitamin A which have free radical-scavenging activity and vitamin K and copper that keeps bone healthy. They are a very good source of enzyme-promoting molybdenum, potassium, niacin, vitamin E, vitamin B_1, vitamin B_6, folate, dietary fibre and blood sugar-balancing manganese. In addition, tomatoes are a good source of magnesium, energy producing iron and phosphorus (Borguini RG and Torres Eafds. [4]).

1.1 Polyhouse and Shade house

Polyhouse farming is an alternative new technique in agriculture gaining foothold in rural India and can be successfully employed for niche areas of agriculture. Polyhouse is a tunnel made of polyethylene (prevent ultraviolet rays that are harmful to plants) usually semi-circular, square or elongated in shape. A typical polyhouse is ranges from 400-10,000 m^2; this makes them suitable for farmers with small land holding also. The interior heats up because incoming solar radiation from the sun warms plants, soil, and other things inside the building faster than heat can escape the structure. Air warmed by the heat from hot interior surfaces is retained in the building by the roof and wall. Temperature, humidity and ventilation can be controlled by equipment fixed in the polytunnel.

This methodology of farming reduces dependency on rainfall and makes the optimum use of land and water resources; typical gains may be three times those of traditional farming. It enables cultivation of regular crops in off-season too. Parameters such as moisture,

soil nutrients, solar flux, air movement, humidity, dry bulb and wet bulb temperature etc. inside a polyhouse needs to be controlled to ensure timely and abundant yields. (Yogesh.R. Sonawane, Sameer Khandekar *et al*. [5]).

A shade house on the other hand is a cheaper enclosure made of shade net or shade cloth which is used to protect crops from excessive heat, light or dryness. Sustainable shade house farming for poverty reduction is the one where water is considered to be a scarce resource and used sparingly. For a crop to grow there are certain factors that must be present and they are many but the main ones are water, optimum temperatures, optimum humidity, light energy/sunshine. All these conditions should maintained by a shade house. Actually shade house is similar to plant growing fields, but the difference is that it protects plants from direct sunlight and thereby harmful UV-rays and provides subsequent watering to control humidity.

1.2 Tomato Varieties

Hundreds of different tomato varieties are there, which may include genetically modified varieties, hybrid varieties and so on. These differ in their characteristic features. Anagha, Sakhtiman and Swaraksha are recently developed tomato varieties. Sakhtiman and Swaraksha are hybrid varieties of tomato developed by Namdhari seeds Pvt Ltd. Anagha is an improved variety and is developed by Kerala Agricultural University. Anagha is a high yielding, bacterial wilt resistant tomato variety, tolerant to leaf curl and mosaic diseases. It is also resistant to both radial and concentric fruit cracking (Dr. P.G. Sadhan Kumar and The Hindu-online Edition of India's National news paper). Sakhtiman is a tomato leaf curl virus tolerant hybrid. Fruits have very good keeping quality and are suitable for long transportation. It is recommended for India during summer (Namdhari Seeds-Seeds for a better future). Swaraksha is a F_1 hybrid, moderately tolerant to bacterial wilt and based for North and South India, with a crop cycle of 125-150 days (Agricultural and research and extension unit of food and agricultural research council, 2011).

1.3 Folins-Lowry Method

The determination of protein concentration is an essential technique in all aspects of protein studies and proteomics. The Lowry protein assay is a biochemical assay for determining the total level of protein in a solution. The total protein concentration is exhibited by a colour change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques (Oliver.H. Lowry. [6]).

The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteay phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids (Dunn, [7]). The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained. The major disadvantage of the Lowry method is the narrow pH range of 10 - 10.5 within which it is accurate.

A variety of compounds will interfere with the Lowry procedure. These include some amino acid derivatives, certain buffers, drugs, lipids, sugars, salts, nucleic acids and sulphydryl reagents. Ammonium ions, zwitter ionic buffers, non-ionic buffers and thiol compounds may also interfere with the Lowry reaction. These substances should be removed or diluted before running Lowry assays (Sapan C.V, Lundblad R.L and Price N. C. [8]).

1.4 SDS-PAGE

SDS-PAGE (sodium dodecylsulphate polyacrylamide gel electrophoresis) is a simple and inexpensive method for resolving proteins in complex mixtures. SDS-PAGE is widely used to analyze the proteins in complex extracts. The most commonly used methods are derived from the discontinuous SDS-PAGE system (Laemmli. [9]). The system actually consists of two gels-a resolving (running or separating) gel in which proteins are resolved on the basis of their molecular weights (MWs) and a stacking gel in which proteins are concentrated prior to entering the resolving gel. Differences in the compositions of the stacking gel, resolving gel and electrophoresis buffer produce a system that is capable of finely resolving proteins according to their MWs.

SDS is an amphipathic molecule, consisting of a hydrophobic 12-carbon chain and a hydrophilic sulfate group. The SDS hydrocarbon chain binds to the many hydrophobic groups in proteins, reducing the protein to a random coil, coated with negatively charges along its length. Denatured proteins bind quite a lot of SDS, amounting to ~1.4 g SDS/g protein, or ~one SDS molecule for every two amino acids (Shapiro AL, Vinuela, E.Maizel. [10]).

Naturally occurring proteins are invisible on SDS-PAGE gels. To visualize the positions of proteins after electrophoresis is complete, stain the gels with various dyes that bind non-covalently and with very little specificity to proteins. The most commonly used dyes are the closely related Brilliant Blue R-250 and G-250 dyes, which bind proteins non-specifically through a large number of ionic and Vander Waals interactions (Steinberg.TH. [11]).

2. MATERIALS AND METHODS

2.1 Glasswares

All the glass wares were purchased from Borosil and GenTech.

2.2 Chamicals and Reagents

The chemicals and reagents used for the study were of reagent grade and were purchased from Himedia, Qualigens, Sigma Aldrich, Chromous Biotech.

2.3 Selection of improved tomato varieties

Based on availability, three improved tomato varieties were collected from Agricultural Research Station of Kerala Agricultural University, Anakkayam, Malappuram. The varieties selected were Anagha (Kerala Agricultural University), Sakhtiman (Namdhari seeds Pvt. Ltd.), Swaraksha (Namdhari seeds Pvt. Ltd.). Selected varieties grown in both polyhouse and shade house were considered for comparative protein profiling study.

2.4 Physiological analysis of selected tomato varieties

The physiological parameters such as growth, yield, disease resistance, and fruit shape of the tomato varieties were observed.

2.5 Collection of samples

Leaves and fruit samples of selected varieties were collected from polyhouse and shadehouse of Agricultural Research Station, Anakkayam, Malappuram. Samples at different stages of plant growth were considered for protein profiling study. They were:

a) Leaves before flowering stage.
b) Leaves at flowering and fruit producing stage.
c) Leaves after flowering stage(at fruit developing stage).
d) Ripened fruit.

2.6 Preparation of samples

i. Fresh samples: Collected fresh leaves and fruits were thoroughly washed with tap water and then with double distilled water and allowed to air dry.
ii. Shade dried sample: Thoroughly washed leaves were shade dried and powdered using mortar and pestle.This powder was wrapped with aluminium foil and stored under sterile condition.
iii. Sample preparation for estimation of protein content: 0.01g of each fresh leaf, shade dried leaf sample and tomato fruit were weighed accurately and ground with 1ml of 5X sample buffer. Vortex mixed and centrifuged at 3000 rpm for 30 minutes at 4°C in a vial. After centrifugation supernatant was transferred into another sterile vial and kept at 4°C.
iv. Sample preparation for SDS-PAGE: 0.01g of each protein sample were weighed accurately and ground with 1ml of 5X sample buffer. Vortex mixed and centrifuged at 3000 rpm for 30 minutes at 4°C in a vial. After centrifugation supernatant was transferred into another sterile vial and kept at 4°C.

2.7 Estimation of protein content

Folins-Lowry method was adopted for the estimation of protein concentration in each selected sample. Different dilutions of BSA (Bovine Serum Albumin) solutions were prepared by mixing stock BSA solution and distilled water (10 mg/10 ml) in the test tubes. The final volume in each test tube made up into 5 ml. 0.1 ml supernatant of each sample was taken in test tubes and made up into 5 ml with distilled water.

From these different dilutions, pipetted out 0.2 ml protein solution (both standard and samples) to different test tubes and added 1 ml copper sulphate reagent (Analytical reagent). Mix the solution thoroughly. These solutions were incubated at room temperature for 10 minutes. Then added 0.1 ml of freshly prepared Folins- Ciocalteau reagent to each test tube and incubated for 30-60 minutes in dark.

After incubation, the spectrophotometer was set to zero with blank and taken the optical density (measure of absorbance) at 660 nm. Plot the absorbance against protein concentration to get a standard calibration curve. Checked the absorbance of unknown sample and determined the concentration of the unknown sample using a standard graph.

2.8 Sodium dodecyl sulphate-polyarylamide gel electrophoresis (SDS-PAGE)

For the separation of proteins present in the selected samples SDS-PAGE technique was employed. The technique include following steps:

i. The glass plates were assembled as described by the manufactures.
ii. Separating gel solution was prepared as per composition.
iii. APS and TEMED were added at last and mixed carefully to avoid formation of bubbles (Note: Polymerization begins as soon as APS was added to the mixture. So all subsequent actions must be performed promptly).
iv. Then poured the gel solution between the glass plates with a pipette, leaving about one fourth of the space free for the stacking gel. Carefully covered the top of the separating gel with ice cold isopropanol and left until the gel polymerizes (~30 minutes).
v. A clear line will appeared between the gel surface and the isopropanol on top when the polymerization was completed.
vi. Discarded the isopropanol gently and washed with double distilled water.
vii. Poured the stacking gel solution (prepared as per composition) carefully with a pipette to avoid formation of bubbles (Note: Polymerization begins as soon as APS was added to the mixture. So all subsequent actions must be performed promptly).

viii. Comb was inserted and allowed the gel to polymerize for at least 60 minutes.
ix. After polymerization removed the comb carefully. Put the gel tank and filled the tank (bottom and top of reservoirs) with fresh 1X tris glycine- SDS buffer and made sure that the gel wells were covered with the buffer.
x. Stored supernatant was taken and diluted according to the protein content estimated.
xi. Loaded 15 µl of protein ladder in the first well and the test samples in the remaining wells.
xii. Set an appropriate voltage (200V-30A). Increased the power when the dye front reaches the running gel.
xiii. Stopped the electrophoretic run when the dye front reached the bottom of the gel. Disassembled the gel sandwich and proceeded with gel staining after removing stacking gel.
xiv. The gel was stained with Coomassie brilliant blue for an hour.
xv. After staining, destained the gel by destaining solution.
xvi. Observed the protein bands under UV-trans illuminator.

3. RESULT AND DISCUSSION

3.1. Result

From this study it was obtained that the selected three tomato varieties were differ in their characteristic features, protein content and nature of proteins. It was observed that polyhouse grown varieties are better in the growth, yield and disease resistance compared to shade house varieties in spite of its nature. Polyhouse varieties were grown very rapidly and they were tall. Yield also high in polyhouse varieties. Shade house varieties were not too tall like polyhouse varieties, they were short and growth is very slow when compared to polyhouse. The yield of such varieties was also less. Fruits of shade house grown Anagha and Swaraksha varieties have high content of seeds. Fruits of remaining varieties were found to be seedless.

3.1.1. Physiological analysis of selected tomato varieties

1. Anagha:

This variety showed high yield with reddish, round, medium-sized fruits of an average weight of 45 g. The plants of this variety grown to an average height of 67 cm and started yielding the fruits from 90th day after sowing and were free from green shoulder and observed resistance to diseases.

2. Sakhtiman:

An outstanding yielder with very good leaf cover. Plants were grown with a height of 100-200 cm and fruits were extremely uniform, smooth, oval shape with an average weight of 90-100g. Excellent fairness and colour were the key traits of this variety. Fruits have very good keeping quality and shoulder colour was light green. It was showed high resistance to diseases.

3. Swaraksha:

The variety with determinate growth habit and showed 60-65 days maturity. Fruits were obtained as round in shape with 4 locules and of uniform size with an average weight of 90-100g. They were moderately tolerant to diseases.

3.1.2. Estimation of protein content

Protein concentration of each sample determined by Folins-Lowry method. It was found that varieties grown in polyhouse have high protein content than the shade house varieties. Among the selected tomato varieties Sakhtiman has high content of protein at each stage of growth in both polyhouse and shade house conditions, followed by Anagha. Comparatively Swaraksha has low protein content at each stage of plant growth in polyhouse and shade house. It was observed that protein concentration were high in shade dried leaf sample than the fresh leaf sample. From this it was clear that protein concentration decreases with the presence of water and thereby dry weight showed more protein content during estimation. Finally it was found that protein concentration was high in leaves at flowering and fruit developing stage and in Ripened fruits (Chart 1 to 4).

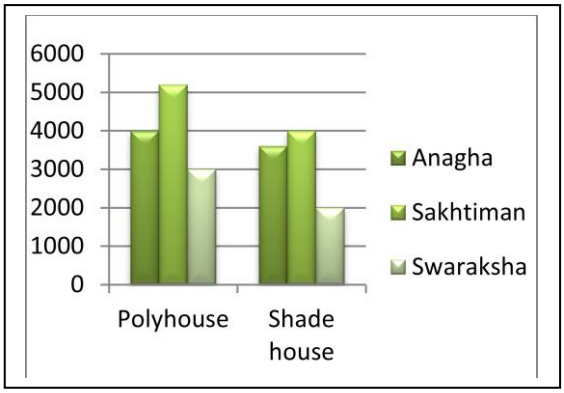

Chart- 1: Difference in protein content of leaf sample grown in polyhouse and shade house before flowering stage.

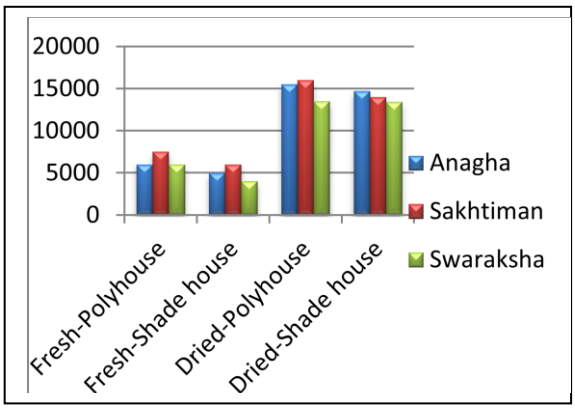

Chart- 2: Difference in protein content of sample grown in polyhouse and shade house at flowering stage fruit developing stage. It also depicit the protein content difference in fresh and dried leaf sample.

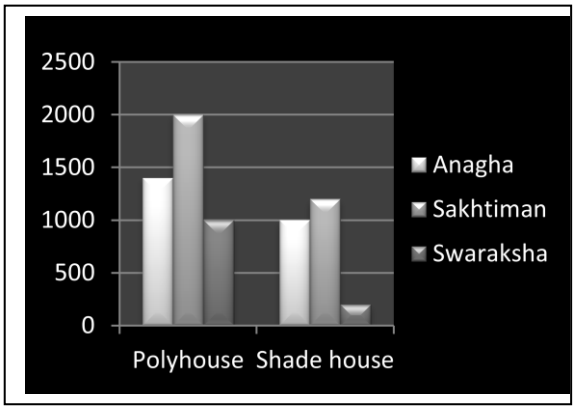

Chart- 3: Difference in protein content of leaf sample grown in polyhouse and shade house at fruit ripening stage (after flowering stage).

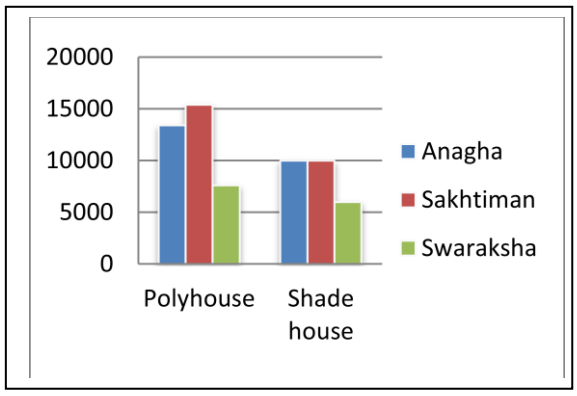

Chart- 4: Difference in protein content of ripened fruit sample grown in polyhouse and shade house.

A colour difference was also observed during sample prepared for protein estimation. Minute colour difference was obtained at the stage of before flowering. Dark colour observed in the case of polyhouse grown varieties than shade house varieties (Figure 1). At flowering and fruit developing stage, greenish colour was observed in all the three polyhouse varieties. But in the case of shade house varieties the colour was blackish brown (Figure 2). Slight colour difference was only observed during estimation of leaf sample after flowering stage (Figure 3). Dark blue colour was observed in fruit sample of polyhouse grown varieties. The colour was green in the case of shade house fruit samples (Figure 4). From observations regarding colour change during sample preparation was a good indication of protein concentration gradient of samples at different stages. Dark colour was observed for polyhouse tomato varieties compared to shade house varieties. Colour change was clearly observable during flowering and fruit developing stage and also in ripened fruit samples. It was also an excellent proof for protein content difference of selected varieties.

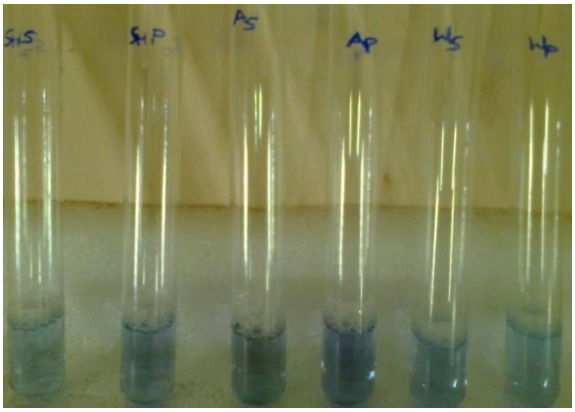

Fig- 1: Colour difference of sample at before flowering stage.

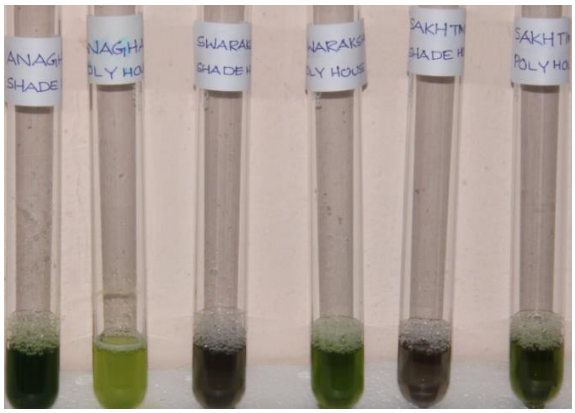

Fig- 2: Colour difference of sample at flowering and fruit developing stage.

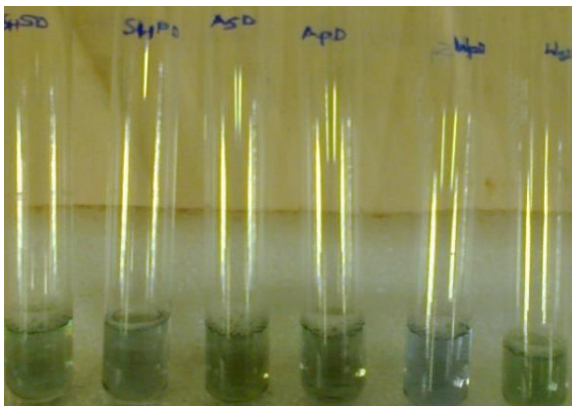

Fig- 3: Colour difference of sample at fruit ripening stage (after flowering stage).

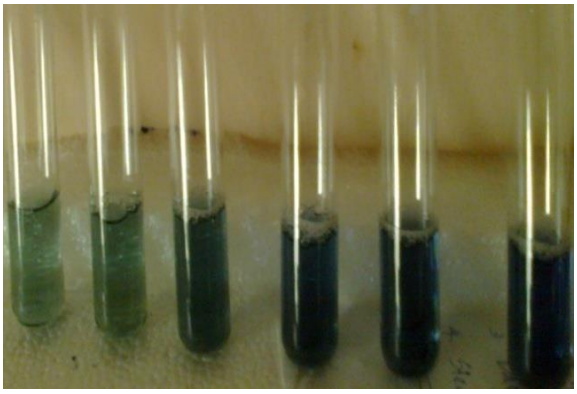

Fig- 4: Colour difference of ripened fruit sample.

3.1.3 SDS-PAGE

Based on the protein content estimated the samples were loaded and separated according to their molecular weight at different stages of plant growth by using SDS-PAGE. Protein ladder of molecular weight ranges from 30-100 kDa was used as standard/control.

Sample of before flowering stage only one band was separated with an average molecular weight of 50kDa in Sakhtiman (shade house and polyhouse) and in Swaraksha grown in polyhouse. In the case of Anagha (both shade house and polyhouse) and Swaraksha (Shade house), separated protein had a molecular weight in between 50-60 kDa and the separated band is thick in polyhouse grown varieties and comparatively thin in shade house plants (Figure 5).

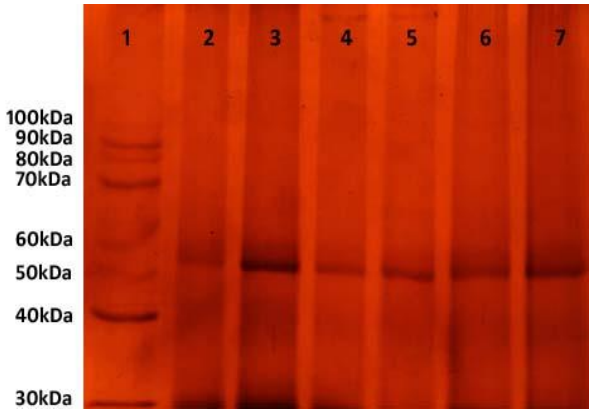

Fig- 5: Protein separated in fresh leaf sample before flowering stage by SDS-PAGE.

Two common protein bands were observed inn fresh leaf sample at flowering and fruit developing stage (Figure 6). One band with molecular weight of 50kDa was found in all varieties and is thick in all polyhouse varieties than shade house grown varieties. Especially it was very thick in Sakhtiman variety. Another common band was obtained with an average molecular weight of 40kDa. It was also thick in polyhouse grown varieties. A number of different protein bands with different molecular weight were obtained in Sakhtiman variety (30kDa, 60kDa etc), especially polyhouse growing Sakhtiman have more protein bands. Three varieties grown in polyhouse have a lot of protein bands.

In case of shade dried sample at flowering and fruit developing satge, a band with an average of molecular weight 50 kDa was observed in all varieties and was thick in polyhouse and thin in shade house grown plant samples (Figure 7). A number of protein bands with different molecular weight were viewed in polyhouse varieties especially in Sakhtiman and Anagha varieties. A band with molecular weight 100 kDa was found in Anagha, Swaraksha and Sakhtiman varieties grown in polyhouse.70 kDa protein band was observed in all the three varieties grown in shade house.

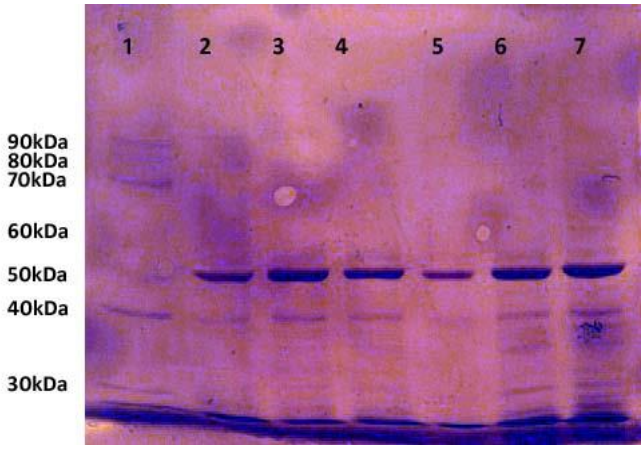

Fig- 6: Protein separated in fresh leaf sample at flowering and fruit developing stage by SDS-PAGE.

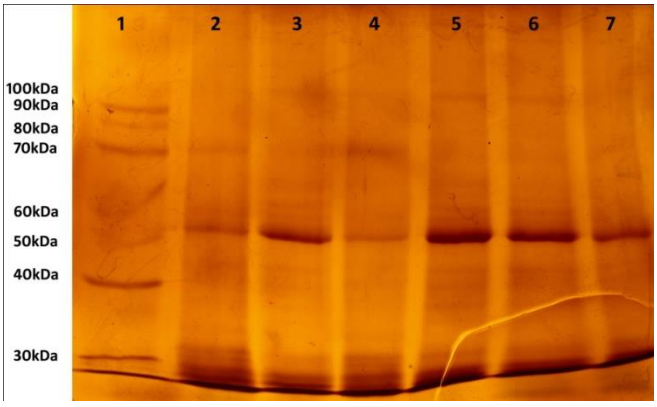

Fig- 7: Protein separated in shade dried leaf sample at flowering and fruit developing stage by SDS-PAGE.

Single protein band with an average molecular weight of 50 kDa were viewed in sample after flowering stage (Figure 8). A number of protein bands with different molecular weight was observed in ripened fruit sample (Figure 9), indicating the sample have a high concentration of protein. In this case also band with molecular weight of 50 kDa was obtained but it was very thin. More number of and thick protein bands were found in Sakhtiman- polyhouse variety. Proteins with

molecular weight 100 kDa, 90 kDa and 80 kDa were viewed in Anagha and Sakhtiman variety. But it was very clear and thick only in Sakhtiman variety grown under polyhouse condition. 70 kDa protein was clearly found in Anagha- polyhouse variety. 60 kDa protein was found in Anagha- polyhouse and Sakhtiman both polyhouse and shade house.

3.2. Discussion

The present work is a comparative study, undertaken to determine the protein concentration and their separation of three improved tomato varieties namely-

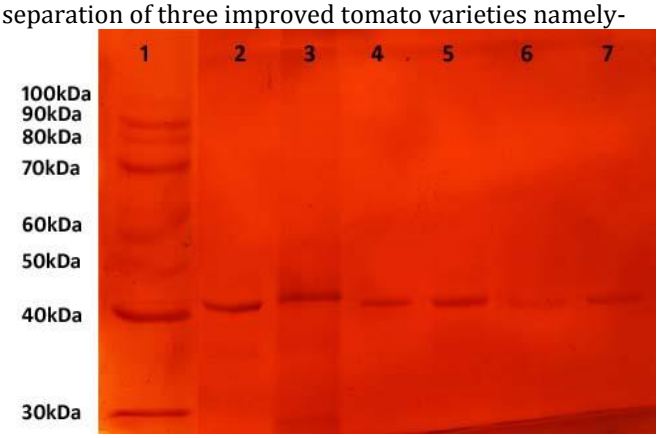

Fig- 8: Protein separated in fresh leaf sample at fruit ripening stage (after flowering stage) by SDS-PAGE.

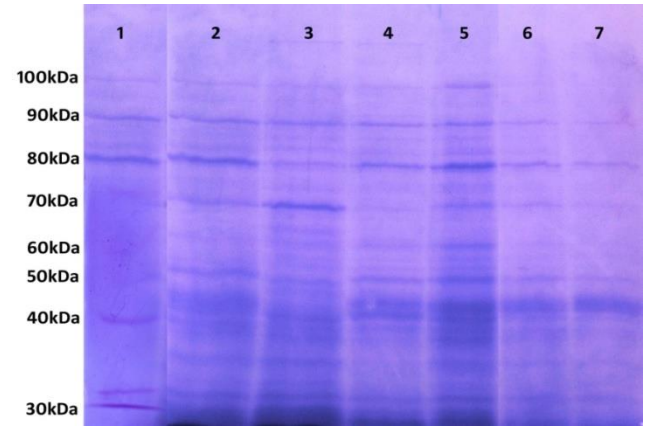

Fig- 9: Protein separated in ripened fruit sample by SDS-PAGE.

Anagha, Sakhtiman, and Swaraksha, grown in polyhouse and shade house to compare them in terms of protein. This may help to predict the growth, yield, and disease resistance of each variety according to their protein concentration.

A colour change was observed during sample preparation is a good indication of protein concentration difference among these varieties. Morphological analysis revealed that Sakhtiman variety is a good yielder and have better growth and disease resistance.

According to Folins-Lowry method, high protein content estimated for Sakhtiman variety grown under both polyhouse and shade house. Less protein content estimated for Swaraksha. Anagha is almost similar in protein content to Sakhtiman.

During the separation of proteins by SDS-PAGE more protein bands also found in the Sakhtiman variety. And in polyhouse grown varieties more proteins were separated according to their molecular weight. In shade house varieties the number of protein bands comparatively less.

An important fact is that, 50 kDa molecular weight of protein observed in all samples at different stages in spite of variety. But it is more potent and thick in leaf sample (particularly at flowering and fruit developing stage). From this it is clear that, it may be an enzyme system or protein having a role in fruit ripening. Because it become thin in fruit sample. May be it have a similar role to 50 kDa protein in tomato, that is, a protein of 50kDa (SBP 50) was identified in plasma membranes of tomato leaves which resembles proteases of the family of Kex2p-like prohormone convertases.

This study show that how protein content of a variety influence its growth, yield, and resistance to diseases. And it is found that Sakhtiman variety has high protein content and by separation got more number of proteins with different molecular weight was obtained. From morphological analysis also it was clear that Sakhtiman variety is a good yielding one and has more disease resistance with rapid growth. So its high protein content may influence all these features.

Anagha variety is almost similar to Sakhtiman, but protein content is not equal to it. Little variation is there in protein concentration. While Swaraksha have low protein content than other two varieties. And number of proteins separated also very low compared to Anagha and Sakhtiman. It is another fact to prove protein content has a great role in influencing growth, yield and disease resistance. Because comparatively growth and yield in Swaraksha and it is mildly tolerant to diseases.

Polyhouse varieties are more proteinaceous than shade house varieties. It may be a cause of high yield and growth in polyhouse than shade house. Growth condition of plants may also have influence in protein content.

4. CONCLUSION

Selected three Tomato varieties- Anagha, Sakhtiman, Swaraksha- differ in their characteristic features and protein content. Sakhtiman is an outstanding yielder with better growth and disease resistance. Protein content is also high in it. Anagha is second most one which shows high protein content and it is in second place in the case of growth and yield also. Swaraksha have comparatively less amount of protein than others and yield and growth of plant also low and is mildly tolerant to diseases.

It was found that polyhouse grown varieties possess high growth, yield and disease resistance than the shade house varieties in spite of its nature. And the protein

content also high in polyhouse varieties than shade house.

A 50 kDa protein was found to be common in all varieties when the proteins of all samples separated by SDS-PAGE, especially in leaf sample. It was thick in leaf sample at all stage when compared to fruit sample and very thick in flowering and fruit developing stage. Similar protein obtained in fruit sample also but it is not much expressive like leaf samples. It may be due to less content of such protein in fruits. The reason may be it is situated in leaves only, it may be an enzyme, having influence in fruit development and ripening. During fruit ripening some extent of it or its components may be transferred to fruits.

Proteins with different molecular weight were observed in all varieties at different stages of plant growth. Protein ladder (molecular weight 30-100 kDa) used to find out the molecular weight of unknown proteins. More proteins also separated in Sakhtiman. Next in Anagha. Swaraksha have less number of protein bands compared to other two varieties.

From all these aspects,it is very clear that protein concentration of each variety influence is growth,yield and resistance to various diseases. Estimation and separation of proteins of each variety at different stages indicates how much protein content of it influence developmental features of of plant at that stage.And it become more helpful to compare the polyhouse varieties and shade house grown varieties,which condition is better to it for good yield nad growth.Another benefit of this study is,it is possible to select more proteinaceous and having high yield,growth,disease resistance interms of protein.So it is more beneficial in the food and agricultural field.

Polyhouse is better for rapid plant growth and for more yield than shade house.And among the selected three improved varieties Sakhtiman is much proteinaceous variety.Anagha is also having almost similar properties like Sakhtiman.But Swaraksha is not much proteinaceous.So for agriculture,food and other purpose Anagha and Sakhtiman are preferable.

5. REFERENCES

[1]Anthon G.E, Le Strange.M and Barrett D.M., "Changes in P^H, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes" J. Sci. Food Agric., 91(7), 2011, pp. 1175-81.

[2]Aldrich H.T, Salandanan.K, Kendall.P *et al*., "Cultivar choice provides option for local production of organic and conventionally produced tomatoes with higher quality and antioxidant content", Sci. Food Agric., 90(15), 2010, pp. 2548-55.

[3]Slimstad. R. and Verheul. M., "Properties of chalconaringrnin and rutin isolated from cherry tomatoes", Agric. Food Chem., 59(7), 2011, pp. 3180-5.

[4]Borguini R.G and Torres Eafds.,"Tomatoes and tomato products as dietary sources of antioxidants", Food Reviews International, 25(4), 2009, pp. 313-325.

[5]Yogesh.R.Sonawane, Sameer Khandekar, Bipin Kumar Mishra, Soundra Pandian K.K., "Environment monitoring and control of a polyhouse farm through internet", 2010.

[6]Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R Slimestad. R and Verheul .M., "Properties of chalconaringenin and rutin isolated from cherry tomatoes", Agric. Food Chem., 59(7), 2011, pp. 3180-5.

[7]Dunn M.J., "Protein determination of total protein concentration", "Protein purification methods", In: Harris ELV, Angal S (eds.) Protein Purification Methods. IRL Press, Oxford, 1992.

[8]Sapan, C.V, Lundblad, R.L, and Price, N.C., "Colorimetric protein assay techniques", Biotechnol. Appl. Biochem., 29, 1999, pp. 99–108.

[9]Laemmli U.K., "Cleavage of structural proteins during assembly of the head of bacteriophage T4", Nature, 227, 1970, pp. 680-685.

[10]Shapiro A.L., Vinuela. E., Maizal J.V., "Molecular weight estimation of polypeptide chains by electrophoresis in SDS- Polyacrylamide gels", Biochem. Biophys. Res. Commun., 28, 1967, pp. 815-820.

[11]Steinberg T.H., "Protein gel staining methods: An introduction and overview", Method Enzymol., 463, 2009, pp. 542-563.