SHRUTI SHARMA

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Career Aspiration

To secure a challenging and rewarding position in a growth-oriented organization, that offers diverse job responsibility in teaching, research and development and fully utilize both my technical and managerial abilities.

Academic/Professional Qualification

Ph.D (Botany), 2020, University of Delhi, N. Delhi.
M.Phil (Botany), 2012, University of Delhi, N.Delhi.
M.Sc (Plant/Crop Physiology), 2010, Allahabad Agriculture University (Under alias of Indian Council of Agriculture Research), Allahabad, Uttar Pradesh.
B.Sc (Hons. Botany), 2007, University of Delhi, N. Delhi.

Career Profile

Project Fellow (Since December' 2018)-In UGC SAP DRS-II project, Department of Botany, University of Delhi.
Project Fellow (Since June'2016- June'2018)- In UGC MRP project, Department of Botany, University of Delhi.

Field of Specialization

Proteomics, Biochemistry, Plant Physiology, Non- model crop proteomics, Deciphering Nitroso-proteome of *Dioscorea,* Identifying and characterizing stress Signal Transduction components and Bioinformatics.

Publications

No. of publications- Six (6) and Two (2) Book Chapters
Publications (in referred journals)

- Sharma, S. and **Deswal, R**. (**2021**). *Dioscorea alata* Tuber Proteome Analysis Uncovers Differentially Regulated Growth-associated Pathways of Tuber Development. *Plant Cell Physiology* .62(1):191–204. doi:10.1093/pcp/pcaa151. *(Impact- 4.07)*
- **Sharma, S**. Gupta, R. and Deswal, R. Deswal (**2017**). *Dioscorea alata* tuber proteome analysis shows multiple dioscorin isoforms and novel tuber proteins. *Plant Physiology and Biochemistry*.114:128-137. *(Impact- 3.7)*
- **Sharma, S**. Sehrawat , A. and Deswal, R. (**2016**). Asada -Halliwell pathway maintains redox status in *Dioscorea alata* tuber which helps in germination. *Plant Science***.** 250 (2016): 20–29. *(Impact- 3.6)*
- Deswal, R., Abat, J. K., Sehrawat, A., Gupta, R., Kashyap, P., **Sharma**, S., Sharma, B., Chaurasia, S., Sougrakpam, Y., Masi, A., Agrawal, G., Sarkar, A., Agrawal, G., Renaut, J. and Rakwal, R. (**2014**). First Systematic Plant Proteomics Workshop in Botany Department, University of Delhi: Transferring Proteomics Knowledge to Next-generation Researchers and Students. *Proteomics.* 14, 1581–1586. *(Impact- 3.25)*
- Arya, M., Chaurasia, S., Sograkam, Y., Babuta, P., Sharma, B., Sharma , S. and Deswal, R. (**2017).** Proteome Analysis of *Brassica,* Seabuckthorn and *Dioscorea* for Dissecting abiotic (Cold/Freezing) Stress Signaling and Redox Modulation. *Botanica.67:24-33.*

- Rakhee, Sethy, N.K., Singh, V. K., **Sharma**, S., Sharma, R.K., Deswal, R., Bhargava, K., and Misra, K. (**2016)**. Phytochemical and Proteomic Analysis of A High Altitude Medicinal Mushroom Cordyceps Sinensis. *Journal of Proteins and Proteomics.* 7(3), 187-197. *(Impact- 1)*

Book Chapters/Proceedings

- Sharma, S. and Deswal, R. (2017). Genomic and Proteomic Tools for Understanding Mysterious Protein Dioscorin for *Dioscorea* Tuber. Zagar Sajid and Rai Vandana (*Eds*). **Plant OMICS and Crop Breeding. Apple academic Press. pp 97-114.**
- Sharma, S. and Deswal, R. (2013). Dioscorea – A wonder plant. Proceedings of National symposium on biotechnology: present status and future prospects (March 15-16, 2013) organized by Department of Biotechnology Deenbandhu Chhotu Ram University of Science & Technology, Murthal – 131039 (Haryana).

Workshops/Conferences

- **Shruti Sharma** & Renu Deswal **(2019).** Poster entitled "Dissecting the *Dioscorea* tuber proteome for a comprehensive understanding of the biological and metabolic changes associated with tuber growth" presented in National Conference on "Neglected and Underutilized Crop Species for Food, Nutrition, Energy and Environment" at National Institute of Plant Genome Research, Delhi, India, 2 August, 2019.
- Poster presented in International conference on Biotechnological innovations in food and health care entitled "Analyzing the role of Nitric oxide (NO) in *Dioscorea alata* tuber for managing its post- harvest shelf life" from **27-28 January, 2019**, at Birla Institute of Technology (BITS) Pilani, Dubai Campus. **(AWARDED THE BEST POSTER).**
- Poster presented in 9th Annual meeting of Proteomics Society of India and International conference on Proteomics in Health and Disease entitles "Change in the redox status triggers tuber germination and showed the involvement of Asada –Halliwell pathway in germination" from **30 November- 2 December, 2017**, at Institute of Life Sciences (ILS), Bhubaneswar, India**. (AWARDED THE BEST POSTER).**
- Poster presented in National Conference "Plant Science research –Looking beyond 21st century for Environmental and agricultural evolution" held from **5-7 Feb, 2016** at Department of Botany, University of Delhi.
- Attended workshop on "Application of Bioanalyzer and off gel Fractionator" at Advanced Instrumentation and Research Facility, JNU held from **17-18 Nov, 2014.**
- Paramananda Barman, Shruti Sharma, Renu Deswal, R Geeta (2013) Alpha Carbonic anhydrases: evolution, expression and activity in a monocot, *Dioscorea* at 7th annual Convention of ABAP and international conference on Plant Biotechnology, molecular medicine and human health from **18 October – 20 October, 2013** at Department of Genetics, University of Delhi, South Campus, Delhi.
- Poster presented on "Purification and Characterization of Dioscorin from *Dioscorea alata* and regulation of its activities by nitric oxide" in the "National Symposium on Biotechnology: Present Status & Future Prospects" held from **15-16 March 2013** in the Department of Biotechnology, DCRUST, Murthal, Sonipat.

Training/ Workshops attended and organized

Organized

- 4thPlant proteomics Workshop/ Training Programme (2019) at Department of Botany, University of Delhi.
- 3rd National Conference of Seabuckthorn Association of India on Seabuckthorn: Translating Research into Sustainable Utilization and Conservation, (2019).
- 3rd Plant proteomics Workshop/ Training Programme (2017) at Department of Botany, University of Delhi.
- 2nd Plant proteomics Workshop/ Training Programme (2017) at Department of Botany, University of Delhi.
- 1st Plant Proteomics Workshop/ Training Programme (2013) at Department of Botany ,University of Delhi.
- Plant Proteomics Society (2014) meeting Delhi Chapter, at Department of Botany University of Delhi.

Professional Experience

- Worked on the changes in the proteome and biochemical analysis of *Diosorea alata* tuber during the tuber growth and development.
- Worked on Purification and biochemical characterization of Dioscorin from *Diosorea alata* tuber from August 2010-March 2012.
- During M.Sc. dissertation worked on "The Physiological response of *Azolla* grown on sewage water" under the guidance of Dr. G. Abraham [The Centre for Conservation and Utilization of Blue Green Algae (CCUBGA)] and Dr. Altaf Ahmad [Jamia Hamdard] from December 2009- May 2009.
- M.Sc mini project : 2 months training in Biopesticides at Allahabad Agricultrural Institute.

Experience in handling Techniques

- Two Dimensional Gel Electrophoresis, SDS-PAGE, Chromatography (Ion exchange, Affinity & Gel filtration)
- Western blotting, Biotin Switch Technique , Affino botting.
- HPLC
- ELISA
- Microtomy, UV-Visible Spectrophotomerty, Off gel fractionator
- Bioinformatic analysis using NCBI, Swiss Prot, Uni Prot, Signal P, Gene Ontology , String, Map Man.

Personal Information

Date of Birth	: 15-12-1986
Languages Known	: English, Hindi and Punjabi

PCP
PLANT & CELL PHYSIOLOGY

Dioscorea Alata Tuber Proteome Analysis Uncovers Differentially Regulated Growth-associated Pathways of Tuber Development

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During its life cycle, the *Dioscorea* tuber undergoes multiple morphological and biochemical changes. To gain a better understanding of the metabolic changes associated with tuber growth, a stage-specific gel-free proteome analysis of four distinct morphological stages namely germinating tuber (S1), degrading tuber (S2), new tuber formation (S3) and tuber maturation (S4) was done and validated by principal component analysis. A comprehensive data set identifying 78.2% of the total 3,681 proteins was generated. PANTHER and KEGG MAPPER revealed both expected (carbohydrate metabolism and redox regulation) and novel biological processes (transcription factors and hormonal regulation) characteristic for each developmental stage. Higher abundance of the enzymes of ascorbate–glutathione cycle and carbohydrate metabolism was detected during tuber germination (S1) and tuber formation stages (S3) in comparison with the mature tuber. The presence of ethylene biosynthesis components during tuber formation hints toward its probable role in postharvest shelf life. The data set comprehensively describes the proteome of *Dioscorea* tuber and provides growth-specific markers for tuber germination (ascorbate peroxidase, monodehydroascorbate reductase, invertase) and tuber formation (sucrose synthase), which were validated by enzyme activity assays and Western blotting. The study provides information that may influence the direction of research for improving the productivity of this under-utilized and largely neglected crop.

Keywords: *Dioscorea* alata • Metabolic pathways • Morpho-staging/phenotypic staging • Proteomics • Tuber formation • Tuber germination.

Introduction

Humans are reliant on plants producing starchy roots and tubers for their nutrition. These crops are cultivated as staple energy sources, second to cereals in the tropical regions of the world (Chandrasekara and Kumar 2016). For example, potatoes and yams are tubers, whereas taro and cocoyams are derived from corms, underground stems and swollen hypocotyls. Cassava and sweet potatoes are storage roots. Yam belonging to the family Dioscoreaceae accounts for 6% of the global tuber production (Adeola et al. 2012). They are rich source of carbohydrates and vitamins E, A and K (USDA NAL 2018). The tubers are used for curing diabetes, neurodegenerative diseases and cancers (Maithili et al. 2011). Dioscorin is the major storage protein constituting 85% of the total tuber protein (1.5 g/100 g, USDA NAL 2018) (Harvey and Boulter 1983). It is a 'multifunctional protein' and possesses α-carbonic anhydrase (CA), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), amylase and trypsin inhibitor activities (Xue et al. 2012). Recently, the existence of the Asada–Halliwell cycle in the tuber was established and its association with tuber germination was shown (Sharma et al. 2017).

A search for *Dioscorea* on National Centre for Biotechnology Information showed 1,255 publications, of which only 5 are related to proteomics (**https://www.ncbi.nlm.nih.gov/pubmed/?term=Dioscorea**) in the last 15 years (till November 15, 2020). Most of the proteomics studies are restricted to the purification and characterization of dioscorin (Tsai et al. 2013). Recently, gel-based analysis (1D and 2DGE) identified >30 dioscorin isoforms in the pI range of 3.2–7.1. The interactome analysis contemplated these to be involved in oxidative stress, carotenoid synthesis and vesicular transport (Sharma et al. 2017).

Tuber growth and development is a complex process. During growth the storage tissue is formed and is supplied with vital biomolecules like carbohydrates, proteins and lipids required for subsequent development (Hannapel et al. 2017). Earlier reports on potato and sweet potato focused on changes in protein abundance profile in microtuber (Desire et al. 1995a), tuber life cycle (Lehesranta et al. 2006) and sink source transition (Borgmann et al. 1994). However, these studies have used plants grown under greenhouse conditions. It is conceivable that there would be many other classes of unidentified proteins, which might be crucial in tuber development in plants grown under natural field conditions. Moreover, despite *Dioscorea* tuber being a promising non-model crop, details about the physiological and biochemical changes associated with the tuber growth and development are missing. To understand

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Asada-Halliwell pathway maintains redox status in *Dioscorea alata* tuber which helps in germination

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ABSTRACT

Reactive Oxygen Species (ROS) are important regulatory molecules governing physiological processes. In the present study a biochemical and proteome level comparison of two contrasting growth stages of *Dioscorea alata* tuber namely germinating and mature tuber was performed in order to understand the tuber physiology and biochemistry. Existence of all the component enzymes [APx (ascorbate peroxidase), GR (glutathione reductase), DHAR (dehydroascorbate reductase), MDHAR (mono-dehydroascorbate reductase)] and major products [ascorbate (ASC) and glutathione (GSH)] of the cycle showed an operational Asada-Halliwell cycle in the tuber. A 2.65 fold increase in ASC content & a 3.8 fold increase in GR activity fortified the redox milieu during germination. In contrast a 5 fold higher H_2O_2 content (due to 3.08 fold lower APx activity) and accumulation of reactive nitrogen species (RNS) such as nitric oxide (NO, 2.4-fold) and S-nitrosothiol (SNO, 2.08 fold) contributed to overall oxidative conditions in the mature tuber. The carbonic anhydrase (CA, 7.5 fold), DHAR (5.31 fold) and MDHAR (7 fold) activities were higher in the germinating tuber in comparison with the mature tuber. GSNO negatively regulated the CA (3.6 & 3.95 fold), MDHAR (7.5 & 1.5 fold) and APx (2.3 & 1.81 fold) while another NO donor, CysNO negatively regulated the DHAR (2.24 & 1.32 fold) activity in the mature and germinating stages respectively indicating again that the lesser inhibition by NO (via nitrosylation) may be because of overall reducing environment in the germinating tuber. Increased SNO leading to S-nitrosylation of dioscorin was confirmed by Biotin switch assay. This is the first report showing dioscorin nitrosylation. The present analysis showed differential redox regulation and also suggests the physiological relevance of CA, DHAR, MDHAR, APx & GR in tuber germination for the first time. These enzymes may be used as potential markers of tuber germination in future.

1. Introduction

Dioscorea (commonly called as yam), a tuberous crop of the family *Dioscoreace* with over six hundred species, is considered a staple crop in Africa, Australia and East Asia. Nutritionally, the tubers are rich in carbohydrate and are valuable sources of vitamins. A lot of medicinal relevance is also associated with the tubers. It is used as herbal medicine for diabetes, aging, neurodegenerative diseases and cancers. Biochemical characterization of the purified dioscorin (85% of the total protein) showed CA, DHAR, MDHAR, trypsin inhibitor (TI) and α-amylase activities [1,2]. The presence of MDHAR and DHAR activity in *D. alata* tuber is already reported indicating the possibility of existence of ASC-GSH cycle which has not been reported from any tuber till date and provided a strong reason for exploring its existence and role in the *D. alata* tuber.

Tuber development and maturation is a highly coordinated process during which the storage tissue is formed and is supplied with vital biomolecules like carbohydrates, proteins and lipids required for subsequent germination. Developmental processes like pinus seed germination [3], wheat kernel maturation [4] and potato tuber development [5] are accompanied with changes in redox status. Upon imbibition the reactivation of metabolism during seed germination may be the source of ROS, so the enzymes and metabolites responsible for ROS scavenging are of particular importance for the success of germination [6]. Ascorbate-glutathione cycle, a set of reactions in which ASC and GSH are regenerated is a vital regulator contributing to redox status [4]. The first enzyme of the cycle is APx which detoxifies H_2O_2 by oxidizing ASC to monodehydroascorbate (MDHA) which is reduced to ASC using nicotinamide adenine

Abbreviations: CA, carbonic anhydrase; DHAR, dehydro ascorbate reductase; MDHAR, mono dehydro ascorbate reductase; NO, nitric oxide; SNO, S-nitrosothiol; 2-DGE, two dimensional gel electrophoresis.

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Research article

Dioscorea alata tuber proteome analysis shows over thirty dioscorin isoforms and novel tuber proteins

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ABSTRACT

In Dioscorea, dioscorin (31 kDa) is the major storage protein constituting 8.5% of the total tuber proteins. An integrated proteomic and biochemical approach was used to understand the physiological role of dioscorin in the two contrasting growth stages (germinating and mature tuber). HPLC analysis showed 3 fold reduction in mannitol and 12.88 and 1.24 fold increase in sucrose and maltose in the germinating tuber. A 1.8 and 3 fold increase in sucrose phosphate synthase and mannitol dehydrogenase activity respectively was observed in the germinating tuber while a 2 fold higher invertase probably lowers the sucrose accumulation in the mature tuber. SDS-PAGE and 2-D maps of the mature and germinating tubers confirmed depletion (more than 50%) of dioscorin on germination. Dioscorin was purified using ion exchange and gel filtration chromatography with 43.32 fold purification and 38.16 yield. Out of a total of 35 spots at 31 kDa only 12 spots (identified as dioscorin isoforms) were present in the 2D gel of the purified fraction. To search for other tuber proteins besides dioscorin, the unbound fractions of DEAE column were analysed by 2DGE. DRB1 1A, caffeic acid 3-O-methyltransferase and Rab-1 small GTP binding protein were identified perhaps for the first time in the Dioscorea proteome. The interactome analysis revealed these to be involved in oxidative stress, carotenoid synthesis and vesicular transport. This is perhaps the first attempt to identify tuber proteome (although limited) and to understand the physiological significance of these proteins.

1. Introduction

Dioscorea (commonly called as yam), a tuberous crop common in Eastern Africa and Asia contributes to 6% of the world's tuber production (FAO 1994). It is regarded as a staple crop and serves as a nutritional supplement (Wanasundera and Ravindran, 1994). Potato, cassava, yam, taro and sweet potato are the major tuber crops cultivated in India, of which yams are the most understudied. *Dioscorea alata* (greater yam), *D. esculenta* (lesser yam) are the major cultivated yams in India (Edison et al., 2006). In China, yams have been traditionally used as a health food and herbal medicine (Liu et al., 2007).

The major storage protein of yam tuber, dioscorin accounts for 80–85% of the total soluble proteins (Harvey and Boulter, 1983). It exhibits carbonic anhydrase (CA), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and trypsin inhibitor (TIA) activities (Hou et al., 1999; Hou et al., 2000). CAs are zinc metalloenzymes that catalyze the interchange of CO_2 and HCO_3^-. In plants, these play an important role in photosynthesis and respiration (Tiwari et al., 1994; Xue et al., 2012). There are at least four distinct CA subfamilies: α, β, γ and δ, with no significant amino acid sequence identities. It is interesting to note that the amino acid sequence of dioscorin is closer to the α-CAs of animals than to plant β-CAs (Hewett-Emmett and Tashian, 1996). On the other hand, DHAR and MDHAR are important enzymes in the ascorbate-glutathione cycle, an antioxidative system for protecting plants from the toxicity of reactive oxygen species (ROS) (Foyer and Noctor 2005, 2011; Gill and Tuteja, 2010). DHAR catalyzes the reduction of DHA to ascorbate (ASC) using reduced glutathione (GSH) as an electron donor (Hou et al., 1999; Yang et al., 2009). Recently, it was shown that CA, DHAR and MDHAR activities are important for germination in *D. alata* tuber (Sharma et al., 2016).

Hou et al. reported that dioscorins isolated from *Dioscorea batatas*, *D. alata* and *D. pseudojaponica* tubers exhibited both CA and TI activities. However, Gaidamashvili et al., 2004 showed that dioscorins DB2 and DB3 from *D. batatas* tubers did not exhibit CA or

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Research Article

PHYTOCHEMICAL AND PROTEOMIC ANALYSIS OF A HIGH ALTITUDE MEDICINAL MUSHROOM *CORDYCEPS SINENSIS*

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Abstract: *Cordyceps sinensis* (*C. sinensis*) is well established as a traditional Chinese medicine (TCM) that has been valued as a health food for centuries. It is an entomopathogenic fungus in Ascomycetes that naturally occurs at high altitude in Himalayan region and has received considerable attention due to the abundance of various biologically active compounds. Despite having reported health benefits and economic importance, qualitative phytochemical analysis, proximate composition and proteome study of Indian isolates of *C. sinensis* grown at high altitude remains untapped. In the present study, qualitative physochemical analysis was carried on powdered whole body of *C. sinensis* (CS_{wb}) and its aqueous extract (CS_{aq}) prepared by accelerated solvent extraction technique which indicated the presence of several bioactive constituents such as alkaloids, amino acids and proteins, carbohydrates, flavonoids and phenols, gums, mucilages and saponins. We evaluated chemical composition of the Indian Himalayan medicinal mushroom *C. sinensis* in terms of its carbohydrate (55.68%) content, crude fiber (6.40%), fat (1.80%), moisture (7.18%), protein (21.46%) and total ash (7.48%). Furthermore, soluble protein identification of both CS_{wb} and CS_{aq} by SDS-PAGE followed by MALDI-TOF-TOF analysis revealed the presence of various types of most abundant proteins such as P-type II A ATPase, TE1b [Blumeriagraminis f. sp. hordei], Chitin synthase Chs [Penicilliummarneffei ATCC 18224], Serine/threonine-protein kinase CLA4, DEHA2C06820p [Debaryomyceshansenii CBS767], YALI0E29887p [Yarrowialipolytica] etc. In conclusion, the present study provides a comprehensive qualitative phytochemical analysis, proximate composition and proteome study on Indian isolate of *C. sinensis* which could endorse its use as a functional food.

Keywords: *Cordyceps sinensis*; phytochemical analysis; proximate composition; proteome study.

Note: Coloured Figures available on Journal Website in "Archives" Section

Introduction

C. sinensis popularly known as "Yartagunbu" or "Dong Chong Xia Cao" (winter worm summer grass) is a high value medicinal mushroom, naturally distributed in China, India, Nepal and Bhutan (Holliday and Cleaver, 2008). *C. sinensis* is a parasitic fungus found at altitude of more than 3,200 meters. It has a characteristic life cycle on the larva of a moth; belongs to *Clavipitaceae* family and the genus *Ascomycetes* (Jang *et al.*, 2015). The wild fungus along with the cultivated varieties as well as cultured mycelia, fruiting body and extracts reportedly possesses diverse medicinal properties (Valverde *et al.*, 2015). Owing to these properties, it has been employed to treat various rehabilitation disorders such as arrhythmias, asthenia after severe illness, bronchitis, cancer, hyperglycaemia, hyperlipidaemia, hyposexuality, liver disease, lungs disorders, night sweating, renal dysfunction and renal failure etc. (Donohue, 1996; Han, 1995; Manfreda *et al.*, 1989; Qiuo and Ma, 1993; Tuli *et*

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Proteome Analysis of *Brassica*, Seabuckthorn and *Dioscorea* for Dissecting abiotic (Cold/Freezing) Stress Signaling and Redox Modulation

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Abiotic stress conditions contribute to almost 70% loss in crop yield. With increasing food demand and limited resources (like land and water), it has become necessary to limit this loss. Although, genomics studies have contributed significantly to abiotic stress biology, the information needs to be complemented by proteome analysis. Proteome analysis of biological systems including plants is significant as proteins are the real executers. Our group is trying to dissect cold/freeze stress signaling using *Brassica juncea* (cold tolerant), tomato (cold sensitive) and Seabuckthorn (cold/freezing tolerant) plants. Anti freeze proteins were purified from Seabuckthorn for cryopreservation of RBCs and Nano biotechnological applications. Cold stress and NO cross talk is also being deciphered focusing on roles of NO based PTMs, S-nitrosylation in particular. To understand stress signaling at subcellular level, cuticle, apoplast and nuclear proteome is being analyzed. Besides, to bridge the supply and demand gap of food, alternate food crops (like tubers) are being explored for their potential usage. Proteome analysis of *Dioscorea alata* to understand the tuber physiology for future crop improvement is undertaken. An overview of groups work is provided in this article.

ANALYSIS OF BRASSICA LEAF CUTICLE PROTEOME

Plant hydrophobic surface "cuticle" provides the largest biological interface which act as a protective barrier against various environmental stresses including drought, temperature extremes, gravity, UV radiations and pathogen infestation as shown in Fig 1. Plant cuticle constitutes cutins, waxes, polysaccharides and minor secondary metabolites (Yeats et al. 2013).

Its synthesis starts in the plastids of the epidermal cells involving complex fatty acid synthesis machinery. Cuticular wax biosynthesis occurs via two distinct pathways, (i) acyl reduction pathway leading to the production of aldehydes and primary alcohols from C20-C34 VLCHCs (Very long chain hydrocarbons), and (ii) decarbonylation pathway which leads to the synthesis of aldehydes, alkanes, ketones and secondary alcohols (Laila et al. 2017). Genetic and environmental factors cause variation in quality (cuticle thickness, composition

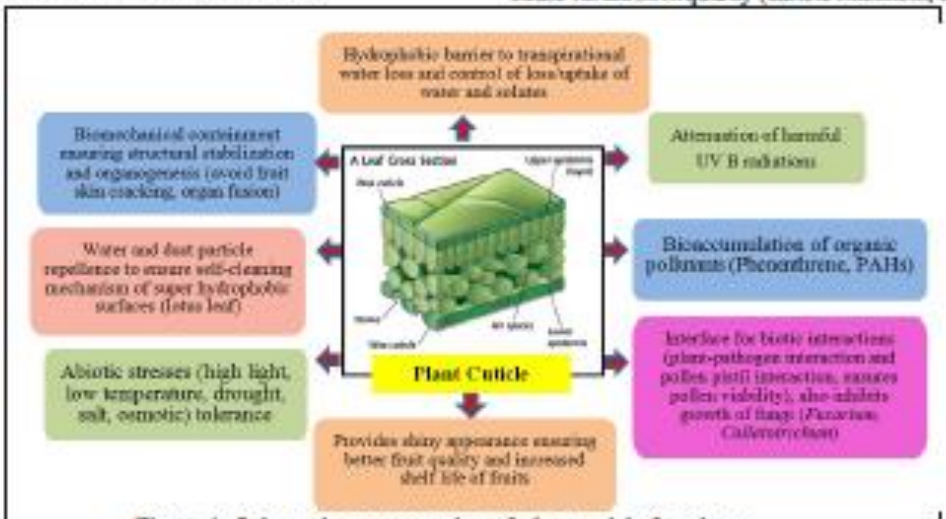

Figure 1: Schematic representation of plant cuticle functions.