Plant cuticular waxes: a review on functions, composition, biosyntheses mechanism and transportation

Hafiz Muhammad Ahmad*[1], Mahmood-Ur-Rahman[1], Qurban Ali[2] and Shahid Iqbal Awan[3]

[1.] Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan
[2.] Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
[3.] Department of Plant Breeding and Molecular genetics, The University of Poonch, Azad Jammu & Kashmir, Rawalakot, Pakistan
Corresponding author's emails: hafizahmad90@yahoo.com, saim1692@gmail.com

Abstract: Plants are simultaneously exposed to various biotic and abitic stresses which effect growth and development of plants causing to reduce yield. The term plant wax is collectively used to describe the lipid components of cuticles covering the areal parts of plants. Cuticular wax layer protects the plants against environmental stresses and work as a waterproof barrier. It reduces the residual transpiration, minimize the non-stomata water loss, shelter plants from ultraviolet radiations, decrease the water deposition on plant surface, minimize the retention of pollens, dust and air pollutants. Plant waxes provide the protection against bacterial, fungal pathogens and reduces plant-insect interactions. Generally cuticular wax is comprised of long chain aliphatic lipids, tritrepenoids, sterols and flavonoids. Aliphetic compounds are synthesized in epidermal cells to from very long chain fatty acids. These very long chain fatty acids are changed to their derivatives to form wax. The aim of present review is to exploit the available information about the composition, functions, biosyntheses process of plant epicuticular wax and factors involved in its regulation so that the information may be helpful for plant biologists to improve the potential of crop plants against environmental stresses.
[Ahmad HM, Rahman MU, Ali Q Awan SI. **Plant cuticular waxes: a review on functions, composition, biosyntheses mechanism and transportation.** *Life Sci J* 2015;12(4s):60-67]. (ISSN:1097-8135). http://www.lifesciencesite.com. 8

Keywords: Cuticular wax, wax composition, drought tolerance, wax biosyntheses

Introduction

Environmental conditions exert prominent effect on the growth and development of plants (Pasha *et al*., 2015). Biotic and abiotic stresses are considered as major factors that cause to eliminate the productivity and yield of agricultural crops. Cuticular wax is a gift of nature for plants to protect them from these stresses, as they don't have the ability of move to protect themselves from the environmental situations. Outer most layer of plants is covered by cuticular wax which is visible as a bluish white colored coating on the surface of stem, buds and leaves, and is termed as waxy bloom. As compared to more glaucous wild type plants, the plants stem in the absence of epicuticular wax layer appear as bright green indicating the lack of wax on its surface. Lipids components of cuticles, covering the areal parts of plants are termed as cuticular wax. In addition to long chain fatty acids, wax is also found in suberin matrix and is associated with underground and wound tissues (Von, 1995). Wax is also thought to be present in lipids of pollen and seed coat (Bianchi *et al*., 1990, Reiter *et al*., 1999).

1-Functions of cuticular waxes

Cuticular wax plays the vital role for the improvement of plant life and protects the plants from both biotic and abitic stresses. To protect the plants from biotic stresses, surface wax plays the role against bacterial and fungal pathogens (Jenks *et al.,* 1994). It also participate in a variety of ways to protect the plants from plant-insect interaction (Eigenbrode & Espelie, 1995). Cuticular waxes eliminate the affection and growth of insects (Muller, 2006) increase resistance of plants against disease causing agents like bacteria & fungi and diminish plant–pathogen interactions (Carver & Gurr, 2006). Waxes have been evolved to repel the droplets of surface water carrying the fungal spores and dust particles (Wagner *et al.,* 2003). Most waxes are derivatives of fatty acids (Kolattukudy, 1973) and work as a hydrophobic barrier between plants and their environment. Leaves are termed as photosynthetic factories in crop plants and are most probably damaged by environmental stresses. Cuticular wax forms continuous hydrophobic barrier to reduce the water loss from plant organs and work as a first line of defense to reduce non-stomatal harmful water loss (Baur, 1998). Outermost hydrophobic layer of cuticle, present on leaves and stems work as drought resistance in plants (Kosma *et al.,* 2009; Bhutta *et al*., 2015). It has been reported that crystalline microstructure of wax have induced the properties of light reflectance in it, which causes to modulate light reflectance hence regulate the temperature and limit the transpiration in plants by regulating the water vaporization (Shepherd *et al.,* 2006). Two components

mainly contribute in plant transpiration i.e stomata conductance which is controlled by regulation of stomata aperture and the residual water loss observed on adaxial leaves deprived of stomata. During water stress, stomata close and cuticular transpiration takes on a significant importance. As an increase of cuticular wax synthesis during water deprivation has been observed in various plants such as tobacco (*Nicotiana glauca* L.) and sesame (*Sesamum indicum* L.), an active role of cuticle in preventing plant desiccation has been proposed (Cameron *et al.,* 2006; Kim *et al*., 2007). It is remained unclear that how drought stress signals are integrated in cuticular wax biosyntheses mechanism and how it is associated at molecular level to response of plant to drought stress (Seo & park, 2011). To shelter the plant from abiotic stresses wax reduces the non-stomatal water loss, reduced amount of epicuticular wax on plant surface shows higher rate of transpiration in crop plants, shelter the plants from ultraviolet radiations (Reicosky & Hanover, 1978; Barnes *et al.,* 1996) and minimize the water deposition on plant surface to reduce the retention of dust, pollen and air pollutants on plant surface (Kerstiens, 1996; Barthlott, 1997). Waxes present in tryphine layer of pollen grains are essential for pollen stigma signaling that is required for fertilization (Preuss *et al.,* 1993). During organ development, cuticle prevents the inappropriate fusion of organ (Sieber *et al.,* 2000). Physiology and quality of fleshy fruits are also disturbed by the presence of cuticle. The external manifestation of fruits such as color, surface, glossiness, and uniformity and post harvest treatments such as storage, transportation and shelf life are also improved due to cuticle barrier (Isaacson *et al.,* 2009; Domínguez *et al.,* 2011). Physical and chemical properties of cuticular wax such as hardness, low surface tension, adhesive strength, high energy content and optical transparency have enabled it for industrial applications. Industrially important products of waxes are plastic, candles, shoe polish and cosmetics in everyday life. It has also gained the importance for the production of biomimetic materials such as superhydrophobic and superhydrophilic tissues and this trend will gear up in future that will make the cuticular wax more important (Javelle *et al.,* 2011).

2-Composition of epicuticular wax

Cuticle consists of cutin, cutan, polysaccharides and organic soluble compounds called as wax (Walton, 1990; Nawrath, 2006; Pollard *et al.,* 2008). Outer layer of cuticle is termed as cuticular wax and physically can be stripped from surface of leaves, stem and fruits by using aqueous glue (Jetter & Schäffer, 2001; Buschhaus & Jetter, 2011). Repeated adhesive application reaches a physical limit at which no additional wax is removed from leaves stem or fruits then subsequent solvent extraction releases additional wax that presumably resided within the cutin and is termed as intracuticular wax. The mechanism of this compartmentalization is still unknown. Cuticular wax is comprised of long chain aliphatic lipids, triterpenoids, sterols and flavonoids are also included as secondary metabolites. Lot of variation is present among the wax composition between species as well as in wax present in same species at different locations. It also has been observed that wax composition is not uniform between two wax layers (Buschhaus *et al.,* 2007, Ji & Jetter, 2008; van & Jetter, 2009). On smallest available scale, we can distinguish different wax layers within two cuticle layers (Jeffree, 2006). Commonly wax comprises long chain linear compounds such as acids, primary and secondary alcohols, aldehydes, ketones and alkanes in varying ratios (Walton, 1990). In addition to linear compounds some cyclic compounds i.e pentacyclic triterpenoids are also found in wax of many species which tends to be accumulate regularly almost entirely in the intra-cuticular wax layers (Jetter *et al.,* 2006). The role of various cuticle substructures and constituents is uncertain yet which needs to exploit.

3-Cuticular wax biosynthesis

At early stage of plant development wax biosyntheses stars by deposition of pro-cuticle in the late globular stage of embryogenesis and is tightly co-regulated with plant growth to provide a constant wax and cutin deposition which has been reported to occur during stem elongation (Tanaka *et al.,* 2002; Such *et al.,* 2005). Cuticular wax biosyntheses is predominantly known to occur in epidermal cells (Li-Beisson *et al.,* 2010). Aliphatic components of cuticular waxes are synthesized in epidermal cells and from very long saturated fatty acids. Formation of very long fatty acid is a complex mechanism which requires the activities of several enzymes in various cellular compartments i.e cytoplasm, plastids and endoplasmic reticulum. A well characterized soluble fatty acid complex present is plastid of stroma is involved for the syntheses of *de novo* fatty acid of C16- C18 (Ohlroggeav & Browseb 1995). Second stage is known as fatty acid elongation stage where multiple elongation steps are involved that are catalysed by enzyme complexes which are associated to membrane. Cuticular wax biosyntheses pathway started with the syntheses of C16 and C18 fatty acids which are synthesized in plastids from where they are exported to cytoplasm for further elongation to form very long chain fatty acids C20 to C34 (Fig.1). A series of enzymes such as 3-ketoacyl-CoA synthetases (KCS), 3-ketoacyl-CoA reductases (KCR), 3-hydroxyacyl-CoA dehydratases and trans-2-enoyl-CoA reductases (ECR) participate in this step (Kunst

& Samuels 2009). These very long chain fatty acids are subsequently changed to their derivatives by decorbonylaation and acyl reduction pathways.

There are two major pathways involved in the biosyntheses of wax components.

(a) Acyl reduction pathway:

First pathway for wax biosyntheses is acyle reduction pathway, primary alcohols and wax esters are the main products of this pathway. Alcohol formation varies within species such as in B.oleracia primary alcohol is produced in two steps and two different enzymes are used in each step i.e. an enzyme NADH independent acyl-CoA redutase is used to reduce very long chain fatty into aldehydes whereas another enzyme NADPH-dependent aldehyde reductase is used for formation of primary alcohol by reduction of aldehydes (Kolattukudy, 1973). It has been reported by (Vioque & Kolattukudy 1997) that in pea plants formation of alcohol take place in single step by an enzyme fatty-CoA reductase and alcohol biosyntheses formation take place via aldehyde intermediate. The second step is the syntheses of wax ester which is catalyzed by a fatty acyle-CoA wax synthase.

(b) Decarbonylation:

Second step is termed as decarbonylation which yields aldehydes, alkanes, secondary alcohol and ketones. Here at first stage acyle CoA ester is reduced to aldhyde by using an enzyme acyle-CoA reductase. Then carbonyl group is removed from aldehyde by enzyme aldhyde decarbonylase to from alkane. Further metabolism of hyrdocorbons is possible by adding the hydroxyl group in carbon chain by enzyme hydroxylase which yields secondary alcohol. Estrification of secondary alcohol yield wax ester on the other hand long chain ketone is formed by oxidizing the hydroxyl group. An association pathway yields β-diketones and 2-alkanols. Last stage of wax ester production from alcohol and fatty acids involves the action of an acyle-CoA alcohol transacylase.

4-Transportation mechanism of cuticular wax

Transportation mechanism of wax is still unknown because it is not yet clear how hydrophobic components of wax moves intra-cellulary and how are exported out of cell. However some molecules have been identified that assists the process of transportation. Various studies have confirmed that various molecules related to wax biosynthesis are synthesized in endoplasmic reticulum (Geer *et al*.,2007; Li *et al.,* 2008). Compounds which are derived from very long chain fatty acids from the cuticular waxes, are synthsised in the endoplasmic reticulum of epidermal cells before being exported to the environmental face of the epidermis (Bernard, 2013). According to Kunst & Samuels (2003) there are two hypotheses about the intracellular transportation of wax.

4(i) Direct transfer of lipids from endoplasmic reticulam to the plasma membrane.

After syntheses, wax components are exported from the site of lipid syntheses to the plastids followed by endoplasmic reticulum then through the plasma membrane to the cell wall where they are deposited. Carrier proteins of soluble acyl transfer the wax compounds from the endoplasmic reticulum before passing through cytoplasm. Carrasco *et al.,* 2011 reported the stable junction between endoplasmic reticulum-plasma membrane in various eukaryotes i.e mammals, yeast, insects and plants. Directly wax transportation from endoplamic reticulum to plasma membrane indicates that the specific proteins are present in ER that contact at specific site on plasma membrane for transportation of wax (Bernard & Joubès 2013).

4(ii) From the plasma membrane to the extracellular matrix

Wax compounds after reaching the plamsmalemma, are exported from membrane to released into the extra cellular matrix. Active proteins of ABC family are involved for the completion of this step. However the mechanism of wax transport from endoplasmic reticulum to plasma membrane is unclear yet that how ABC transporters handle wax components. The first ABC transporter identified required f or wax transport was found to be encoded by CER5 and was named ABCG12 according to the nomenclature of ABC transporters. A reduction in the wax load of the cer5 mutant was characterized a long time ago as it exhibited the shiny stem phenotype typical of wax-associated mutants (Samuels *et al.,* 2008). ATP-binding cassette (ABC) proteins are ubiquitously associated with transport across membranes of a broad range of molecules in prokaryotes and eukaryotes (Verrier, 2008). Likewise, a role for ABCG11 was found in the transport of cutin monomers in flowers, siliques and seeds as well as in the transport of suberin in roots, accounting for the pleiotropic effects triggered by a loss of ABCG11 (Panikashvili *et al.,* 2007). In addition, the remaining export of 50% of the waxes on the plant surface in the absence of ABCG11 and ABCG12 (Bird *et al.,* 2007) indicates that other ABC-transporters might be required to export wax compounds. Alternatively, another yet to be described transport machinery could participate in that process. Consistent with the first idea, numerous putative ABC transporter encoding sequences were found up-regulated in the stem epidermis (Suh *et al.,* 2005).

5-Regulation of wax biosynthesis

Cuticular wax biosyntheses regulation is a complex mechanism that involves signaling networks

associated with abiotic stress reponses, pathogen responses and on the integrity and structure of cuticle itself. As the cuticle is entirely synthesized by epidermal cells so the regulation of epidermis identity during the development can play a regulatory role in cuticle development. In recent studies an association between cuticle biosyntheses and developmental process such as epidermal cell formation has been reported by (Hen-Avivi *et al..,* 2014). In this review we will elaborate transcriptional and environmental factors of cutin and wax biosyntheses.

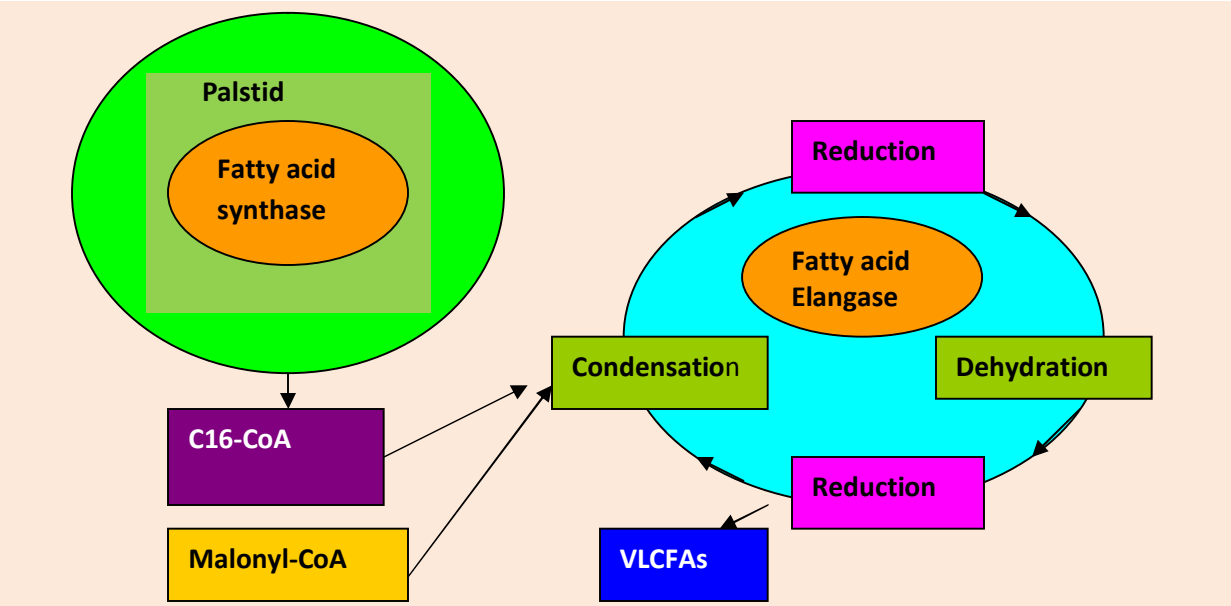

Figure.1 BIOSYNTHESES OF VERY LONG CHAIN FATTY ACIDS

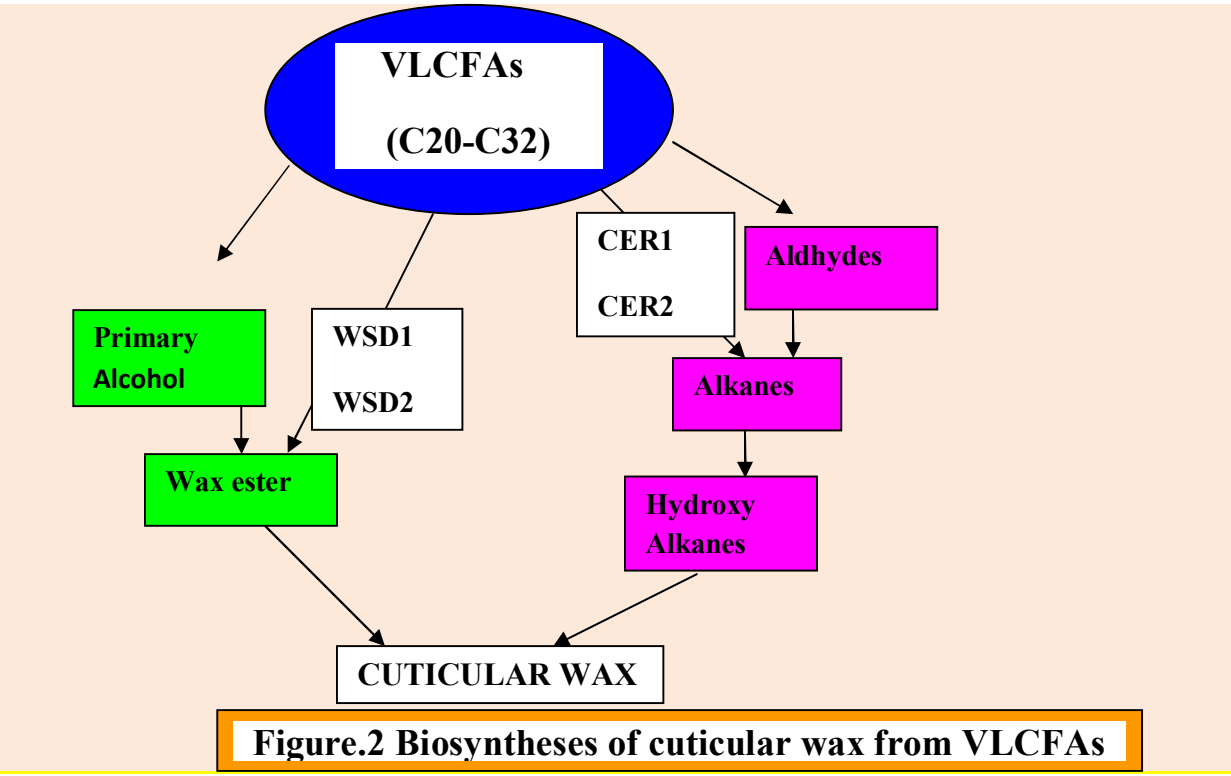

Figure.2 Biosyntheses of cuticular wax from VLCFAs

5(i) Transcriptional factors

Regulation of cuticular wax biosyntheses is a complex network that occurs on multiple levels and is not limited to transcriptional factors; it also includes post transcriptional regulation along with epigenetic factors. First gene identified that have role in cuticle biosyntheses regulation was AP2 domain containing WAX INDUCER1/SHINE1 (Aharoni *et al.,* 2004; Broun *et al.,* 2004). Studies have shown that WINI/SHN1 over-expression causes to increase the cutin level of plants indicating that genes up-regulating cutin biosyntheses enzymes proceeds the induction of wax biosyntheses genes (Kannangara *et al.,* 2007). It has been observed that a transcriptional factor AP2/ERFtype work as transcriptional repressor of cuticular wax biosyntheses (Go *et al.,* 2014).

Four genes CER2, CER3, GL2, GL15 cloned from mutation collections are considered to code for regulatory proteins (Negruk *et al.*, 1996; Xia *et al.*, 1996). Out of these genes GL15 was recommended to function as transcriptional factor for the regulation of leaf epidermal cell identity. Whereas the identities of *CER2, CER3 and GL2* gene products could not be deduced from their primary sequences, and their predicted functions in regulation of wax deposition remain to be confirmed. Similarly, *WXP1*, an *AP2/EREBP* domain transcription factor from *Medicago truncatula*, increases leaf cuticular wax accumulation and results in a glossy leaf phenotype when over expressed under the control of the 35S promoter in alfalfa (*Medicago sativa*; Zhang *et al.*, 2005).

5(ii) Environmental factors

Regulation and deposition of cuticular wax biosyntheses comprises on a regulatory network that control the differentiation of epidermal cells and environmental stress responses. It has been observed that wax biosyntheses is thought to be induced by drought related traits, sodium chloride, and abscisic acid treatments (Kosma *et al.,* 2009). Absisic acid regulates cuticular wax biosynthesis by binding directly to the promoters of genes encoding fatty acid elongating enzymes, i.e KCS, KCR, ECR (Seo & Park 2011). Seo *et al*, 2011 reported that cuticular wax deposition has correlation with plant responses to cellular dehydration. As compare to wax biosyntheses, cutin biosyntheses is only dependent to drought not on abscisic acid or sodium chloride (Kosma *et al*., 2009). Regulation of abscisic acid for cuticle biosyntheses is an interesting region for drought resistance and stomatal aperture in crop plants (Lee and Luan, 2012). Resistance to pest and pathogens due to cuticular wax biosyntheses is not understood yet, however is has been observed that numerous wax producing genes have been induced in plants by bacterial pathogens (Raffaele *et al.,* 2008). Similar results also have been reported in wheat due to Hessian fly by (Kosma *et al.,* 2010).

Conclusion

Plant cuticular waxes play significant role against various biotic and abiotic stresses. Hence there is dire need of the hour to study the mechanism of its biosyntheses and transportation so that plant biologists may be unable to understand this phenomenon at molecular level to increase the resistance against various stresses in plants. This review will help the plant physiologists to understand the basic mechanism that how wax is synthesized in plants, factors involved for its transportation and regulation along with various properties of plant waxes related to biotic and abiotic stresses.

Authors:

Hafiz Muhammad Ahmad*[1], Mahmood-Ur-Rahman[1], Qurban Ali[2] and Shahid Iqbal Awan[3]

[1.] Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

[2.] Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

[3.] Department of Plant Breeding and Molecular genetics, The University of Poonch, Azad Jammu & Kashmir, Rawalakot, Pakistan

Corresponding author's emails:
hafizahmad90@yahoo.com, saim1692@gmail.com

References

1. Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A, 2004.The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell, 16:2463-80.
2. Barnes JD, Percy KE, Paul ND, Jones P, McLaughlin CK, Mullineaux PM, Creissen G and Wellburn AR, 1996. The influence of UV-B radiation on the physicochemical nature of tobacco (Nicotiana tabacum L.) leaf surfaces. J Exp Bot. 47: 99-109.
3. Barthlott W, Neinhuis C, 1997. Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta. 202: 1–8.
4. Baur P,1998. Mechanistic aspects of foliar penetration of agrochemicals and the effect of adjuvants. Recent Resch Dev Agric Food Chem. 2: 809–837.

5. Bernard A, Joubès J,2013. Arabidopsis cuticular waxes: Advances in synthesis, export and regulation**.** Progress in Lipid Research**.** 52(1):110–129.
6. Bhutta MA, Muhammad KQ, Muhammad KS, Mahmood A, Malook SU Ali Q. Oxidative damage caused by Reactive Oxygen Species under drought stress in *Gossypium hirsutum***.** *Life Sci J* 2015;12(4s):51-59.
7. Bianchi G, Murelli C, Ottaviano E,1990. Maize pollen lipds. Phytochemistry. 29:739–44.
8. Bird D, Beisson F, Brigham A, Shin J, Greer S, Jetter R, Kunst L, Wu X, Yephremov.
9. Samuels AL, 2007. Characterization of *Arabidopsis* ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. Plant J. 52: 485–498.
10. Broun P, Poindexter P, Osborne E, Jiang CZ, Riechmann JL, 2004. WIN1, a transcriptional activator of epidermal wax accumulation in Arabidopsis. Proceedings of the National Academy of Science, USA 101:4706–4711.
11. Buschhaus C, Herz H, Jetter R, 2007. Chemical composition of the epicuticular and intracuticular wax layers on adaxial sides of Rosa canina leaves. Ann Bot (Lond) 100: 1557–1564.
12. Buschhaus C, Jetter R, 2011. Composition differences between epicuticular and intracuticular wax substructures: how do plants seal their epidermal Surfaces. J Exp Bot. 62: 841–85.
13. Cameron KD, Teece MA, Smart LB, 2006. Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol. 140:176–83.
14. Carrasco S, Meyer T. STIM, 2011. proteins and the endoplasmic reticulum–plasma membrane junctions. Ann Rev Biochem.80:973–1000.
15. Carver TLW, Gurr SJ, 2006. Filamentous fungi on plant surfaces. In MRiederer, C Muller, eds, Annual Plant Reviews23: Biology of the Plant Cuticle. Blackwell, Oxford, 2006. pp 368–397.
16. Domínguez E, Cuartero J, Heredia A, 2011. An overview on plant cuticle biomechanics. Plant Sci. 181: 77–84.
17. Eigenbrode SD, Espelie KE, 1995.Effects of Plant Epicuticular Lipids on Insect Herbivores. Annu Rev Entomol. 40:171–194.
18. Gniwotta F, Vogg G, Gartmann V, Carver TLW, Riederer M, Jetter R, 2005. What do microbes encounter at the plant surface? Chemical composition of pea leaf cuticular waxes. Plant Physiol 139: 519–530.
19. Go YS, Kim H, Kim HJ, Suh MC, 2014. Arabidopsis cuticular wax biosynthesis is negatively regulated by the DEWAX gene encoding an AP2/ ERF-type transcription factor. Plant Cell.doi: 10.1105/tpc.114.123307.
20. Greer S, Wen M, Bird D, Wu X, Samuels L, Kunst L, 2007. The cytochrome P450 enzyme CYP96A15 is the midchain alkane hydroxylase responsible for formation of secondary alcohols and ketones in stem cuticular wax of Arabidopsis. Plant Physiol.145:653–67.
21. Guhling O, Kinzler C, Dreyer M, Bringmann G, Jetter R, 2005. Surface composition of myrmecophilic plants: cuticular wax and glandular trichomes on leaves of Macaranga tanarius. J Chem Ecol. 31: 2323–234.
22. Hall DM, Jones RL, 1961. Physiological significance of surface wax on leaves. Nature. 191: 95-96.
23. Hen-Avivi S, Lashbrooke J, Costa F and Aharoni A, 2014. Scratching the surface: genetic regulation of cuticle assembly in fleshy fruit. J Exp Bot, doi:10.1093/jxb/eru225.
24. Isaacson T, Kosma DK, Matas AJ, 2009. Cutin deficiency in the tomato fruit cuticle consistently affects resistance to microbial infection and biomechanical properties, but not transpirational water loss. The Plant J. 60: 363–377.
25. Javelle M, Vernoud V, Rogowsky PM, Ingram GC, 2011. Epidermis: the formation and functions of a fundamental plant tissue. New Phytol. 189:17–39.
26. Jeffree CE, 2006. The fine structure of the plant cuticle. In M Riederer, C Müller, eds, Biology of the Plant Cuticle, Annual Plant Reviews, Vol. 23. Blackwell, Oxford, pp:11–144.
27. Jenks, M.A., R.J. Joly, P.J. Peters, P.J. Rich, J.D. Axtell and E.A. Ashworth, 1994. Chemically-induced cuticle mutation affecting epidermal conductance to water vapor and disease susceptibility in Sorghum bicolor (L.) Moench. Plant Physiol., 105: 1239-1245.
28. Jetter R, Kunst L, Samuels AL, 2006. Composition of plant cuticular waxes. In M Riederer, C Müller, eds, Biology of the Plant Cuticle, Annual Plant Reviews, Vol. 23. Blackwell, Oxford, pp 145–18.
29. Jetter R, Schäffer S, 2001. Chemical composition of the Prunus laurocerasus leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant Physiol 126: 1725–1737.
30. Ji X, Jetter R, 2008. Very long chain alkylresorcinols accumulate in the intracuticular

wax of rye (Secale cereale L.) leaves near the tissue surface. Phytochemistr, 69: 1197–1207.
31. Joubès J, Raffaele S, Bourdenx B, Garcia C, Laroche-Traineau J, Moreau P, Domergue F, Lessire R, 2008. The VLCFA elongase gene family in Arabidopsis thaliana: phylogenetic analysis, 3D modelling and expression profiling. Plant Mol Biol 67: 547–566.
32. Kakani VG, Reddy JR, Zhaq D, Mohammad AR, 2003. Effect of ultraviolet-B radiation on cotton [Gossypium hirsutum L.] morphology and anatomy. Ann Bot 2003; 91: 817-826.
33. Kannangara R, Branigan C, Liu Y, Penfield T, Rao V, Mouille G, Höfte H, Pauly M, Riechmann JL, Broun P, 2007. The transcription factor WIN1/SHN1 regulates Cutin biosynthesis in Arabidopsis thaliana. Plant Cell 19:1278–1294.
34. Kerstiens G, 1996. Cuticular water permeability and its physiological significance. J Exp Bot 47: 1813–1832.
35. Kim KS, Park SH, Jenks MA, 2007. Changes in leaf cuticular waxes of sesame (Sesamum indicum L.) plants exposed to water deficit. J Plant Physiol 164:1134–43.
36. Kolattukudy, P.E., Buckner, J.S. and Liu, T.Y.J, 1973. Biosynthesis of secondary alcohols and ketones from alkanes: Arch. Biochem. Biophys 156: 613–620.
37. Kosma DK, Bourdenx B, Bernard A, Parsons EP, Lü S, Joubès J, Jenks MA, 2009. The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiol 151: 1918–1929.
38. Kunst L, Samuels AL, 2009. Plant cuticles shine: advances in wax biosynthesis and export. Curr Opin Plant Biol 12:721-7.
39. Kunst, L, Samuels AL, 2003. Biosynthesis and secretion of plant cuticular wax. Progress in Lipid Res 42:51–80.
40. Lee SC, Luan S, 2012. ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant Cell Environ 35: 53–60.
41. Li F, Wu X, Lam P, Bird D, Zheng H, Samuels L 2008. Identification of the wax ester synthase/acyl-CoenzymeA: diacylglycerol acyltransferase WSD1 required for stem wax ester biosynthesis in Arabidopsis. Plant Physiol 148:97–107.
42. Li-Beisson, Y., Shorrosh, B., Beisson, F., Andersson, M., Arondel, V., Bates, P., Baud, Bird, S, D DeBono, A Durrett, T, 2010. Acyl-lipid metabolism. The Arabidopsis Book/American Society of Plant Biologists. 8, e0133.
43. Muller C, 2006. Plant-insect interactions on cuticular surfaces, In M, Riederer., C, Mu¨ller, eds, Annual Plant Reviews23: Biology of the Plant Cuticle. Blackwell, Oxford, 2006; 398–422.
44. Nawrath C, 2006. Unraveling the complex network of cuticular structure and function. Curr Opin Plant Biol 9: 281–287.
45. Ohlroggeav J, J Browseb, 1995.Lipid Biosynthesis. The Plant Cell 7:957-970.
46. Panikashvili D, Savaldi-Goldstein S, Mandel T, Yifhar T, Franke RB, Hofer R, Schreiber L, Chory J, Aharoni A, 2007. The *Arabidopsis* DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. Plant Physiol 145: 1345–1360.
47. Pasha MFK, HM Ahmad, M Qasim, I Javed, 2015. Performance evaluation of zinnia cultivars for morphological traits under the Agro-climatic conditions of Faisalabad. Eur. J Biotech. Biosci 3 (1): 35-38.
48. Pollard M, Beisson F, Li Y, Ohlrogge JB, 2008. Building lipid barriers: biosynthesis of cutin and suberin. Trends Plant Sci 13: 236–246.
49. Preuss D., Lemieux B., Yen G., Davis R.W, 1993. A conditional sterile mutation eliminates surface components from Arabidopsis pollen and disrupts cell signaling during fertilization. Genes Dev **7**, 974–985.
50. Raffaele S, F Vailleau, A Léger, J Joubès, O Miersch, C Huard, E Blée, S Mongrand, F Domergue, D Roby, 2008. A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis. Plant Cell 20: 752–767.
51. Reicosky DA, Hanover JW, 1978. Physiological effects of surface waxes I. Light reflectance for glaucous and nonglaucous Picea pungens. Plant Physiol. 62: 101–104.
52. Reiter B, Lechner M, Lorbeer E, Aichholz R, 1999. Isolation and characterization of wax esters in fennel and caraway seed oils by SPE-GC. HRC Journal of High Resolution Chromatography. 22(9):514–520.
53. Samuels L, Kunst L, Jetter R, 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells. Ann Rev Plant Biol **59**: 683–707.
54. Seo PJ, CM Park, 2011. Cuticular wax biosynthesis as a way of inducing drought resistance. Pl. Signal. Behav, 6(7):1043-1045
55. Seo, PJ, SB Lee, MC Suh, MJ Park, YS Go, and CM Parka, 2011. The MYB96 Transcription Factor Regulates Cuticular Wax Biosynthesis under Drought Conditions in Arabidopsis The Plant Cell 23: 1138–1152.

56. Shepherd T, Wynne Griffiths D, 2006. The effects of stress on plant cuticular waxes. New Phytol. 171:469–99.
57. Sieber, P., Schorderet, M., Ryser, U., Buchala, A., Kolattukudy, P., Metraux, J.-P. and Nawrath, C, 2000. Transgenic Arabidopsis plants expressing a fungal cutinase show alterations in the structure and properties of the cuticle and postgenital organ fusions. Plant Cell 12, 721–738.
58. Suh MC, Samuels AL, Jetter R, Kunst L, Pollard M, Ohlrogge J, 2005.Cuticular lipid composition, surface structure, and gene expression in Arabidopsis stem epidermis. Plant Physiol 139:1649–65.
59. Tanaka H, Watanabe M, Watanabe D, Tanaka T, Machida C, Machida Y, 2002. ACR4, a putative receptor kinase gene of Arabidopsis thaliana, that is expressed in the outer cell layers of embryos and plants, is involved in proper embryogenesis. Plant Cell Physiol 43:419–28.
60. Van MC, Jetter R, 2009. Composition of the epicuticular and intracuticular wax layers on Kalanchoe daigremontiana (Hamet et Perr. Dela Bathie) leaves. Phytochemistry 70: 899–906.
61. Vioque J., Kolattukudy PE, 1997. Resolution and purification of an aldehyde-generating and an alcohol-generating fatty acyl-CoA reductase from pea leaves (Pisum sativum L.). Arch. Biochem Biophys 340: 64–72.
62. Von Wettstein-Knowles P 1995. In: Hamilton RJ, editor. Waxes: chemistry, molecular biology and functions. Dundee: Oily Press, p. 91–129.
63. Wagner P, Furstner R, Barthlott, Neinhuis, C, 2003. Quantitative assessment to the structural basis of water repellency in natural and technical surfaces. J Exp Bot 54, 1295–1303.
64. Walton TJ, 1990. Waxes, cutin and suberin. In JL Harwood, JR Boyer, eds, Methods in Plant Biochemistry, Lipids, Membranes and Aspects of Photobiology, Vol 4. Academic Press, London, pp 105–158.
65. Negruk, V., Yang, P., Subramanian, M., McNevin, J.P. and Lemieux, B, 1996. Molecular cloning and characterization of the CER2 gene of Arabidopsis thaliana, The Plant J 9:137–145.
66. Xia Y, Nikolau, BJ Schnable PS, 1996. Cloning and characterization of CER2, an Arabidopsis gene that affects cuticular wax accumulation, The Plant Cell, 8, 1291–1304.
67. Zhang JY, Broeckling CD, Blancaflor EB, Sledge MK., Sumner LW and Wang ZY, 2005. Overexpression of WXP1, a putative Medicago truncatula AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (Medicago sativa), The Plant J 42:689–707.

9/8/2015