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PLANT EPICUTICULAR WAX : CHARACTERISTICS AND ROLES

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INTRODUCTION

In plants, the protective role is played by a polymer layer composed of lipid components called cuticle. The outer surface of the leaf epidermis is covered with a substance called cutin, which is impregnated with wax; together they comprise the cuticle. Since the lipids are present on and above the cuticle layer and are made of a long chain carbon esters, the name Epicuticular wax (ECW) is coined. With the advent of modem techniques, research investigations on the structure, composition and physiological significance of ECW have gained momentum. In this paper an attempt is made to review the work carried out so far on different aspects of ECW.

CHARACTERISTICS OF WAX

Composition

ECW is non-polar in nature. The wide range of wax constituents are alkanes, esters, β-diketones, aldehydes, primary and secondary alcohols, ketones, free acids and terpenoids. The predominant range of carbon chain length is nC_{19} - nC_{33}. The major compounds are divided into (a) hydrocarbons, (b) oxygenated compounds and (c) cyclic compounds.

(a) Hydrocarbons: These are the skeletal compounds in wax. The predominant hydrocarbons are paraffins with nC_{25} - nC_{33} iso and anteiso dimethyl alkanes and internally branched chain compounds. Olefins are the type of hydrocarbons abundantly present in algae.

(b) **Oxygenated** compounds: These are the derivatives of hydrocarbons. Most common occurrence of this group are primary and secondary alcohols, β-diketones and hydroxy β-diketones as observed in *Brassica oleracea* (Purdy and Truter, 1963) and wheat (Tulloch, 1973).

(c) Cyclic compounds: Pentacyclic triterpenes are predominant among this group. Urosolic acid and oleonolic acid in apple fruits (Silva Fernandes *et al.*, 1964) and grape berries (Chambers and Possingham, 1963) account for 50% of surface wax. In addition to thick cuticle, presence of terpenoid components makes extraction difficult. Flavones, terpenoid hydrocarbons, phenolic compounds and aromatic cyclic compounds are present in small quantities in Eucalyptus and banana.

The chemical composition of the lipids as well as the total amount are of major importance (Grncarevic and Radler, 1967). For instance, the leaf and stem wax of *Panicum virgatum* contained predominantly β-diketones (69%), while other compounds were in low quantities- hydroxy β-diketones (6%), hydrocarbons (4%) esters (3%), triterpenoid alcohols (2%), free acids (2%), free alcohols (1%) (Tulloch and Hoffman, 1980). Skoss (1955) reported the relationship between composition of cuticle and environmental factors. The chemical composition of ECW varied in four mutants of *Pisum sativum* (Holloway *et al.*, 1977).

Ultrastructure

Some of the early reports on the fine structure of surface wax were in relation to environment (Juniper, 1960), in Sultana grapes (Chambers and Possingham, 1963) and in four mutants of *Pisum sativum* (Holloway *et al.*, 1977). Epicuticular waxes of five cultivated varieties of *Picea pungens* and six other species of conifers were examined directly with a scanning electron microscope (Hanover and Reicosky, 1971). The quality, quantity and distribution patterns of surface wax deposits varied between species, within species; within plants and within individual leaves. The authors found two basic types of wax namely structural and amorphous. Structural wax is associated with glaucousness and the blue foliage in *Picea pungens* and other species. The degree of occlusion of the stomatal pore by surface waxes also varied from heavily occluded to completely free of wax.

The scanning electron microscopy of the surface of needless of four coniferous species revealed the complex nature of wax filaments related with the ultraviolet and blue reflectance (Clark and Lister, 1975) and in sorghum leaves (Sanchez *et al.*, 1972). Hull *et al.* (1978) studied the leaf surface ultrastructure on four drought tolerant and three drought susceptible lines of Lehmann lovegrass, *Eragrostis tabmaniana*. Electron microscopic observations revealed that leaves of all lines were covered with a basic type of ECW structure comprising small crystalline bodies in combination with various types of ribbons and tubes. Drought tolerant lines were characterised by the presence of the large wax plates on leaf surfaces. This showed that the wax morphology could serve as a valuable characteristic for selection of germplasm for seedling drought tolerance.

Thair and Lister (1975) studied the distribution and fine structure of ECW of *Pseudotsuga* sp. Scanning electron microscopic examination of ECW formation was done in many higher plants (Davis, 1971).

The chemical and structural characterization of the needle ECW was studied in two clones of *Pinus strobus* differing in sensitivity to ozone (Trimble *et al.,* 1982). From the scanning electron microscopic examination of samples exposed to different levels of ozone, it was clear that either fibrillar wax structure or an amorphous plate like wax structure were present in the samples regardless of exposure to ozone. In a recent paper, Tuomisto and Neuvonen (1993) concluded that changes in the ECW of ***Picea abies*** needles could be used to compare wax degradation at sites exposed to different degrees of air pollution.

Synthesis

The synthesis of ECW components takes place in the epidermal cells (Kolattukudy, 1968). There are three hypothesis to explain the synthesis of long chain fatty acids and related compounds, namely head to head condensation hypothesis, acceptor-donor hypothesis and elongation-decarboxylation hypothesis (Kolattukudy, 1975, Tulloch, 1986). The most accepted hypothesis for the formation of various compounds of ECW is elongation-decarboxylation hypothesis. According to this palmitic acid is the substrate to which C_2 units are added till the required chain length is attained. Final formation of hydrocarbon results through decarboxylation of this acid.

The incorporation of (2-^{14}C) malonate and (1-^{14}C) acetate into internal lipid and surface wax by cotton leaves having leaf water potential of -15 bars (control) and -19 to **-32** bars (water stressed) was compared (Weete *et al.,* 1978). Lipids from stressed leaves contained a mean of 57% more radioactivity than corresponding controls. Acetyl CoA carboxylase was not limiting to fatty acid synthesis in water stressed cotton leaves at the water potential levels tested, whereas fatty acid synthetase was stimulated. The experiments provided the evidence to show that after a suitable period of rehydration, previously stressed cotton leaves produce more wax than leaves prior to stressing. Leek ***et al.*** (1977) demonstrated that the primary effect of water stress on wax and lipid synthesis is at the site of incorporation of ^{14}C malonate into fatty acids.

METHODS OF ISOLATION AND IDENTIFICATION OF WAX

Extraction

The ECW can be readily extracted in organic solvents such as chloroform, hexane. benzene or petroleum ether for 10 to 30 seconds. The extraction differs from plant to plant and even among plant pans. Chloroform was used for surface wax extraction (Silva Fernandes *et al.*, 1964), from barley (Larsson and Svenningsson, 1986), sorghum (Ebercon *et al.*, 1977), rice (O'Toole *et al.*, 1979), cocoa (Balasimha *et al.*, 1985). coconut (Voleti and Rajagopal, 1991), whereas hexane extracted the wax from *Panicum virgatum* (Tulloch and Hoffman. 1980). Drastic treatments are necessary for materials having thick cuticles as in apple fruits and grape berries.

Identification

For qualitative study, the wax components were fractionated by thin layer chromatography on silica gel G plates (Holloway and Bauer, 1968). Barrick *et al.* (1979) described procedures followed for dissolving the wax and the solvent used for developing the TLC plates. Identification of individual wax components was carried out after spraying the dried plates with 5% $K_2Cr_2O_7$ in 40% H_2SO_4 and charred at 150°C (Radler, 1965). These methods are used in many cases as in barley (Larsson and Svenningsson, 1986), coconut (Voleti and Rajagopal, 1991 and Kurup *et al.*, 1993) and rubber (Gururaja Rao *et al.*, 1988).

Quantification

The gravimetric method of isolation (Silva Fernandes *et al.*, 1964) requires larger leaf samples, a larger quantity of chloroform to extract wax and longer period of time to evaporate the chloroform extract. Thus, this method is too slow for routine selection work involving many genotypes. Realising this problem, Ebercon *et al.* (1977) developed a rapid colorimetric method for ECW of sorghum leaves. The authors compared the gravimetric with colorimetric method and found no significant differences in wax content from leaf samples taken at various growth stages. The advantage of colorimetric method was that the number of samples which could be processed per day was ten times greater than that with gravimetric method.

The development of the rapid colorimetric method was based on colour change produced due to the reaction of wax with acidic potassium dichromate, $K_2Cr_2O_7$ (Bradgon, 1951). While in the gravimetric method the residue, after evaporation of chloroform, was weighed and the quantity of wax calculated on the basis of leaf area, in the colorimetric method the colour developed was read in spectrophotometer and the wax content quantified with the help of a standard curve prepared with carbowax or similar wax components. The rapid method where the leaves are dipped in solvent for ten seconds, is in vogue in many recent investigations as it helps screening large number of samples for drought tolerance. Although generally and widely used for extraction of lipid components from leaf surface there is always the risk that the dipping method extracts not only the soluble surface lipids (ECW), but also more or less of the soluble lipids from the interior of the cuticle Le. intracuticular lipids (ICL). The disadvantages of dipping method in interpreting the data are highlighted by Svenningsson and Liljenherg (1986) and described more under the section on cuticular transpiration.

ROLES OF WAX

Various functions and adaptive roles have been attributed for the surface lipids of plant leaves. These include modification of water loss (Schonherr, 1976), wettability of leaf surfaces (Troughton and Hall, 1967), exchange of gases with the atmosphere (Jeffree *et* al., 1971), protection against mechanical and pest damage (Martin, 1964), protection against ultraviolet radiation (Clark and Lister, 1975) and air pollutants (Swiecki *et al.*, 1982). The physiological significance of surtace wax on leaves was described much earlier by Hall and Jones (1961)

Cuticular transpiration rate (CTR)

Earliest reports on the reduction of moisture by cuticular wax were on apple (Horrocks, 1964), grapes (Radler, 1965) and *Brassica* (Denna, 1970b). Later Bukovac e *al.* (1979) studied in peach the changes in wettability, retention, cuticular permeability and epicuticular wax chemistry during expansion with special reference to spray application. Nagarajah (1979) observed the differences in cuticular resistance in relation to transpiration in tea. The effect of ECW load on CTR in sorghum bloom and bloomless lines has been studied (Jordan *et al.*, 1984). The control of transpiration rate by ECW was found in clover (Hamilton. 1975).

The relationship between the quantity and composition of ECW and the CTR was studied in six varieties of oat (Bengtson *et al.*, 1978). Unstressed seedlings of the most drought resistant variety, Stormogul II, showed the highest cuticular transpiration rate. After stress treatment the CTR was most strongly reduced in this variety with largest increase in the amount of ECW. In soybean also there was positive relationship between CTR and ECW (Clark and Levitt, 1956). However, in barley the reduction in CTR was not accompanied by any increased lipids (Larsson and Svenningsson, 1986). The authors studied 20 cultivars of barley and 15 eceriferum mutants and inspite of variation in transpiration rate, most of the cultivars had'almost the same amount of epicuticular lipids (EL) and only a weak correlation was found between CTR and total amount of EL. None of the analysed lipid components namely alkanes, aldehydes, primary alcohols, esters, fatty acids, was better correlated with CTR than the total EL.

The explanations for the absence of correlation between CTR and EL were advanced by Larsson and Svenningsson (1986). According to them the substances extracted by the method of dipping leaves in a solvent also include a greater or smaller fraction of the soluble lipids located deeper in the cutin matrix of the cuticle. the intracuticular lipids, ICL (Schonherr, 1982). The true EL, present in a very thin layer, are probably extracted during the first few seconds. Thus, the dipping method gives a mixture of the EL and fraction of ICL and depending on the variation of ICL fraction the relation between EL and CTR will be determined. Another possible explanation for the correlation between CTR and EL may he related to the structure and distribution of ELs on the leaf surface. All these point to the fact that the barrier against diffusion of water through the cuticle depends not only on the EL hut also on ICL. The evidence for this hypothesis is provided by the observations that a lot of species with thick layer of ELs transpire approximately as much as species with a thinner layer of lipids as in *Brassica oleracea* (Denna, 1970a, h) and weeds and crops (Ramdas *et* al., 1979).

An exhaustive study was undertaken for three successive years on the composition of soluble cuticular lipids and water permeability of cuticular membranes from citrus leaves (Haas and Schonherr, 1979). Cuticular membranes are characterized by a great variability in water permeability between years. Both water permeability of individual membranes and variability between membranes are shown to be determined by soluble cuticular lipids contained within the cuticular membranes. The soluble cuticular lipids of citrus leaves consist of fatty acids, primary alcohols, esters and hydrocarbons. According to the authors, the absence of relationship between composition of cuticular lipids and water permeability could he due to the fact that the lipid composition observed represented the averages of 20 to 30 membranes so much so the differences between individual membranes might have been leveled out. Thus, the role of wax on transpiration rate is a complex phenomenon and the variations observed could be due to more than one reason. Besides drought, NaCl salinity also was found to affect the amount and composition of ECW in groundnut (Rao *et al.*; 1981).

Glaucousness and reflectance

Glaucousness is the waxy covering which imparts a dull white or bluish-green cast commonly referred to as bloom. Glaucousness is one characteristic that has been cited as a plant adaptation to drought (Johnson *et al.*, 1983). Isogenic lines of sorghum with or without glaucousness or waxy bloom has been developed (Blum, 1975). Glaucous lines had greater adaxial leaf reflectance in the visible and near infra red wavelengths than non-glaucous lines. Higher reflectance of radiant energy will result in lower leaf temperature, thus reducing thermal injury (Loomis, 1965 and Eller and Willi, 1981). To identify genetic and environmental sources of variation in residual transpiration of wheat, the effects of glaucousness. ECW, leaf age, plant height and growth environment were determined using excised leaves (Clarke and Richards, 1988). Glaucousness reduced residual transpiration by an average of 10%. Differing quantities of ECW and glaucousness in an isogenic pair of genotypes affected residual transpiration, the rate being 33% greater in low wax non-glaucous line (AUS 2499) than in the high wax glaucous line (AUS 2598). ECW level of AUS 2598 was found to be 28% greater than that of AUS 2499 (Johnson *et al.*, 1983).

Non-glaucous sorghum under controlled conditions exhibited higher rates of photosynthesis and transpiration than glaucous lines 23 days after sowing; however, water use efficiency was higher in the glaucous compared to non-glaucous lines (Chatterton *et al.*, 1975). It is possible that the difference in yield between bloom and bloomless sorghum under semi-arid conditions, as reported by Ross (1972), is a result of the difference in gas exchange ratio. The importance of glaucousness in wheat was suggested by Fischer and Wood (1979), according to whom the best prediction of yield under drought from traits measured in the absence of drought was given by a linear model containing an index for degree of leaf waxiness or glaucousness. Near isogenic lines that differ in the presence or absence of glaucousness were developed in durum wheat (Tnticum turgidum) and common wheat (T. aestivum) grown in plots in three field environments, varying in three soil water availability (Johnson *et* al., 1983). The glaucous elections yielded significantly more grain and dry matter than non-glaucous selections in the two higher yielding environments but not in the very dry environment. Lines selected for glaucousness varied in ECW of the different plant parts. Leaf reflectances increased linearly with the amount of ECW and were greater in the driest environments. Richards *et* al. (1986) demonstrated, based on detailed investigation on isogenic wheat, that glaucousness can be yield positive character under water limited conditions, primarily through its effect on water use efficiency, on an extended period of transpiration and on the timing of deposition of wax. Nizam-Uddin and Marshall (1988) evaluated cultivars of common and durum wheat for ECW and studied its relationship with spectral reflectance. Mulroy (1979) recorded the spectral properties of heavily glaucous and non-glaucous of a succulent rosette plant.

Even in coconut, a plantation crop, there was a relationship between ECW and nut yield in that the hybrids LO x **GB** and LO x COD, relatively tolerant to drought, had high ECW on the surface of leaflets and nut yield higher than the other genotypes (Rajagopal *et* al., 1992). Accumulation of ECW resulting in high reflection of radiant energy appeared to be an adaptive feature under drought in mhber clones **GI 1**, RRII 308 and RRII 105 (Gururaja Rao *et* al., 1988).

According to Jensed and Driscoll (1962), glaucousness in wheat was controlled by a multiple allelic system of two dominant inhibitors on chromosomes **2B** and 2D. These major genes are subject to considerable modification by the environment.

Protection from pathogens

The role of cuticle in the defence against plant disease was reviewed more than three decades ago (Martin, 1964). The plant cell walls must be resistant to microbial decomposition and ECW acts as a natural barrier in higher plants. The waxes often contain fungistatic or toxic compounds, which inhibit spore germination, as reported on beet root and broad bean.

Rumen microbes more readily penetrate the surfaces of bloomless leaves of sorghum than the surface of bloomed ones (Powell *et ol.,* 1977). However, there are examples of micro-organisms like micrococcus and *Pseudomonas* which decompose wax and cause damage to the tissue. Leece (1978) reported the nature and development of surface wax barrier.

Indicator of pollution

The role of surface wax in susceptibility of plants to air pollutant injury was studied in *Phaseolus vulgaris* by Swiecki *et al.* (1982). In this paper, the relationship between quantity of ECW and plant sensitivity to HCI gas was reported. Multi-regression analysis showed that surface wax quantity was negatively related to percent of leaves glazed. Tuomisto (1988) studied the ECW morphology in *Picea abies* needles which were used as indicators of air pollution. The weathering of Scots pine ECW in clean air and SO_2- polluted air was studied (Crossley and Fowler, 1986). Needles at the polluted site were heavily contaminated with particulate debris and tubular wax showed alterations during weathering. There are other reports on the response of wax to air pollution (Percy and Riding. 1978. Cape and Fowler, 1981, Turunen and Huttunen, 1990).

Effects of ozone and acid rain on red spruce needle ECW ultra-structure were reported (Percy *et al.,* 1990). Earlier, Trimble *et al.* (1982) found the chemical and structural changes in the ECW of needles of two clones of *Pinus strobus* differing in sensitivity to ozone. Ozone was also found to accelerate structural degradation of ECW of Norway spruce needles (Barnes *et al.,* 1988).

GENETIC VARIABILITY IN WAX

Genotypic differences

There are many reports in the literature on the extent of variation in ECW among the genotypes tested and also interspecific variation (Hall *et al.,* 1965). Earlier reports in this regard were from maize (Dube *et al.,* 1975), sorghum (Blum, 1975 and Ebercou *et al.,* 1977). The ECW composition of hexaploid and octaploid triticales was studied which revealed that the wax compositions of triticales are closer to that of wheat than that of rye (Bianchi *et al.,* 1982). Genotypic variation in ECW among 30 rice cultivars from varying hydrological regions was reported by O'Toole and Cruz (1983). The relative amount of an unknown wax constituent, appeared as peak 3 in GLC, was found to be associated with adaptation of rice cultivars to hydrologically drier habitats. As ECW has direct relationship with cuticular resistance (Cr) Yoshida and Reyes (1976) investigated the Cr in rice varieties. High Cr appears to account for the drought resistance of some rice varieties.

Screening method for drought resistance/tolerance

In view of the role of ECW in controlling cuticular transpiration (CT), several authors have used the determination of ECW as an effective method of screening varieties for drought resistance/tolerance in different crops. Six varieties of oat were screened for CT and ECW and were arranged according to decreasing order of drought resistance (Bengtson *et* al., 1978). Unstressed seedlings of the most drought resistant variety (Stormogul II) showed the highest CT. After stress treatment, the CT rate most strongly reduced in this variety and showed the largest increase in ECW. In the variety Pendek, with least increase in ECW, the CT rate was only 5% lower after stress treatment. Thus, the possibility of using the above parameters in a screening test for drought resistance was suggested from this study. However, Larsson and Svenningsson (1986) were cautious about using the ECW as selection in breeding for drought resistance in barley.

In cocoa, the drought tolerant accessions showed higher content of ECW than the drought susceptible ones (Balasimha, 1982 and Balasimha *et* al., 1985). Among the accessions screened, NC 23, NC 29 and Amel x Na 33 were identified as highly tolerant to drought; water stress decreased total lipid content to an extent of 30% in tolerant and 38% in susceptible accessions.

Highly significant clonal variations were found in the levels of ECW in the young rubber plants (Hevea sp). Among the clones, maximum ECW occurred in GI 1 followed by RRII 105, which were classified as fairly drought tolerant clones (Gururaja Rao et al., 1988). The amount of ECW in these clones was correlated with high reflectance values (r = 0.963). Thus, high levels of ECW observed in the tolerant groups are associated with high reflectance of heat energy and reduced transpirational water loss. These results indicate that measurements on ECW or optical properties can serve as useful tools for screening for drought tolerance in Hevea.

Rajagopal ***et al.*** (1990) have screened 23 coconut genotypes for drought tolerance using the estimation of ECW content as one of the methods. They found variations in ECW more during stress period than during non-stress period. The genotypes identified as drought tolerant, based on high stomatal resistance with low transpiration rate and high leaf water potential, also had higher content of ECW than those classified as drought susceptible. Thus, the hybrids LO x GB, LO x COD and WCT x COD exhibited high ECW levels. The relationship between ECW and transpiration rate was clearly shown in coconut (Rajagopal ***et*** al., 1991). The coconut hybrids which possessed the desirable traits to withstand drought, including high ECW content, also proved to be high yielders of nuts (Rajagopal ***et*** al., 1991).

Likewise, changes in ECW occurred in leaves of two groundnut cultivars during and after relief of drought (Vakharia ***et*** al., 1993). Cultivars of common wheat and durum wheat were screened for ECW (Nizam-Uddin and Marshall, 1988). Highly significant differences were found in the amount of ECW among the cultivars. ECW content under stress and coqtrol conditions were positively correlated, suggesting that selection for this trait could be practiced in either environment. The study has highlighted that ECW is associated with drought tolerance. Baker and Procopiou (1980) studied the effect of soil moisture on ECW in some drought resistant species. Rajagopal *et al.*(1989) reported the influence of irrigation levels on ECW in coconut.

Genetics of wax

The ability to accumulate wax under stress conditions appears to have a genetic basis (Jordan *et al.,* 1983). As for genetic modifications of ECW loads, high and low wax parents can he identified and used in crosses. The data collected by the authors indicate the possibility of producing higher ECW loads or specific hybrids.

The presence of bloom was reported to be conditioned by two genes bloom (Bm) and bloom (H), which are completely dominant over bloomless (bm) and sparce bloom (h) (Ayyangar and Ponnaiya, 1941, Ayyangar *et al.,* 1937). However, the studies by Peterson *et* al. (1982) reveals that more genes are involved in the above. Blum (1975) studied two isogenic lines of Sorghum *bicolor* cv. Combine Kafir- 60, one possessing bloom (Bm Bm) and the other devoid of bloom (bm bm). The total amount of ECW was significantly higher in leaf blades of the Bm

Bm than the bm bm genotypes. Reflectance of radiation in the visible and near infra-red region over the adaxial leaf surface was about 4 to 5% greater in the Bm Bm than in the bm bm genotype. There was marked difference in the ultrastructure of wax, as revealed by the scanning electron microscope, between the genotypes.

A detailed study on the effect of genotype and environment of the *ECW* load on sorghum leaves was undertaken by Jordan *et al.* (1983). Entries in the tests included normal bloom type sorghums, with a range of lines and hybrids. General and specific combining abilities were determined in investigations .involving 30 F1 hybrids from crosses between six male and five female parents in five environments. General combining abilities of males and females were significant ($P < 0.001$), but specific combining abilities were not. The portion of genetic variance contributed by females was 73%, while that by males 27%. The best parental lines for increasing the *ECW* loads in hybrids were RT x 430 among the males and RT x 623 among females. RT x 7078. RT x 7000 and 4 AR generally decreased the *ECW* load of their hybrids.

WAX CONTENT IN RELATION TO GROWTH STAGE AND SEASON

The amount and composition of the lipids differ from one plant organ to another (Scheiferstein and Loomis, 1956, Purdy and Truter, 1963, Hanover and Reicosky 1971 and Tulloch 1973), with season, location and plant age (Haas, 1977, Baker *et al.*, 1979, Bukovac *et al.*, 1979). The two sides of the same leaf differ significantly in both morphology and chemistry of lipids (Holloway *et al.*, 1977, Baker *et al.*, 1979, Bukovac *et al.*, 1979).

The lipid quality of two high yielding dwarf wheat varieties, S308 and Kalyan Sona 227, was studied at different growth stages under soil moisture stress conditions (Singh *et al.*, 1971). The stress at the tillering and early boot stages mostly decreased the lipid content per grain. The ratio of polar:non-polar lipids was not affected at any growth stage during stress. A considerable decrease in triglyceride fraction of non-polar lipids was recorded between the late dough stage and late boot stage.

In spring wheat and durum wheat, the composition of wax varied at different stages of growth and in different plants parts (Tulloch, 1973). The major components of wax identified from leaf blades were alcohols, while it was β-diketones from leaf sheaths. Prior to the formation of leaf sheaths and flag leaf i.e. 50 days after germination, octosanol was the major wax component in leaf blades, while at later stages with complete development of sheath β-diketone content was highest. Atkin and Hamilton (1982) also found changes in ECW with age in sorghum

The ontogenic variations in wax composition was reported in apple (Silva Fernandes *et al.*, 1964), cypress (Dyson and Herbin, 1970), coffee (Stocker and Wanner, 1975), Ivy (Haas, 1977) and pine (Franich *et al.*, 1978). Developing leaves and fruits of blue berry showed variations in waxes (Freeman *et al.*, 1979).

The youngest leaves of peach exhibited triterpenoid acids as major components (84 to 95%) of the waxes, whereas in expanded leaves the proportion of these constituents decreased (Baker *et al.*, 1979). The authors also found differences between the waxes of abaxial surface of expanded leaves, with hydrocarbons and triterpenoid acids, and adaxial surface with large proportion of primary alcohol and esters. Variations were also noted between fully expanded leaves of different ages. For example, the abaxial surface waxes of the oldest leaves contained highest proportion of hydrocarbons, while the adaxial surface waxes of corresponding leaves had the largest amounts of esters, sitosterols and hydrocarbons. In *Rhododendron*, alkanes varied with leaf age (Sala Soo, 1983).

The gas chromatographic studies in maize revealed 1- dotriacontanol to be the dominant component (GC peak of the waxes) from young leaves, while wax from mature leaves consisted predominantly of odd numbered alkanes (Blacker and Greyson, 1988). The study also indicated that the amount of wax extracted per unit area increased during the first 20 days, declined rapidly until 50 days and decreased gradually at later stages.

In coconut, the leaves from seedlings had less ECW content than those from adult palms (Voleti and Rajagopal, 1991). There was also distinct difference in ECW between seasons; the three hybrids tested showed three fold increase in ECW during dry season, as compared to wet season. Kurup *et al.* (1993) noticed differences in ECW between the first and sixth leaf in coconut, with differential response to stress periods. Rao and Reddy (1980) reported seasonal variation in ECW of some semi-arid shrubs.

INFLUENCE OF ENVIRONMENTAL FACTORS ON WAX

Light and Temperature

The environmental factors like light. temperature, relative humidity and rainfall influence the content and composition of surface wax. Literature survey indicated that the lipids vary due to changes in light intensity (Juniper 1960, Whitecross and Armstrong, 1972, Baker, 1974, Giese, 1975), photo period (Wilkinson and Kasperbauer, 1972, Giese, 1975) and changes in temperature (Whitecross and Armstrong, 1972, Baker, 1974, Giese, 1975, Haas, 1977, Wilkinson and Kasperbauer, 1972). Rainfall also alter the content and composition of ECW (Mayeux and Jordan, 1987, Wilkinson and Mayeux, 1987, Turunen and Huttunen, 1991, Kurup *et al.,* 1993). Hull *et al.* (1975) reported the environmental influences on cuticle development and resultant foliar penetration.

Wilkinson and Kasperbauer (1972) investigated the effect of photoperiod, temperature and leaf age on epicuticular alkane content in tobacco grown under controlled conditions. In leaves of same physiological age, leaf epicuticular alkane content was influenced by the photoperiod and temperature conditions under which the leaves developed. Leaves of different ages collected at the same time from field-grown plants differed in alkane content. An increase in radiant energy rate, a decrease in humidity or a decrease in temperature was found to induce the largest deposits of wax in the normal and four mutant forms of brussels sprout (Baker, 1974). In this study, the environmental conditions showed only limited effect on the composition of the waxes of the normal and mutant lines, but modify the size, configuration and distribution of surface wax structures.

In Bonus barley, the amount of wax per unit area of expanding primary leaves depends on both the photo- and thermo-periods in which the seedlings were grown (Giese, 1975). Transfer of dark grown Leaves, with a temperature cycle of 15-10°C, to the light stopped leaf expansion and after 24 hours contained more wax than the light grown plants. This demonstrated that wax synthesis and extrusion are not directly correlated with leaf expansion. Another interesting observation of this study was that the pathways of wax formations are the same in light and dark. However, chain length composition of wax is influenced by the two conditions, with one group of chain length dominating over the other.

In a well conceived experiment Wilkinson and Kasperbauer (1980) showed that generally long photoperiod and cool temperature were associated with highest long aliphatic carbon chain production on leaf area basis in tobacco. At 18°C leaves from plants grown under long photoperiods had more wax than leaves from short photoperiods. However, there was no difference in total wax of leaves from either long or short photoperiods grown at 28°C. At both photoperiods, increased temperature resulted in low content of total wax. Alkane and fatty acids decreased on leaves under long photoperiods at 18°C and increased at 28°C. but fatty alcohols showed reverse trend. Light-induced changes in leaf ECW was observed in C_4 weeds (Reddy and Rao, 1982). A temperature dependent variation in the quantity of wax deposited on the leaves was noticed in three ecotypes of ***Eucalyptus virminalis*** (Banks and Whitecross, 1971).

The ECW content was estimated in rainfed coconut genotypes, with simultaneous measurement of weather parameters viz. light, temperature and relative humidity at three distinct stages- pre-stress (prior to monsoon), stress (dry condition) and post-stress (after monsoon) (Kurup ***et al.,*** 1993). In all the three genotypes tested, the first leaf had higher ECW during pre-stress and showed marginal increase during stress period. In contrast, the wax content of sixth leaf exhibited a sharp increase during stress in all the types. There was rapid decline in ECW in both the leaves of the three genotypes during post-stress period. High evaporative demand prevailed in the atmosphere during stress period, when ECW also recorded marked increase. Thus, high ECW content during stress period coincided with high light intensity, temperature and low relative humidity. The major components of ECW are esters of higher fatty acids and fatty alcohols. In general, the number of components of ECW was more in both leaf positions during stress period than either pre or post-stress periods. Stress period was characterized by detection of hydrocarbons and esters, while fatty acids occurred only during the post-stress period. The wax components like β-diketones, secondary alcohols and aldehydes were detected in monsoon season in semi-arid shrubs (Rao and Reddy, 1980).

Mechanism of action

Investigations were also directed towards the mechanism of action of light and temperature on the biosynthesis of ECW. Based on the experiments with labelled precursors, Kolattukudy (1966, 1967) concluded that light had no effect on the synthesis of hydrocarbons in

Brassica. However, Macey (1970) observed that light increased the synthesis of hydrocarbons and secondary alcohols at the expense of aldehydes and esters. His study also revealed that formation of the ketones was almost light independent. Kolattukudy (1968) noticed the synthesis of ECW components in the epidermal cells. Light regulates wax biosynthesis and extrusion on to the surface of barley primary leaves.

Both light and temperature influence the chain length distribution in wax groups. For example, the C_{29}, C_{31}, C_{33} homologues dominate the hydrocarbons in the light, while C_{21}, C_{23} and C_{25} group is much higher in the dark. This. would meaq that the enzyme carrying out a given reaction in wax biosynthesis select specific chain lengths as substrates. Similarly, the temperature also influence chain length composition of the hydrocarbons and free fatty acids, but has little influence on the composition of aldehydes, free and esterified primary alcohols. This also indicates that two sets of enzymes **with** different chain length specifications exist in wax biosynthesis.

Rainfall

Another environmental factor that is closely linked with ECW is the rainfall. According to Mayeux and Jordan (1987) rainfall removes wax content from leaf surfaces. They found 15 to 35% decrease in ECW of *Isocoma* leaves during rainy period as compared to dry period. Simulated rain of 5 cm removed about 5% of the wax from common gold weed leaves (*Isocoma coronopifolia*) and upto 36% of wax from Drummond's gold weed (*I. drummondii*). The predominance of short chain free fatty acids and alcohols suggests that the ECW of the above plants are relatively hydrophilic compared with other plants (Wilkinson and Mayeux, 1987). This partially explains the observed loss of ECW in rainfall. The significantly low ECW content in coconut genotypes observed during post-stress period was attributed to the rainfall (1500 mm) between June and August (Kurup *et* al., 1993).

WAXINESS AS A CRITERIA FOR BREEDING FOR DROUGHT RESISTANCE

A number of mechanisms can contribute to drought resistance in crop plants (Parsons, 1979). Parker (1968) has listed about seven plant characteristics that impart drought resistance and factors that could be considered in a breeding programme. Leaf cuticle finds third place in the list. It is well recognized that a thick or waxy cuticle is advantageous in reducing water loss in plants. As early as 1975, Blum reported that the Bm Bm genotype for ECW increased the drought resistance of sorghum. The improved drought resistance was due to increased reflection of solar energy and decreased cuticular permeability and this resulted in higher leaf water potential. The advantage of bloom on sorghum in semi-arid regions with high yields was reported by Chattenon *et* al. (1975).

Breeding for greater waxiness to produce a less permeable cuticle may help slightly in postponing desiccation, provided tight stomatal closure also occurs in the species. during saess (Parsons, 1979). According to him breeding for this character could be difficult because of the strong environmental effects on wax content. It is true that there are not many studies to prove unequivocally that waxiness could be used in all breeding programmes involved with drought resistance of plants. This itself could form a future thrust for combined efforts by plant physiologists and plant breeders to develop crosses between parents differing in waxes. A beginning has been made in the author's laboratory to screen coconut crosses made between parents with variation in ECW content.

CONCLUSIONS

The research efforts on ECW passed through many areas as divergent as biochemistry of waxes, genetic variability, responses to environment and drought resistance. Considerable progress has been made in most of the aspects, although there are some contradictory results obtained with plant species. While the basic research that centered around the characteristics of wax like electron microscopic studies on ultra structure of wax showed satisfactory progress, the investigations on applied aspects, particularly the use of waxiness as a criteria for breeding for drought resistance are limited. This throws an open challenge to tackle the problems of plant species under drier conditions. The fact that ECW has larger control both on heat load on leaf surface and transpiration rate shows that these are promising areas for exploiting genetic variability. Another area that needs to be taken up sooner than later is the molecular basis of wax synthesis in response to adverse situations. The latest biotechnological developments open up new vistas in this direction.

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