
I N T R O D U C T I O N

The mysterious minute pores in the aerial parts of the green world, above the evolutionary level of the sporophyte generation of mosses and hornworts, stomata, are the sites of two important processes - diffusion of water vapour and diffusion of gases. Since their discovery major advances have been made in understanding their characteristics and behaviour under normal and water stress conditions. Though this subject was reviewed from time to time by large number of workers (Meidner and Mansfield, 1968; Zelitch, 1969; Raschke, 1975, 1979; Hsiao, 1976 and Zeiger, 1983) much lacuna is still left out in the minds of people to understand stomatal mechanics under water stress conditions. The principle aim of the present work is to understand the stomatal movements in purple pigmented leaves of plants growing under water stress conditions. Purple pigmentation was observed in some dicot plant species including Euphorbia hirta, growing on soils with different levels of soil moisture. These purple pigments may be either anthocyanins or betacyanins. In Euphorbia hirta, these pigments were reported as anthocyanins (Veeranjaneyulu and Das, 1982) and their intensities varied depending on the soil moisture levels. Accumulation of these

anthocyanins in leaves may be an adaptation of the survival of the plant to severe water stress and to the high natural irradiation. These anthocyanins in flowers may aid in pollination, in fruits help in dispersal and in leaves may form a defence mechanism against radiation stress. Anatomical observations of pigmented leaves of E. hirta, revealed the presence of purple pigments in the epidermal cells, and their conspicuous absence in guard cells. Hence the present investigation is taken up to understand the influence of anthocyanin accumulated subsidiary cells on the guard cell movements.

Water is an essential component of the plant life. It comprises approximately 85 to 90% of the total fresh weight in physiologically active herbaceous plants. If the water content in moist species falls below this level, many physiological activities of the plant are impaired. Environmental stress such as drought causes the plant water deficits, which affect the plant growth.

Water stress in plants affects at the cellular level, on cell division and expansion on the whole plants and its components and on plant communities on crops. In many species cell expansion is one of the plant

processes most sensitive to drought. Cell wall synthesis and cell expansion are dependent on the turgor pressure. Cell division is considered to be less sensitive to water stress. Water stress causes the closure of stomata, reduce leaf surface area and rate of photosynthesis per unit leaf area. If plant water deficits become more severe the photosynthetic machinery is damaged. Water stress reduces the protein synthesis, chlorophyll synthesis, leaf area, water transport, and seed size. Water deficit affects the plant growth and yield only by affecting the physiological processes and conditions in plants, as outlined below.

ENVIRONMENTAL FACTOR

Water Stress



PHYSIOLOGICAL PROCESSES AND CONDITIONS

Water absorption, ascent of sap, transpiration, plant water balance as reflected in water potential, turgor, stomatal opening and cell enlargement. Photosynthesis, carbohydrate and nitrogen metabolism and other metabolic processes.



GROWTH

Size of cells, organs and plants, Root-shoot ratio, succulence, kinds and amounts of various compounds accumulated Economic yield.

A wide range of mechanisms of adaptation to water deficits exists among plants. In natural communities many of these mechanisms appear to be more important for plant survival than for high productivity (Turner, 1979; 1981; 1982). When the role of the various mechanisms of adaptation to water deficits was evaluated, it was initially assumed that several physiological and morphological responses to water stress were transduced by cell turgor pressure (Begg and Turner, 1976; Hsiao et al., 1976). The adaptive mechanisms that aid in the maintenance of turgor, such as osmotic adjustment (Hsiao et al., 1976; Turner and Jones, 1980; Morgan, 1984), were considered important in maintaining plant growth through maintenance of stomatal opening, photosynthesis, leaf growth and root growth.

Purple pigmentation in leaves of plants under water stress conditions, may be considered as an adaptive mechanism to drought (Veeranjaneyulu and Das, 1984). These purple pigments may be either anthocyanins or betacyanin. The anthocyanins are water soluble, vacuolar pigments, responsible for the violet, blue, purple, dark red and scarlet colouration of fruits, flowers, stems and leaves in all orders of higher plants (Swain, 1976),

with one exception, the order centrospermas, where the violet red colours are due to a different group of water soluble vacuolar pigments, the betacyanins (Piattelli, 1976). It has been reported that about 1.5% of the carbon fixed annually in photosynthesis is used for the synthesis of flavonoids (Smith, 1972). The amount of pigment formation in plants is affected by environmental factors including water stress.

Purple pigmentation is frequently observed in some dicotyledonous weed species, subjected to high solar radiations and water stress. It was reported that in some local weeds such as Euphorbia, Mollugo and Lippia, the purple pigments are anthocyanins and in Portulaca, Alternanthera and Gomphrena, they are betacyanins (Veeranjaneyulu and Das, 1984). Varying intensities of anthocyanin pigments have also been reported in the leaves of Euphorbia hirta growing at different soil moisture levels. The physiological significance of these pigments in the leaves of the plants is poorly understood.

Spyropoulos and Mavrommatis (1978) studied the effect of water stress on pigment formation in three Quercus species, Q. coccifera, Q. ilex and Q. robin.

They have observed an increased anthocyanin levels with decreasing relative water content in these three species, the largest increase occurring in Q. coccifera. Action of light on anthocyanin production was studied by many (Christopher, 1939; Hoftman, 1937; Mayness, 1928; Schneider and Marth, 1931; Walter, 1966; Proctor and Creasy, 1971). The general characteristics of the action of light on anthocyanin production are those typical of the high irradiance responses of plant morphogenesis (Mancinelli 1980, Mancinelli and Rabino, 1978; Mohr 1972, Shropshire, 1972; Smith, 1975).

The intensely coloured anthocyanins are most abundantly present in petals, fruits and leaves of some plants. Their occurrence in petals aid in pollination, in fruits for dispersal and in leaves as a defence mechanism against radiation stress by acting as a screen against damaging UV radiation (Harborne, 1976). Anthocyanin pigmented leaves reflect more radiation in the visible spectrum of the light (Howard, 1966).

In E. hirta purple pigmentation is confined to the epidermis of purple pigmented leaves. Various intensities of pigmentation in leaves were observed and

accordingly the plants were categorized as light, moderate and heavy pigmented leaves. Epidermal peelings from these leaves observed under research microscope revealed the presence of purple pigments in the epidermal cells except in the guard cells. The significant absence of anthocyanins in guard cells and their presence in surrounding epidermal cells may cause changes in turgor pressures, since these pigments are water soluble compounds.

Stomatal opening and closing ensues because of relative turgor changes in the guard cells with respect to the surrounding epidermal tissue (Hsiao, 1976; Meidnes and Mansfield, 1968; Raschke, 1979). Within the epidermis of a leaf, the individual cells compete for space because of their turgor. Stomatal movements usually occur as a result of turgor changes in the guard cells. The turgor differences between the guard cells and their immediate neighbours determines the opening and the size of the stomatal pore. In fully opened stomata, the guard cell turgor pressure is always higher than the turgor pressure of surrounding epidermal cell. Thus the aperture changes depend on turgor differentials in the epidermis. Since the anthocyanins, the water

soluble glycosides accumulate only in the epidermal cells and not in the guard cells, there may be interesting variations in the water relations between epidermal cells and guard cells.

Responses of stomata to water stress can be regarded as fundamental both to plant survival and efficient crop production. Closure of stomata in water stressed plants is necessary to reduce transpiration, to restore turgor and growth, and to protect the organ cells of the leaf that are sensitive to water stress. There are many reports of stomatal adjustments to humidity, low leaf water potential and high temperatures in plants under water stress conditions (Ludlow, 1980). It is reported that osmotic adjustment within the leaf and particularly in the guard and subsidiary cells of plants undergoing stress assists the maintenance of turgor by partially offsetting the decline in water potential and allowing partial stomatal opening. The osmotic adjustment is achieved by organic compounds such as organic acids, amino acids and soluble sugars, and by inorganic elements such as Na, Ca and K. The stomatal closure is a powerful tool for reducing the water loss and it is reported to be under the control of ABA. The first response of stomata to the onset of water stress is to open slightly

temporarily. But this opening phase is transient and the stomata close progressively with increasing water stress under well irrigated or dry land conditions throughout the season. Stomatal closure was observed in younger sorghum and maize plants at -14 to -16 bars under much faster water stress (Fereres, et al., 1978). Wright (1969) and Wright and Hiron (1969) observed an increased ^dogenous levels of abscisic acid within about 30 min of wilting in water stressed wheat leaves. Wright (1977) showed that there is a smooth sigmoid relationship between ABA production and decreasing water potential in wheat. Decrease in photosynthesis, respiration and stomatal conductance was observed in two species of Brassica under water stress (Sunita and Sheoran, 1984). In many plants ABA synthesis stimulated by water stress results in stomatal closure. But in Sorghum, all trans farnesol synthesis initiated by water stress may lead to stomatal closure without the need for synthesis of new ABA (Ogunkanmi et al., 1974). Chloroplasts to be damaged by water stress are sites of ABA synthesis (Boyer, 1976). Modern immunoassay techniques can detect farnol (10^{-15}) quantities of ABA in chloroplasts (Weyers and Hillman, 1979). This amount of ABA slowly narrows down the stomatal opening in the early stages of water

stress. ABA needed for further closure of stomata is supplied from mesophyll cells. There is now much experimental evidence that ABA is synthesized in mesophyll cells, probably in the cytoplasm, and then trapped in chloroplasts. This 'stored' ABA is found in the chloroplasts of well watered plants and is available for release when cellular stress is experienced as a result of water stress.

The ABA induced inhibition of stomatal opening in isolated epidermal strips of Commelina benghalensis, in light was reversed under conditions conducive for cyclic photophosphorylation and remarkably reversed by ATP in the presence of pyruvate (Raghavendra et al., 1976). It is proposed that the direct effect of ABA on stomatal opening was mediated in two ways. (1) By inhibition of cyclic photophosphorylation activities of guard cell chloroplasts and (2) by blocking organic acid formation in guard cells. Stomatal closure by ABA could be achieved by ABA interfering with K^+ , Cl^- ionic exchanges of guard cells or with the metabolic events necessary for interconversions between starch and malate. The outcome of action of the action of ABA is the

inhibition of both the uptake of K^+ ions and the disappearance of guard cell starch, under conditions normally conducive to stomatal opening (Mansfield and Jones, 1971). It was reported that ABA stimulates an efflux both of Rb (analogous to K^+) and of Cl^- out of the guard cells, apparently acting at both the tonoplast and at the plasmalemma. Raschke (1975) concluded that ABA exerts its effect on an H^+ expulsion mechanism in the plasmalemma of the guard cells. Later, Dittrich and Raschke (1977) showed that ABA could also stimulate the release of malate from the guard cells of isolated pieces of epidermis, in the surrounding medium. Potassium loss from stomatal guard cells at low water potentials was observed by Ehret and Joyer (1979). They noticed the loss of guard cell potassium and closure of stomata in turgid leaf segments floated on an ABA solution.

From the above studies it is clear that

1. Purple pigmentation occurs in leaves of plants under water stress conditions
2. Purple pigments in leaves of E. hirta are anthocyanins
3. Stomatal adjustments occur in plants under water stress conditions

4. Stomatal movements depend on the differences in the turgor potentials between guard cells and subsidiary cells.
5. Stomatal closure occurs in plants under stress due to released ABA from mesophyll chloroplasts.

In the literature, the significance of accumulation of anthocyanins in the epidermal cells was not reported, although there are reports on purple pigmentation in leaves as a defense mechanism against radiation stress. Further, their conspicuous absence in the guard cells and accumulation in subsidiary cells was not reported in the literature. The present study reports the preliminary observations on the movements of stomata in purple pigmented and green leaves of Euphorbia hirta.