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**D I S C U S S I O N**

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The present preliminary investigation on the stomatal characteristics of purple pigmented leaves is believed to have opened a new direction in the field of stomatal physiology of plants under water stress conditions. In the present investigation an attempt has been made to study the stomatal characteristics of plants, which accumulated purple pigments in the leaves under water stress conditions. Although much work has been done on stomatal physiology, and many reviews are available on stomatal physiology under water stress conditions (Ludlow, 1980; Mansfield and Davies, 1981). Little was mentioned on the characteristics and movements of stomata in purple pigmented leaves growing under water stress condition. Hence the present study.

The present investigation has revealed the

1. presence and accumulation of purple pigments in the epidermal cells and subsidiary cells of stomata,
2. absence of purple pigments in guard cells,
3. occurrence of defunct guard cells,
4. low transpirational rate and
5. presence of higher levels of free acids in purple pigmented leaves of Euphorbia hirta

Euphorbia hirta, a dicotyledonous C<sub>4</sub> species was seen growing on soils with different soil moisture levels. Purple pigmentation was observed in leaves of plants growing on soils with low moisture levels and the intensity varied with soil moisture content. The leaves of plants growing on soils with high moisture levels are green and normal. A negative correlation was observed between the intensity of purple pigmentation and soil moisture content. The purple pigmentation in E. hirta was reported as anthocyanin (Veeranjaneyulu and Das, 1982). The anthocyanin content was increased with decrease in soil and leaf moisture content. Initially the anthocyanin pigmentation was observed on the adaxial surface of the leaf, and as the stress intensity increased, both adaxial and abaxial surfaces developed the intense purple pigmentation. In the light pigmented leaves, at the initial stage of anthocyanin development, the pigmentation was observed only in the subsidiary and other neighbouring epidermal cells of guard cells. This observation indicates that the purple pigmentation in leaves of water stressed plants has a role in stomatal movements. In heavy pigmented leaves, pigmentation is seen in all the epidermal cells except in guard cells. The conspicuous absence of

purple pigments in guard cells and presence in subsidiary cells in leaves of water stressed plants are interesting.

The water soluble, vacuolar anthocyanin pigments occur in fruits, flowers, stems and leaves in all orders of higher plants (Swain, 1976) except in the order centrospermae. Their occurrence in reproductive organs may aid for pollination and dispersal but their importance in vegetative organs is yet to be understood. The anthocyanin synthesis and accumulation is reported to be influenced by environmental factors including water stress. Spyropoulos and Mavromatis (1978) studied the influence of water stress in three species of Quercus, such as Q. coccifera, Q. ilex and Q. robur. They have observed the increased levels of anthocyanins in leaves with decreasing relative water content. Action of light on anthocyanin production was studied by many (Christopher, 1939; Hoftman, 1937; Magness, 1928; Proctor and Creasy, 1971; Walter, 1966). The activities of key enzymes such as phenylalanine ammonia lyase and cinnamic acid 4-hydroxylase, which are involved in biosynthesis of flavonoids (Russel, 1971; Hanson and Havir, 1972) increase by light, ethylene and ABA treatments

(Amrein and Zank, 1970; Cracker et al., 1971; Hyodo and Yang, 1971; Rhodes and Wooltorton, 1971; Wong, 1976). Ethylene and ABA levels are reported to increase in plants subjected to water stress (Hsiao, 1973; Loveys and Kriedmann, 1973; Zabadal, 1974).

Anthocyanin occurrence in leaves is reported as a defence mechanism against radiation stress by acting as a screen against damaging UV radiation (Harborne, 1976). Further, it is reported that anthocyanin pigmented leaves reflect more radiation in the visible spectrum of the light (Howard, 1966).

The radiation, water and temperature stresses occur together in summer months under tropical arid environments, and the plants in natural communities during summer months exhibit intense pigmentation. Several adaptive mechanisms develop in plants under stress and many of these mechanisms appear to be more important for plant survival than for high productivity (Turner, 1979; 1981; 1982). One of such physiological and morphological adaptations to water stress may be the synthesis and accumulation of anthocyanins specifically in the epidermal cells of leaves.

The significance of specific accumulation in subsidiary and other epidermal cells of leaves of water stressed plants may be understood by explaining the turgor regulated movements of guard cells.

Stomatal opening and closing ensues because of relative turgor changes in the guard cells with respect to the surrounding epidermal tissue (Hsiao, 1976, Meidner and Mansfield, 1968, Raschke, 1979). Within the epidermis of a leaf, the individual cells compete for the space because of their turgor. Stomatal movements usually occur as a result of turgor changes in the guard cells. The turgor difference between the guard cells and their immediate neighbours determines the opening and size of the stomatal pore. Because of the cell wall mechanics of the guard cells (Raschke, 1979; Wu and Sharpe, 1979) increase in turgor of the guard cell increases the aperture of the pore. In a full opened stomata, the guard cells turgor pressure is always higher than the turgor pressure of the surrounding epidermal cell. In the closed stomata guard cell turgor pressure is less than the subsidiary cells. Thus the aperture changes depend on turgor differentials in the epidermis.

The turgor differentials are related to the water potentials of the cells, since turgor pressure is a component of water potential as shown below :

Water potential = Osmotic potential + Pressure potential

The lasting turgor differences between guard cells and subsidiary cells are maintained along the osmotic gradients. Thus the turgor pressure depends on the osmotic potential of the cell.

The anthocyanins, water soluble glycosides present in the vacuoles, contribute for the osmotic potential. Since the anthocyanins were observed only in subsidiary cells, the osmotic potentials of these cells will be low; this state of condition results in the higher turgor pressure. Thus the anthocyanins in subsidiary cells contribute for higher turgor pressure by way of osmotic adjustment in water stressed plants. This higher turgor pressure presses the guard cells, and causes the stomata to close totally or partially.

The frequency of stomata with defunct guard cells has also increased with increase in the intensity

of purple pigmentation. The occurrence of defunct guard cells causes the stoma partially closed. Their occurrence can be explained by the higher turgor pressure in the subsidiary cells, which will push the guard cells for the available space. This partial closure of stomata may also reduce the transpirational water loss.

The significance is evident from the observed lower transpirational rates in the purple pigmented leaves than in the green leaves.

The confirmatory observation for the above conclusion is that the frequency of stomata with one or two or three pigmented subsidiary cells is nearly same in light pigmented cells. But as the pigmentation intensity increased due to increase in water stress all the three subsidiary cells of stomata accumulated purple pigmentation and the frequency of stomata with single subsidiary pigmented cell decreased.

Stomata, based on their movements, can be categorized as photoactive stomata and scotoactive stomata. A general understanding of the pattern of stomatal movement is that in the mesophytic plants,

stomata open during day time and close during night under natural conditions. (Loftfield, 1921, Rees, 1958; Meidner and Mansfield, 1968). In contrast, a few xerophytic plants show night opening and day closure of stomata. These plants possess a specialized carbon fixation pathway and are CAM plants. The scotoactivity of stomata is considered as an adaptive water saving mechanism for CAM plants under continuous drought conditions (Kluge and Ting, 1978). Induction of such water saving CAM metabolism by dark opening of stomata in  $C_3$  plant Mesembryanthemum under water stress conditions has been reported (Winter and Troughton, 1978; Winter et al., 1978). In the present investigation, the purple pigmented plants of E. hirta have shown a tendency of dark opening of stomata. In green plants maximum stomatal opening was observed at 11 and 14 hours. While in purple pigmented plants maximum opening was at 20 and 23 hours. Studies on diurnal variations in pH revealed low pH during day time in green plants, and during night in purple pigmented leaves. Further, diurnal fluctuations in free acid levels were observed in both green and purple pigmented leaves. In purple pigmented leaves the higher levels of free acids during night may suggest the presence of CAM metabolism in leaves.

However, studies on malic acid levels indicated the absence of CAM pattern of  $\text{CO}_2$  fixation. In CAM plants dark fixation of  $\text{CO}_2$  into malic acid and its accumulation is well established (Kluge, 1982). During day time malic acid is decarboxylated and the liberated  $\text{CO}_2$  is refixed by Calvin's cycle of photosynthesis. This diurnal variations in malic acid levels with dark accumulation is significant feature of CAM phenomenon. The absence of dark accumulation of malic acid indicates absence of CAM phenomenon in purple pigmented E. hirta. However, the higher levels of free acids in purple pigmented leaves may be for the osmotic adjustment to maintain the turgor under water stress condition (Turner, 1986).

ABA and proline accumulate in plants under water stress conditions. It is well established that endogenous levels of ABA raise as the leaf water potential falls (Wright 1977, Kannangara et al., 1981, Durley et al., 1981). ABA is reported to be associated with stomatal movements (Tal and Imber 1970, 1981) under water stress conditions. ABA causes the turgor changes of guard cells by regulating the movement of  $\text{K}^+$ ,  $\text{H}^+$ ,  $\text{Cl}^-$

and malate. ABA is synthesized and released from mesophyll chloroplasts and travels to the guard cell (Loveys, 1977), where it inhibits  $K^+$  uptake (Mansfield and Jones 1971, Weyers and Hillmann, 1980),  $H^+$  release (Raschke, 1977) and promotes malate leakage from the guard cells (Dittrich and Raschke, 1977; Vankirk and Raschke, 1978). These effects result in the loss of turgor in guard cells and cause the stomata to close. In the present investigation stomata of both green and purple pigmented leaves were closed under ABA treatments. But stomata of purple pigmented leaves have shown quick response to ABA and stomatal closure was higher than that of the green plants. Similar response to proline treatments in both<sup>h</sup> green and purple pigmented leaves was observed. Although proline accumulation in water stressed plants is well documented (Aspinall and Paley, 1982; Stewart, 1982), its role in stomatal movements is little known. Proline caused the closure of stomata in both green and purple pigmented leaves. Response of stomata in purple pigmented leaves to proline is more than that of green leaves and is similar to the response to ABA.

ATP and ADP enhanced the stomatal opening in both green and purple pigmented leaves. Maximum stomatal opening was in ATP incubated stomata indicating the requirements of energy for stomatal opening.

Potassium is widely utilized for turgor build up in the majority of plant cells (Hastings and Gutknecht, 1978; Mengel and Kirkby, 1980). Guard cells also use  $K^+$  as a major osmoticum (Hsiao, 1976; Raschke, 1979). Several investigations have revealed that in fully opened stomata,  $K^+$  content of guard cells is several fold higher than that of the surrounding tissue (Hsiao, 1976; Mac Robbie and Lettan, 1980 a,b; Penny and Bowling, 1974; Raschke, 1979). The correlation between  $K^+$  content and degree of aperture is usually high (MacRobbie, 1981; Raschke, 1979) and is reported that  $K^+$  contributes about half of expected increased in osmotic potential needed on the build up of turgor. In the present investigation, wide stomatal opening was observed in green plants in low concentrations of KCl (50 and 100 mM), while in purple pigmented plants maximum opening was observed in 200 mM KCl solution. This indicates the requirement of higher levels of osmoticum ( $K^+$ ) for the guard cells of purple pigmented

plants, for maximum opening, since the subsidiary cells possessed higher levels of anthocyanins contributing for higher osmoticum. The requirement of higher concentrations of  $K^+$  for the wide opening of stomata in purple pigmented leaves indirectly revealed the higher turgor in subsidiary cells of stomata, and the role of anthocyanins in the regulation of stomata during water stress conditions.

Thus from the present preliminary investigation, it may be stated that, the accumulation of anthocyanins in subsidiary and other epidermal cells has a role in controlling the water loss through transpiration. Further it may also be stated that purple pigments in leaves under water stress conditions besides offering protection against radiation stress, also help the plant to conserve water by regulating the stomatal movements. Much work is needed in this direction to understand the stomatal movements in purple pigmented leaves and further work is in progress.

Thus purple pigmentation in leaves may be an adaptation of the survival of plant under extreme drought condition.