INTRODUCTION

During the past two decades major advances have been made in the field of stomatal physiology in understanding the mechanism of stomatal movements. This work was reviewed from time to time by large number of workers (Meidner and Mansfield, 1968; Zelitch, 1969; Raschke, 1975; Heath and Levitt, 1976; Zeiger, 1983). Inspite of large number of articles published, no satisfactory mechanism has been envisaged regarding the opening and closing of stomata.

Stomatal movements occur usually as a result of turgor changes in the guard cells and the surrounding epidermal cells. Tugor changes are resulted from water fluxes due to osmotic gradients. The maintenance of osmotic gradients between guard cells and surrounding epidermal cells involves the energy, and the source of which is a matter of discussion. Hence the present study was intended to study the role of photophosphorylation energy in stomatal movements of Vigna unguiculata.

Stomatal opening and closing occur because of relative turgor changes in the guard cells with respective to the surrounding epidermal tissue (Meidner and Mansfield, 1968; Hsiao, 1976; Raschke, 1979). Because of the mechanics

of the guard cell walls (Raschke, 1979; Wu and Sharpe, 1979) increase in turgor increases the aperture of the pore, while decrease in guard cell turgor causes the closure of stomata. Hence stomatal movements are the result of turgor changes in the guard cells. In fully opened stomata guard cell turgor is always higher than the turgor of surrounding epidermal cells. Thus aperture changes depend on turgor differentials in the epidermis, which are related to the water potentials of the cells. These turgor differences can only be maintained along the osmotic gradients, which generate water fluxes and turgor changes.

The importance of the osmotic changes in stomatal movements was recognised early in stomatal physiology. The origin and nature of solutes causing the osmotic changes are of considerable importance in the stomatal physiology.

Several investigators have reported that K^+ is one of the major solutes of guard cell that contribute for osmotic changes. It was reported that in fully opened stomata K^+ content of the guard cell is several fold higher than that of the surrounding tissue, indicating the operation of a concentration mechanism against diffusion

gradients. (Hsiao, 1976; McRobbie and Letten, 1980; Penny and Bowling, 1974; Raschke, 1979). The dominant participation of K^+ in stomatal movements was well established in many species (Hsiao, 1976; Raschke, 1979). Selective uptake of K^+ in the presence of other monovalent ions has also been demonstrated (Humble and Hsiao, 1971). A positive correlation between K^+ content and the size of the aperture was also observed (McRobbie, 1977; Raschke, 1979), and it was reported that K^+ contributes about half of the expected increase in osmotic potentials needed for build up of turgor. Thus it may be concluded that K^+ accumulation in guard cell is an essential feature of stomatal opening.

One possible consequence of the massive accumulation of K^+ in the guard cells during stomatal opening would be the development of a large electrical charge. To balance the change and maintain the electroneutrality, two basic mechanisms may be involved. The electroneutrality might be achieved i) by the simultaneous transport of negatively charged anions, along with K^+ into the guard cells or ii) by the simultaneous transport of osmotically inactive cations out of the guard cells.

It was reported that majority of the species, except Allium cepa have no specific anion requirement for stomatal opening (Raschke, 1975). In species having starch in the guard cell chloroplasts, organic acids mainly malate were reported as anions to maintain the electroneutrality (Allaway, 1981; Outlaw, 1982; Raschke, 1979). Besides malate, citrate, glutamate and aspertate were also reported to serve as counter ions of K^+ (Allaway, 1981; Outlaw, 1982). Several investigators reported that the increase in organic acid content found in guard cells upon on stomatal opening can be attributed to the de novo synthesis in the guard cells (Outlaw and Manchester, 1979; Outlaw et al., 1979; Allaway, 1981; Outlaw, 1982; Raschke, 1979). The postulated biochemical pathway utilizes PEP from starch degradation (Outlaw and Manchester, 1979). The cytoplasmic enzyme PEP carboxylase (Outlaw et al., 1979) catalyzes CO_2 fixation into PEP, generating oxaloacetic acid, which is then reduced to malate by malic dehydrogenase (Allaway, 1981; Outlaw, 1982; Raschke, 1979).

Thus uptake of ions against diffusion gradients has been demonstrated during stomatal opening, implying that energy is utilized in the movement of ions.

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The source and nature of the energy required for K^+ transport between the cells of the stomatal complex has been a subject of major speculation and debate (Zeiger, 1983), although it is generally assumed that energy in the form of adenosine triphosphate (ATP) is involved.

The guard cell bioenergetics has been a controversial aspect of stomatal physiology (Hsiao, 1976; Raschke, 1975, 1979). Available evidences indicate that in guard cells which contain chloroplasts and mitochondria, both oxidative phosphorylation and photophosphorylation are potential energy sources during stomatal movements (Hsiao, 1976; Melis and Zeiger, 1982; Zeiger et al., 1981; Zeiger et al., 1977, 1978; Melitch, 1965). The concept of former two distinct energy sources driving ion transport in guard cells was well documented.

Ultrastructural studies clearly indicate that mitochondria exist in large numbers in guard cells (Hsiao, 1976, Meidner and Mansfield, 1968; Raschke, 1975). The ratio of mitochandria to chloroplasts is often reported to exceed the ratio in the mesophyll cells by several fold. Guard cells contain demonstrable dehydrogenase activity and the respiratory inhibitors such as azide, cyanide and

DNP also inhibit stomatal opening (Hsiao, 1976, Lurie, 1978, Pallaghy and Fischer, 1974; Raghavendra, 1981; Turner, 1973; Walker and Zelitch, 1963; Wilson et al., 1978). This evidence suggests that respiratory activity is one of the major sources of ATP within the guard cells.

Several lines of evidence point to a role of photophosphorylation by guard cell chloroplasts in stomatal movement. Studies on the wavelength dependence of stomatal opening showed that at moderate to high light intensities, the spectral sensitivity of opening closely resembles that of photosynthesis (Kuiper, 1964; Hsiao et al., 1973). This indicates an important role of guard cell chloroplasts in stomatal movements (Levitt 1974, Ogawa et al., 1978, Pallas and Dilley, 1972, Zeiger et al., 1981). Using immunological, micro histochemical and functional approaches, it was shown that guard cell chloroplasts lack the key enzymes of photosynthetic CO_2 reduction (Outlaw and Lowry, 1977, Outlaw et al., 1979, Schnabl, 1981).

Studies with inhibitors of photophosphorylation indicate that ATP produced in this process is utilized in stomatal mechanism. Dichlorophenyl dimethyl urea, an inhibitor of noncyclic photophosphorylation, was reported

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to have no effect on stomatal opening under light, while salcylaldoxime an inhibitor of cyclic photophosphorylation suppresses the light stimulated stomatal opening (Zeiger, 1983). Thus it was concluded that cyclic photophosphorylation also contributes the energy required for transport of K^+ into the guard cells.

Blue light photosystem involvement in stomatal movements has been demonstrated in many species. Stomatal opening was reported to be consistently higher in blue light than in red light, indicating that in addition to the photoresponses of chlorophylls, a blue light system has been activated (Jarvis and Morison, 1981; Lusie, 1978; Ogawa, 1981; Pemadasa, 1982; Sharkey and Raschke, 1981; Travis and Mansfield, 1981; Zeiger and Field, 1982). The swelling of onion guard cell chloroplasts in blue light but not in red light, the wavelength dependence of malate biosynthesis and increase in transpiration by blue light in the graminae (Hrogardh and Johnson, 1975; Ogawa et al., 1978; Skaar and Johnson, 1978; Zeiger and Hepler, 1977) provide experimental evidence for a blue light effect independent of the photosynthetic active radiation dependent system of guard cells.

It was concluded that each energy source could be connected with specific stomatal responses, such as blue light photosystem with the blue light dependent opening at dawn (Zeiger et al., 1981), oxidative phosphorylation with VPD responses (Losch and Tenhunen, 1981) and the possible energy requirements of stomatal closing (Hsiao, 1976) and photophosphorylation with stomatal openiny at moderate to high light intensities.

Thus requirement of energy for stomatal opening was well documented. And the cyclic photophosphorylation as source of energy was also demonstrated. However, since cyclicphotophosphorylation was centred around PSI activity, the significance of the presence of the functional PSII was not clearly understood. Hence in the present study an attempt was made to understand the role of PSII of guard cell chloroplasts in stomatal opening with PSII electron inhibitors and donors.

Further, cowpea (Vigna unguiculata) being an important semiarid pulse crop, stomatal responses to KCl and ABA, were studied.