# DISCUSSION

It may be stated that the present investigation has offered some clues for the role of PSII in light induced stomatal opening in Vigna unguiculata. The studies with PSII electron donors such as DPC, $MnCl_2$ hydroxylamine hydrochloride and sodium ascorbate have shown that opening was enhanced. Similarly DCMU, an inhibitor of photosynthetic electron transport, inhibited the stomatal opening at high concentration, although it showed little effect at low concentrations. Further the stomatal opening was enhanced by $K^+$ and closed by ABA.

Imamura (1943) first suggested a positive correlation between the intensity of stainable stomatal aperture and guard cell osmotic potential under a variety of conditions. Later Fischer (1967) reported that stomata in the epidermal strips of Vicia faba open readily in light only when floated on solutions containing $K^+$. He estimated the potassium uptake with $^{86}Rb^+$ and found it sufficient to account for the observed change in guard cell osmotic potential, if counter ion for $K^+$ ion is assumed (Fischer, 1968, Fischer and Hsiao 1968). Later several investigators have estimated the $K^+$ ion content of guard cells by using electron micro probe (Pallaghy 1971,

Rashke and Fellow, 1971), and by flame photometry of quasi isolated guard cells (Allaway and Hsiao, 1973). From these observations it was deduced that $K^+$ ion accounts for a major part of the osmotic potential change.

Similarly the present investigation has confirmed the $K^+$ ion requirement for the stomatal opening in Vigna unguiculata. $K^+$ enhanced stomatal apparture size over control in light. Further it is also interesting to note that $K^+$ ion caused slight increase in aperture size over control in darkness. The enhancement in light may involve the participation of photochemical energy for the accumulation of $K^+$. However in darkness, oxidative phosphorylation may provide the necessary energy for the opening of stomata.

In general, it was reported that $Ca^{2+}$ is known to reduce or inhibit the stomatal opening. Willman and Mansfield (1969) reported that $CaCl_2$ and $MgCl_2$ at 1 mM concentration suppressed the stomatal opening in strips of Commelina communis, C. sikkimensis and Vicia faba. Calcium chloride at low concentration of 0.1 mM reduced the aperture size in Vicia faba (Fischer, 1972) and

18

C. communis (Fujino 1967). But in the present study, the divalent cation $Mn^{++}$ enhanced the stomatal opening in light. This enhancement may be due to its ability to donate electrons to PSII, which may generate ATP through photophosphorylation.

In darkness, $MnCl_2$ treatments neither enhanced stomatal opening over control nor closed the stomata. Thus it may be stated that $MnCl_2$ induced enhancement in stomatal opening in light is through its involvement in photochemical reactions of PSII.

Although there area a few reports on the influence of photosynthetic electron transport inhibitors on stomatal movements (Das and Raghavendra, 1974, 1982) little information is available on the role of electron donors of PSII on stomatal movements. In the present study, besides $MnCl_2$, ascorbate diphenylcarbazide and hydroxylamine hydrochloride were also studied for their influence on stomatal movements in light and darkness. Similar to $MnCl_2$, DPC, ascorbate and hydroxylamine hydrochloride have stimulated the stomatal opening in light. The DPC, ascorbate and hydroxylamine hydrochloride are electron donars of PSII (Trebst, 1974; Vernon and

Shaw, 1969; Katoh et al., 1971) and thus their influence on stomatal movements may be through the enhanced PSII activities. This may indicate the participation of non-cyclic photophosphorylation as on energy source in stomatal movements. In darkness, these donors showed little influence on stomatal movements.

Fluorescence spectroscopic studies of guard cells demonstrated the presence of light harvesting pigments of PSI and PSII (Melis and Zeiger, 1982; Ogawa et al., 1982; Zeiger et al., 1981). Electron transport in guard cell chloroplasts from Vicia faba has also been demonstrated (Ogawa et al 1982; Shimazak et al., 1982). However a negative evidence for the activity of PSII in guard cell chloroplasts was reported in a study comparing oxygen exchange rates from chloroplasts isolated from guard cell protoplasts of Vicia with their mesophyll counterparts (Schnable and Zeiger 1977). However this discrepancy was attributed to the damage of PSII by digestion during isolation protoplasts (Shimazaki et al., 1982).

Thus, although Calvin's cycle enzymes are absent in guard cell chloroplasts, functional PSI and PSII

involving in the generation of ATP are present. The enhancement of stomatal opening by DPC, $MnCl_2$, hydroxylamine hydrochloride and sodium ascorbate may indicate the involvement of PSII generated energy for stomatal opening.

DCMU is an inhibitor of photosynthetic electron transport and inhibits the electron flow between Q and Cyt B559 (Izawa and Good, 1972). In white light, DCMU was reported to have no influence on stomatal opening and opening was observed in the presence of DCMU (Huble and Hsiao 1969). It was assumed that this opening could be sustained by blue light photosystem on oxidative photophosphorylation, inspite of fully inhibition of PSII. Schwaltz and Zeiger (1982) showed that stomatal opening under red light in which energy pool would be expected to depend solely on photophosphorylation, was drastically inhibited by DCMU, both in Vicia and Commelina.

In the present investigation DCMU was found to inhibit the stomatal opening in light at 1 mM concentration and has no effect at 0.1 mM concentration. In darkness it showed little influence on the stomatal movements. Thus DCMU inhibition of stomatal opening in Vigna in light

demonstrates the involvement of PSII activity in stomatal opening.



DNP inhibition of stomatal opening was well documented in the literature (Hsiao, 1976; Das and Rahavendra, 1974). Since DNP is an inhibitor of respiration and cyclic photophosphorylation, it suggests that cyclic photophorylation and oxidative phosphorylation are sources of ATP for stomatal opening. In the present study also, DNP inhibition of stomatal opening was observed both in light and darkness. But in light, total inhibition in stomatal opening, as observed in darkness was not observed in the present study. This indicates the role of photochemical energy other than cyclic photophosphorylation in stomatal opening.

ABA prevents stomatal opening and causes closure (Mittelheurer and Van steveninck, 1969). It is currently receiving much attention for one of its postulated roles, as a regulator of plant behaviour under stress conditions. ABA was reported to cause stomatal closure, within minutes when it is applied to the leaves (Arntzen et al., 1973). It was reported that ANA inhibits stomatal opening by inhibiting the uptake of $K^+$ by guard cells (Mansfield

and Jones 1971; Hortow and Moran, 1972; Arntzen et al., 1974). This inhibition was readily reversible (Horton, 1971). In the present investigation ABA inhibited the stomatal opening in Vigna unguiculata. This stomata of cowpea are sensitive to low concentrations of ABA.

From the present investigation it may be concluded that $K^+$ ion stimulates the stomatal opening in Vigna unguiculata. Further the enhancement of stomatal opening in light by sodium ascorbate, hydroxylamine hydrochloride, DPC and $MnCl_2$, electron donors of PSII suggests the involvement of PSII in stomatal movements. This was confirmed by the observation that DCMU, an inhibitor of photosynthetic electron transport, inhibited stomatal opening at high concentrations although it had little effect at low concentration. Thus the stomata of V. unguiculata showed responses to KCl, ABA and electron donors and inhibitors of PSII.