International Journal of Trend in Scientific Research and Development (IJTSRD)
International Open Access Journal
ISSN No: 2456 - 6470 | www.ijtsrd.com | Volume - 1 | Issue – 6

Studies on Stomatal Movement in *Dolichus biflorus* Linn. (Fabaceae)

Dr.G.Meerabai
Assistant Professor, Department of Botany,
Rayalaseema University, Kurnool -7, A.P., India

B.Koteswari
Assistant Professor, Humanities & Sciences,
Brindavan Institute of Science & Technology,
Chinna Tekur Road, Kurnool, A.P., India

ABSTRACT

Usually relative turgor changes which maintain osmotic gradients in the guard cells cause stomatal movements. In many species the dominant participation of K+ in stomatal movements was well established. Organic acids such as malate, citrate, glutamate and aspertate were reported to serve as counter ions of K+ in many species. Both oxidative phosphorylation and photo phosphorylation are potential energy sources. In earlier studies, the functional role of PS II was not clearly understood. Hence the present study is aimed to understand the role of PS II in guard cells of *Dolicus biflorus* Linn. a Fabaceae member. Influence of PS II electron acceptors and donors such as potassium ferricyanide, hydroxylamine hydrochloride, MnCl2, Sodium ascorbate, diphenyl carbazide, ATP, ADP, ATP + MnCl2 and ADP + MnCl2, MnCl2 + NADP and Hydroxylamine hydrochloride + NADP is studied to understand the role of photo system II on stomatal movements,

Keywords: Turgor changes, guard cells, stomata movements, organic acids, K+ ions, PS II, ATP, MnCl2

I. INTRODUCTION

To understand the mechanism of stomatal movements, major advances have been made during the past two decades. Large number of workers such as Meidner and Mansfield, 1968[(1);] Zelitch, 1969[(2)]; Raschke, 1975[(3);] Zeiger, 1983[(4)] reviewed this work from time to time. No satisfactory mechanism has been envisaged regarding the opening and closing of stomata.

Stomatal movements usually occur because of relative turgor changes in the guard cells with respective to the surrounding epidermal tissue. (Meidner and Mansfield, 1968[(1);] Hsiao, 1976[(5)]: Raschke, 1979[(6)]; Wu and Sharpe, 1979[(7)]). These turgor differences can only be maintained along the osmotic gradients. The origin and nature of solutes causing the osmotic changes are of considerable importance in the stomatal physiology. The dominant participation of K^+ in stomatal movements was well established in many species (Hsiao, 1976[(5)]; Raschke, 1979[(6)]). To balance the change and maintain the electroneutrality, two basic mechanisms may be involved. The electroneutrality might be achieved 1) by the simultaneous transport of negatively charged anions, ii) by the simultaneous transport of osmotically inactive cations out of the guard cells. In majority of the species starch, organic acids such as malate, citrate, glutamate and aspertate were also reported to serve as counter ions of K^+ (Allaway, 1981[(8)]; Outlaw, 1982[(9)]). The energy required for K^+ transport between the cells of stomatal complex is generally available in the form of ATP. Evidences indicate that both oxidative phosphorylation and photo phosphorylation are potential energy sources during stomatal movements (Hsiao, 1976[(5);] Melis and Zeiger, 1982 [(10)]; Zelitch, 1965[(2)]: Zeiger et al.

1977[11], 1978 [12]& 1981[13]). Cyclic photophosphorylation was centered around PS I activity and it was demonstrated as source of energy. The functional role of PS II was not clearly understood. Hence the present study is aimed to understand the role of PS II in guard cells of *Dolicus biflorus* Linn. a Fabaceae member.

II. METHODOLOGY

Seeds of *D.biflorus* were procured and raised in earthenware pots containing red soil mixed with formyard manure in the ratio of 3:1. Three plants in each pot were maintained under natural photoperiod. Fully expanded 2nd and 3rd pair of leaves from the apex of one month old plants were selected for the study of stomatal movements. The green leaves were collected and washed in distilled water. Epidermal peelings were removed from the lower surface in the middle portion or on the side of the midrib region. The peelings were placed in 50mm. phosphate

Buffer P^H 7.0. Uniform peelings were selected for the study. The peelings were mounted on the slide and stomatal aperture was measured under a microscope with a precalibrated ocular micrometer. Each time an average of 30 stomata were selected at random for calculating the pore width.

For studying the influence of various test chemicals, epidermal strips of about 1.0 x 5.0 mm. size were prepared and incubated in 50ml. phosphate buffer P^H 7.0. The required concentrations of test compounds were prepared in phosphate buffer. The epidermal strips were floated on about 15 ml. of incubation medium in petri dishes under illumination or in total darkness. The light source was a bank of incandescent bulbs (100 w x 8) and the light intensity after passing through water filter was 12 fluxes. The temperature was maintained at 27^o +/- 2^oC by circulating cold water through the water filter. Observations were made at regular intervals of 4 hours i.e. 8.00 A.M. to 12.00 noon and 4 P.M. The epidermal strips from the incubation medium were removed and mounted on the slide with same medium and observed under an Olympus research microscope. The size of the stomatal aperture was measured with the help of a precalibrated ocular micrometer. Each time an average of thirty stomata at random was taken for calculating the width. The experiments were repeated thrice on different days. The averages of these readings are presented in results. Observations in buffer were taken as control.

In order to understand the role of photo system II on stomatal movements, influence of PS II electron acceptors and donors is studied.

III. RESULTS

Table 1 indicates the response of stomata to potassium ferricyanide, an electron acceptor of PS II in light and darkness. In presence of potassium ferricyanide, the stomatal opening was totally inhibited both in light and darkness. A little opening was observed in 1 µl potassium ferricyanide solutions in light. It is due to inhibitory effect of ferric cyanide.

Table 2 indicates the influence of different concentrations of hydroxylamine hydrochloride, an electron donor of PS II on stomatal movement. It enhanced the stomatal opening in light over control. It showed little effect in darkness. Maximum stomatal opening was observed in 1 µl solution in light.

Table 3 indicates the influence of MnCl2, an electron donor of PS II on stomatal movement. It enhanced stomatal opening over control in light. In dark, a slight stimulation in stomatal opening was observed in 1 µl. MnCl2 solution after 4 hours of incubation.

Table 4 indicates the influence of Sodium ascorbate, an electron donor of PS II on stomatal movement. It enhanced the stomatal opening over control in light. In dark, it enhanced slight opening of stomata at high concentrations. A very slight stimulation in stomatal opening was observed at 1 µl. concentration.

Table 5 indicates the influence of diphenyl carbazide, an electron donor of PS II on stomatal movement. It enhanced stomatal opening in light.

Table 6 indicates the influence of ATP, ADP, ATP + MnCl2 and ADP + MnCl2 on stomatal movements. All of them enhanced stomatal opening both in light and dark. The enhancement in light is greater than the enhancement in dark.

Table 7 indicates the influence of MnCl2 + NADP and Hydroxylamine hydrochloride + NADP on stomatal movements. Both of them enhanced the stomatal opening in light.

IV. DISCUSSION

Imamura (1943)[14] first suggested a positive correlation between the intensity of stainable stomatal aperture and guard cell osmotic potential under a variety of conditions. Later Fischer(1968) [15], Fischer

and Hsiao (1968) [16] reported that stomata in the epidermal strips of *Vicia faba* open readily in light only when floated on solutions containing K^+, if counter ion for K^+ is assumed. From the studies of Pallaghy (1971) [17], Rashke and Fellow (1971) [18] and Allaway and Hsiao (1973) [19], it was deduced that K^+ ion accounts for a major part of the osmotic potential change.

In general, from the studies of Fujino (1967) [20], Willmer and Mansfield (1969) [21] and Fischer (1972) [22] it was reported that a divalent cataion Ca^{2+} is known to reduce or suppress or inhibit the stomatal opening. But in present study the divalent catiaon Mn^{++} enhanced the stomatal opening in light. This enhancement may be due to its ability to donate electrons to PS II, which may generate ATP through photophosphorylation. In dark, $MnCl_2$ may enhance stomatal opening or closed the stomata. But in light, it induces opening of stomata through its involvement in photochemical reactions of PS II.

There are a few reports on the influence of photosynthetic electron transport inhibitors on stomatal movements (Das and Raghavendra, 1974[23] & 1982[24]). Little information is available on the role of electron donors of PS II on stomatal movements. In present study, besides $MnCl_2$ influence of Sodium ascorbate, Diphenyl carbazide and Hydroxylamine hydrochloride on stomatal movements in light and dark are studied. Similar to $MnCl_2$, Sodium ascorbate, Diphenyl carbazide and Hydroxylamine hydrochloride also have stimulated the stomatal opening in light. Since these are electron donors of PS II, their influence on stomatal movements may be through the enhanced PS II activities. In dark, these donors showed little influence on stomatal movements. This may indicate the participation of non cyclic photophosphorylation as on energy source in stomatal movements

REFERENCES

1) Meidner H, Mansfield TA - Physiology of stomata. Mc Graw Hill, London, 1968, 179.
2) Zelitch I - Environmental and Biochemical control of stomatal movement in leaves. **Biological Reviews** 40(4), 1965, 463 – 481.
3) Raschke K - Stomatal action. **Ann.Rev. Plant Physiology** 26, 1975, 309 – 40.
4) Zeiger E - The Biology of stomatal guard cells. **Ann. Rev. of Plant physiology**, 34, 1983, 441 – 474.
5) Hsiao TC - Stomatal ion transport. In: Transport in plants II, Ed V. Luttge, M.G Pitman **Encycl.Plant Physiol** (N.S) 2 B, 1976,195 – 221, Berlin, Springer.
6) Raschke K - Movements of stomata. In. **Physiology of movements** (ed.) W. Haupt and M.E Feinleib. **Encycl.Plant Physiol.** (N.S.) 7: 383 – 441, 1979, Berlin; Springer.
7) Wu H, Sharpe PJH - Stomatal mechanics II: Material properties of guard cell walls. **Plant, Cell and Environment** 2, 1979, 235 – 44.
8) Allaway WG - Anions in stomatal operation. In **Stomatal Physiology** (ed.) Cambridge: Cambridge Univ. Press. 1981, PP. 295.
9) Outlaw WH Jr - Carbon metabolism in guard cells. In: **Cellular and Sub cellular localization in plant metabolism**. (eds.) L.L.Creasy and G. Hazdina. 1982.
10) Melis A, Zeiger E - Chlorophyll - a fluorescence transient in mesophyll and guard cells. Modulation of guard cell photophosphorylation by Co_2. **Plant Physiology**, 69, 1982, 642 – 47.
11) Zeiger E, Hepler PK, Varela F - Light sensitive membrane potentials in onion guard cells. **Nature** 270, 1977, 270-71.
12) Zeiger E, Bloom AJ, Hepler PK - Transport in stomatal guard cells. A chemico – osmotic hypothesis. **What is new in Plant Physiology**, 9, 1978, 29 – 32.
13) Zeiger E, Field C, Mooney HA - Stomatal opening at dawn possible roles of the blue light response in nature. In: H.Smith (ed.) **Plants and Day light spectrum**, Academic press, London.1981, 391 – 407.
14) Imamura S - Untersuchungen uber den mechanismus der turgorschwandung der spaltoffnungesschliesszellen. **Jpn. J. Bot.** 12, 1943, 82–88.
15) Fischer RA - Stomatal opening in isolated epidermal strips of *Vicia faba*, L. Response to light and to Co_2free air. **Plant Physiology** 43: 1968, 1947 – 52.
16) Fischer RA, Hsiao TC - Stomatal opening in isolated epidermal strips in *Vicia faba* II; Responses to KCl concentration and the role of potassium absorption. **Plant Physiology** 43: 1968, 1953 – 58.
17) Pallaghy CK - Stomatal movement and potassium transport in epidermal strips of Zea Maize. The effect of Co_2. **Planta** 101: 1971, 287 – 295.
18) Raschke K, Fellows MP - Stomatal movements in Zea Maize. Shuttle of potassium and chloride

between guard cells and subsidiary cells. **Planta** 101,1971, 296 – 316.

19) Allaway WG, Hsiao TC - Preparation of rolled epidermis of *Vicia faba*, L. So that stomata are the only viable cells: analysis of guard cell potassium by flame photometry. **Aust. J. of Biol. Sci**. 26, 1973, 309 – 318.
20) Fujino M - Role of adenosine triphosphate and adenosine triphosphatase in stomatal movements. **Science Bulletin,** Fac.Educ. Nagasaki Univ. 18, 1967, 1-47.
21) Willmer CM, Mansfield TA - A critical examination of the use of detached epidermis in studies of stomatal physiology. **New Physiologist** 68, 1969, 363 – 375.
22) Fischer RA - Aspects of potassium accumulation by stomata of *Vicia faba*. **Aust.J.Biol.Sci.** 25, 1972, 1107 – 1123.
23) Rama Das VS, Raghavendra AS - Control of stomatal opening by pyruvate metabolism in light. **Indian journal of Experimental Biology,** 12, 1974, 425 – 428.
24) Rama Das VS, Raghavendra AS The physiology and biochemistry of their regulation in leaves. **Current Science** 51, 1982, 586 – 593.

Table 1 – Influence of potassium ferricyanide on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment Solution	Initial size at 8.00 am	Stomatal LIGHT 12 noon	Stomatal LIGHT 4 P.M.	Size DARK 12 noon	Size DARK 4 P.M.
Buffer	2.2	4.3	4.3	3.2	3.2
Potassium ferricyanide					
10 µl.		closed	closed	closed	closed
1 µl.		2.5	2.5	closed	closed

Table 2 – Influence of hydroxylamine hydrochloride on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment solution	Initial size at 8.00 am	Stomatal LIGHT 12 noon	Stomatal LIGHT 4 P.M.	Size DARK 12 noon	Size DARK 4 P.M.
Buffer	2.2	5.0	5.0	3.9	3.9
Hydroxylamine hydrochloride					
10 µl.		6.0	5.5	3.9	3.9
1 µl.		7.1	7.5	3.9	3.4

Table 3 – Influence of different concentrations of $MnCl_2$ on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment solution	Initial size at 8.00 am	Stomatal LIGHT 12 noon	Stomatal LIGHT 4 P.M.	Size DARK 12 noon	Size DARK 4 P.M.
Buffer	2.2	5.0	5.0	3.9	3.9
$MnCl_2$					
10 µl.		6.0	6.0	3.9	3.4
1 µl.		7.6	7.6	5.0	3.9

Table 4 – Influence of different concentrations of Sodium ascorbate on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment solution	Initial size at 8.00 am	Stomatal LIGHT		Size DARK	
		12 noon	4 P.M.	12 noon	4 P.M.
Buffer	2.2	5.0	5.0	3.9	3.9
Sodium ascorbate					
10 µl.		5.0	5.0	3.8	3.8
1 µl.		6.0	5.0	1.3	1.3

Table 5 – Influence of different concentrations of Diphenyl carbazide on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment solution	Initial size at 8.00 am	Stomatal LIGHT		Size DARK	
		12 noon	4 P.M.	12 noon	4 P.M.
Buffer	2.2	3.3	3.3	3.3	3.3
Diphenyl carbazide					
10 µl.		3.0	3.0	1.8	1.8
1 µl.		4.4	4.4	1.7	1.7

Table 6 – Influence of ATP, ADP, ATP + $MnCl_2$ and ADP + $MnCl_2$ on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment solution	Initial size at 8.00 am	Stomatal LIGHT		Size DARK	
		12 noon	4 P.M.	12 noon	4 P.M.
Buffer	2.2	3.2	4.3	3.2	3.2
ATP 1 µl.		7.5	8.6	5.9	5.9
ADP 1 µl.		8.6	8.6	8.0	6.5
ATP + $MnCl_2$ 1 µl.		8.6	10.7	6.5	6.5
ADP + $MnCl_2$ 1 µl.		8.0	8.0	6.5	6.5

Table 7 – Influence of $MnCl_2$ + NADP and Hydroxylamine hydrochloride + NADP on stomatal movements in isolated epidermal strips of *D.biflorus* in light and dark (Pore size in µm.)

Treatment solution	Initial size at 8.00 am	Stomatal LIGHT		Size DARK	
		12 noon	4 P.M.	12 noon	4 P.M.
Buffer	2.2	5.5	5.5	3.2	3.2
$MnCl_2$ + NADP		8.6	8.6	4.9	4.4
Hydroxylamine hydrochloride + NADP		8.6	7.6	4.9	4.4