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STOMATAL RESPONSES OF SOME ARID ZONE PLANT SPECIES

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ABSTRACT

Stomatal responses in intact and isolated epidermis of some arid zone plant species like *E. neriifolia, C. colocynthis* and *P. cineraria* in relation to epidermal turgor, cation exchange, growth regulators and antitranspirants have been studied. It is concluded that the case of each species and its prevalent environmental conditions must be examined specifically. Recent hypotheses of the stomatal regulation are discussed in relation to our new observations.

INTRODUCTION

WATER deficits in the field are expected to develop when transpiration exceeds water uptake by plants, and the induced deficits will, in turn, cause a compensatory closing of the stomata. The velocity of stomatal response is presumed to be dictated by the external conditions, although this is not always true. The relationship between the relative water content and water potential of leaves is more commonly termed the resistance of leaf tissue to desiccation and is taken to indicate one aspect of drought resistance of the particular species[1-3]. The species with smaller change in relative water content for each interval of water potential is considered to be more drought resistant[4].

Stomata serve two conflicting needs : first, to conserve water, and second, exchange of CO_2 and O_2 for metabolic processes to go on smoothly. In general it is accepted that in light CO_2 concentrations are reduced, which result in a rise of pH in the guard cells ; the higher pH allegedly stimulates the conversion of starch to sugar so that an increase in the osmotic value of the guard cell occurs ; water is accordingly taken up and the stomata open. This process is reversed in the dark. This may be right or wrong, but it is certain that for opening of the stomata there must be an uptake of water resulting in an increase in the turgidity of the guard cells. Most commonly, stomatal opening and increased osmotic value in the guard cell occurs in light. This may result due to organic solutes produced in the guard cells or from the "pumping" of ions into the guard cells.

The present work is intended to provide some specific information on the relative contribution of the guard cells and the epidermal cells by which stomatal opening is caused. Behaviour of stomatal regulation is also observed in plants growing in natural environment. The effects of different sugars, cations, growth regulators, antitranspirants and water on the stomatal regulation in isolated epidermal peelings of a few arid zone plants are presented here.

MATERIALS AND METHODS

Plant species included in the present study were : *Euphorbia neriifolia* L., *Citrullus colocynthis* (L.) Schrad. and *Prosopis cineraria* L. Epidermal peelings from the leaves of these species were immersed in different concentrations of test solutions : sugars (glucose, sucrose, mannitol), potassium chloride (KCl), calcium chloride ($CaCl_2$), growth regulators (B_9, CCC, GA, kinetin, ethrel), antitranspirants (phenylmercuric acetate—PMA), etc. The peelings were either incubated in continuous light (about 1,000 lux) or in total darkness. For control, the peelings were kept accordingly in distilled water. The incubation period for different tests varied from 3 to 24 hours.

The histochemical detection of potassium was performed by employing cobalt-sodium nitrite as described[5-7]. The presence of starch in guard cells was estimated by usual iodine test or by Heath's reagent. The width of the stomatal pore was measured by precalibrated microscope. Plasmolysis, if doubted, was observed with the help of neutral red.

EXPERIMENTAL RESULTS

The following results were noteworthy as regards the effect of different surrounding media on the regulation of stomatal aperture in different species.

A. *Euphorbia neriifolia* Linn.

(*a*) *Natural course.*—As for many other fleshy plant species, the stomata in this species under natural conditions remained close during the day and so transpiration was extremely low. The guard cells lying parallel close to each other were fully packed with starch. When tested with iodine, plastids were hardly visible in the guard cells (Fig. 1 A). When placed in water, guard cells swelled considerably but did not cause any effect on the opening of the stomatal pore.

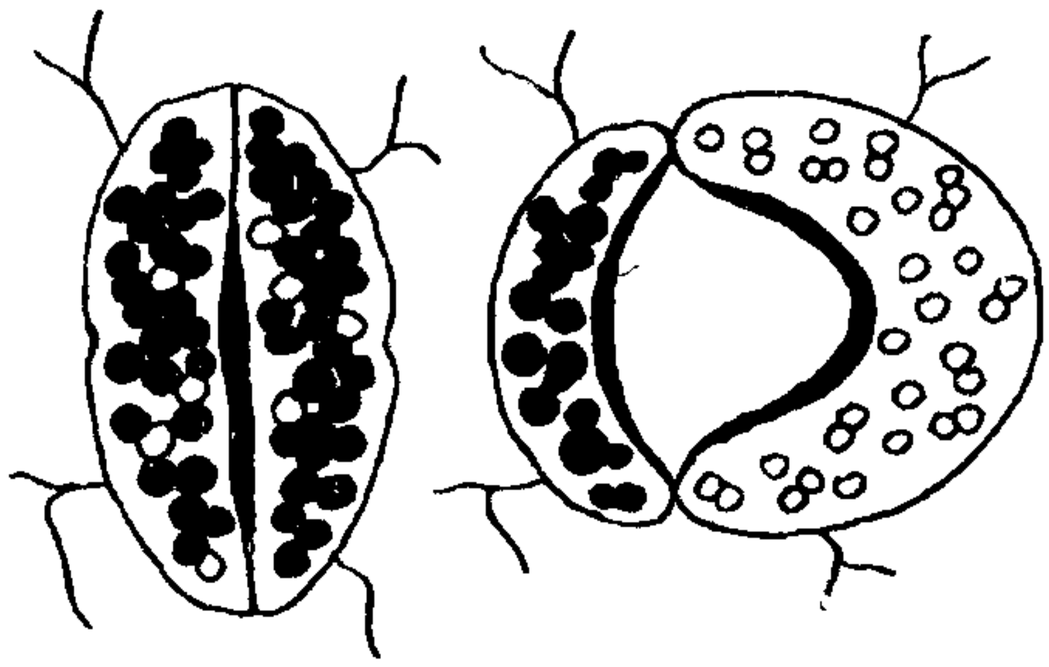


FIG. 1. Diagrammatic diagram of stomata from the isolated epidermal peelings of *E. neriifolia* leaf. A, Stomata in initial condition where plastids are hardly visible due to starch grains; B, Open stomata after incubation in KCl, showing disappearance of starch grains from the functioning guard cell only. Black and white circles represent starch and chloroplasts, respectively.

(*b*) *Influx of Potassium.*—The influx of potassium was tremendous within 3 hours of immersion in KCl solution of as low as 10^{-1} M. The turgor pressure on the guard cells was so high that the width of the stomata appeared to be the actual length, and the pore become as wide as 15 microns. This change was associated with nearly complete disappearance of starch in the guard cells. Such a change was not observed in stomata with a non-functioning guard cell (Fig. 1 B). Thus it could be concluded that the influx of K^+ caused hydrolysis of starch resulting in an increase of osmotic amounts of guard cells. This led to the uptake of water by the guard cells and the opening of stomata in the proportion of starch hydrolysis. After the influx of K^+ in guard cells, the plastids became distinct due to hydrolysis of starch. Potassium uptake was light-stimulated, as the peelings kept in dark did not show open stomata and also gave negative test for K^+ and positive test for starch. A solution of $CaCl_2$ (10^{-2} and 10^{-3} M) did not cause any effect on the opening of stomatal pore.

(*c*) *Efflux of K^+ as an effect of PMA.*—When peelings with KCl-induced open stomata were transferred to PMA solution (10^{-3} M) in light, the aperture nearly closed down within half an hour. This was associated with simultaneous appearance of starch in guard cells, as if condensation phenomenon had taken place together with withdrawal of K^+ ions from guard cells. Efflux of K^+ ions from guard cells was lesser in dark. It was evident from this observation that more uptake of PMA resulted in nearly complete efflux of K^+, and the guard cells with lesser uptake of PMA in dark still had some K^+, thus lowering the closing effect of PMA.

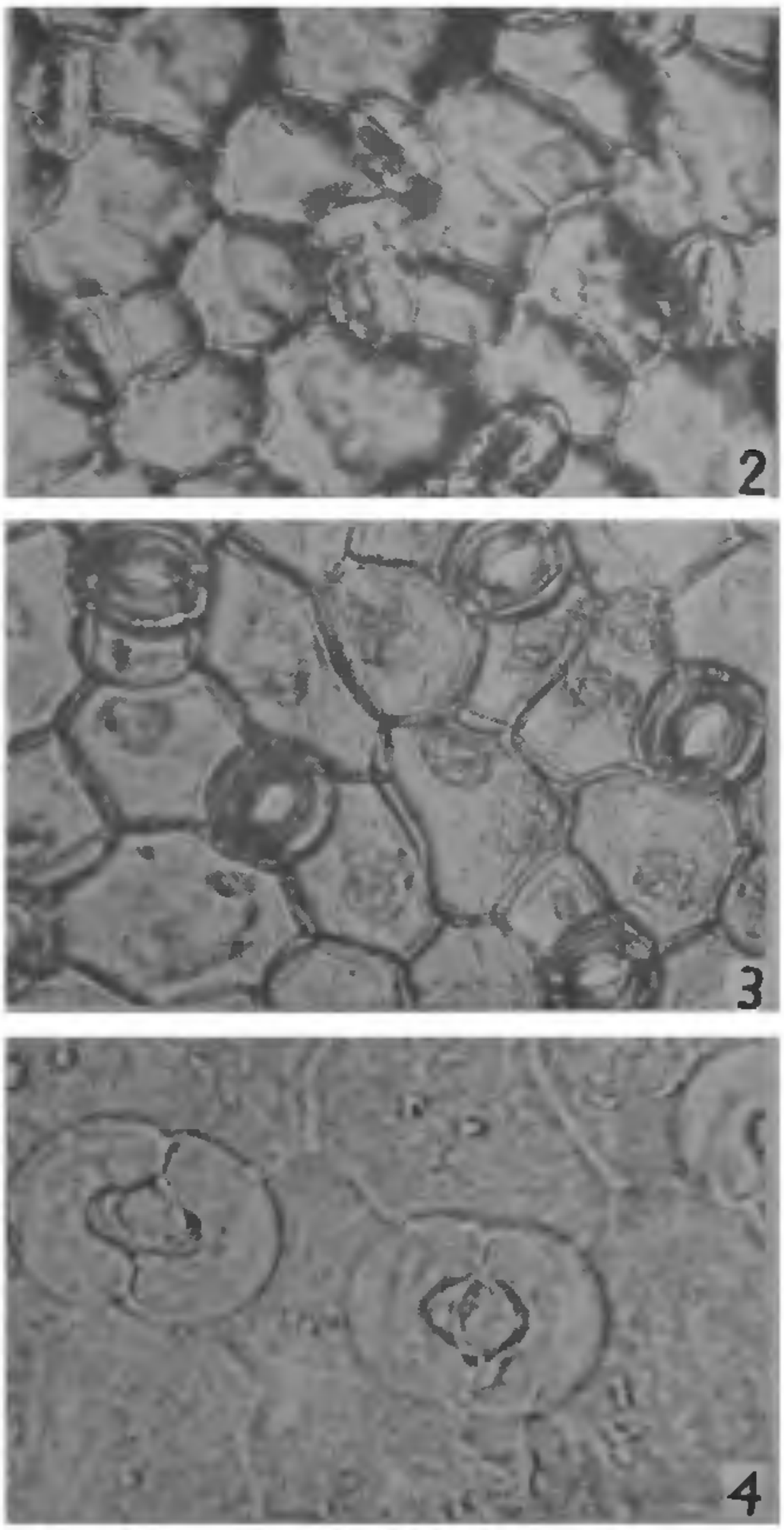
B. *Citrullus colocynthis* (L.) Schrad.

(*a*) *Natural Course.*—The stomata in this species normally opened to a maximum in nature in the afternoons between 1–3, under nearly full insolation and at low atmospheric humidity (30–35%). They remained close in total darkness and under cloudy days. In cold season the width of the pore was lesser than after rainy season. Thus it appeared that in nature the opening of stomata was also light and temperature dependent.

(*b*) *Role of Epidermal Cells.*—Epidermal peelings with closed stomata (Fig. 2) when kept in sucrose solution (0·5 M) showed nearly a cent percent plasmolysis in the epidermal cells, which was associated with maximum width of the stomatal pore (Fig. 3). In more concentrated solution of sucrose, stomatal pore showed a decline in width due to plasmolysis which set in the guard cells as well. In sucrose solution of 1·0 M, both epidermal as well as guard cells were plasmolysed, resulting in the closure of stomata. The above opening and closing of stomata was light independent. Starch test gave a positive result in closed whereas it was negative in open stomata. This observation further proved that there existed very different absorbing capacities to accumulate solutes.

(*c*) *Influx of Potassium.*—Isolated epidermal peelings, when incubated in water, caused turgidity in the guard cells, but associated with a very slight opening of the stomata. The incubation of epidermal strips in KCl solution (10^{-1} M) for 3 hours stimulated the opening of the pore to the maximum in light, while it failed to do so in dark. This opening of the stomata was associated with nearly complete disappearance of starch in guard

cells. This potassium uptake was light-dependent. The influx of K^+ in *C. colocynthis* was very similar to *E. neriifolia* described earlier.



FIGS. 2–4. Fig. 2. Epidermal peeling showing closed stomata in *C. colocynthis,* after incubation in water for 24 hours (× 380). Fig. 3. Epidermal peeling showing open stomata with plasmolysed epidermal cells in *C. colocynthis* after incubation in 0·5 M sucrose solution for 24 hours (× 380). Fig. 4. Epidermal peeling with fully open stomata in *C. colocynthis* showing the lining layer of the pore and that of the guard cells separated distinctly after incubation in kinetin (50 ppm) solution for 24 hours (× 850).

(d) Effect of Growth Regulators.—Peelings were transferred to differently concentrated solutions of a few selected growth regulators (in continuous light) for a period of 24 hours. The behaviour of epidermal and guard cells was variable, which has been summarised in Table I.

TABLE I

Effect of different growth regulators on the epidermal and guard cells of isolated epidermal peelings in C. colocynthis

Growth regulators	Conc. in ppm	Epidermal cells	Stomata (width of pore in microns)
B_9	500	Plasmolysed	3
	1000	do	Closed
CCC	500	Not plasmolysed	4·5
	1000	do	Closed
IAA	50	do	1·5
	100	do	Closed
GA	50	Plasmolysed	do
	100	do	do
Kinetin	50	Not plasmolysed	12–18
	100	do	10–15
Ethrel	500	do	2
	1000	do	Closed

It is evident from Table I that kinetin had a remarkable influence on the turgidity of the guard cells, causing a tremendous pressure on the external wall, due to which they bent into a stretched "C". The lining layer of the pore and that of the guard cells separated distinctly, which

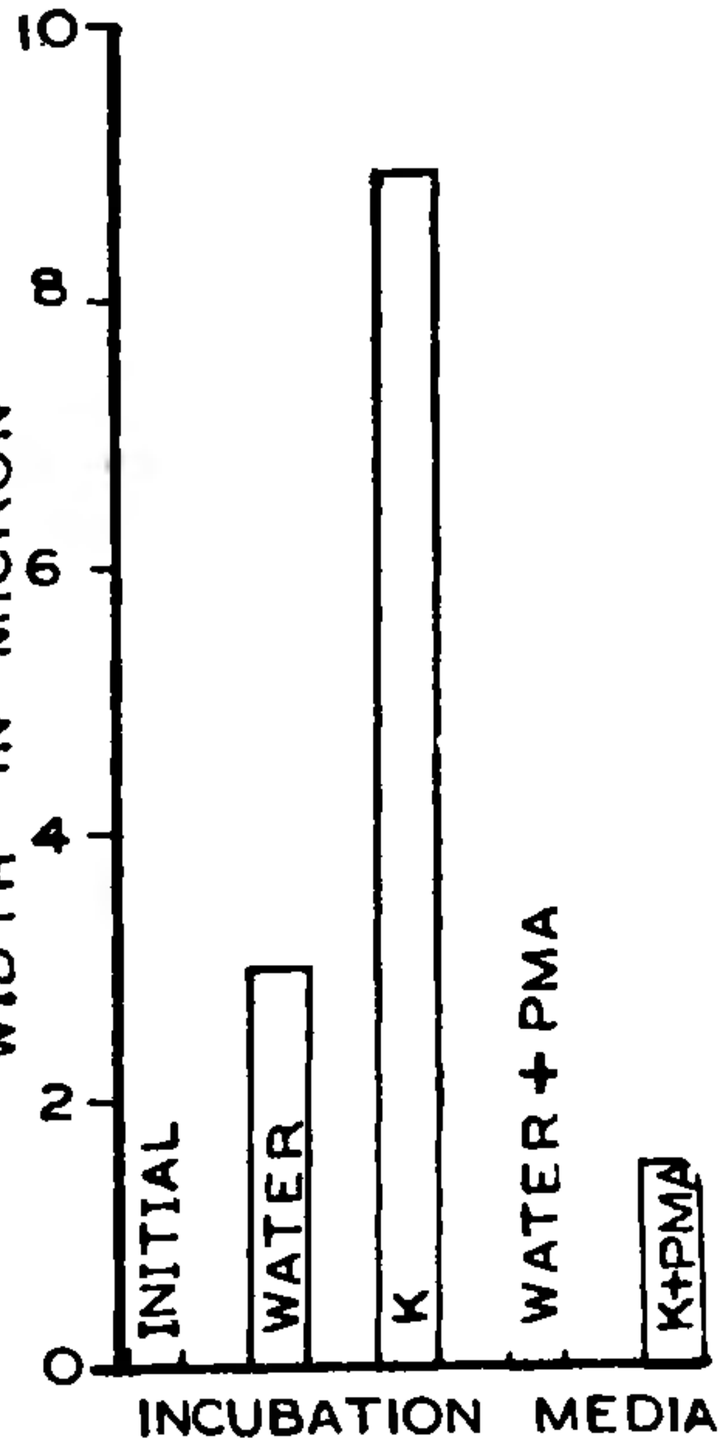


FIG. 5. Effect of different incubation media (K = KCl, PMA = phenylmercuric acetate, and water) on the width of stomatal pore in isolated leaf epidermal peeling of *P. cineraria,* with initially closed stomata.

might have been the effect of this cytokinin (Fig. 4). A negative test for starch was detected in such turgid guard cells.

(*e*) *Effect of PMA on KCl-Induced Open Stomata.*—PMA solution (10^{-2} M) caused the closure of KCl-induced open stomata in continuous light but it failed to do so in total darkness. A slight closure occurred in total darkness, but peelings with KCl-induced open stomata did not show a complete closure when transferred to PMA in light. This may be due to the accumulation of K^+ ions in guard cells which prevented the closure. KCl could not induce opening of the pore to the original size when once treated with PMA.

C. *Prosopis cineraria* Linn.

(*a*) *Natural Course.*—The stomata in this species generally remained close throughout the year. The maximum stomatal pore width reached was 3 microns in November which remained so nearly the whole day. A noteworthy feature was that stomatal movement was much lesser in an adult tree as compared to a seedling 1–2 years old. When peelings with closed stomata were immersed in water, the guard cells became turgid, and the pore became 3 microns wide in both types of plants.

(*b*) *Role of Epidermal Cells.*—Peelings when incubated for 24 hours in glucose (1·0 M) indicated maximum width (9 microns) of the pore, with all the epidermal cells plasmolysed. In 0·5 M glucose solution, the stomata remained close when plasmolysis in epidermal cells just started, which increased with the increasing concentrations of glucose solutions, leading to wider stomatal pore. However, the light and dark conditions for the effect of glucose on stomatal opening remained neutral like *C. colocynthis*.

In 0·5 M sucrose solution, the peelings exhibited quicker plasmolysis in epidermal cells as compared to guard cells. This plasmolysis in epidermal cells did not lead to the opening of the pore. In lower concentrations than 0·5 M sucrose, no effect on either epidermal or guard cells was caused.

(*c*) *Influx of Potassium.*—Peelings from a seedling only responded to incubation in KCl solution. Incubation of peelings in KCl solution (0·5 M) for 3 hours indicated an overall expansion of the guard cells, with the result that the stomata ultimately appeared completely circular in outline. The influx of K^+ ions into the guard cells was positively testified. This influx of K^+ ions was indifferent to light or dark. The high concentration of KCl solution (0·5 M) for causing the maximum width of the pore (9 microns) in *P. cineraria* is noteworthy in comparison to the other two species studied here.

(*d*) *Effect of PMA.*—PMA solution (10^{-3} M) brought about a closure of stomata in peelings with either water or KCl-induced open stomata. However, the closure caused by PMA in KCl-induced open stomata was not complete as compared to water-induced ones (Fig. 5). When peelings with PMA-closed stomata were transferred to KCl, no opening was caused.

Discussion

It has been argued that a rapidly increasing epidermal water deficit leads to increased stomatal aperture, and a rapidly decreasing epidermal water deficit leads to decreased stomatal aperture[8]. The driving force for the change in stomatal aperture is the development of a difference in turgor pressure between the guard cells and the epidermal or accessory cells; it is accentuated by the lack of intercellular spaces in the epidermis[9]. Adjacent epidermal cells have been postulated as the source of water for opening guard cells[10].

Davenport *et al.*[11] have summarised the correlations of stomatal opening and closing with a number of factors. It is generally agreed, however, that stomatal movement is brought about by changes in the turgor of guard cells[12], and neighbouring epidermal cells, and that these changes may be caused by osmotic gradients of the two. Glinka[13] found that the maximum aperture was obtained when epidermal cells were at about incipient plasmolysis and that any increase in their turgor pressure brought a decrease in stomatal aperture of illuminated leaf discs of *Vicia faba*. These findings again emphasised the importance of epidermal cells in determining the width of the stomatal pore. It has been proved conclusively that the reduction in epidermal turgor alone was not the cause of stomatal opening[6]. However, they[6] hold the opinion that reduction in turgor of the epidermal cells, owing to the solutes in the incubation medium, may also contribute to the wide stomatal openings.

It has been stated that the idea[14] about difference between pressures of the guard cells and epidermal cells, which determine the stomatal aperture, was based on the assumption that the efficiency of the two pressures is equal, which was not felt so in the experiments on the same species[13]. The present study supported the work of Glinka[13]. Any effect on the opening of stomata was not found by exogenously supplied sucrose in 48 species studied, except for one in *Rheum rhaponticum* where stomata opened on treatment with glucose-1-phosphate, and accumulation of starch was also not noticed in the guard cells[15].

It was observed that the disappearance of starch might be intimately connected with cation uptake. They further added that the uptake of K^+ by guard cells immersed in KCl was a totally independent contribution to the osmotic pressure increase in the guard cells, leading to the opening of stomatal aperture[17]. The specific requirement of potassium for light activated stomatal opening was confirmed by several workers[16,18,19,6,20,21]. Consequent disappearance of starch on K^+ uptake and opening of the aperture appeared to be correlated in *E. neriifolia*[22]. Influence of potassium ions into the guard cells has been reported to be independent of light in *C. procera*[23].

Kinetin has been reported to influence stomatal aperture[24,25], but it has also been reported to exert little effect[26]. It has been suggested that kinetin effect stomatal aperture by altering epidermal turgor[27]. Potassium concentrations in guard cells were reported to be reduced by abscisic acid treatment, while the starch content of the chloroplasts increased, resulting in closure of the stomata[28]. Kinetin had a remarkable effect on the opening of stomatal pore in *C. colocynthis*, whereas it produced no effect on *P. cineraria*. A negative test for starch was also indicated by the guard cells of treated peelings in the former species. In case of treatment with B_9 and GA, the epidermal cells, although plasmolysed, did not induce any opening, whereas with kinetin, the case was just reverse. Thus the role of epidermal cells in bringing about stomatal opening becomes doubtful.

Phenylmercuric acetate has been reported to retard stomatal closure and also to retard stomatal closing as well as opening[11]. This was in contradiction with the present study where KCl-induced open stomata of *E. neriifolia* indicated a variable effect of PMA in light and darkness.

Recently it has been argued that starch $\rightleftharpoons$ sugar hypothesis for stomatal regulation should not be rejected on the ground that it has been with us for far too long without any unequivocal evidence in its favour[28]. Evidence from our work suggests that starch disappearance occurs simultaneously with K^+ ions accumulation in guard cells. Kinetin induced maximum opening of stomata in *C. colocynthis*, with negative starch test in the guard cells, whereas no such effect was observed in *P. cineraria*. The interaction of KCl with PMA on *C. colocynthis* and *E. neriifolia* also suggest that when stomata closed down, the test for K^+ was negative but that of starch was positive. Low turgor pressure of the epidermal cells also led to the opening of stomata in certain cases when incubated in sugar solutions. The isolated epidermal peelings with plasmolysed epidermal and guard cells caused by sugar solutions, when incubated in water, had no source of water from the epidermal cells but for the surrounding medium and then also stomata opened.

The suggestion that ATP from oxidative phosphorylation does not play an essential part in the opening of stomata has been repudiated[29], who not only suggest a direct involvement of light energy in the stomatal opening but also ascribe a possible role for the cyclic photophosphorylation *in vivo*.

It is concluded that for the regulation of stomatal opening starch hydrolysis is obligatory, but light, potassium ions, kinetin and epidermal turgor are the contributory factors.

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