

Stomatal Responses towards Neutral Red Uptake and Particulate Movement in Some Arid Zone Plants

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Abstract. The stomata and their behaviour towards neutral red uptake in a few arid zone plant species under stress conditions have been investigated. Variable patterns of accumulation or non-accumulation and retention of this vital dye, by various cells of leaf epidermis have been observed. The neutral red uptake by the cells of the epidermal strip, coalescence of particles and their movement appear to be connected with the open/closed condition of the stomata.

For the study of stomatal structure and behaviour, use of epidermal strips has been the most common. The usefulness of these findings have been questioned (HEATH 1959, WILLMER and MANSFIELD 1969) as well as supported (FISCHER 1968). Epidermal strips are useful in stomatal studies but the investigator must be careful throughout the experiment if the guard cells are functioning normally (PALLAS 1964, 1966, 1971).

Marked differences in the uptake of neutral red into the cells of the epidermis in *Vicia faba* and *Commelina* species have been found (WILLMER and MANSFIELD 1969). In *Vicia faba*, the uptake occurs in both epidermal and guard cells, with the result that there is little, if any, change in the stomatal aperture. In *Commelina*, the subsidiary cells surrounding the guard cells do not themselves increase in turgor, but pass solutes to the guard cells where they accumulate and increase the turgor of guard cells relative to subsidiary cells, thus leading to stomatal opening. However, WILLMER and MANSFIELD (1969) found difficulty in accepting FUJINO's (1967) suggestion that normal opening in light, in an intact leaf, is a result of active cation transport into the guard cells from the other leaf cells, including those of mesophyll.

The observation of PALLAS (1966) that the guard cells have more absorbing capacity of solutes than the epidermal cells, have been confirmed for *Merremia aegyptia* but not for *Merremia dissecta* by SHARMA and SEN (1976), although both these species have distinct subsidiary cells.

Two reliable microscopic observations that have been recommended to identify living guard cells were protoplasmic streaming and neutral red

uptake (PALLAS 1971). Except for the report by SEN *et al.* (1975), with regard to protoplasmic streaming and neutral red uptake, there does not appear to exist any other literature on this aspect in epidermal, subsidiary or guard cells. Different patterns of absorption, accumulation or non-accumulation and retention of the vital dye, neutral red, by the different cells of the epidermal strip and the subsequent appearance of particulate movement have been investigated and reported here in a few plant species growing under stress conditions of the arid zone.

MATERIAL AND METHODS

a) Plant Material

As many as eight species growing mostly in the New Campus of the University of Jodhpur, from which the epidermis could be easily removed, were selected. Generally, the mature, fully expanded leaves were picked up for preparing strips. The leaves were brought from nature straight to the laboratory for observations.

b) Treatment of Epidermal Strips

Epidermal strips from the leaf were mounted in a drop of water on the slide and a cover glass placed over it. A drop of neutral red chloride ($100\text{ }\mu\text{g g}^{-1}$) was placed on one side of the cover glass and this was drawn in by means of a filter paper from the other end. Observations with regard to the uptake of this vital dye into epidermal, subsidiary and guard cells; and the appearance of protoplasmic streaming by moving, variously stained particles/droplets or globules were continuously made for a period ranging from 5–20 min. This was essential because there were cases where streaming movements in cells subsided after a very short time, or the moving particles coalesced to larger masses resulting in slowness and ultimate loss of their movements. At times the staining of different cells in the peeling was successive and so this took a long time. Diagrammatic sketches were drawn to show the staining pattern in different cells of the epidermal peelings.

RESULTS

1) *Borreria articularis* (L.) F. N. WILL.

Neutral red first stained most of the epidermal cells which looked brownish-red leaving a few of them colourless. Very soon some of them surrounding the subsidiary cells assumed a uniform light pink colour. At this stage two types of colour tones were observed: some cells showed a uniform light pink colour, while others brick red. Till then subsidiary and guard cells did not pick up the stain and remained colourless. Then a gradually uniform bright pink stain was picked up by the subsidiary cells also. Some dark red stained particles of varying sizes were observed moving very fast inside the pink stained epidermal and subsidiary cells. These particles showed a tendency to coalesce and become larger, gradually losing their movement (Fig. 1). Last of all, the guard cells picked up the red colour very slowly and appeared much deeper at the polar ends due to cytoplasmic accumulation. By that time the brick-red cells had lost their stain and turned colourless.

2) *Convolvulus microphyllus* SIEB. ex SPRENG.

At first neutral red accumulated in all the epidermal cells. Very soon it was differentiated into two types of colour tones. Either one or both subsidiary cells developed a bright pink colour followed by the appearance of a large number of dark violet particles in them (Fig. 2A), while other epidermal cells retained only a very light pink colour (Fig. 2B). Then the stain was gradually picked up by the guard cells also. It was noted that the staining pattern of the guard cells in a stoma with a single brightly coloured subsidiary cell (Fig. 2C) differed from those stomata with two such cells (Fig. 2D). In the former case, the less turgid guard cell with brightly coloured subsidiary cell picked up the stain very slowly in comparison to the corresponding more turgid guard cell which picked up the bright red stain quickly and perfectly with the lightly stained subsidiary cell (Fig. 2C). In the later case where the stomata had two brightly coloured subsidiary cells, the flaccid guard cell hardly picked up any stain with completely closed stomata (Fig. 2D). Gradually after about twenty minutes, the guard cells picked up the bright pink stain and their protoplasm accumulated into two polar bodies. Particulate movement in the brightly stained cells continued without any coalescence.

3) *Dicoma tomentosa* CASS.

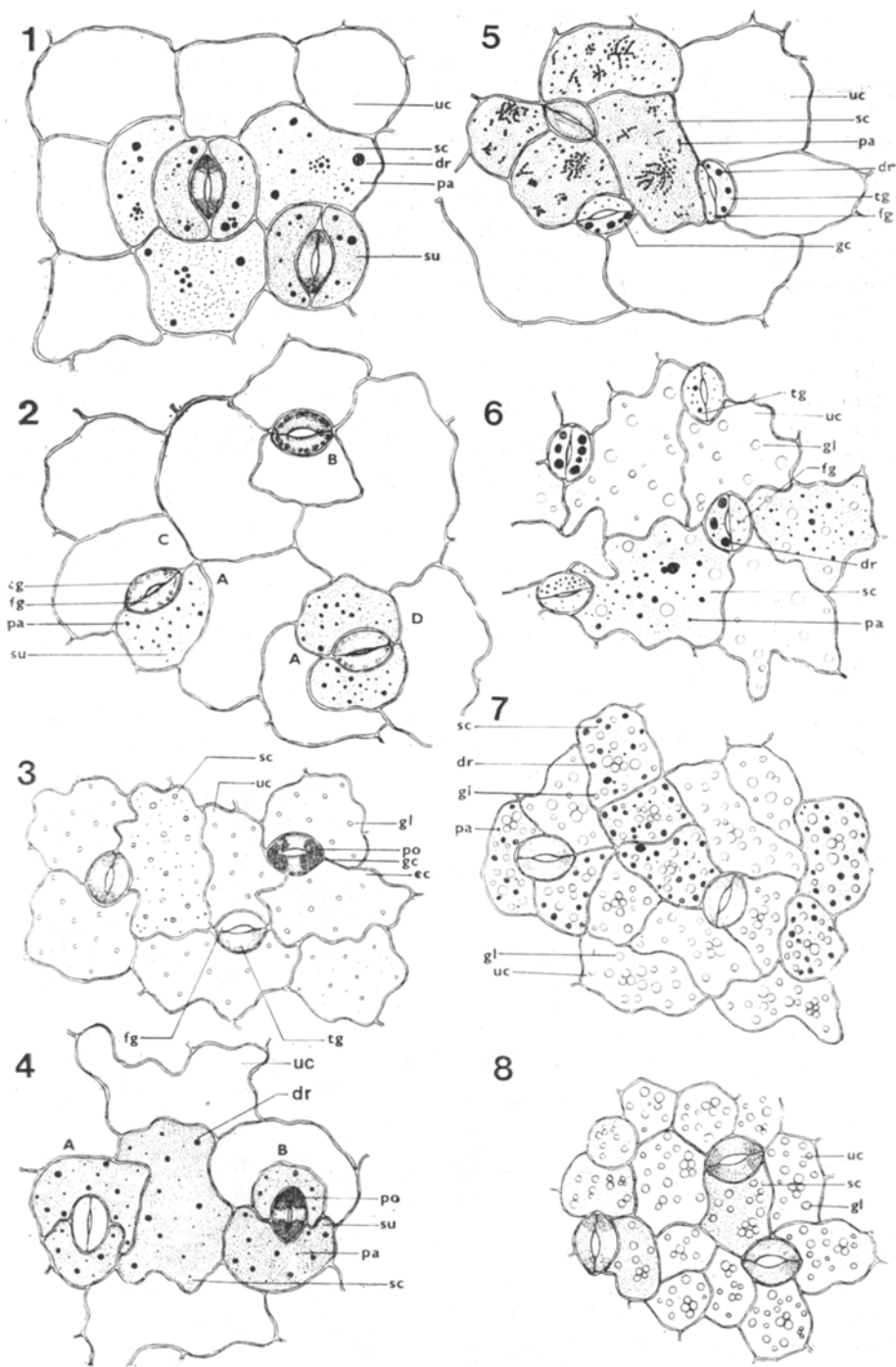
Only a few epidermal cells adjacent to the stomata picked up a very light pink colour while other cells remained colourless. Then the deeply stained particles were observed in the stained cells without visible movement. Gradually the stain appeared in the guard cells also, which showed a bright pink colour with the development of deeply stained polar bodies (Fig. 3). At certain places only one guard cell picked up the stain.

4) *Justicia vahlii* ROTH.

At first a neutral red stain accumulated in the epidermal cells. After some time most of the epidermal cells appeared colourless but a few cells neighbouring on the stomata retained a uniformly pink colour. Gradually the stain passed on to the two unequal sized subsidiary cells, the smaller one partially enclosed by the larger one. These cells showed a very uniform bright pink colour. Then darkly stained droplets of varying sizes appeared in the coloured epidermal and subsidiary cells. Droplets of smaller size showed movement while larger ones did not. Guard cells either did not pick up the stain at all (Fig. 4A) or when they did so, their protoplasm formed deeply stained polar bodies at both ends (Fig. 4B).

5) *Launaea procumbens* (ROXB.) RAMAYYA and RAJGOPAL

At first some of the epidermal cells neighbouring on the stomata developed a light uniform pink colour, while other cells remained colourless (Fig. 5). After some time a large number of fast moving deep violet particles were observed inside the stained cells. These particles exhibited a tendency to move closer to each other forming clusters but did not merge together completely and retained their individual identity. Then the guard cells picked up a light uniform pink colour at the beginning which eventually exhibited the formation of bigger droplets in turgid and smaller droplets in flaccid ones.



6) *Pulicaria crispa* (CASS.) BENTH. et HOOK. f.

First of all neutral red accumulated in all the epidermal cells. Gradually it became faint in all but a few epidermal cells adjacent to each stoma (Fig. 6). A large number of violet coloured moving particles appeared in the stained cells, which formed larger droplets by mutual merging. Then the stain passed on to the guard cells and after some time violet coloured moving

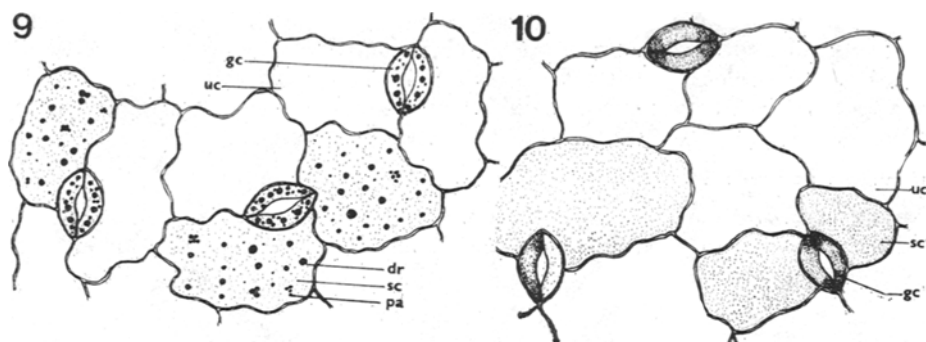


Fig. 9. *Solanum surattense* — lower epidermis.

Fig. 10. *Solanum surattense* — upper epidermis.

bodies were observed inside them as well. In most of the stomata, one of the guard cells (the turgid one) picked up the stain very quickly showing a greater number of coloured particles/droplets while the corresponding flaccid guard cell showed only a brick red colour very late with a few particles inside them.

7) *Fagonia cretica* L.

The staining pattern in the epidermis from the lower and upper surface showed some remarkable differences (Figs. 7 and 8).

a) Lower epidermis: First of all a bright pink stain was picked up by some of the epidermal cells only, so that two different shades of stained and unstained cells became distinct. A large number of globules of different sizes were seen inside them. In the stained as well as unstained cells these globules remained unstained, while in the stained cells small particles developed a deep violet colour, coalesced and formed larger droplets (Fig. 7). Particles/glob-

Fig. 1. *Borreria articularis*.

Fig. 2. *Convolvulus microphyllus*.

Fig. 3. *Dicoma tomentosa*.

Fig. 4. *Justicia vahlII*.

Fig. 5. *Launaea procumbens*.

Fig. 6. *Pulicaria crispa*.

Fig. 7. *Fagonia cretica* — lower epidermis.

Fig. 8. *Fagonia cretica* — upper epidermis.

Abbreviations used: dr — droplets, ec — epidermal cells, gc — guard cells, gl — globules, fg — flaccid cells, tg — turgid cells, pa — particles, po — polar bodies, sc — stained cells, su — subsidiary cells, uc — unstained cells.

ules of smaller size showed movement both in coloured as well as in colourless cells. Last of all, the guard cells also picked up a uniform bright pink stain which gradually became deeper in the polar than in the vacuolar region.

b) Upper epidermis: Here also epidermal cells showed two different shades of stained and unstained cells. In the stained cells the cytoplasm picked up a uniform pink colour throughout. A large number of globules of varying sizes were observed inside both types of the epidermal cells, smaller ones exhibiting movement. Unlike the lower epidermis, stained particles and droplets were not observed at all even in the stained cells (Fig. 8). Guard cells picked up a uniform bright pink colour at first which later on showed more accumulation in the polar region.

8) *Solanum surattense* BURM. f.

Neutral red at first accumulated in all the epidermal cells. Very soon it got differentiated into two shades. The staining pattern in the lower and upper epidermis showed some differences (Figs. 9, 10).

a) Lower epidermis: Some of the epidermal cells picked up a very light pink stain, while others remained colourless. Inside the stained cells a large number of particles of different sizes were seen to pick up a dark red colour (Fig. 9). These particles showed a very fast movement and tended to coalesce forming larger droplets. Then the light pink stain was picked up by the guard cells also which showed uniform distribution for a while and very soon moving particles/droplets appeared in them.

b) Upper epidermis: A very deep uniform stain was picked up by most of the epidermal cells but some of them surrounding either single or a group of stomata did not do so at all and formed colourless patches in between (Fig. 10). Then a very deep pink stain was picked up by the guard cells. At certain places only one of the guard cells was well stained. No movement of particles was observed here.

DISCUSSION

In the present investigation, *Dicoma tomentosa* showed that the guard cells have more absorbing capacity of solute than the epidermal cells, agreeing with PALLAS (1966). In contrast to this, observation on *Borreria articularis* and *Justicia vahlii* support the findings of BHANDARI and SEN (1973) where epidermal cells appeared to have more absorbing capacity for neutral red, and hence they assumed that the guard cells and epidermal cells have different osmotic potentials and absorbing capacities. Other species studied showed a successive uptake and accumulation of neutral red. Remarkable variability observed in *Fagonia cretica* and *Solanum surattense* in the upper and lower epidermal cells of the same leaf in their behaviour towards neutral red uptake might be due to the differences in their osmotic potentials.

Convolvulus microphyllus exhibited a unique pattern of neutral red absorption and accumulation. When the guard cells were turgid with open stomata, very little neutral red was retained by the subsidiary cells and accumulation took place mostly in the guard cells. On the other hand, in closed stomata neutral red mostly accumulated in the subsidiary cells only. In such cases where one guard cell of the same stoma was turgid it got

stained perfectly and its subsidiary cells showed very little stain accumulation; while its corresponding flaccid guard cell hardly absorbed any stain and its subsidiary cell showed more stain accumulation. These observations indicate that there exists a relationship in the turgidity of the guard cells and neutral red uptake by them. These results agree with the observations of SEN and HARSH (1974). It was further confirmed by the neutral red uptake patterns of the guard cells in *Pulicaria crispa*, where most frequently one of the guard cell of some stomata picked up the stain perfectly while the corresponding ones did not. These unstained guard cells were less turgid in comparison to their corresponding stained guard cells. It is evident that the neutral red is first absorbed by the epidermal cells and then successively by subsidiary and guard cells. Absorption of stain is followed by its retention and subsequent accumulation by different cells. The stain showed accumulation in the epidermal cells only when it was not readily accepted by the subsidiary cells. It showed more accumulation in the subsidiary cells when it was not accepted by the guard cells.

In the guard cells the stain showed a uniform distribution at first and gradual appearance of deeply stained polar ends or big droplets on quick and ready absorption. On the other hand it took the form of small droplets when absorbed slowly and reluctantly as was observed in *Launaea procumbens*.

The appearance of particles, their movement and coalescence forming droplets showed a great variability. It was observed mostly in the epidermal, subsidiary and guard cells when the stomata were closed. Clear movements were visible in the cells adjacent to the closed stomata. It may be due to the fact that stain tends to enter the guard cells where its entry is denied under the influence of certain factors and thus caused the streaming of protoplasm. These observations agree with the assumption of PALLAS (1971) that the neutral red uptake is possibly associated with the protoplasmic streaming. The association of protoplasmic streaming with neutral red accumulation could be explained by either photo- or oxidative phosphorylation providing the energy source for streaming, and that the alkaline conditions of the medium compared to the guard cell may generate energy for stomatal movement and streaming chemo-osmotically. BARTELS and SCHWANTES (1957) have reported the neutral red accumulation in the epidermal cells of *Allium cepa* to be pH dependent. The different shades of red and violet are probably due to pH factor.

These studies indicate that the neutral red uptake, its retention, accumulation, appearance, movement and coalescence of the particles exhibited by the various cells of the leaf epidermis are connected with the open/closed conditions of the stomata.

Acknowledgements

One of the authors (H. D. L.) is most grateful to the authorities of Tribhuvan University, Nepal and the Government of India for the fellowship under T. C. S. of Colombo-plan, and also to Prof. H. C. Arya of this department and the University of Jodhpur for providing facilities.

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BOOK REVIEW

IARC MONOGRAPHS ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS. SOME METALS AND METALLIC COMPOUNDS. — IARC, Lyon, Volume 23, 1980. 438 pp., Sfr. 50.—, US \$ 30.—.

Ten years ago (1971) the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic hazards of chemicals to humans with the objective of producing monographs on individual chemicals. The 23rd volume of the IARC Monographs contains evaluation of the carcinogenic effects of metals and their compounds. The following elements and compounds are considered: arsenic and arsenic compounds (arsenic pentoxide, sulphide, trioxide etc.), beryllium and beryllium compounds (beryllium acetate, oxide, silicate etc.), chromium and chromium compounds (barium chromate, calcium chromate, chromic chloride etc.) and of lead and lead compounds (lead acetate, carbonate, naphthenate etc.). Each chapter includes chemical and physical data of the individual chemicals, methods of synthesis, production, use, occurrence and analysis, as well as biological data relevant to the evaluation of carcinogenic risks to humans, and mutagenic and other short-term tests. Case reports and epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are also described. The individual chapters comprise the overall evaluation of the carcinogenic risk of the studied chemicals to humans. According to the IARC Working Group (which met in Lyon 23–30 October 1979) there is sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in humans and that beryllium should be considered suspect of being carcinogenic. There is sufficient evidence of respiratory carcinogenicity in men occupationally exposed during chromate production, and that lead subacetate, acetate and phosphate are carcinogenic to rats. In the absence of adequate human data it is reasonable, for practical purposes, to regard the lead compounds as a potential carcinogenic risk to humans.

This 23rd volume of IARC Monographs will be valuable not only to those interested in carcinogens but also to all studying the effects of environmental mutagens.

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