FUNCTIONAL STOMATA ON THE PODS OF THE ARGENTEUM MUTANT OF PISUM SATIVUM

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Many fruits, including pods of the Leguminosae, have stomata on their outer surface; however, very little is known about the movements and functions of the stomatal apparatus. Some stomata may open and close normally but many remain open permanently, especially when they are present in the inner epidermis of fruits. The function of stomata on pods such as those of Pisum sativum is of great importance when considering the carbon economy of the pod.

Recently a mutant of Pisum. Argenteum (Arg.), was described (2,3), the leaves of which have an easily detachable epidermis. This makes it ideal for the study of stomatal behavior. We have recently investigated the behavior of stomata on the upper and lower epidermis of this mutant (1), and we were also interested in the behavior of stomata on the epidermis of the Arg pod. The pod epidermis does not detach as readily as that of the leaves but it appears to be easier to remove from young pods of Arg than from those of other Pisum lines.

The *in situ* behavior of the pod stomata was investigated in two ways. The first experiment (Table 1) was a "split pod" experiment in which pods were removed from the plant, split in half, and the seeds were removed. Each half was kept either in the light or dark for 4 hours and at the end of this period strips were removed from the pods and the stomata] apertures were determined under the microscope. Table 1 shows that in this type of experiment the stomata were slightly more open in the light than in the dark (about 1 mkm). The second experiment involved taking epidermal samples from pods still attached to the plant at various times in a light/dark cycle; stomatal apertures were determined by microscopic measurements. Table 2 shows that under these conditions the stomata on the pods had a wider aperture in the light period than in the dark period.

Table 1. Stomatal apertures on "split pods" in the light or dark. Three 8-day old pods were taken from the plant and split into two halves. Each half was placed onto water, inner surface down, in a Petri dish. One dish was kept in the light (140 mkmol m^{-2} s^{-1}) 400-700 nm, and the other in the dark for 4 hr at 20 C. At the end of this period three strips of epidermis were taken from each pod half and 10 apertures were measured on each strip (90 apertures in total per treatment). The results were analyzed by a paired t-test and showed a significant difference between light and dark (P=0.025, df=2).

Treatment	Mean aperture μ	S.E.
Light	4.5	0.2
Dark	3.5	0.2

Table 2. Stomatal apertures of pods from plants in the light or dark period of a diurnal rhythm.
Three strips of epidermis were taken from each of three 9-day-old pods on plants in either the light or dark period of their diurnal rhythm. Ten stomatal apertures were measured on each strip (90 apertures in total per treatment). The results were analyzed with an unpaired t-test and were found to be significant (P=0.001, df=4).

Treatment	Mean aperture µm	S.E.
Light period	3.6	0.06
Dark period	2.4	0.1

Since these data showed evidence of functional stomata, further investigations were carried out to see if changes in K^+ and starch were involved in the movements. Histochemical studies with epidermal strips taken from pods on plants either in the light or dark showed that K^+ does appear to accumulate in the pod guard cells in the light (Fig. 1 A, B) and also that starch which is present in the guard cell chloroplasts in the dark (Fig. 1D) disappears during the light period (Fig. 1 C).

These reactions are well documented for stomata on leaves of many species but so far relatively little work has been carried out on the functions of pod stomata. From these studies it would appear that the stomata on the pods of the Arg mutant function in a similar way to leaf stomata, although they do not open as wide as those on the leaf. A more detailed study of the function of pod stomata with age would be useful to enhance our knowledge of the role of pod stomata in the development of the seed.

1. Donkin, M. E., A. J. Travis, and E. S. Martin. 1982. Z. Pflanzenphysiol. 107:201-209.
2. Marx, G. A. 1978. PNL 10:34-37.
3. Marx, G. A. 1982. J. Heredity 73:413-420.

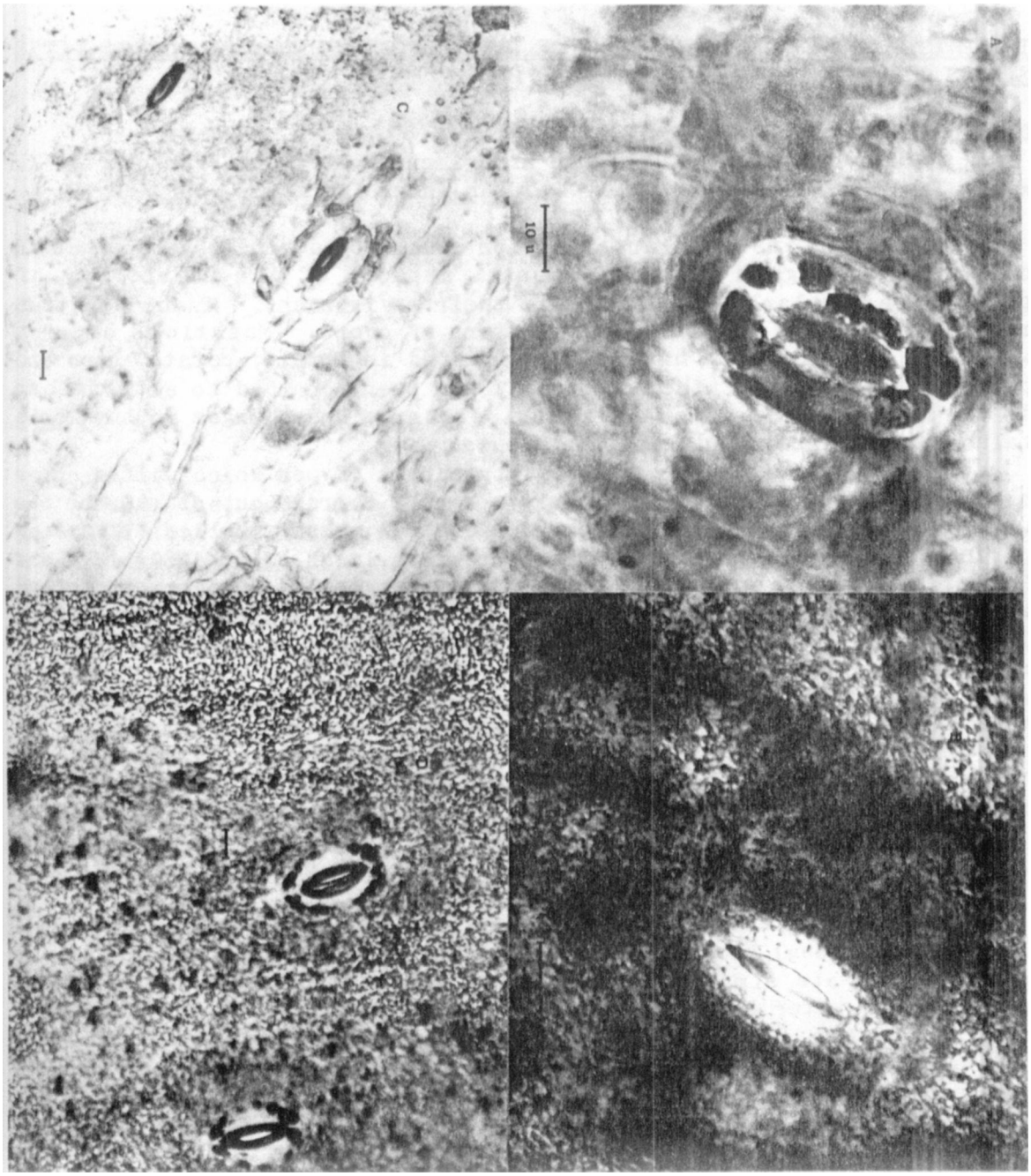

Fig. 1. Light micrographs of K^+ and starch staining in Argenteum pod guard cells in the light or dark.
A and B show staining for K in the guard cells by the MaCallum stain.
A = light, B = dark
C and D show staining for starch in guard cell chloroplasts by the I_2/KI stain.
C = light, D = dark
The dark deposits on the epidermal surface in B and D are cuticular wax. The bar on the micrographs is equal to 10 um.