New Phytol. (1979) **83**, 623–626

SILICA DISTRIBUTION IN *EQUISETUM HYEMALE* VAR. *AFFINE* L. (ENGELM) IN RELATION TO THE NEGATIVE GEOTROPIC RESPONSE

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(*Accepted 20 February 1979*)

SUMMARY

In young growing *Equisetum* shoots silica deposition proceeds basipetally in the internodes. The surface of the mature internode, including the stomata, becomes encrusted with silica. Only young shoots with active intercalary meristems and very little silica deposition show a negative geotropic response. As the internodes mature, the difference in silica deposition between intercalary meristem and upper part diminishes so that the failure of mature internodes to respond geotropically is most likely due to significant silica deposition in the once active intercalary meristem sites.

Initiation of negative geotropic curvature in young *Equisetum* shoots takes 36 to 48 h, and six to nine internodes are involved. Control of the geotropic response is auxin-mediated as shown by experiments with the auxin transport inhibitors, triiodobenzoic acid (TIBA) and methyl-2-chloro-9-hydroxy fluorene (CFM, a morphactin). The former partially inhibited the response while the latter completely inhibited curvature.

INTRODUCTION

Some species of *Equisetum* are annual, e.g. *E. arvense*, whilst others are perennial, e.g. *E. hyemale* var. *affine*, *E. hyemale* var. *hyemale* and *E. lavigatum*. Kaufman *et al.* (1973) and Dayanandan (1977) found that in the annual species, silica ($SiO_2 . nH_2O$) occurred almost exclusively in the papillae on the surfaces of the stomata and surrounding epidermal cells, whereas in the perennial species, it appeared in the outer tangential walls of the subsidiary cells of the stomata, on the epidermal papillae, and in both radial and tangential walls of the long epidermal cells. These observations suggested that silica distribution was related to the relative longevity of the shoots of different species of *Equisetum*. Species with less silica, localized only on the surfaces of stomata, are annuals; those with a high silica content, distributed over the entire periphery of the shoot are perennials.

Silica also appears important in connection with the negative geotropic response of the shoots of higher plants. In *Avena sativa* and other grasses, Dayanandan, Hebard and Kaufman (1976) found that silica (as well as lignin) is excluded from, or occurs in extremely low amounts in the geosensitive sheath pulvini. The mechanism for this exclusion is not known.

The present study was undertaken to determine whether the shoot geotropic response could be related to the distribution of silica in a perennial species of *Equisetum*, namely, *E. hyemale* var. *affine*.

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0028-646X/79/060623+05$2·00/0

MATERIALS AND METHODS

Plants of *E. hyemale* var. *affine* were collected along the roadside in Washtenaw County, Michigan. They were cold-hardened outdoors in pots for about 3 months (November 1977 to January 1978), and then brought into the greenhouse to force the shoots to develop. These actively growing shoots were used for geotropic studies.

Silica distribution was followed with a scanning electron microscope (SEM) JEOL Model No. JSM-U3. The specimens were prepared as follows: (a) fresh or dried stem portions were placed on carbon stubs using carbon paint and then coated with carbon, (b) they were observed under the SEM at 15 keV with variable magnification to study epidermal features in the secondary electron image mode, (c) a multichannel analyser (MCA) (Northern Scientific, Model No. 710) attached to the SEM was employed for X-ray mode of analysis of silicon in the specimens, (d) with the MCA, silicon distribution maps were obtained, and for semi-quantitative analysis of silicon in the samples, point counts were made for 200 s, (e) secondary electron images were photographed with Polaroid black and white film (type 55, p.n. 4×5 in.).

Geotropism experiments were set up by placing young intact growing shoots (8 to 10 cm long) horizontally. A protractor was used to measure the angle of curvature of geostimulated shoots (Plate 1, No. 1). In the auxin transport inhibitor experiments, we used, 2,3,5-triiodobenzoic acid (TIBA) (gift of Professor Han Burström, Lund, Sweden) and Methyl-2-Chloro-9-hydroxy Fluorene (Celamerck, Germany).

RESULTS

In young growing *Equisetum* shoots, silica deposition starts from the shoot tip and proceeds downwards in the internodes (Plate 1, Nos. 2, 3). As the stem matures, the surface of the internode, including the bands of stomata, become completely encrusted with silica. Silica deposition starts first on the outer surface of stomata and eventually the apertures become occluded.

Silicon counts for the basal intercalary meristem (Plate 1, No. 2) of young internodes of *Equisetum* are much lower than those for the upper part of the same internode (Table 1; cf. also Plate 1, No. 4). However, the difference between the two locations gradually becomes less marked as one proceeds downwards to successively older internodes (Table 1). Thus, the former intercalary meristem zone in the older internodes becomes as silicified as the upper parts. Highest amounts of silicon were found in the upper part of the internodes, nearest to the shoot apex.

A negative geotropic response was first recorded 36 to 48 h after geostimulation; it took about 6 to 8 days for the shoots to attain 60° curvature. The primary curvature occurred in the intercalary meristem region of each responding internode and many internodes (six to nine) were involved. When comparatively mature shoots (25 to 40 cm long) were geostimulated, they did not show any significant negative geotropic response.

Control of negative geotropism in *Equisetum* is auxin-mediated as shown by experiments with the auxin transport inhibitors, TIBA and CFM and application of exogenous IAA (0·05 %) to upright *Equisetum* shoots. Both TIBA and CFM inhibited negative geotropic curvature, though not quite to the same extent; the former partially inhibited the response (80 %), while CFM completely blocked it (Plate 1, No. 5). IAA applied unilaterally to upright, rapidly elongating shoots at five intercalary

Table 1. *Silicon counts in young and mature internodes of* Equisetum hyemale *var* affine

Intercalary meristem	Silicon count (200 s)	Upper part of the same internode	Silicon count (200 s)
3rd Internode*	191	3rd Internode*	12573
10th Internode*	1087	10th Internode*	8838
25th Internode*	6470	25th Internode*	6957

* From shoot apex.

meristems caused significant shoot curvature similar to that in control shoots (Plate 1, No. 5).

Hand sections of portions of ten young geostimulated internodes were cut and stained with acetocarmine. Under the light microscope, the new cells immediately above the active interclary meristem on the outer side of the internodes appeared elongated longitudinally. Comparisons of the length of the parenchymatous cells outside the vascular bundles showed a mean cell size on the inner side of the geostimulated internode of 21 μm as against 25 μm the outer side. On the outer side no cell division could be seen in any of the elongating cells.

Discussion

The present study shows that in *E. hyemale* var. *affine*, the intercalary meristem at the base of the internode of a young shoot (Plate 1, No. 2) is the site of the negative geotropic response. In this region there is very little silica compared with that in the upper part of the same internode. However, as the internode matures, this difference between intercalary meristem and upper zone gradually diminishes, and in a fully mature internode, there is virtually no difference (Table 1). High amounts of silica in the intercalary meristems of mature internodes would greatly interfere with the shoot curvature response, and this could explain why they do not respond geotropically. In other words, only the relatively unsilicified intercalary meristems of younger shoots can be geostimulated. A similar situation occurs in festucoid grasses, except that the primary geosensitive site is the sheath pulvinus, not the intercalary meristem (Dayanandan *et al.*, 1976). In the panicoid grass, *Phragmites*, the internode has a geosensitive pulvinus which controls the geotropic response (Lau *et al.*, 1978) and this compares with the internodal intercalary meristem of *Equisetum*, which becomes gradually silicified on aging.

In *E. hyemale* var. *affine*, many internodes (6 to 9) are involved in geotropic curvature unlike festucoid cereal grasses (e.g. *Avena*, *Triticum*, *Hordeum*) where usually just two of the sheath pulvini participate. In contrast, in *Phragmites*, a panicoid grass, as many as six to eight pulvini show a negative geotropic response, which is more like *Equisetum*.

In *E. hyemale* var. *affine*, the geotropic response is much slower (beginning after 36 to 46 h) than in cereal grasses (*Avena*, *Hordeum*, *Triticum*) which require only 20 to 30 min (measured with an angular recording transducer, Dayanandan *et al.*, 1976). *E. hyemale* var. *affine* took 6 to 8 days to exhibit 60° curvature, whereas *Avena* *shoots* took 2 days. This might be related to the slow growth of the *Equisetum* plant itself.

The basic mechanism underlying the geotropic response appears to be similar in both *Equisetum* and grasses, involving auxin as one of the primary regulating hormones. It would be of interest to determine the levels of endogenous free and conjugated auxin in upright and geostimulated *Equisetum* shoots and to investigate auxin transport in the internodal intercalary meristems of young shoots.

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EXPLANATION OF PLATE

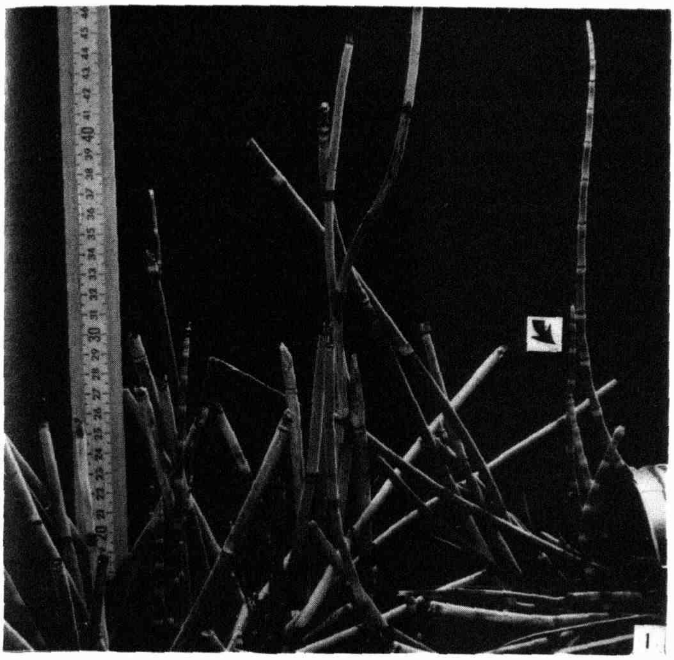
No. 1. Geostimulated shoots (intact) back in almost upright position after 10 days. Reduced 1·7 times.

No. 2. Secondary electron image of the basal intercalary meristem (IM) of a young internode of *E. hyemale* var. *affine.* In the middle of the picture is a slim ridge; between two such ridges lie two rows of stomata. Stomata in the uppermost region are mature and a gradation in development can be seen from the extreme base upwards. ×33.

No. 3. Silicon X-ray image of intercalary meristem from very young internode of a young *E. hyamale* var. *affine* showing very little silica deposition. ×65.

No. 4. Silicon X-ray image of upper part of internode of a young *E. hyemale* var. *affine* shoot showing significant silica deposition over the epidermis. Black 'holes' represent stomatal crypts where the electron beam does not penetrate. ×52.

No. 5. Picture of excised shoots of *E. variegatum* showing results of geostimulation experiment with auxin transport inhibitors, TIBA (triiodobenzoic acid) and CFM (Methyl-2-Chloro-9-hydroxy Fluorene). Note, the latter completely blocked the curvature while the former partially inhibited the curvature. Time for geostimulation was 72 h. Reduced 0·33 times.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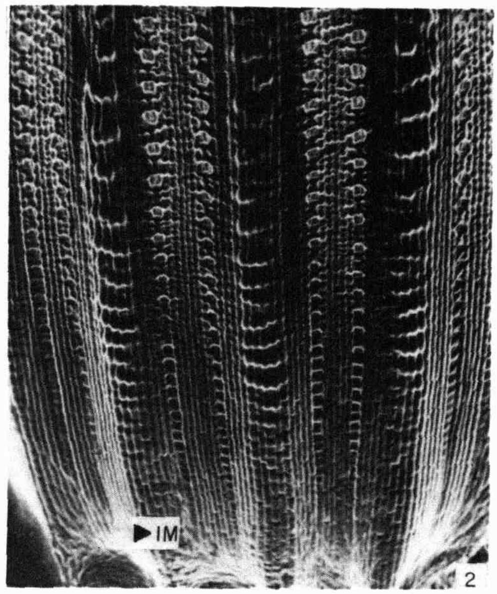
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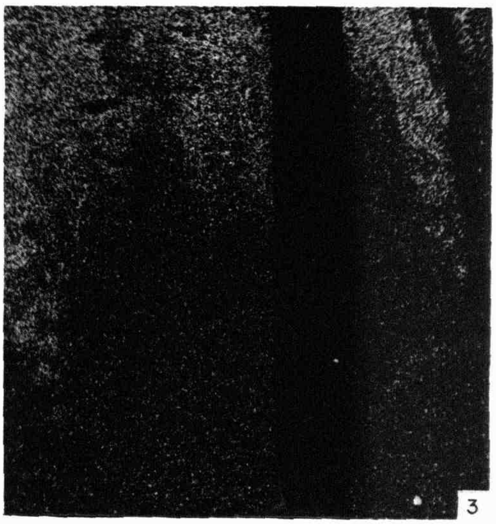
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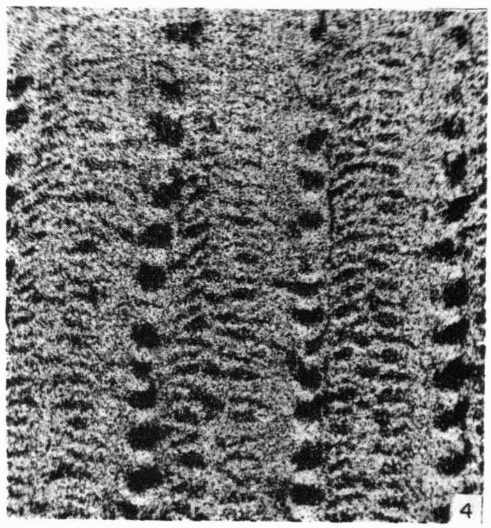
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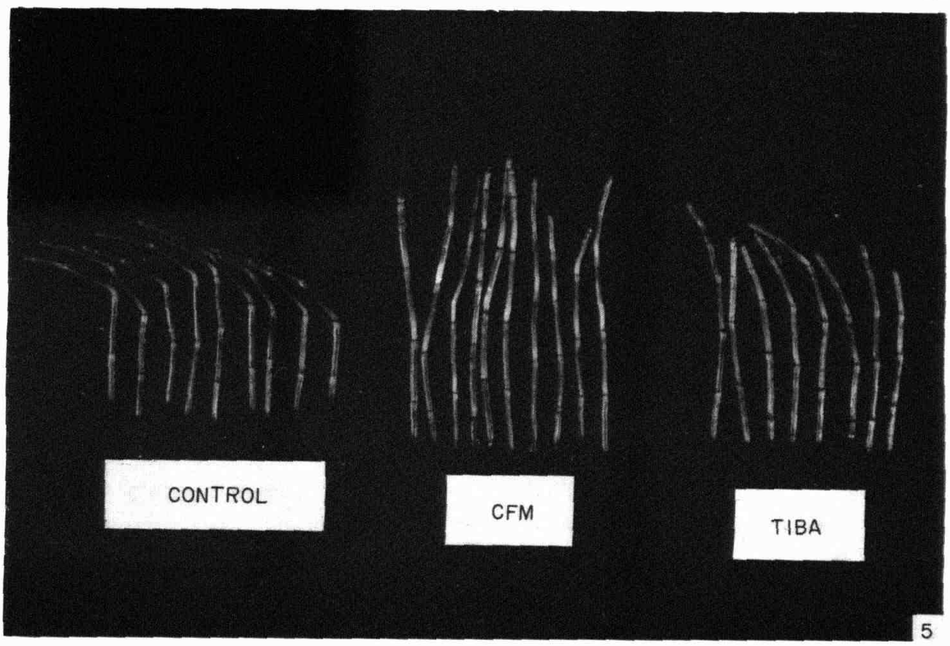
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