EFFECT OF pH OF POLLEN GERMINATION MEDIUM ON POLLEN DIFFUSATES AND pH REGULATION IN *IPOMOEA CAIRICA* L

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GERMINATING pollen releases aminoacids[1], proteins[2] and a range of other chemical moieties[3] into the culture medium. Studies of Speranza and Calzoni[4] on *Malus domestica* and Southworth[5] on *Lilium longiflorum* indicate a role of pollen diffusates in pH regulation of the germination medium. In the present investigation, an attempt is made to assess whether the pH of the external medium has any effect on the release of proteins together with aminoacids and their subsequent role in regulation of the pH of the medium in *Ipomoea cairica*.

Pollen of *I. cairica* was collected from freshly dehisced anthers of field grown plants. To determine the change in pH of the germination medium as a function of time, 60 mg of pollen was cultured in 10 ml medium consisting of 10 % sucrose, 100 $\mu g\ ml^{-1}$ boric acid and 300 $\mu g\ ml^{-1}$ calcium nitrate, in 50 ml flasks. The flasks were hand shaken and the pH was measured at regular time intervals ranging from 1 to 30 min. Similar replicates, at varying time intervals were used for protein and aminoacid estimation after removing the pollen from the medium by filtering through a milli pore filter (0.45 μm pore). To determine the effect of pH of the germination medium, on amino acids and protein release, 60 mg of pollen was cultured in 10 ml of the medium, adjusted to different pH 3–10 using 0.1 N NaOH or 0.1 N HCl. The flasks were shaken for 30 min and the final pH was recorded. The proteins released into the germination medium were estimated by Lowry's[6] method using casein as the standard. The total free aminoacids were estimated by the method of Rosen[7] using isoleucine as standard. The aminoacid content is expressed as micrograms of isoleucine equivalent per milligram of pollen.

Pollen of *I. cairica* affected the pH of the external germination medium within a few minutes of incubation. The pH shift of the medium is completed within the first 10 min after shaking the pollen in the medium. Prolonged shaking of pollen in the medium however did not change the pH any further. At initial pH values between 3 and 6, the pH of the medium increased and became less acidic, while the media with pH values of 7 to 10 decreased becoming less basic (table 1). The least pH change is observed at pH 6.

Table 1 *Effect of initial pH on change in pH, release of amino acids and proteins, within the first 30 min of shaking in pollen germination medium.*

Initial pH	Final pH	pH Difference	Protein content µg/mg pollen	Amino acid content µg/mg pollen
3	3.6	+0.6	15.99	6.87
4	5.5	+1.5	17.14	3.34
5	6.2	+1.2	18.65	4.16
6	6.2	+0.2	22.44	5.24
7	6.4	−0.6	23.96	6.42
8	7.4	−0.6	22.06	5.06
9	8.0	−1.0	21.69	4.25
10	9.5	−0.5	25.09	4 16

Although pH values of 5, 6 and 7 change toward pH 6.2, the media with initial pH values 3, 4, 8, 9 and 10 did not reach this level (table 1).

The amount of aminoacids and proteins released into the media of different pH values was not the same. The data on proteins and aminoacids leached into the media after 30 min of incubation are also shown in table 1. Leaching of aminoacids was maximum at pH 3 and 7, moderate between pH 6, 8, 9 and 10 and minimum at pH 4. The release of protein was maximum at pH 7 and minimum at pH 3 (table 1).

Buffering capacity of the diffusates was tested by titrating the fresh medium (pH 6.3) and filtrate with 0.1 N HCl or 0.1 N NaOH until the pH was changed to either pH 3 or 10 respectively. The amount of HCl or NaOH required to shift the pH of filtrate was twice that required for fresh medium (table 2).

Table 2 *Buffering capacity of Pollen diffusates of Ipomoea cairica L.*

		Amount of 0.1 N HCl/0.1 N NaOH required to change the pH (in ml)	
	Final pH	HCl	NaOH
Fresh control medium, (pH 6.3)	3	0.7	–
	10	–	0.1
Filtrate of medium after pollen culture (pH 6.3)	3	1 4	–
	10	–	0 3

Although pollen in germination media at pH 7–10 lowers the pH, the increase in pH in acidic media towards a value of pH 6.2 suggest that the pH change is not controlled by proton fluxes alone but involves release of buffering compounds. Pollen of *Malus*

domestica[4] and *Crotalaria retusa*[8] also modified the pH to 6 and 6 25 respectively. The release of leachates from *Ipomoea* pollen (present investigation) does not alter the pH further, after 30 min of incubation. This indicates that substances responsible for pH shift diffuse within the first 10 min and continued incubation (or release of substances from pollen tubes) do not significantly change the pH. This indicates that the substances responsible for pH change within the first 10 min of incubation originate from the pollen wall.

Our data in table I indicate that the rate of release of aminoacids and proteins are pH-dependent. The amount of material lost by the pollen is a function of pH of elution medium. The relation between pH of the medium and the release of proteins and aminoacids is not clear. The reverse is unlikely and does not seem to be the case, since the maximum release of proteins at pH 7 and aminoacids at pH 3 does not correspond with maximum pH shift at pH 4.

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TRACHEARY ELEMENTS IN *CERATOPTERIS THALICTROIDES* (L) BRONG

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OCCURRENCE of the vessels in pteridophytes is uncommon, though in recent years, vessels have been described in a number of them *e.g. Selaginella*[1,2]; *Equisetum*[3,4]; *Helminthostachys zeylanica*[5]; *Pteridium aquilinium*[6–12]; *Pteridium esculentum*[11]; *Athyrium felix-femina*[12]; *Actiniopteris radiata*[13]; *Adiantum incisum*[14]; *Marsilea quadrifolia*[15–17] and *Regnellidium diphyllum*[18]. The present study describes vessels and branched tracheids for the first time in *Ceratopteris thalictroides*, a common water fern of Parkeriaceae. The vessels occur chiefly in the rhizomatous parts, and show either circular or elliptical perforations in their end walls and their lateral walls show scalariform, reticulate and spiral thickenings.

The plants of *C. thalictroides* were collected from Roxburgh Botanical Garden, Botany Department, University of Allahabad, Allahabad and from Garhwa lake (in Shankargarh), a locality nearly 50 km south west from Allahabad. Rhizomes, roots, stipes, rachis and leaves were macerated by the techniques of Jeffrey[19] and Ghouse and Sabir[20] and their tracheary elements were thereafter stained in safranin and were mounted in glycerine jelly. Some of the rhizome pieces were also sectioned and stained in safranin to confirm the occurrence of vessels in the respective parts.

The tracheary elements of the rhizomatous parts of *C. thalictroides* consist of tracheids and vessel elements. The tracheids are simple or branched showing variations in shape and size measuring, 225–800 μm long and 10–37 μm wide, having tapered end walls with scalariform, spiral and reticulate thickenings on the lateral walls. Some of the tracheary elements possess perforations at their end walls forming the vessel elements and perforation plate which measures, 143–300 μm long and 35–49 μm wide. The vessels are simple and unbranched having scalariform, spiral and reticulate thickenings in their lateral walls. The perforations are simple and oblique and generally at one end of the vessel element. It is usually terminal or subterminal in position. The perforations are oblique, circular or elliptical in form and vary from 38–50 μm $\times$ 24–30 μm in dimension (figures 1–11).

Branched tracheids also occur at the transitions of