

groups of bivalves are shown during Callovo–Oxfordian time (Figure 1). Among the three groups of bivalves, the epifaunal–infaunal groups represent their greater abundance from the Callovo–Oxfordian beds, whereas the semi-infaunal bivalves show only 2% of the population from the Callovian beds. The abundance of epifaunal–infaunal groups of bivalves from the Callovo–Oxfordian beds (which could be furnished as a result of marine cyclic transgressive–regressive events) is significant not only with the associated lithology but also with the marine cyclic events.

Finally, on the basis of the present study, it is interesting to note that the Oxfordian bed (in which the marine transgressive event took place) is characterized by the greater abundance of infaunal bivalves, whereas the Callovian beds (in which marine regressive event took place) constitute predominant epifaunal bivalves.

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## Electromagnetic field-induced *in vitro* pollen germination and tube growth

M. P. Alexander and S. Ganeshan

Division of Plant Genetic Resources, Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bangalore 560 089, India

**The effect of electromagnetic pulsing on pollen germination and tube growth of *Carica papaya* L., cv. 'Washington' was studied. Pollen germination and tube length showed significant increase over controls. The results show that the pollen grains exposed to magnetic field germinate faster and produce longer pollen tubes than the controls. The technique of electromagnetic excitation provides a new tool for evaluating a large number of cryopreserved pollen grains rapidly and would be of immense value in pollen storage and breeding strategies.**

MAGNETIC field induces morphological<sup>1–3</sup>, physiological<sup>4</sup> and biochemical<sup>2,5,6</sup> changes when biological systems are exposed to it. The effect of magnetic field on biological systems generally deals with morphological changes in germinating seeds<sup>7,8</sup> and seedlings<sup>9,10</sup>. Magnetic response in mice<sup>11</sup>, mud-snails<sup>12</sup>, eggs of sea-urchins<sup>13</sup>, micro-organisms<sup>14,15</sup> and mitotically dividing plant cells<sup>16</sup> has also been studied earlier. All these

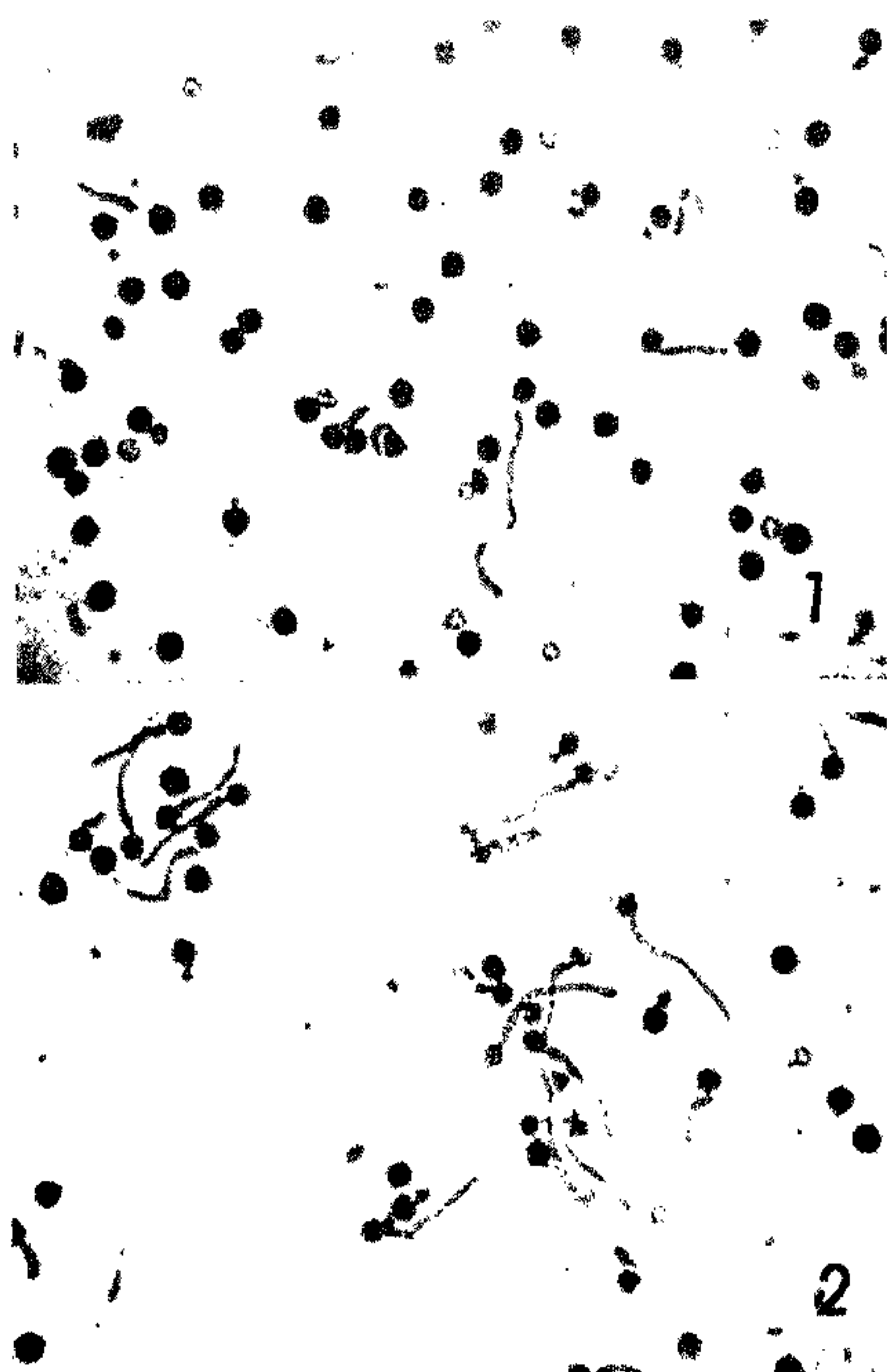
relate to effects at organ, cellular and molecular levels. However, with reference to plant gametophyte (pollen), information is not available. Therefore, a series of experiments were conducted on the pollen of horticultural crops during 1986–88. The present results pertain to studies on the pollen of papaya exposed to a magnetic field of 96 G for 10 min at 12 DC and 0.5 A (strength and time were prestandardized in preliminary experiments).

Two identical bar electromagnets were fabricated by winding 720 turns of 22 gauge enamelled copper wire on 50 mm core. The power supply consisted of a 2 A step-down transformer with tappings at 3, 6, 9, 12, 15, 18, 21 and 24 V AC which was converted into DC by a 5 A bridge rectifier and by a 50 MFD 150 V DC electrolytic capacitor. The electromagnets were connected in parallel circuit to the DC supply and placed one above the other with opposing polarity keeping an air gap of one inch between them.

The pollen of *Carica papaya* L. cv. 'Washington' was mounted in hanging drops containing 6% sucrose. The cavity slides with hanging drops were placed in a hardboard tray, specially fabricated, to enable correct positioning and uniform exposure in the region of maximum flux. After exposure to the magnetic field, the slides were incubated at 25 ± 1°C along with controls in a chamber with 100% relative humidity. Fourteen replicates, each with a parallel control, constituted the experimental set-up. The hanging drops were stained with a versatile stain<sup>17</sup> after 2 h of incubation, remounted on a flat slide and sealed with epoxy resin. Observations on pollen germination and tube lengths were recorded from 10 randomly selected microscopic fields using a Leitz Neopromar projection microscope, following standard procedures<sup>18</sup>. Tube lengths were measured from 10 germinated pollen grains randomly selected from treated and control sets.

Figures 1 and 2 show normal germination and germination of pollen grains exposed to the magnetic field. Pollen germination and tube growth were significantly higher ( $P < 0.01$ ) in magnetic field-induced germination. The mean germination recorded for the induced set was 23.1%, compared to 12.7% for controls. Likewise the tube length was 154.8 µm in the induced set, compared to 104.3 µm for controls. Pollen tubes in the induced set were healthy and normal. Initiation of pollen tubes in the induced set took place after 30 min, while it was after 45 min in the controls. Papaya pollen which takes 3 h *in vitro* to record 25% germination showed a germination of 23.1% in 2 h under the influence of electromagnetic field.

Some of the magnetic influences reported are enhancement of germination and seedling growth (barley)<sup>19</sup>, chromosomal aberrations<sup>16</sup>, enlargement of nucleus<sup>20</sup>, increase in RNA content at growth zone (barley)<sup>20</sup>, triggering of enzymatic activity<sup>6,21</sup>, and



Figures 1 and 2. 1, *In vitro* germination of control *Carica papaya* pollen ( $\times 250$ ). 2, *In vitro* germination and tube growth of *Carica papaya* pollen exposed to magnetic field for ten minutes at a strength of 96 G ( $\times 250$ ).

increase in reducing sugars (wheat)<sup>5</sup>. Pittman<sup>3,5</sup>, based on numerous experiments, found an increase in the efficiency of metabolism on account of stimulus, applied to quiescent seeds and growing seedlings. Commoner *et al.*<sup>22</sup> demonstrated *in vitro* presence of unpaired electrons in enzymatic reactions; they attributed the influence of magnetism on the reaction rate of enzymes to paramagnetic molecules and free radicals in the system. He concluded that for a given magnetic stimulus, the metabolic process that normally occurs before DNA duplication or those processes that are intimately associated with cell division are influenced. These studies, therefore, suggest that pregermination or postgermination magnetic exposure affects plant metabolism, leading to enhanced metabolic growth processes. It may therefore be inferred that the role played by electromagnetism in promoting pollen germination and tube growth could be due to an accelerated metabolic process during germination *in vitro*.

The outcome of this study on electromagnetic stimulus to pollen provides scope for evolving new techniques that would help plant breeders in inducing

gametic selection. Pollen cryobanks which handle large numbers of samples can also effectively apply this technique to assess viability *in vitro* and protract the time taken for pollen to germinate.

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## A method for the rapid isolation of hypocotyl protoplasts of *Eruca sativa*

Amla Batra and Mukta Dhingra

Department of Botany, University of Rajasthan, Jaipur 302 004, India

Protoplast fusion can successfully overcome the difficulties in hybridization between two genera. Hypocotyl explants from ten-day-old seedlings of *Eruca sativa* were taken for isolation of protoplasts. Cellulase 1%, Macerozyme 0.5% and Drieselase 0.2% combinations with 0.6 M mannitol and 2%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (osmotic stabilizers) at pH 5.8 were found to be most suitable. Freshly isolated protoplasts were spherical, colourless and of various sizes.

UNTIL recently, sexual crossing was the only means of