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STRUCTURAL CHANGES ASSOCIATED WITH DORMANCY BREAKING TREATMENTS IN CARDAMOM

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*Seeds of cardamom (*Elettaria cardamomum *Maton) variety* Malabar *were found to exhibit dormancy due to their hard seed coat. Experiments were conducted to study the effect of various physical and chemical treatments for breaking seed dormancy. Treatment of seeds with sulphuric acid (25 %) for ten minutes and absolute alcohol (80 %) for 30 minutes were found to be the best amongst all the treatments in breaking seed dormancy. Scanning electron microscopy of seed coat surface revealed the presence of mucilaginous layer over the control seed and irregular shape lesions of various sizes over the seed coat of treated seeds.*

Germination in cardamom (*Elettaria cardamomum* Maton) is reported to be often poor, delayed and very irregular with some seeds taking over a year to germinate despite favourable conditions (Purseglove, 1972). Under natural conditions, the seeds remain dormant for considerable period of time (Abraham, 1958). The seeds of cardamom are aromatic, dark brown angled with thin mucilaginous aril, bulky white perisperm and small embryo. The hard stony seed coat is reported to be responsible for the delay in seed germination and hence any procedure which enables the seed to imbibe moisture, promotes germination (Kologi *et al.*, 1973; Reddy *et al.*, 1973). Therefore, an attempt has been made to soften the hard seed coat of cardamom by giving various physical and chemical treatments. Surface structural changes which led to increased per cent germination were studied using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Seeds of cardamom, variety Malabar (clone-37) obtained from National Research Centre for Spices, Calicut (Kerala) were used in the

present investigations. For conducting germination test, the seeds were surface sterilized with 0.1 per cent mercuric chloride for ten minutes, washed thoroughly 2-3 times with distilled water and then plated over two layers of moistened filter paper in a petriplate (11 cm diameter). Four replicates of 25 seeds each were used per treatment. All the petriplates were maintained in a germinator at 20°/30°C for 16/8 hr photoperiod. Triphenyl Tetrazolium Chloride (TTC) test for viability following various chemical treatments were carried out based on the earlier reports (Anonymous, 1978; Reddy *et al.*, 1973 and Kololgi *et al.*, 1973). Chemical treatments included acetic acid 25 per cent (10 min), nitric acid 25 per cent (10 min.), hydrochloric acid 50 per cent (10 min), sulphuric acid 25 per cent (10 min.), gibberellic acid 200 ppm (20 min), cellulase 1 per ent (20 min.), ethyl alcohol 80 per cent (30 min.) and potassium nitrate 0.2 per cent (30 Min.). The seeds were thoroughly washed under running tap water after each chemical treatment and then were plated for germination. Physical treatment was given by chipping the seeds at micropylar end and soaking in water for 6 days. All the experiments were repeated thrice and average value for percentage germination was recorded.

Since the cardamom seeds take two to three weeks for germination, a quick viability test was standardised to record the viability after various treatments. The seeds were pre-soaked in water for 18 hrs, then bisected and the half portion of seed containing embryo was put in 1 per cent solution of TTC for 8 hrs at 30°C. After washing, the seeds were observed for the development of red colour stain by embryo which indicated the viability of seed.

The seed coat surface structure studies were performed using scanning electron microscope Model Jeol JSM 840A. Treated and untreated seeds of various samples were cleaned and dried for 48 hrs. at room temperature. Selected seeds were cut with a sharp razor into halves at the raphe region. Both complete and cut seeds were mounted on brass stubs using silver adhesive and gold coated using ion sputter. The coated samples were examined at an accelerating voltage of 5 to 10 kv. The surface was uniformly scanned in all the samples and observations were recorded.

RESULTS AND DISCUSSION

The germination pattern of cardamom seeds after various treatments is depicted in Fig 1. Untreated (control) seeds showed 60 per cent germination whereas, the per cent germination was improved by 23, 28, 20, 35, and 32 per cent after treating with acetic acid, nitric acid, hydrochloric acid, sulphuric acid and ethyl alcohol, respectively (Table 1). However, the maximum increase in germination percentage was

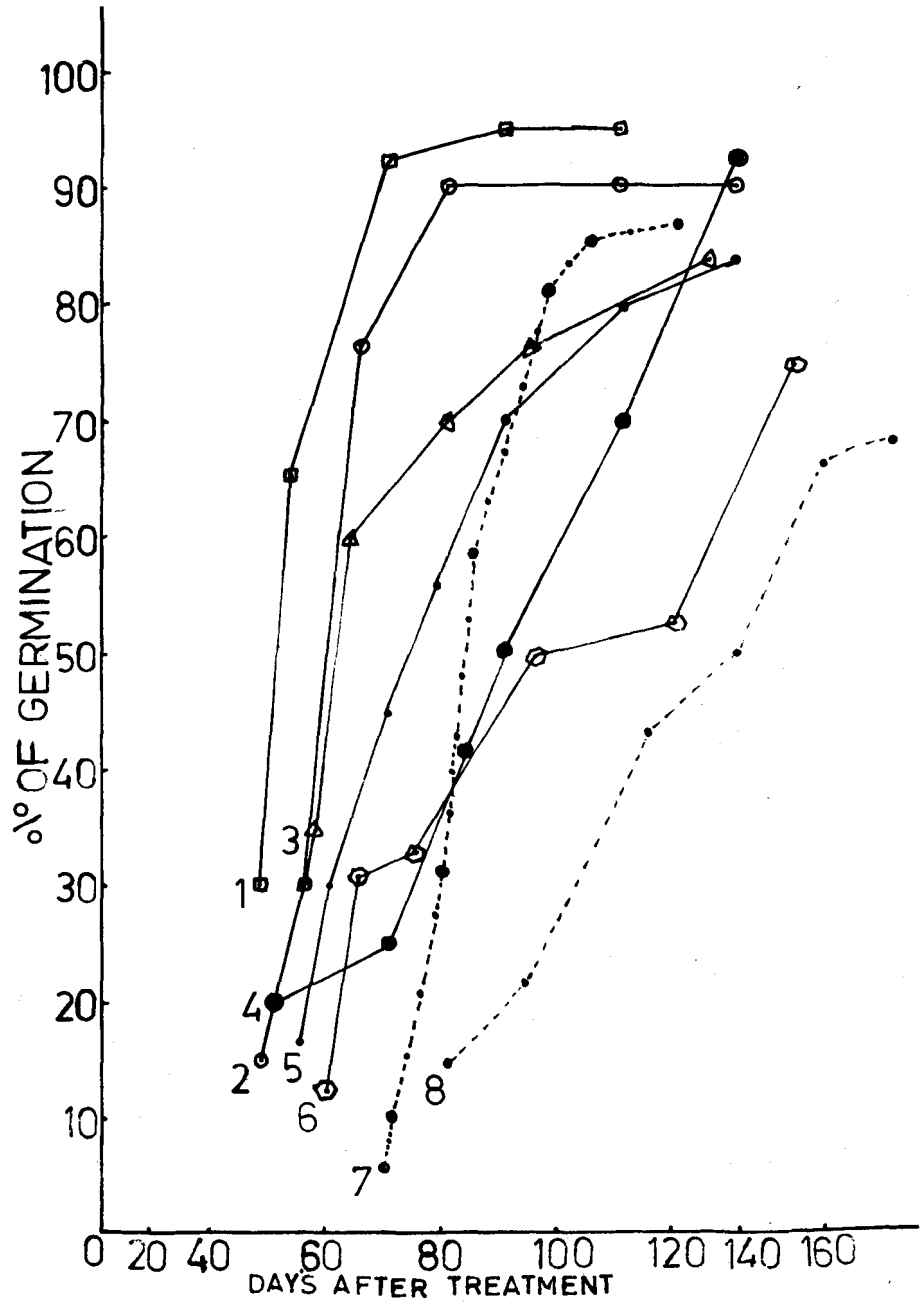

Fig. 1. Germination pattern of cardamom seeds after various treatments 1 = Control, 2 = AA 20, 3 = HNO_3 10, 4 = HCl 10, 5 = Water washed (1 day), 6 = Water washed (6 days), 7 = KNO_3, 8 = H_2SO_4 + GA_3.

achieved with sulphuric acid and ethyl alcohol. Other treatments i.e., potassium nitrate, gibberellic acid and cellulase did not show any significant increase whereas, continuous washing of seeds with water for 6 days showed 15 per cent increase in germination over the control. Also there was a significant effect of acid treatment on speed of germination. Control seeds germinated after 135 days of plating, whereas the treated seeds germinated within 50 days of plating. Although acid treatment improved germination percentage and rate of germination, yet the seedlings treated with ethyl alchohol, potassium nitrate and the control showed higher vigour index (Table 1). Seeds chipped at the micropylar end did not show any germination.

Table 1. Percentage germination and vigour index of seeds under various treatments

Treatments	Germination (%)	Vigour Index (% germination x root length)
Dark treatment	62	80.4
Potassium nitrate	68	75.4
Gibberellic acid	62	78.5
Cellulase	65	77.8
Acetic acid	83	62.0
Sulphuric acid	95	59.5
Hydrochloric acid	80	60.6
Nitric acid	88	98.2
Absolute alcohol	92	184.0
Chipped seeds from one side	0	0
Low temperature treatment (4°C)	50	72.0
Water washing for 6 days	75	88.5
Control	60	79.7

*Average value of 3 replicates.
Difference between treatment and control highly significant (P=0.01) for all treatments except dark treatment, gibberellic acid and chipped seeds.

Result of the TTC test for viability showed a reddish-pink staining pattern in the embryo. Ninety five per cent of the seeds showed stain which closely paralleled with the viability of cardamom seeds after breaking dormancy. SEM studies were done to find out the micromorphological changes in the seed coat surface pattern after various treatments. Seed coat surface of control cardamom seeds was characterised by elongated hexagonal cells with indistinct cell boundaries. Thick deposition of mucilage in the form of fibres was observed along the cell boundaries (Fig. 2A). The seed coat surface of treated

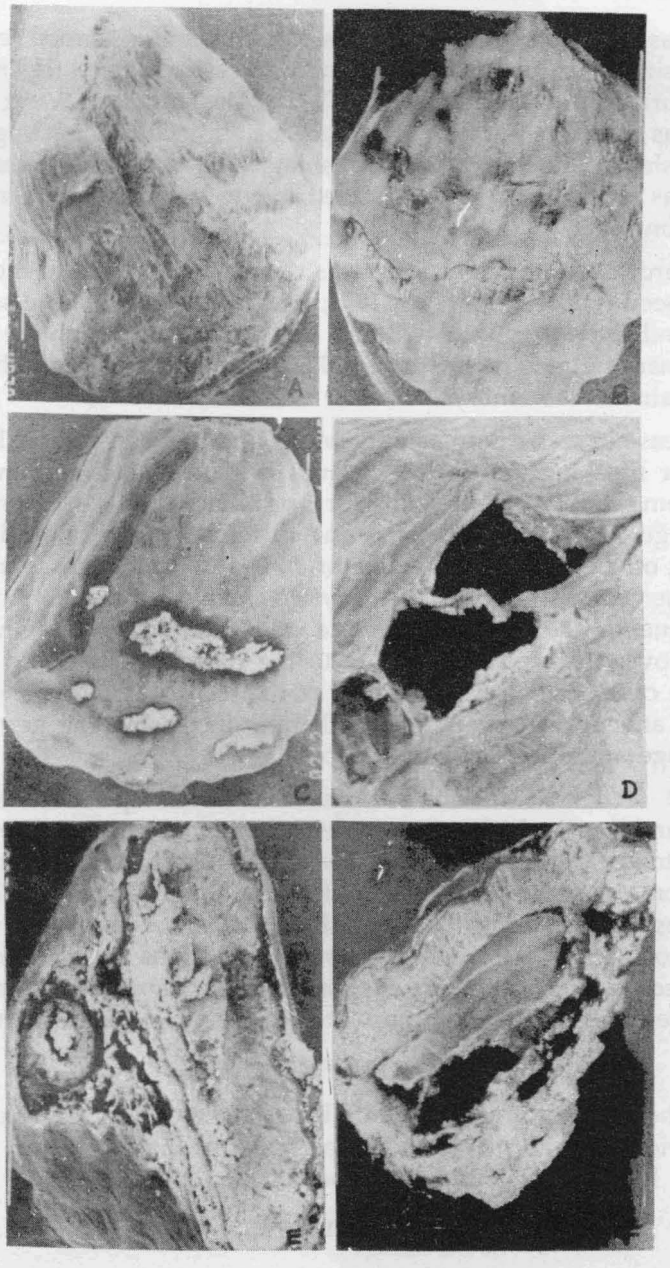

Fig. 2. A-F. SEM micrographs of cardamom seeds. A. Seed coat surface structure of normal seed. B. Seed coat surface of treated seeds, arrow showing lesions, C. Small pores on seed coat surface after treatment, D. Showing deep depressions on seed coat after treatment, E. Longitudinal section of normal seed showing embryo (arrow), F. Longitudinal section of water soaked seed arrow showing flaccid and distinct embryo. (A, B, C = 50x, D = 600x, E, F = 100x).

seeds was free of mucilage and possessed irregular shaped lesions of various sizes (Fig 2B). Small pores and depressions were often recorded on the surface of treated seeds (Fig. 2C,D). The surface of water washed seeds was completely free of mucilaginous white aril which might have been washed off during repeated washings. The embryo in water soaked seeds was found to be much enlarged due to imbibition in comparison to the control (Fig. 2E,F).

In cardamom, the dormancy was reported to be due to the impermeable seed coat (Kologi *et al.*, 1973; Reddy *et al.*, 1973). The seed coat was found to be very thick (28 µm) and hard which might be causing the dormancy due to its impermeability to water, gases or mechanically a constraint to the embryo.

Increase in water permeability was found to be an essential prerequisite for breaking the dormancy and increasing seed germination in cardamom seeds. Water washing for 6 days increased seed germination percentage which may either be due to the softening of seed coat or washing off of germination inhibitor(s), if any. The increase in germination percentage after various physico-chemical treatments could be either due to the removal of extra deposition of mucilaginous aril present over the seed coat or due to the rupture of the seed coat. In both the cases, chemical component of the seed coat is involved which directly affects the water uptake, thereby increasing the germination percentage and speed of germination.

SEM seed coat surface studies revealed the presence of white mucilaginous aril which was completely removed in treated seeds. The mucilaginous aril is reported to play a vital role during germination of cardamom seeds. In the presence of water, the mucilage swells up by absorbing water and may become a diffusion barrier for oxygen (Sulikeri and Kologi, 1977, 1978). Various pores and lesions were found on the treated seeds which might be allowing the free imbibition of water and exchange of gases, thereby leading to increase in per cent germination.

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