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EFFECT OF CHEMICAL AND PHYSICAL TREATMENTS ON PROMOTING GERMINATION OF CERTAIN WOODY LEGUME SEED

BY

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B.S., Chinese Culture College, 1974

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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Manhattan, Kansas

1980

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LITERATURE REVIEW

Impermeability of the seed coat to water is a major factor in maintaining seed dormancy in many woody legumes (5, 25, 26). The embryo is quiescent but is sealed inside a water impermeable covering that can preserve the seed at low moisture contents for many years even at warm temperature (16).

Some researchers (4, 22, 25) reported that impermeability in legume seed was attributed to either the cuticle or the macrosclereid layer, also known as the palisade layer or the Malphigian layer. Brant et al. (6) found that impermeability of the seed coat was due to the macrosclereid cells, the cuticle was not the sole source of impermeabiltiy. Burns (7) also reported that in blue lupine, Lupinus angstifolius, dye penetrated the cuticle but not the macrosclereid layer.

The macrosclereid layer occurring as a single layer of cells except at the hilum where a double layer exists immediately adjacent to the hilar fissure (30). The hilum of a legume seed contains a fissure known as the hilum valve. This valve closes when atmospheric humidity is higher than the moisture content inside the seed, but opens when the reverse conditions prevail (19). The light line which lies at the base of the caps of the macrosclereid layer near the apical termination of the lumens is considered by some to be associated with permeability (7). However, Hamly (15) thought the light line was likely an optical occurrence and not closely associated with permeability. Directly below the macrosclereid layer is a layer of

loosely packed osteosclereid cells which, in sweet clover at least, are believed to be permeable to water (14).

Many woody legume seeds will not germinate if planted immediately after maturation (11, 12). They require seed coat degradation and/or a period of low temperature in order to break dormancy (9, 23). Seed coat degradation is necessary so that imbibition and gaseous exchange may occur. The cold period is necessary for the breakdown of-abscisic acid (ABA), which acts as a germination inhibitor, and the activation of gibberellin synthesis (5).

Mechanical scarification, utilizing abrasion by rough grift, is the most common method of scarification, but seed so treated are often injured with a resulting decrease in vigor and viability (6).

However, scarification with sulfuric acid has been tried with varying degrees of success on species such as beach pea, Lathyrus maritimus (21). A 10 to 15-min soak in commercial sulfuric acid was effective in breaking hard seed dormancy in commercial samples of crownvetch, Cornilla varia L. (20). Weisehugel (33) reported that 24-hour water soak followed by 2 hours in concentrated sulfuric acid at room temperature was effective in promoting germination of Kentucky coffeetree, Gymnocladus dioicus (L.) K. Koch.. **Frett** and Dirr (13) also found that Kentucky coffeetree seed proved easy to germinate with 2, 4, and 8 hour concentrated sulfuric acid treatment resulting in 93, 100 and 95% germination, respectively. Soak

seed in sulfuric acid from 1 to 2 hours at room temperature has been used to make honeylocust, Gledistia L., permeable (17, 31), and a constant temperature of 20°C for 21 days is recommended for germination (3).

Seeds of redbud, Cercis canadensis L., have hard impermeable seed coats in addition to internal dormancy. Both scarification and cold stratification are needed before adequate germination will occur (1, 18, 31, 34). The usual recommendation for redbud germination is sulfuric acid treatment of 25 to 60 minutes followed by cold treatment of 2 to 5°C for 5 to 8 weeks (2), however, an optimum germination occurred after 60 days cold and 15, 30, 60 minutes of acid scarification (13).

Wilson (35) found that immersion of legume seed in boiling water was successful to make hard seed permeable in species such as black locust, Robinia spp.. For honeylocust, when the hot water treatment is used, seeds are placed in 3 to 4 times their volume of water at about 88°C, then seeds and water are allowed to cool to room temperature or until the seeds swell (31). Submerging seeds in boiling water for 1 minute or hot water of 82°C has been used to treat impermeable redbud seeds (2, 32), but, after treatment seeds should be stratified promptly, they can not be stored (8, 10, 24, 34).

Peto (27) successfully used enzymes such as hemicellulase and pectinase to loosen the seed cap on sugar beet, Beta vulgris.. Works and Erickson (36) postulated that infra-red radiation disrupted the continuous cuticular layer of the seed coat and fractured small areas in the macrosclereid layer.

Rincker (28, 29) found that treatment at 42°C for 1 hour or at 105°C for 90 sec. was effective on hard seed of sweet clover. Immersion of seeds such as sweet clover and alfalfa in liquid air (-95.6°C) or liquid N (-198.5°C) successfully reduced the percentage of hard seed (4). Brant et al. (6) also reported that treatment in liquid N reduced hard seed of crownvetch to 15% and increased readily germinable seed to 65%.

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MANUSCRIPT

This manuscript is written in the style of and for publication in Journal of the American Society for Horticultural Science.

Effect of Chemical and Physical Treatments on Enhancing Germination of Certain Woody Legume Seed.[1]

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Additional index words: Impermeability, scarification, stratification, scanning electron micrography, macrosclereid cells.

Abstract: Various chemical and physical treatments were applied to seed of Kentucky coffeetree (Gymnocladus dioicus (L.) K. Koch.), honeylocust (Gleditia triacanthos var. inermis L.) and redbud (Cercis canadensis L.), in an attempt to increase water permeability and speed germination. Kentucky coffeetree seed germinated maximally after treatment for 120 or 150 minutes with concentrated sulfuric acid scarification. Maximum honeylocust germination occurred following 60, 90, or 120 minutes concentrated sulfuric acid scarification while redbud germinated best following 30, 60, or 90 minutes concentrated sulfuric acid scarification followed by a 60-day stratification. Scanning electron micrographs of acid scarified Kentucky coffeetree, honeylocust, and redbud seed indicated that lumens of the macrosclereid cells were exposed thereby permitting imbibition of water. Boiling water treatment ruptured the macrosclereid layer while seed treated in liquid N had fissures present which did not appear to penetrate

the macrosclereid layer.

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[1]Received for publication_____. Contribution NO._____
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Kentucky coffeetree, honeylocust, and redbud are all cultivated as ornamental trees. All have hard and impermeable seed coats that prevent or delay germination (9, 2).

The impermeability of the seed coat is a major factor in maintaining seed dormancy in many woody legumes (5, 16, 17). Impermeability of the seed coat is due to a layer of palisade-like macrosclereid cells, especially thick-walled on their outer surface with a waxy, external cuticle (6). The macrosclereid layer occurring as a single layer of cells except at the hilum where a double layer exists immediately adjacent to the hilar fissure (18). Disintegration of the caps or tops of the macrosclereid cells or mechanical stress separating these cells allows water to enter and induce germination (6). Besides seed coat degradation, some woody legumes also require a period of low temperature in order to break dormancy (7, 16). The cold period is necessary for the breakdown of abscisic acid (ABA), which acts as a germination inhibitor, and the activation of gibberellin synthesis (5).

Conventional hulling and scarification equipment commonly utilizes a grit-impregnated disc or bowl against which the seeds are forced. This procedure hulls and scarifies the seed, but seed so treated are often injured with a resulting decrease in vigor and viability (6).

Weisehuegel (20) reported that 24-hour water soak followed by 2 hours in concentrated sulfuric acid at room temperature was effective in promoting germination of Kentucky coffeetree. Frett

and Dirr (10) also found that Kentucky coffeetree seed proved easy to germinate with 2, 4, and 8 hours concentrated sulfuric acid treatments resulting in 93, 100, and 95% germination, respectively. For honeylocust, soaking seeds in either concentrated sulfuric acid from 1 to 2 hours at room temperature or hot water (88°C) has been used to make them permeable (14, 3). Redbud seed germination requirements have been adequately covered in the literature (2, 12, 13) and the usual recommendation is sulfuric acid treatment of 25 to 60 minutes followed by cold treatment of 2 to 5°C for 5 to 8 weeks (15). Maximum germination of redbud occurred after 60 days cold and 15, 30, 60 minutes of acid scarification (10). In addition, immersion of certain legume seeds such as sweet clover and alfalfa in liquid N (-198.5°C) or liquid air (-95.6°C) successfully made them permeable to water (4).

The objectives of this research were 1) to evaluate selected scarification treatments which would promote seed germination of Kentucky coffeetree, honeylocust, and redbud and 2) to observe differences caused by scarification treatments on the seed surface using the SEM.

Materials and Methods

Germination tests. Kentucky coffeetree seeds were collected from a single tree in Kansas State University campus, during the spring of 1980. Viable seeds were separated from the lighter and dead seeds by flotation (8). Seeds that sank to the bottom were tested for seed viability using the triphenyltetrazolium

chloride test (TTC) (11). Viable seeds were subjected to the following procedures:

1) Seeds were treated with concentrated sulfuric acid for 30, 60, 90, 120, and 150 minutes (with agitation) followed by washing them thoroughly in distilled water.
2) Seeds were treated in boiling water for 1 minute, then removed the heat immediately and allowed to soak in the gradually cooling water for 24 hours.
3) Seeds were immersed in liquid N (-195.8°C) by two 2-minute immersions with a 1-minute exposure to room air between immersions.

After treatments, seeds were grown in a growth chamber maintained at 25°C/21°C day/night temperature regime. Twelve hours of light was provided daily. Final germination counts were taken 30 days after germination. Each treatment was replicated 4 times and each replication contained 25 seeds.

Viable honeylocust seeds received the same scarification treatments as Kentucky coffeetree seeds did. After treatments, seeds were planted in a germinator set at a constant temperature of 20°C for 21 days with a 12-hour photoperiod. Each treatment consisted of 4 replicates and each replicate contained 25 seeds.

Redbud seeds were also collected from a single tree on the KSU campus, during the spring of 1980. Seed separation and scarification treatments were same as those of Kentucky coffeetree. Seeds were then stratified at 5°C for 60 days followed by growing

them in a greenhouse of 20°C to 27°C for 14 days.

SEM observation. Scanning electron micrographs of untreated and treated seed coat of Kentucky coffeetree, honeylocust, and redbud were taken with an ETEC Autoscan U-1 SEM in Kansas State University Scanning Electron Microscope Laboratory. The specimens were coated with 200A° carbon followed by 200A° gold-palladium (60-40), and an accelerating voltage at 10KV was used on the SEM. The micrographs produced by the SEM were on Polaroid Type 55 positive and negative film.

Results and Disscussion

Germination tests. Germination percentages of Kentucky coffeetree increased significantly as the sulfuric acid scarification period increased. Seed germination was 95% and 99% with 120 or 150 minutes acid treatment, respectively (Table 1), however, germination in the boiling water or liquid N treatment was poor. Honeylocust seed germinated best with 60, 90, or 120 minutes concentrated sulfuric acid treatments resulting in 97, 98, and 91% germination, respectively (Table 1). Germination results also showed no significant differences between the control and the boiling water or liquid N treatment. Maximum germination of redbud occurred following 30, 60, or 90 minutes sulfuric acid scarification followed by a 60-day stratification at 5°C, while treatment with either boiling water or liquid N yielded the worst germination. According to the TTC tests, boiling water treatment periods of 1 minute killed nearly all of the Kentucky coffeetree

and redbud seeds while some of the honeylocust seeds were also killed by boiling water scarification.

SEM observation. Scanning electron micrographs (15x, 300x, 3000x) of unscarified seed of Kentucky coffeetree, honeylocust, and redbud clearly show a cracked surface and remnants of the waxy substances (Figures 1, 3, and 5). It is apparent from the photographs taken at 15x magnification that sulfuric acid etches the seed coat and locally dissolves the caps or tops of the macrosclereid cells of Kentucky coffeetree, honeylocust, and redbud (Figures 1, 3, and 5). With increasing magnification (3000x) the columnar structure of the macrosclereid cells become evident. Scanning electron micrographs (15x, 300x, and 3000x) of seed treated with liquid N indicate that the surface of the seed coat does not differ greatly from that of unscarified seed; the seed coat also shows a cracky surface and still has remnants of the waxy substances (Figures 2, 4, and 6). Photographs (15x, 300x, and 3000x) of 1 minute boiling water scarified seed suggest that mechanical stress induced by termal expansion ruptures the seed coat and separates the macrosclereid cells (Figures 2, 4, and 6).

Consideration of the above germination results in conjunction with the scanning electron micrographs indicate that concentrated sulfuric acid treatment results in etch pits which penetrate the caps of the macrosclereid layer and expose the lumens of the macrosclereid thereby permitting imbibition of water. A brief dip in boiling water results in thermal expansion which separates or

destroys the columnar cells of the macrosclereid layer, permitting water to penetrate, but this treatment also seriously damages the seed reducing its ability to germinate. In contrast, seed treated in liquid N has fissures not penetrating the macrosclereid layer, which means, liquid N treatment apparently does not induce sufficient mechanical stress to make hard seed permeable.

The effect of concentrated sulfuric acid on enhancing germination has been demonstrated for Kentucky coffeetree (10, 20), honeylocust (14, 3), and redbud (10, 15). The germination results of these studies also indicate that concentrated sulfuric acid treatment is effective to make hard seed permeable to water and induce good germination. In this experiment, the optimum soaking time in acid was 120 or 150 minutes for Kentucky coffeetree, 60, 90, or 120 minutes for honeylocust, and 30, 60, or 90 minutes for redbud. As documented in previous studies (1, 19), submerging seeds in boiling water for 1 minute was effective in increasing germination of redbud. However, germination results in this study suggest that a one minute boiling water treatment resulted in a poor germination, not only for redbud, but also for Kentucky coffeetree and honeylocust. Although treatment in liquid N (two 2-min dips separated by a 1-min interval) has been used to increase germination of crownvetch (6), it apparently can not increase germination of Kentucky coffeetree, honeylocust and redbud seed. Some researchers have reported that impermeability in legume seed is attributed to both cuticle and the macrosclereid layer (6).

This experiment supports the premise that in legume seed the region of impermeability lies in the area of the macrosclereid cells and that the cuticle is not the sole source of impermeability.

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Table 1. Germination of Kentucky coffeetree, honeylocust, and redbud seed after scarification.

Treatment[y]	Germination (%)		
	Kentucky coffeetree	Honeylocust	Redbud[x]
Control	4e[z]	32d	1d
30-min Acid	53d	80c	67a
60-min Acid	84c	97ab	72a
90-min Acid	93b	98a	70a
120-min Acid	95ab	91ab	31b
150-min	99a	90b	19c
Boiling water	1e	36d	0d
Liquid N	3e	34d	0d

[z]Mean separation in columns by Duncan's multiple range test, .05 level.

[y]All treatment means of 4 replicates of 25 seeds.

[x]Scarification followed by a 60-day stratification at 5°C.

Figure 1. Scanning electron micrographs of Kentucky coffeetree seed showing scarification effects on seed coats. Left: unscarified seed. Magnification: A)15x; B)300x; C)3000x. Note the cracky surface at arrows and remnants of the waxy substances at arrowheads. Right: after 150-min acid scarification. Magnification: D)15x; E)300x; F)3000x. Caps of macrosclereid cells have been destroyed by acid, exposing the lumens of the cells at arrows. Bar length (A and D) = 100 μm; Bar length (B and E) = 10 μm; Bar length (C and F) = 5 μm.

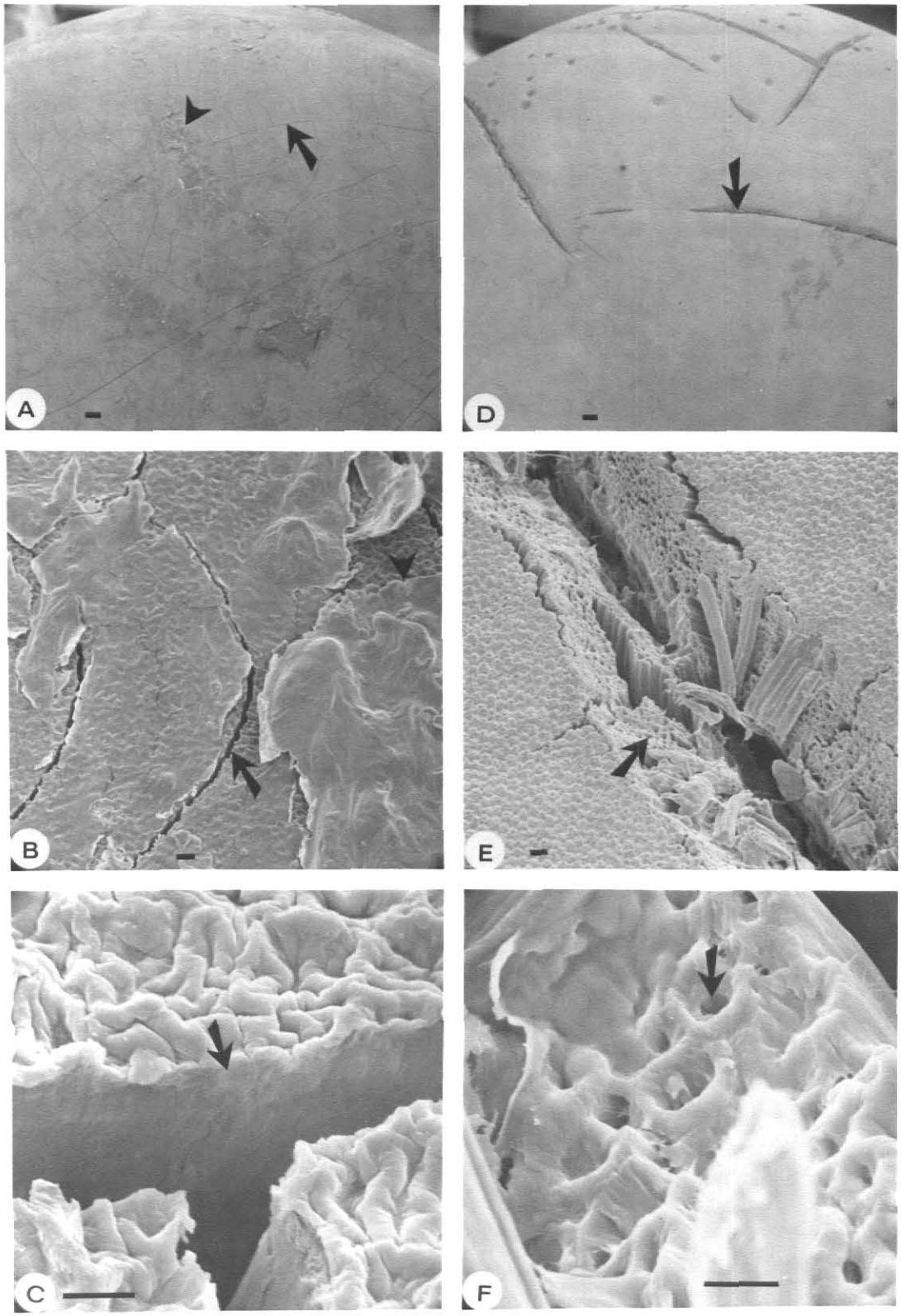
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Figure 2. Scanning electron micrographs of Kentucky coffeetree seed showing scarification effects on seed coats. Left: after liquid N scarification. Magnification: A)15x; B)300x; C)3000x. Note the cracky surfact at arrows and the seed still has remnants of the waxy substances at arrowheads. Right: after seed immersion in boiling water for 1 minute. Magnification: D)15x; E)300x; F)3000x. The seed coat has been destroyed by boiling water, separating the macrosclereid cells at arrows. Bar length (A and D) = 100 μm; Bar length (B and E) = 10 μm; Bar length (C and F) = 5 μm.

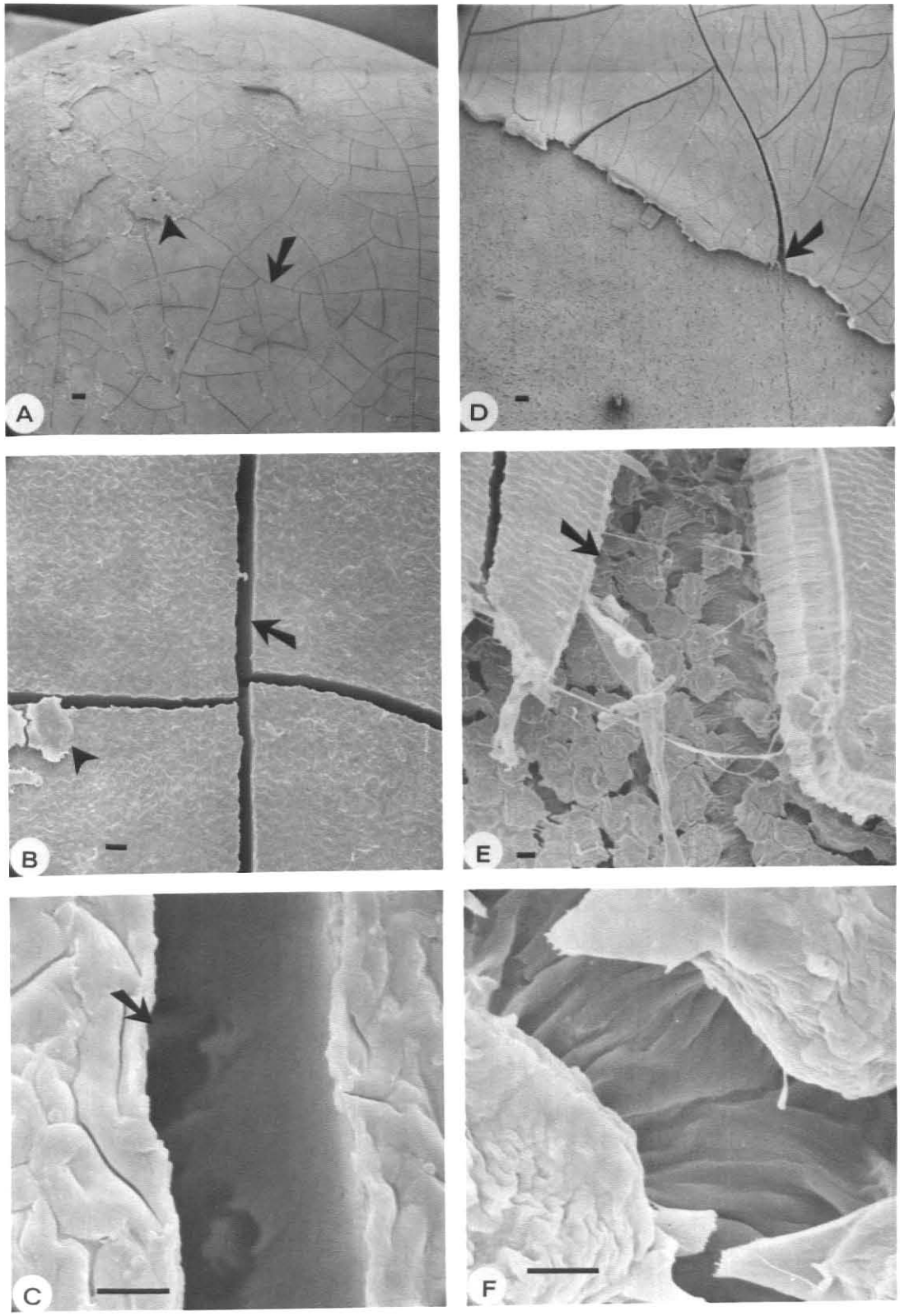
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Figure 3. Scanning electron micrographs of honeylocust seed showing scarification effects on seed coats. Left: unscarified seed. Magnification: A)15x; B)300x; C)3000x. Note the cracky surface at arrows and remnants of the waxy substances at arrowheads. Right: after 90-min acid scarification. Magnification: D)15x; E)300x; F)3000x. Caps of macrosclereid cells have been destroyed by acid, exposing the lumens of the cells at arrows. Bar length (A and D) = 100 μm; Bar length (B and E) = 10 μm; Bar length (C and F) = 5 μm.

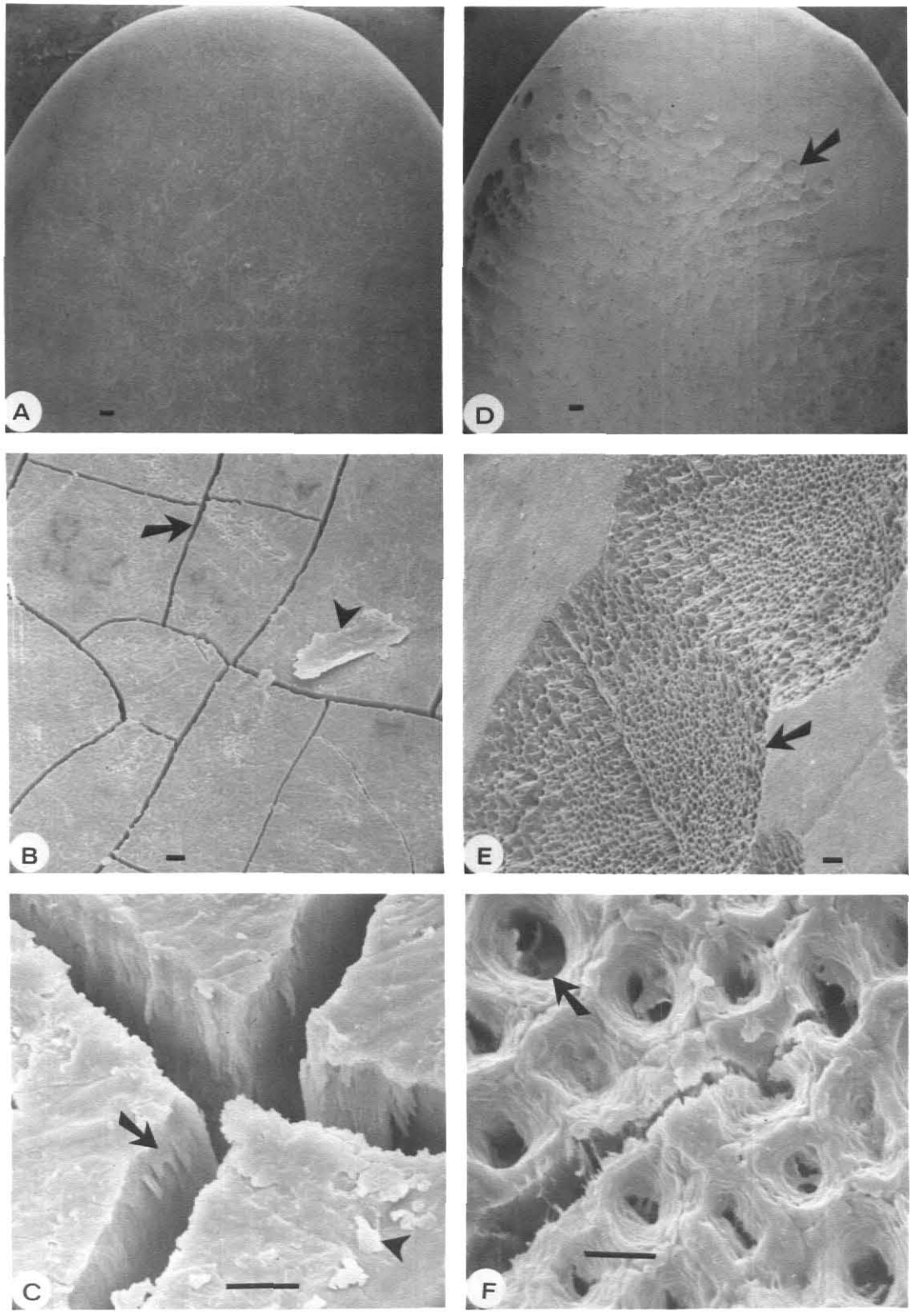
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Figure 4. Scanning electron micrographs of honeylocust seed showing scarification effects on seed coats. Left: after liquid N scarification. Magnification: A)15x; B)300x; C)3000x. Note the cracky surface at arrows and remnants of the waxy substances at arrowheads. Right: after seed immersion in boiling water for 1 minute. Magnification: D)15x; E)300x; F)3000x. The seed coat has been destroyed by boiling water, separating the macrosclereid cells at arrows. Bar length (A and D) = 100 μm; Bar length (B and E) = 10 μm; Bar length (C and F) = 5 μm.

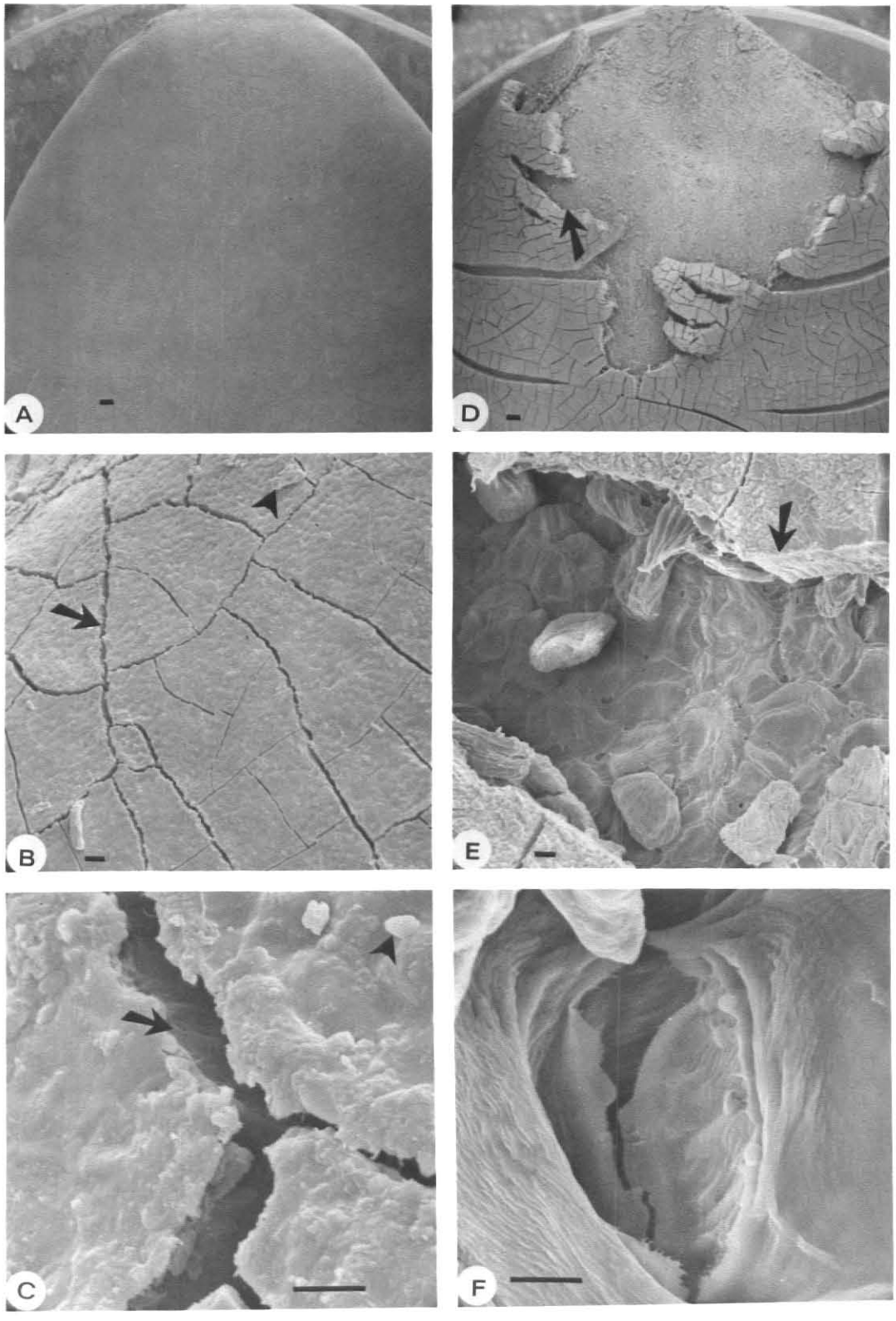
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Figure 5. Scanning electron micrographs of redbud seed showing scarification effects on seed coats. Left: unscarified seed. Magnification: A)15x; B)300x; C)3000x. Note the cracky surface at arrows. Right: after 60-min acid scarification. Magnification: D)15x; E)300x; F)3000x. Caps of macrosclereid cells have been destroyed by acid, exposing the lumens of the cells at arrows. Bar length (A and D) = 100 μm; Bar length (B and E) = 10 μm; Bar length (C and F) = 5 μm.

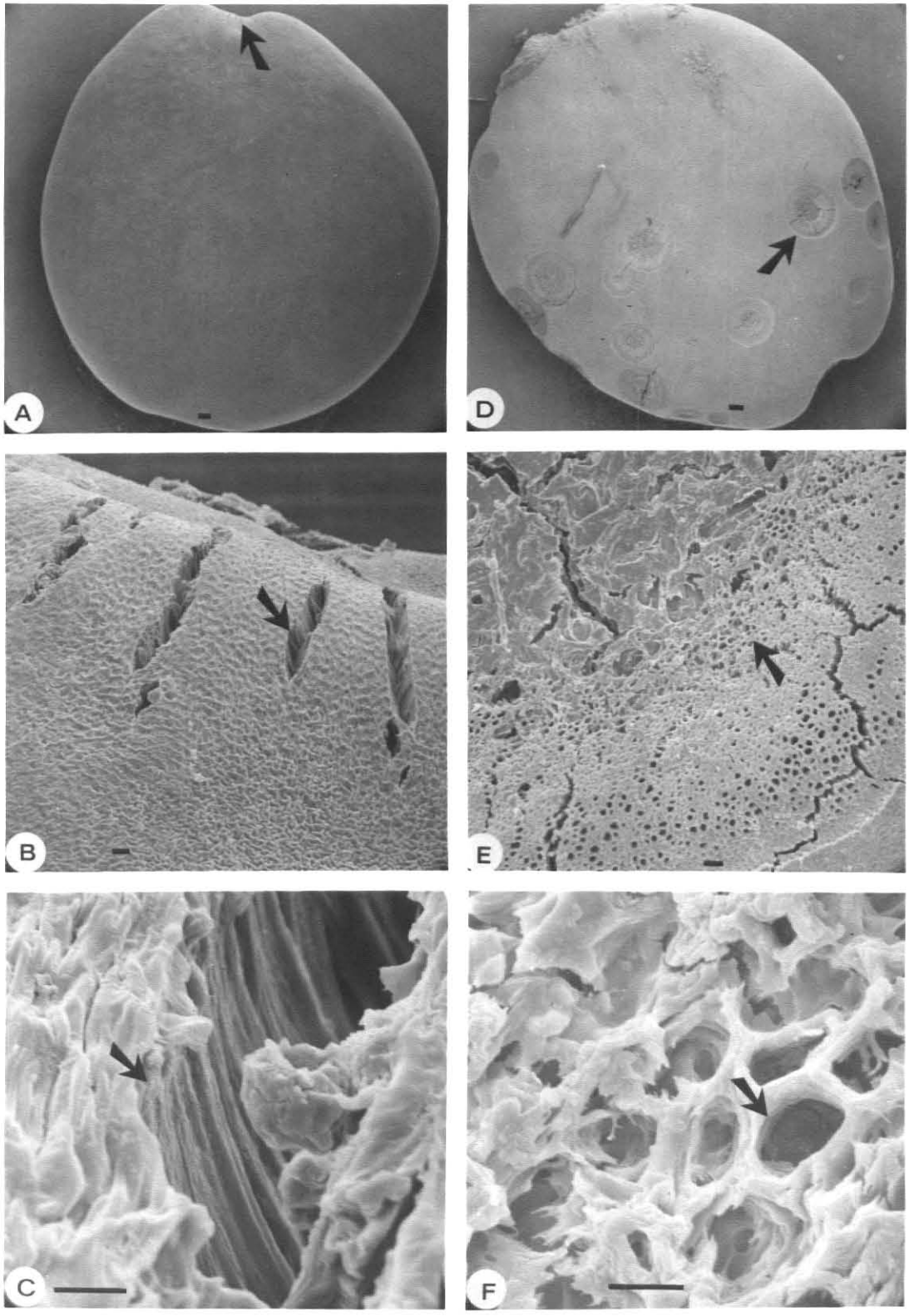
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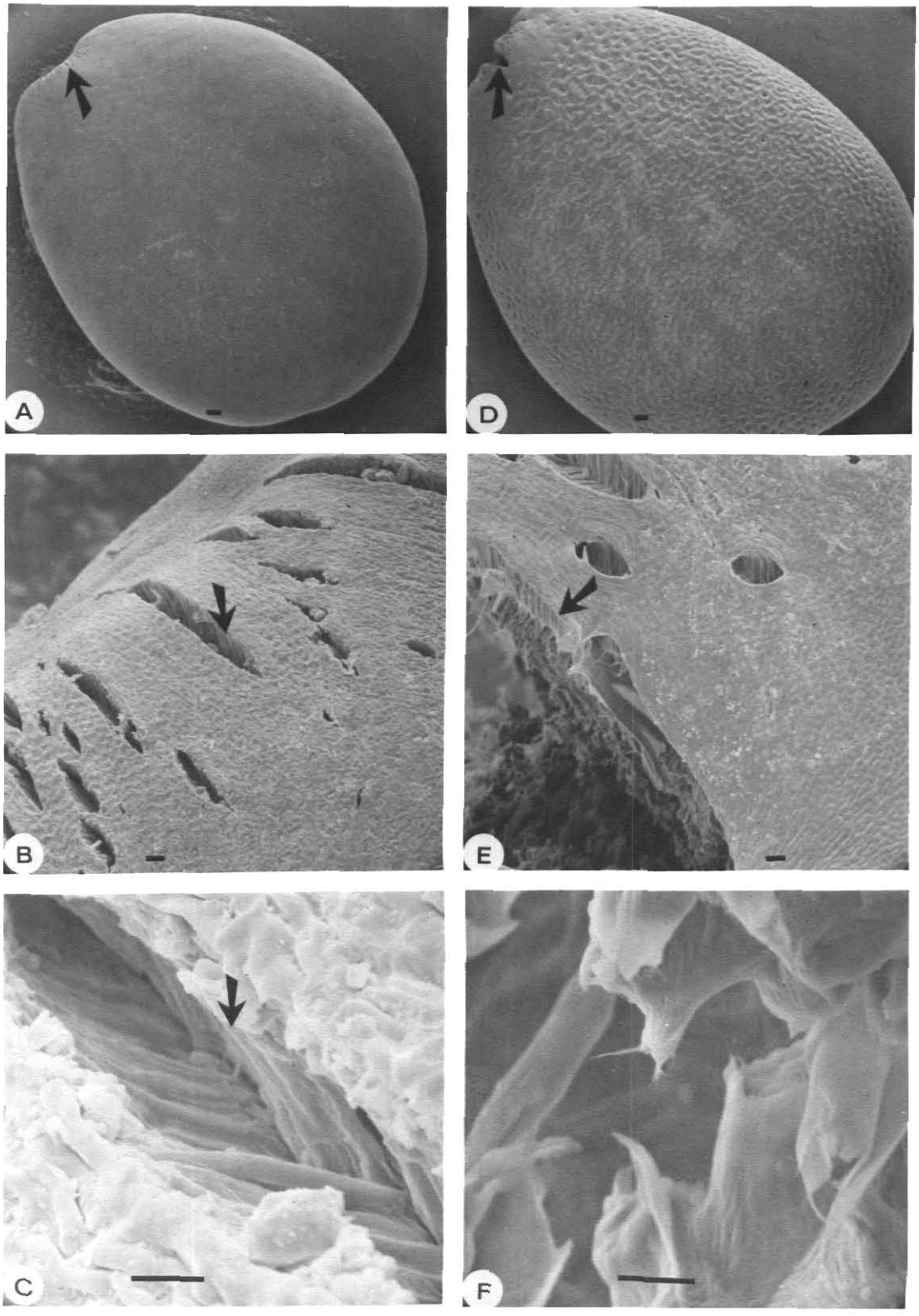
Figure 6. Scanning electron micrographs of redbud seed showing scarification effects on seed coats. Left: after liquid N scarification. Magnification: A)15x; B) 300x; C)3000x. Note the cracky surface at arrows. Right: after seed immersion in boiling water for 1 minute. Magnification: D)15x; E)300x; F)3000x. The seed coat has been destroyed by boiling water, separating the macrosclereid cells at arrows. Bar length (A and D) = 100 μm; Bar length (B and E) = 10 μm; Bar length (C and F) = 3 μm.


A
D
B
E
C
F

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my appreciation to my major Professor, Dr. Houchang Khatamian, Department of Horticulture, for his guidance, patience, and encouragement throughout my graduate study.

Sincere appreciation is also extended to Dr. Thomas A. Fretz, Head of the Department of Horticulture, and Dr. Peter P. Wong, Associate Professor of Physiology, for their advice as members of my academic committee.

Special thanks to Dr. Frank D. Gibbons III, Assistant Professor of Horticulture, and Mr. John Krohma for their help on the scanning electron micrographs. I also wish to express my appreciation to all others who have helped me in this research in numerous ways at one time or another.

I wish to acknowledge the financial support provided by the Department of Horticulture.

Finally, I am forever indebted and grateful to my parents whose encouragement made my graduate studies possible.

EFFECT OF CHEMICAL AND PHYSICAL TREATMENTS ON ENHANCING GERMINATION OF CERTAIN WOODY LEGUME SEED

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

Kansas State University

Manhattan, Kansas

1980

Various chemical and physical treatments were applied to seed of Kentucky coffeetree (Gymnocladus dioicus (L.) K. Koch.), honeylocust (Gleditia triacanthos var. inermis L.) and redbud (Cercis canadensis L.), in an attempt to increase water permeability and speed germination. Kentucky coffeetree seed germinated maximally after treatment for 120 or 150 minutes with concentrated sulfuric acid scarification. Maximum honeylocust germination occurred following 60, 90, or 120 minutes concentrated sulfuric acid scarification while redbud germinated best following 30, 60, or 90 minutes concentrated sulfuric acid scarification followed by a 60-day stratification. Scanning electron micrographs of acid scarified Kentucky coffeetree, honeylocust, and redbud seed indicated that lumens of the macrosclereid cells were exposed thereby permitting imbibition of water. Boiling water treatment ruptured the macrosclereid layer while seed treated in liquid N had fissures present which did not appear to penetrate the macrosclereid layer.