STRUCTURAL FEATURES OF LEAVES OF BLOOM, BLOOMLESS, AND SPARSE-BLOOM VARIETIES OF SORGHUM AS RELATES TO DROUGHT TOLERANCE AND GREENBUG RESISTANCE

By

ISSAGA DIT MORIBA KONATE

Ingenieur des Sciences Appliquees

Institut Polytechnique Rurale du Mali

Katibougou, Mali

1975

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1983

STRUCTURAL FEATURES OF LEAVES OF BLOOM, BLOOMLESS, AND SPARSE-BLOOM VARIETIES OF SORGHUM AS RELATES TO DROUGHT TOLERANCE AND GREENBUG RESISTANCE

Thesis Approved:

Dale E. Weibel
Thesis Adviser

Paul E. Richardson

Lawrence G. Morrill

Norman N. Durham
Dean of Graduate College

PREFACE

Mali, like many countries in the Third World, is experiencing a severe drought causing food shortages. External aid has been helpful but for continuous self-sufficiency our own people need training in diverse facets of agriculture such as Plant Breeding, Crop Physiology, Soil Science, etc. Therefore, I have been studying at Oklahoma State University to receive advanced training in Plant Breeding so that I may be able to contribute in some way to the agricultural self-sufficiency of my country. My degree could not have been completed without the help of many people.

First, I owe appreciation and deep gratitude to my major adviser Dr. Dale E. Weibel for his support, patience, indulgence, sound guidance, constant and enthusiastic suggestion; and to Dr. Paul E. Richardson for his advice and the use of his laboratory facilities. Appreciation is also extended to Dr. Lawrence G. Morrill and Dr. Richarson, who served on my graduate committee, for their constructive suggestions and reviews of my manuscript.

I wish to specially thank all the people who participated in some way or another in my education:

- The government of Mali for selecting me for this type

of training.

- The government of the United States, through the U.S. Agency for International Development (U.S./AID), especially Dr. Larry Littlefield and Kareen Utterback.
- The Office of International Programs at Oklahoma State University, especially Mr. Hugh Rouk, Mrs. Janita Legako, and Mrs. Gemma McCornick.
- The sorghum crew of the Oklahoma State University Agronomy Department.
- My wife Fanta, my son Abdoulaye and my daughter Djenebou for their patience and encouragement.
- My brother Mamadou Konate and his family who came to the United States from Mali to encourage me.

I wish to thank Mrs. Melanie Bayles for typing the final copy of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sorghum bicolor (L.) Moench has been a well established economic crop since the time of earliest civilizations. The grain is used for human food as well as feed for animals, and the stems and leaves are used for green chop, hay, silage, and pasture.

Grain sorghum is cultivated throughout Africa and extensively in India, Manchuria, and the United States. It is also grown in Asia Minor, Iran, Pakistan, Korea, Japan, and some islands of both the East and the West Indies. It is an important crop in the United States, particularly in the Southwest where the climate is too hot and dry for corn. The states leading in grain sorghum production are Texas, Kansas, Nebraska, Oklahoma, California, and Missouri.

Sorghum secretes a waxy coating known as "bloom" (epicuticular wax) on the leaf blades and leaf sheaths. Three types have been recognized: a heavy coating known as "bloom", a light coating or "sparse-bloom", and no covering which is termed "bloomless".

Greenbugs, Schizaphis graminium (Rondani), a major pest of sorghum since 1968, have been reported to show nonpreference for the bloomless types of sorghum, but few

studies have been done to explain this nonpreference. The objectives of this study have been to:

1. Describe the surfaces of leaves of the three types and to record the relative amounts and distribution of wax on bloomless, sparse-bloom and normal bloom types of sorghum.
2. Study the comparative morphology of the three types of leaves at the tissue level.
3. Relate these features to preference or nonpreference of greenbugs.

CHAPTER II

LITERATURE REVIEW

Ayyangar et al. (3) and Ayyangar and Ponnaiya (4), working with the World Collection of Sorghum at the Millet Breeding Station at Coimbatore, India, reported that all sorghum plants exude a form of epicuticular wax known as "bloom". This bloom is a white powdery appearing epicuticular wax which is readily observed on leaf blades and leaf sheaths. The authors recognized three types: 1. the heavy covering or "bloom"; 2. the light covering or "sparse-bloom"; and 3. the absence of covering termed "bloomless". They (3) mentioned also that both cultivated and wild sorghums of Asiatic and African origins showed this waxy secretion in some degree, and on careful examination bloomless types of sorghum revealed traces of wax. The appearance of the waxy secretion began as early in the life of the plant as the third or fourth day after germination. The secretion increased until the plant approached the flowering phase and then there was a gradual decline in deposition. The deposit was more pronounced on the intraveneous region, whether on leaf sheath, leaf blade or any other region.

Ayyanguar and Ponnaiya (4) indicated that an African

variety, "Vigage", was of the bloomless type. They reported inheritance studies when the bloomless type of sorghum was crossed with both heavy-bloom and sparse-bloom types of sorghum. In the cross with the heavy-bloom, all F_1 had heavy bloom, and the F_2 segregated into 252 plants with heavy bloom and 84 bloomless plants. This gave a ratio of 3:1, indicating a single recessive gene for bloomlessness. In the cross with the sparse-bloom, all F_1 had heavy bloom, and the F_2 segregated into 108 heavy-bloom, 35 sparse-bloom, and 43 bloomless plants, giving a 9:3:4 ratio. The authors concluded that bloom was completely dominant over bloomless, and that bloomless was epistatic to sparse-bloom. The symbols used were BmBm for bloom, bmbm for bloomless, hh for sparse-bloom, and HH for bloom.

In another study, Peterson et al. (12) investigated the relationship among five bloomless and four sparse-bloom mutants. The mutants were crossed in all combinations, and with two normal bloom lines. The results showed that bloomlessness was controlled by homozygous recessive alleles at either one of two loci, and the gene symbols bm_1bm_1 and bm_2bm_2 were assigned. Sparse-bloom was conditioned by three independently inherited homozygous recessive alleles at either one of three loci. The gene symbols assigned were h_1h_1, h_2h_2, and h_3h_3. The fourth mutant was not positively identified.

Wilkinson and Commins (17) extracted leaf epicuticular waxes with chloroform from bloom and bloomless near-isogenic

lines of Redbine-60. The chloroform extract was esterified, separated, and quantified. They used the first leaf below the flag leaf at the dough stage of maturity. The results showed that for equal density of leaf, the leaves of the bloomless near-isogenic line had 73% of the epicuticular fatty acid, 118% of the fatty alcohol, and 1% of the alkane content of those constituents of the bloom leaves. It appeared that there was a 57% reduction on the bloomless leaves of the fatty acids + fatty alcohols + alkanes, compared to the bloom leaves.

Other studies have been done which demonstrated that bloomless forage sorghums have better digestibility than bloom types of forage (6, 7). Cummins and Dobson (6) compared the digestibility of bloom and bloomless sorghums with an *in vitro* technique. The three near-isogenic bloomless sorghum lines had 22% higher digestibility than the three near-isogenic bloom sorghum lines. The study showed that the absence of the waxy deposit on the bloomless lines facilitated the penetration of the microorganisms in the rumen, and the waxy coating on the bloom lines slowed down the penetration process. Hanna et al. (7) applied a similar method to that used previously by Cummins and Dobson (6). They concluded that the bloomless lines had higher digestibility. From other studies they indicated that the bloomless types had more water loss and so less drought tolerance, due to the absence of the waxy bloom on the

plant.

Blum (5) reported a study to relate the forms of epicuticular wax to the resultant effect on the spectral characteristics of the leaf. He used two near-isogenic lines of sorghum, one bloomless (bmbm) and the other normal (BmBm), to study the role of epicuticular wax as a possible drought resistance factor in sorghum. Samples from leaf sheaths and leaf blades were examined by scanning electron microscopy after being treated and frozen in liquid nitrogen and gold plated in a vacuum chamber. Some samples were dewaxed with chloroform. Leaf blades were used to make the quantitative determinations of epicuticular wax, and the reflectance was measured by the mean of spectrophotometer equipped with a chromatographic scanning attachment. Blum (5, p. 51) observed the bloom genotype by using the scanning electron microscopy and noted (1) "appearance of waxy bloom in the form of fine filaments over the leaf sheaths and the central basal region of the abaxial leaf blade surface"; (2) "a possible increase in thickness of the homogeneous-amorphous epicuticular wax"; (3) "an excessive formation of waxy flakes over the epicuticular wax layer". He also noted that "the total amount of chloroform-extracted epicuticular wax was significantly greater in the leaf blades of bloom than the bloomless genotype." Finally he (5, p. 51) mentioned, "The reflectance of radiation in the visible and near-infrared region over the adaxial leaf blade surface was about 4-5% greater in the bloom than in the bloomless

genotype." The bloom genotype was considered to be drought resistant.

Greenbugs have been a major pest of sorghum since 1968 (15). Greenbugs are plant aphids which inject toxin that kills leaf tissue as they feed. They fed only on small grains prior to 1968, but now they feed on sorghum also. Considerable effort has been directed to the study and characterization of the responsible biotypes, as well as to the search for resistant germplasm. Peiretti (11) studied greenbug resistance in sorghum as related to the bloomless character, since greenbugs exhibited a high degree of nonpreference for bloomless sorghums. He studied a bloomless line (RWD3-Weskan), a normal resistant line (Shallu Grain), their F_1, F_2, and a susceptible check (RS 610). He reported that the bloomless character was regulated by a single recessive pair of genes, with the expression of bloom being dominant to bloomless. The greenbug nonpreference was associated with a trait from bloomless RWD3-Weskan which was inherited independently from alleles which regulated the expression of tolerance to damage from Shallu Grain, and the tolerance to damage was regulated by a single pair of alleles with partial or no dominance. Amini (1) also studied the nature of resistance of bloomless sorghum to greenbugs. He confirmed the nature of the inheritance of the bloomless character and also reported that the two types of resistance from RWD3-Weskan

(nonpreference) and from IS 809 (tolerance) were regulated by independent factors. He concluded that the bloomless sorghum appeared to increase in the trait for nonpreference with increasing the age of the plants. Starks and Weibel (14) conducted a study of the nonpreference of greenbugs for bloomless sorghum. Near-isolines were used for comparison in three different tests: natural infestations or field conditions, leaf cage tests, and large cage tests. The results showed that the bloomless and sparse-bloom conditions in sorghum reduced the reproduction of greenbugs and damage from natural infestation. However, when the aphids were confined in cages on leaves, damage and reproduction were not reduced. This led them to suggest that the mechanism of resistance was mainly nonpreference by the aphid instead of antibiosis or tolerance to injury by the plant. Some other suggestions were that in the early plant growth stages the resistance due to nonpreference for bloomless and sparse-bloom genotypes was not effective. However, there was protection 45 or more days after emergence.

CHAPTER III

MATERIALS AND METHODS

Field Conditions

Three near-isogenic lines (R OKY62 bm_1bm_1, R OKY62 h_2h_2, and R OKY62 BmBm) were grown at the Perkins Agronomy Research Station during the summer of 1982 under two different sets of environmental conditions in single row plots. The first treatment was supplemental irrigation. Seeds were planted June 14, while seeds for the second treatment were sown June 19 and grown without irrigation. The leaf samples were collected at four different intervals. The first sample was collected at approximately growth stage #3, which corresponds to the "growing point differentiation" of Vanderlip (16). The second sample was collected at approximately growth stage #4, or "final leaf visible in whorl", and the last two samples were collected at growth stage #6 or "half bloom" and growth stage #7 or "soft dough". In each treatment at each sampling date, one representative plant was chosen at random in the row. It was pulled from the ground with roots still attached, and taken to the laboratory for observation. The removal of wax was carefully avoided.

Scanning Electron Microscopy (SEM)

The plant samples were examined with a JOEL JSM 35 Scanning Electron Microscope (SEM). One leaf was chosen at random by numbering and drawing a number from among the first to the last fully expanded leaf. The same leaf was used for both scanning electron microscopy and light microscopy. An area of 3 x 5 cm was cut from the fresh leaf with a razor blade, and mounted on an aluminum stub with silver paint. The usual pretreatment of materials was not used because the solvent in standard SEM procedures removes epicuticular wax (8). Samples were taken from leaf sheath and leaf blade of each type of sorghum. The surfaces of bloom leaf sheath and leaf blade samples were examined directly with the SEM, whereas the surface of sparse-bloom and bloomless had to be coated with gold palladium and dehydrated at the same time in a vacuum chamber, because their more regular surfaces caused electrical charging.

The SEM distinguishes the features of the surface of the cuticle, while light microscopy shows the cross sectional features of the tissue surface.

Light Microscopy

From the same leaf collected for scanning electron microscope observation, some larger samples were collected from the leaf sheath and the leaf blade for observation with the light microscope. Standard procedures of microtechnique

were used (13). For killing and fixing purpose, the pieces of tissue of each type were immersed and stored in 50% FPA solution (formalin-propronic acid-alcohol). The material can be stored in FPA indefinitely.

The preparation of the material for light microscopy included dehydration followed by embedding in paraffin. The T-butyl alcohol (a solvent of paraffin) served as both a dehydratant and a clearing agent. Six different solutions contained increasing concentrations of t-butyl alcohol and decreasing concentrations of distilled water and ethyl alcohol (13).

Samples were left in solutions #1 to 5 for one hour, and there were three changes of solution #6. The third change was aspirated and left overnight. The next step was infiltration in paraffin, which consisted of dissolving the paraffin in the solvent containing the samples, and gradually increasing the concentration of paraffin while decreasing the concentration of the solvent. After the material was infiltrated, it was embedded in melted paraffin in a plastic boat. The paraffin was allowed to solidify to act as supportive matrix for sectioning. The embedded samples were mounted on wood blocks and sectioned with a rotary microtome at twelve micrometers. The sectioned ribbons were mounted to slides (13). Ten slides were made for each specimen and they were stained in an electro-mechanical stainer.

After the staining, cover slips were mounted to the

slides with some drops of "Adams histoglav" and the leaf tissues were ready for observation. During the observation of the tissue, one hundred measurements were made from each slide for the thickness of the cell wall of the lower epidermis and the upper epidermis. Since there were no replications, the means, the range, and the standard deviation were calculated for each type.

CHAPTER IV

RESULTS AND DISCUSSION

Scanning Electron Microscopic Observation

R OKY62 bm_1bm_1 (Bloomless Isogenic Line)

The observations of four samples of the bloomless line R OKY62 supported the belief that there were no new anatomical features of the adaxial cross section including the epidermis of the leaf sheath and leaf blade tissue other than those observed by Metcalff (10) and Artschwager (2). The structure of the adaxial surface of the leaf sheath revealed prominent major veins covered mostly by five to six single rows of silica cells associated with cork cells (Figure 1). The stomates, subsidiary cells and guard cells were arranged at either side of the vein. The long cells were arranged along with the single row of stomates. They had thin cell walls and were elongated and narrow (Figure 2). Silica cells and cork cell groups alternated with short cells (Figure 3). More often micro-hairs which were composed of two rounded parts occurred with the long cell (Figure 2), and prickle hair cells which were not divided appeared occasionally along long cells (Figure 4). There

Figures 1-4. Scanning Electron Micrographs Showing Main Features of the Leaf Sheath Adaxial Epidermis and Leaf Blade Abaxial Epidermis of Bloomless Sorghum R OKY62 bm_1bm_1

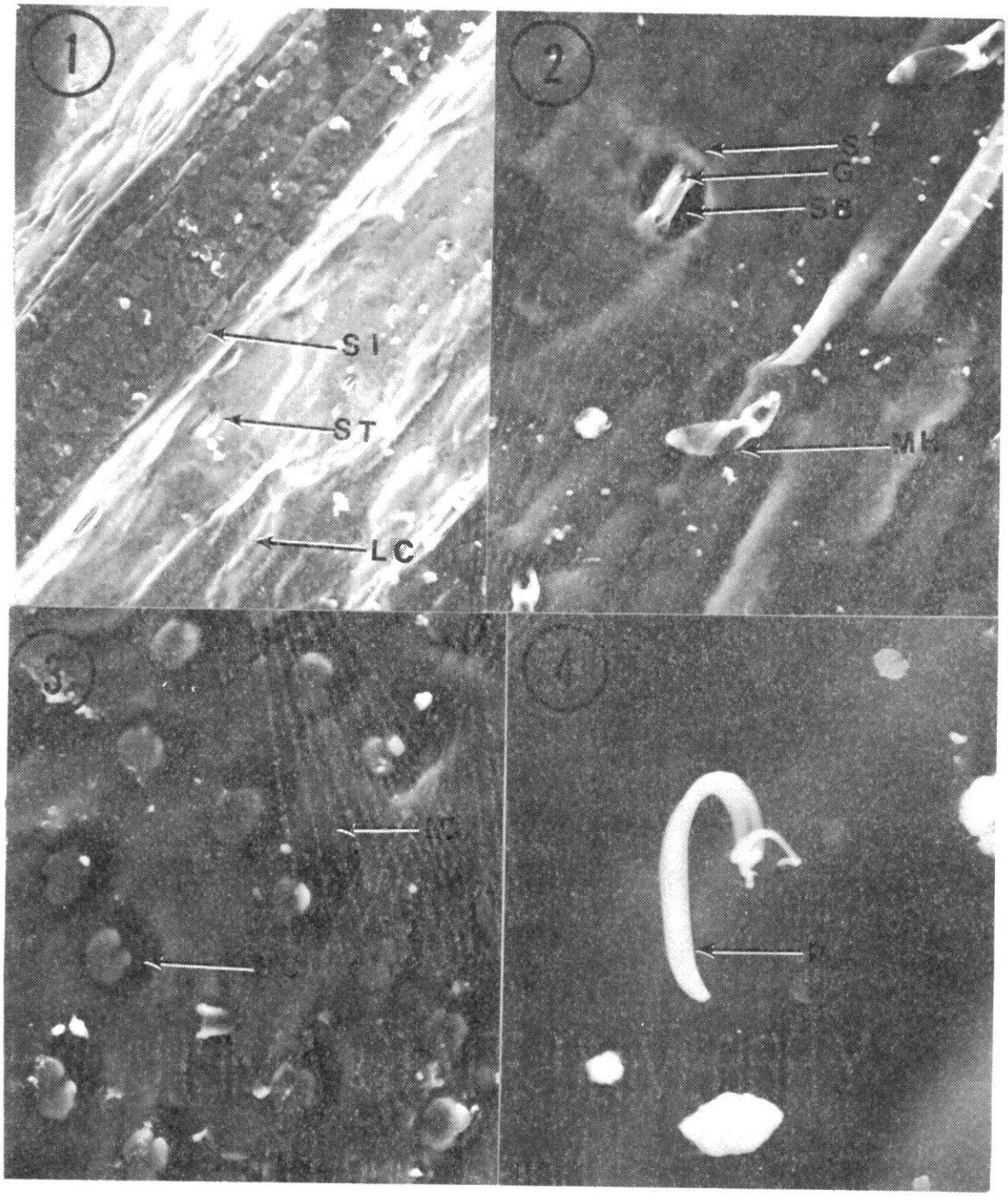
SI = silica cells
MV = major veins
C = cork cells
SC = silica-cork cell groups
MH = micro-hairs
P = prickle hairs
LC = long cells
ST = stomates
G = guard cells
SB = subsidiary cells

Figure 1. Leaf Sheath at "Eight Leaf" Stage Showing Major Veins and Interveinal Area x200

Figure 2. Leaf Blade at "Half Bloom" Stage Showing Epidermis Cells x540

Figure 3. Leaf Sheath at "Half Bloom" Stage Showing Features of Major Veins x540

Figure 4. Figure 1 Showing Prickle Hair in Leaf Sheath x2000


1
SI
ST
LC
2
S
G
SB
MH
3
LC
4
P

appears to be a thickening along long cells, at the base of silica-cells, stomates, micro-hairs, and prickle hairs that Blum (5) called amorphous wax (Figure 4). This amorphous wax is different from the filamentous epicuticular bloom wax. However, there seemed to be little difference in amorphous wax deposition between leaf sheath and leaf blade surface under supplemental irrigation. The structure of the leaf blade abaxial epidermis showed mainly the same features as the leaf sheath, except that the adaxial apidermis of the leaf sheath had more prominent veins. The contrast may be seen in Figure 5 and Figure 7, compared with Figure 6 and Figure 8. Also the dumbbell shaped silica cells associated with cork cells were more abundant on leaf sheath surfaces (Figures 5 and 7). However, there was no appearance of bloom wax on the surface of any of the four samples of bloomless tissue (Figures 1 through 8). The micro-hairs were less abundant on the leaf sheath (Figure 9) compared to the leaf blade surface (Figure 10) in all three types of sorghum. The dumbbell shaped silica cells showed a depression in the middle and there seemed to be a groove traversing the long axis of each silica cell (Figure 11). The micro-hairs had a longitudinal depression (Figure 12), and their bases became thickened as they entered a thick deposition of amorphous wax. In conclusion, the observations of the bloomless sorghum did not reveal the presence of any filamentous epicuticular wax deposit on leaf sheaths nor on leaf blades during the vegetative growth of

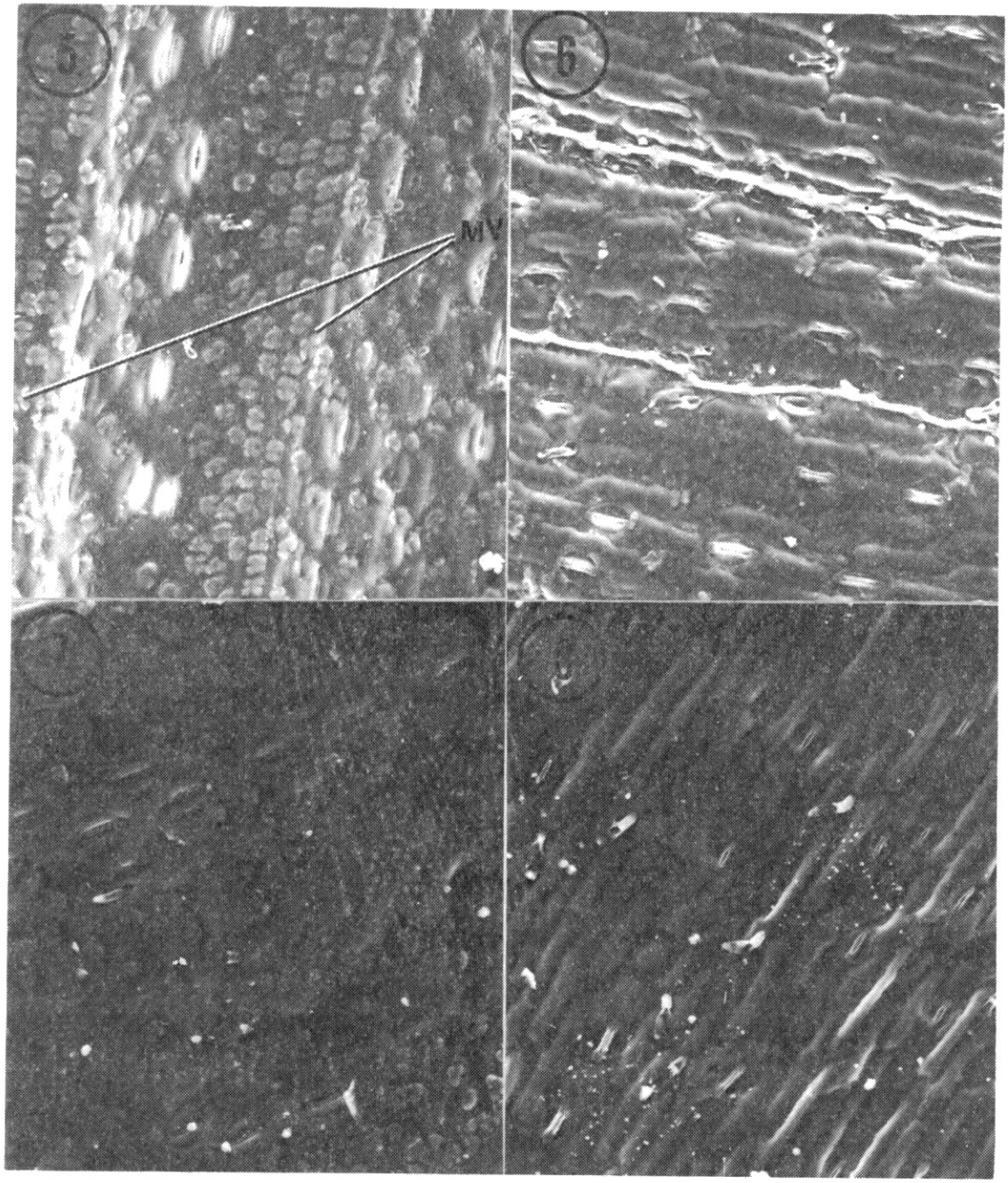
Figures 5-8. Scanning Electron Micrographs Showing Contrast of Epidermal Features of Leaf Sheath and Leaf Blade at "Final Leaf" Stage and at "Boot" Stage of Bloomless Sorghum R OKY62 bm_1bm_1

Figure 5. Leaf Sheath Structure at "Final Leaf" Stage Showing Two Close Veins, High Frequency of Silica Cells and Stomates x200

Figure 6. Leaf Blade Structure at "Final Leaf" Stage Showing Long Cell Walls Slightly Raised and Sparse Single Rows of Stomates x200

Figure 7. Leaf Sheath Structure at "Boot" Stage Showing the Same Features as for Figure 5 x200

Figure 8. Leaf Blade Structure at "Boot" Stage Showing the Same Features as for Figure 6 x200


5
MV
6
7
8

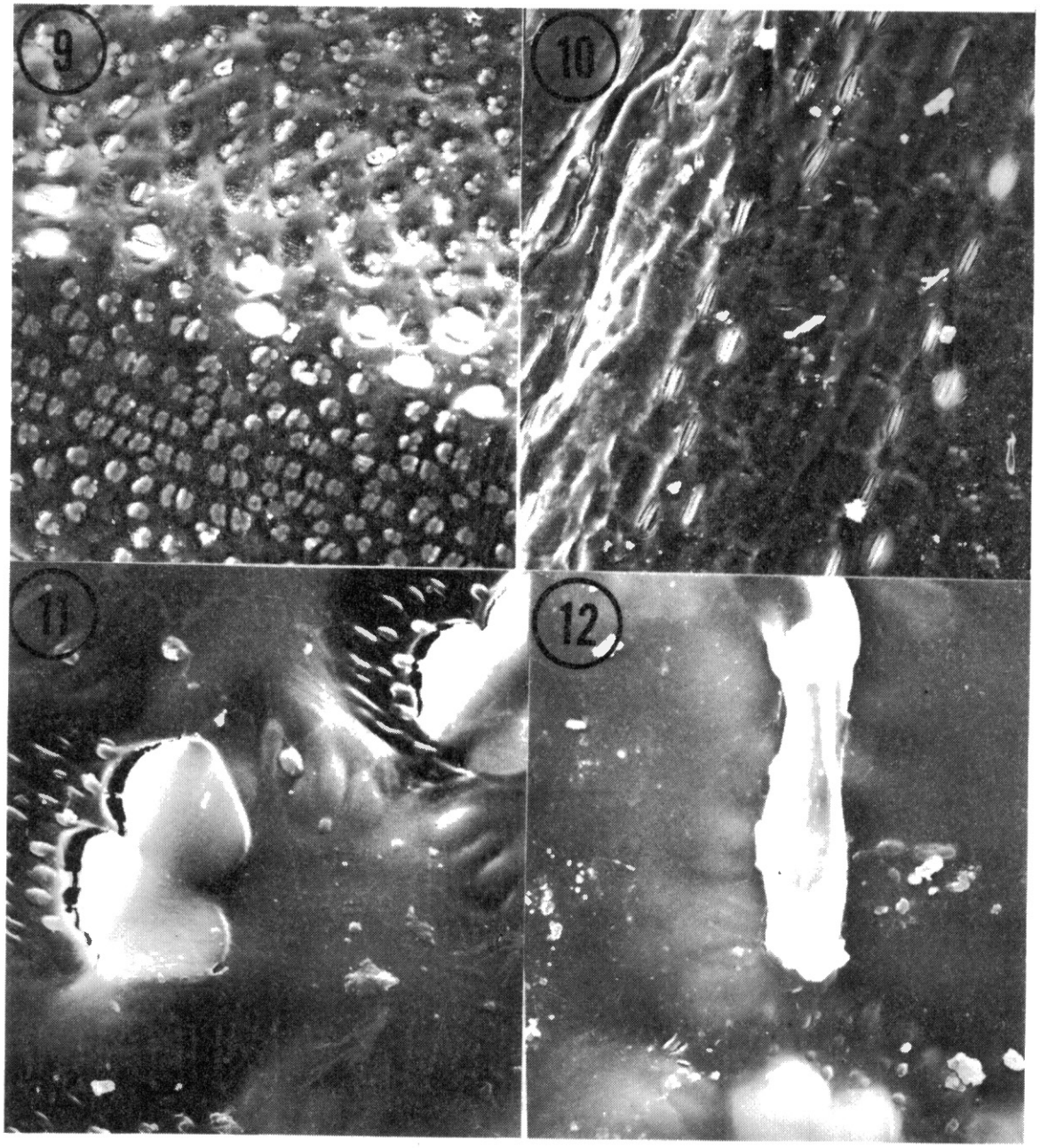
Figures 9-12. Scanning Electron Micrographs Showing Contrast of Leaf Sheath and Leaf Blade at "Half Bloom" Stage and High Magnification of Silica Cells and a Micro-hair of R OKY62 bm_1bm_1

Figure 9. Leaf Sheath at "Half Bloom" Stage Showing Major Vein, Silica Cells and Stomates x200

Figure 10. Leaf Blade at "Half Bloom" Stage Showing Multiple Micro-hairs on Abaxial Epidermis x200

Figure 11. Figure 9 Showing Silica Cells and Grooves x2000

Figure 12. Figure 10 Showing a Micro-hair and Silica Cork Group of Cells x2000


9
10
11
12

plants in the dryland condition or under supplemental irrigation. The structures of the leaves included high concentrations of silica cells and micro-hairs on leaf sheaths. However, no differences in wax deposition were observed among samples in the four different dates of sampling (Figures 1 through 12).

R OKY62 h_2h_2 (Sparse-bloom Isogenic Line)

The scanning electron micrographs showed an increase of filamentous epicuticular wax fragments on the leaf sheaths from the "eight leaf" stage to the "soft dough" stage. On the leaf blades wax filaments appeared only by the "soft dough" stage. When the samples were collected at "eight leaf" stage, there was no visible bloom on the upper and lower surfaces of leaf blades, but there was some bloom on the leaf sheaths. This appeared in the scanning electron micrographs as thin filaments of wire-like material which was found mainly over the veins (Figures 13 and 15). This wax extruded from the epidermal cells and accumulated predominately near the veins (Figure 13). The filaments of bloom wax appeared to be shorter near the surface of the cuticle and longer in the uppermost layer. Stomates were distinguishable beneath the thick covering (Figure 15). In general the filamentous wax bloom did not cover all the cuticle surfaces. On the contrary the scanning electron micrographs of the abaxial epidermis of the leaf blade did not show any filaments of wax on the surface of the

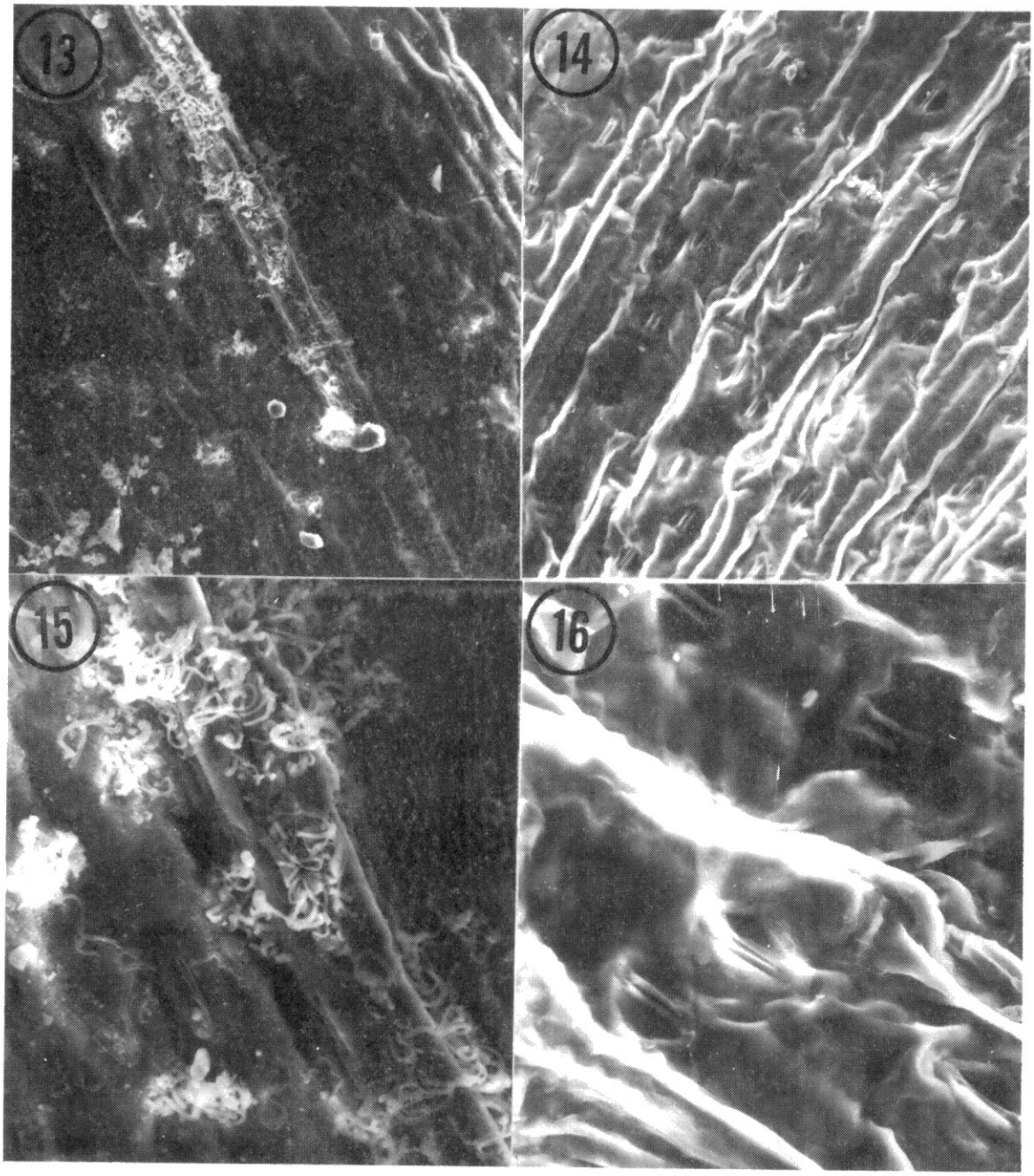
Figures 13-16. Scanning Electron Micrographs Showing Leaf Adaxial Epidermis and Leaf Blade Abaxial Epidermis at "Eight Leaf" Stage Under Two Magnifications of R OKY62 h_2h_2

Figure 13. Leaf Sheath at "Eight Leaf" Stage Showing Appearance of Filamentous Waxes Over Vein x200

Figure 14. Leaf Blade at "Eight Leaf" Stage Showing Long Cell Walls and Stomates But No Wax Filaments x200

Figure 15. Figure 13 Showing Enlarged Wax Filaments x540

Figure 16. Figure 14 Showing No Trace of Filaments x540


13
14
15
16

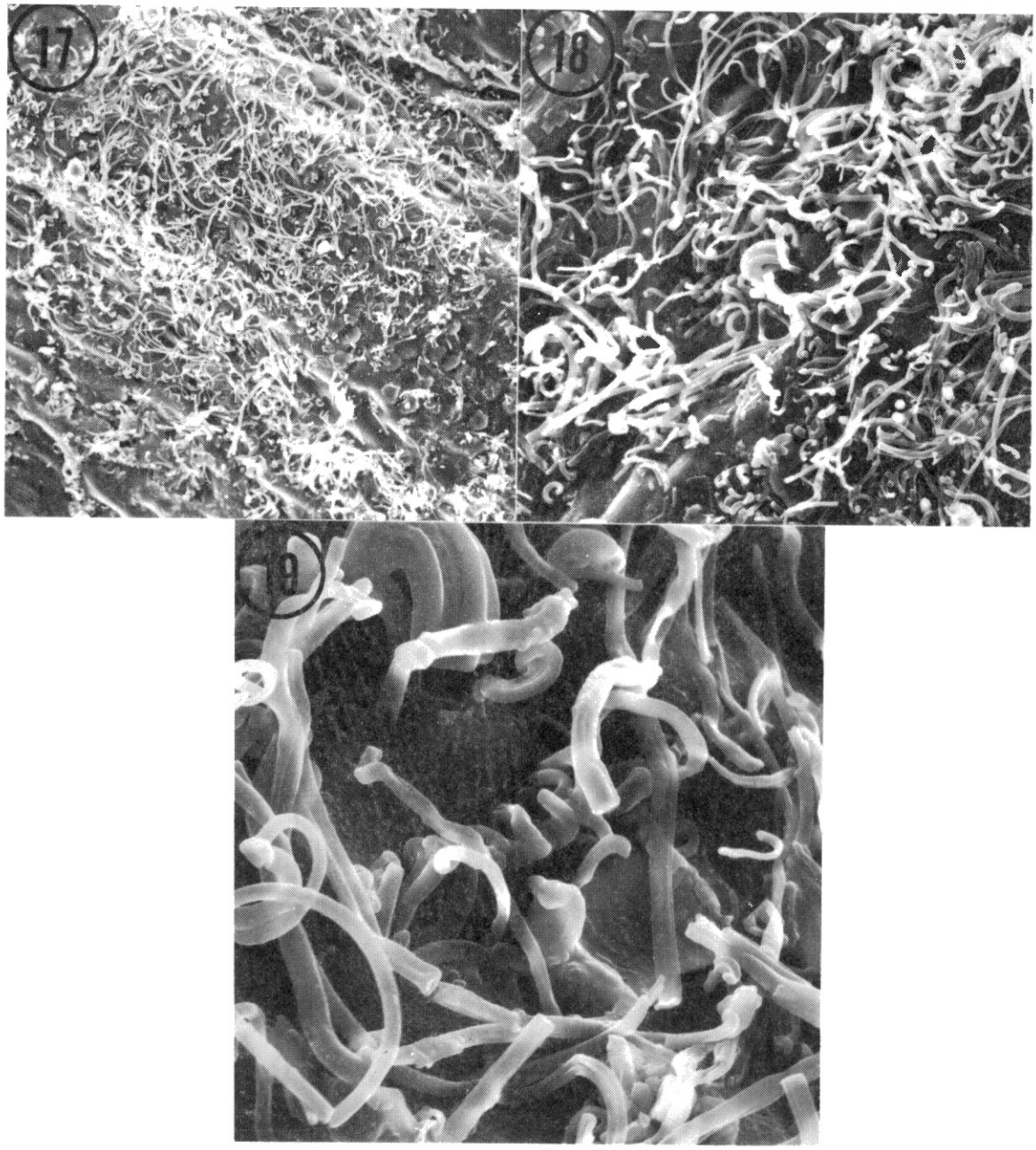
epidermis (Figures 14 and 16). The cell walls of the epidermis and the stomates appeared clearly. The structure of the waxy bloom of this type of sorghum was that of rodlike long filaments (Figure 17). Except in areas with much artifactual material, long cells and silica cells are visible. At higher magnification only silica cells could be observed under the wax filaments (Figure 18). The filaments appeared curved and hook-like, and their interiors appeared transparent or hollow (Figure 19). They seemed to be extruded from the epidermal surface. Just before the "half bloom" stage, the wax filaments on the leaf sheaths appeared to be smaller in diameter and more intertwined (Figure 20). The wax layer on the top of the cuticle became dense. Scanning electron micrographs of the leaf blade at the "half bloom" stage still showed no wax filaments over the adaxial epidermis (Figure 21). However, at the "soft dough" stage there appeared an extensive amount of wax on the leaf sheath and leaf blade (Figures 22 and 23). The wax runs parallel to the silica cells. There did not seem to be a high concentration of filaments in areas near stomatal openings. There seemed no great difference from the two environmental conditions. Scanning electron micrographs of leaves of plants collected before "half bloom" stage under dry land conditions showed a little bloom on the leaf sheath and none on the leaf blade (Figures 24 and 25), but both parts of the leaves showed epicuticular wax filaments at the "soft dough" stage (Figures 26 and 27).

Figures 17-19. Scanning Electron Micrographs Showing Leaf Sheath Wax Filaments at "Final Leaf" Stage at Different Magnifications of R OKY62 h_2h_2

Figure 17. Wax Filaments Accumulated Over Veins of Leaf Sheath x200

Figure 18. Figure 17 Showing Wax Filaments and Silica Cells x540

Figure 19. Figure 17 Showing Wax Extruded From Epidermal Surface x2000


17
18
19

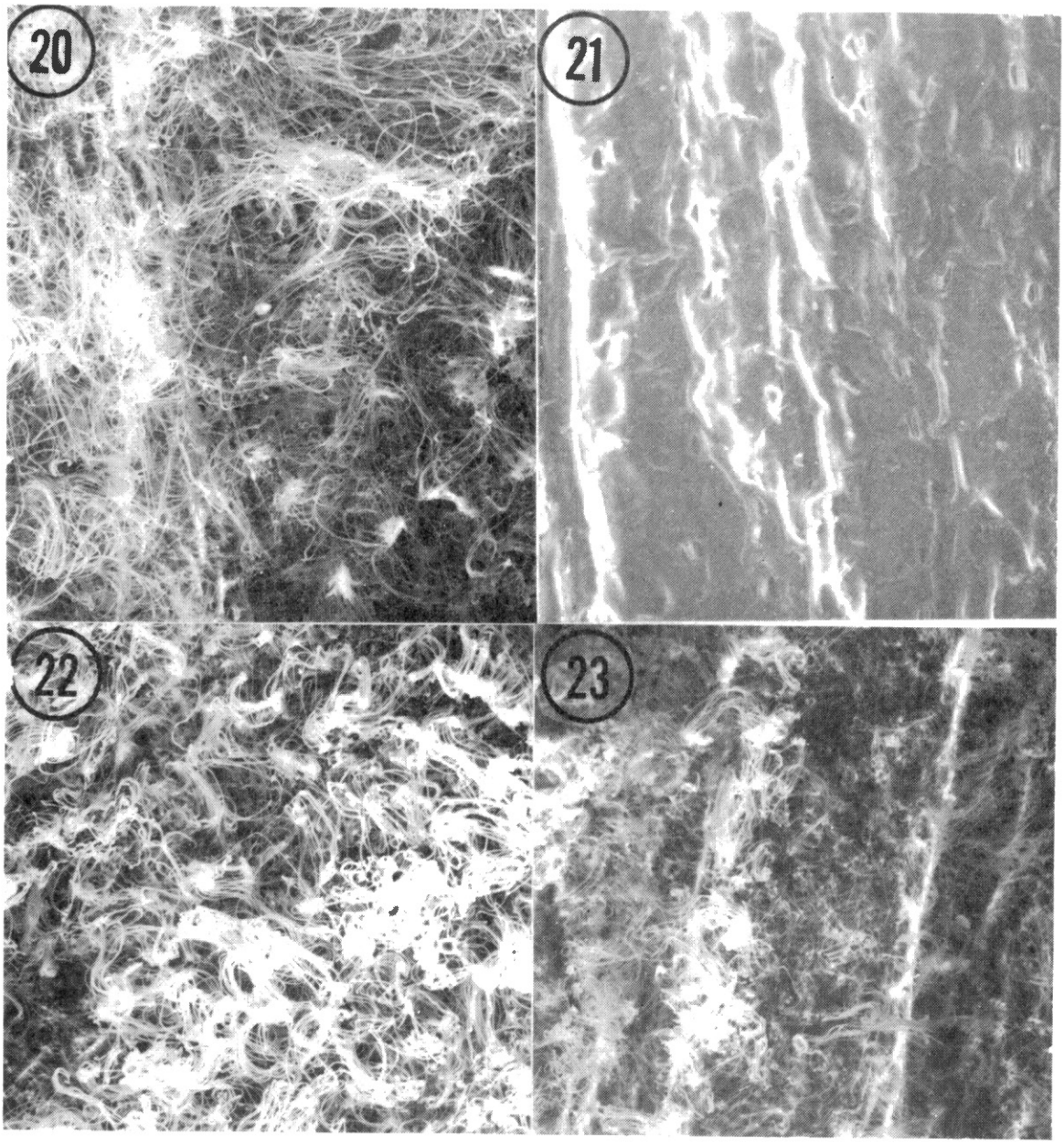
Figures 20-23. Scanning Electron Micrographs Showing Leaf Sheath and Leaf Blade Structures at "Final Leaf" Stage and at "Boot" Stage

Figure 20. Leaf Sheath at "Final Leaf" Stage Showing Extensive Wax Filaments x200

Figure 21. Leaf Blade at "Final Leaf" Stage Showing No Wax Filaments x200

Figure 22. Leaf Sheath at "Boot" Stage Showing Matted Wax Filaments x200

Figure 23. Leaf Blade at "Boot" Stage With Extensive Wax Filaments x200


20
21
22
23

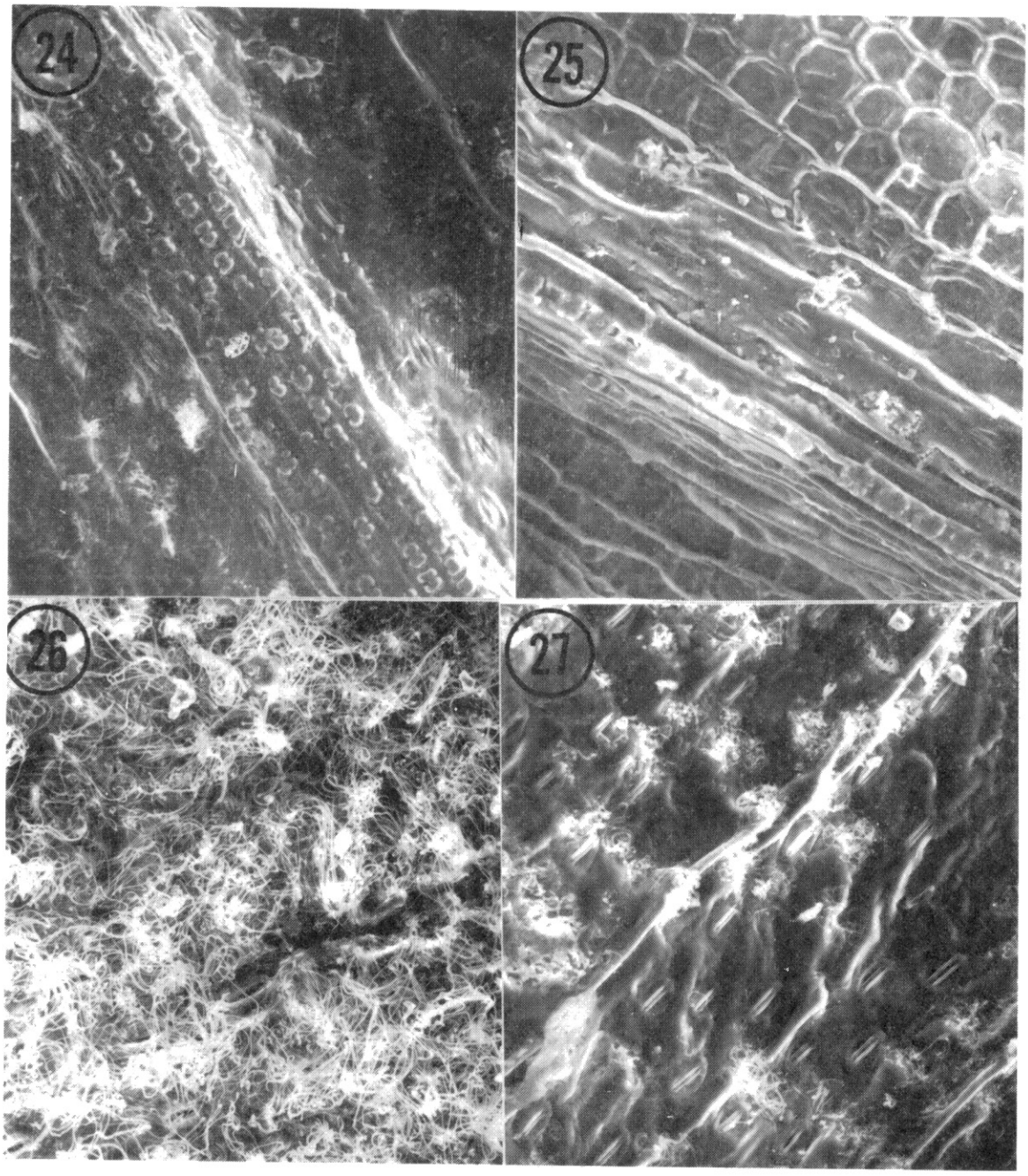
Figures 24-27. Scanning Electron Micrographs Showing Contrast of Leaf Sheath and Leaf Blade Under Dryland Conditions at "Half Bloom" Stage and at "Soft Dough" Stage of R OKY62 h_2h_2

Figure 24. Leaf Sheath Structure at "Half Bloom" Stage Showing Sparse Bloom x200

Figure 25. Leaf Blade Structure at "Half Bloom" Stage Showing No Bloom x200

Figure 26. Leaf Sheath at "Soft Dough" Stage Showing Matted Wax Filaments x200

Figure 27. Leaf Blade at "Soft Dough" Stage Showing Slight Bloom on Adaxial Epidermis x200


24
25
26
27

R OKY62 BmBm (Bloom Isogenic Line)

In all samples of bloom sorghums there were wax filaments over the abaxial surface of the leaf sheaths and leaf blades. The filaments of extruded epicuticular wax were found over veins (Figure 28). At the "eight leaf" stage, scanning electron micrographs showed long strands and coils of filaments over silica cells (Figure 29). The leaf sheath samples observed contained more accumulated filaments which were thinner and more coiled from the "eight leaf" stage (Figures 28 and 29), to the "final leaf" stage (Figures 30 and 31).

Leaf blade surfaces contained less wax filaments than leaf sheaths under the same environmental conditions but the amount of bloom increased during the plant growth (Figures 32 and 33 versus Figures 34 and 35). There seemed to be no difference between the wax filaments observed under the two environments except there was more bloom on the leaf blade of plants grown under the dryland condition (Figures 34 and 35). There was less wax accumulation after two week development uder irrigated conditions at "soft dough" stage (Figures 36 and 37) compared to dryland conditions at "half bloom" stage (Figures 38 and 39). In late stages of growth (Figures 40 and 41) wax filaments formed densely over the epidermis. They appeared to be hollow and to have a knob at the end. In the earlier stage the wax filaments appeared shorter and transparent (Figures 42 and 43).

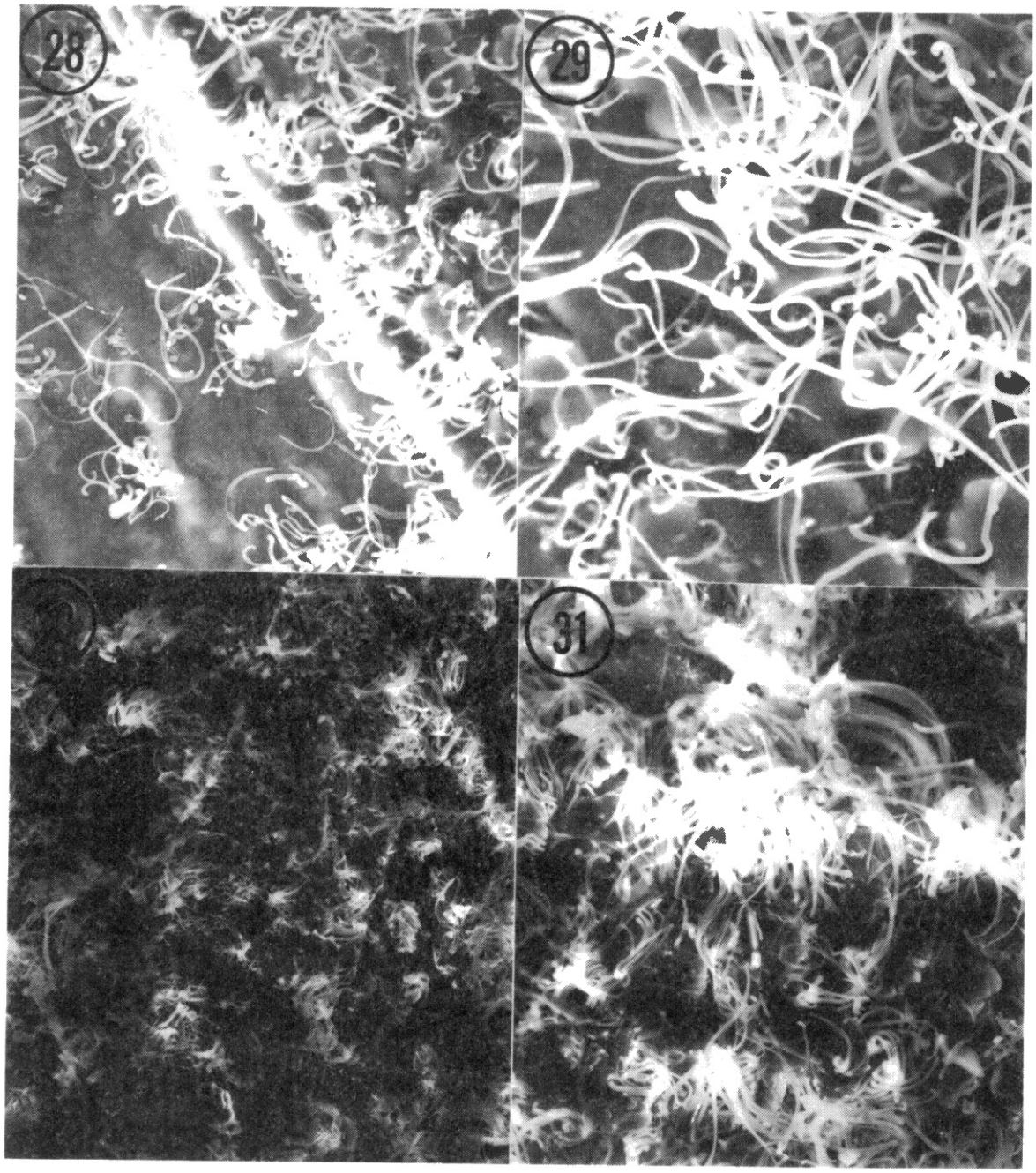
Figures 28-31. Scanning Electron Micrographs Showing Wax Bloom Filaments at Two Different Intervals of Growth on Leaf Sheath Abaxial Epidermis of R OKY62 BmBm

Figure 28. Leaf Sheath at "Eight Leaf" Stage Showing Wax Filaments x540

Figure 29. Figure 28 Showing Silica-Cells Under Wax Filaments x1000

Figure 30. Leaf Sheath at "Final Leaf" Stage Showing Intensive Wax Layer x200

Figure 31. Figure 30 Showing Silica-Cells and Veins Under Wax Filaments x540


28
29
31

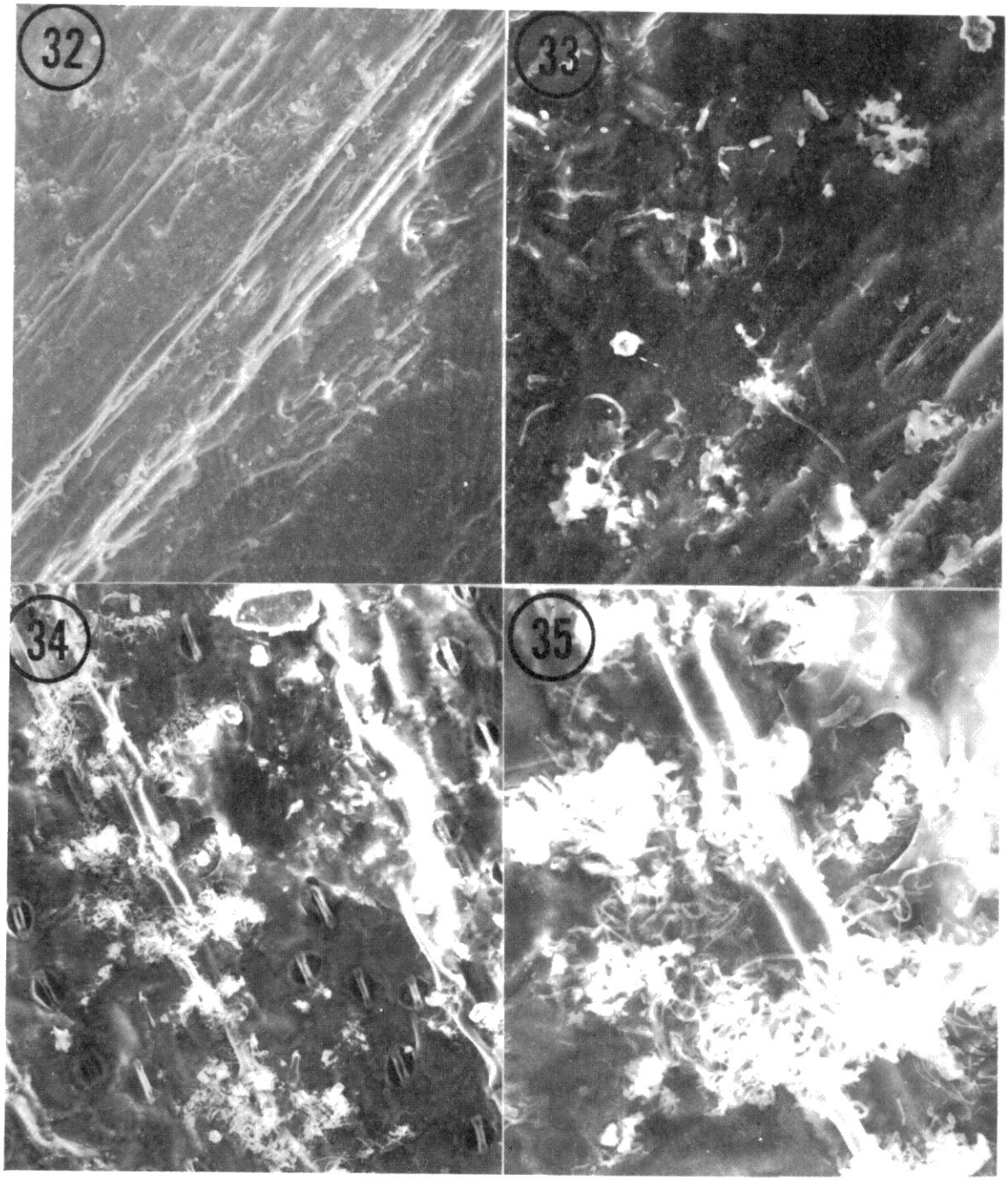
Figures 32-35. Scanning Electron Micrographs of Leaf Blades at Two Different Stages of Growth of R OKY62 BmBm

Figure 32. Leaf Blade at "Boot" Stage Under Supplemental Irrigation Showing Small Amount of Bloom x200

Figure 33. Figure 32 at x540

Figure 34. Leaf Blade at "Half Bloom" Stage Showing More Wax Than in Figure 32 x200

Figure 35. Figure 34 at x540


32
33
34
35

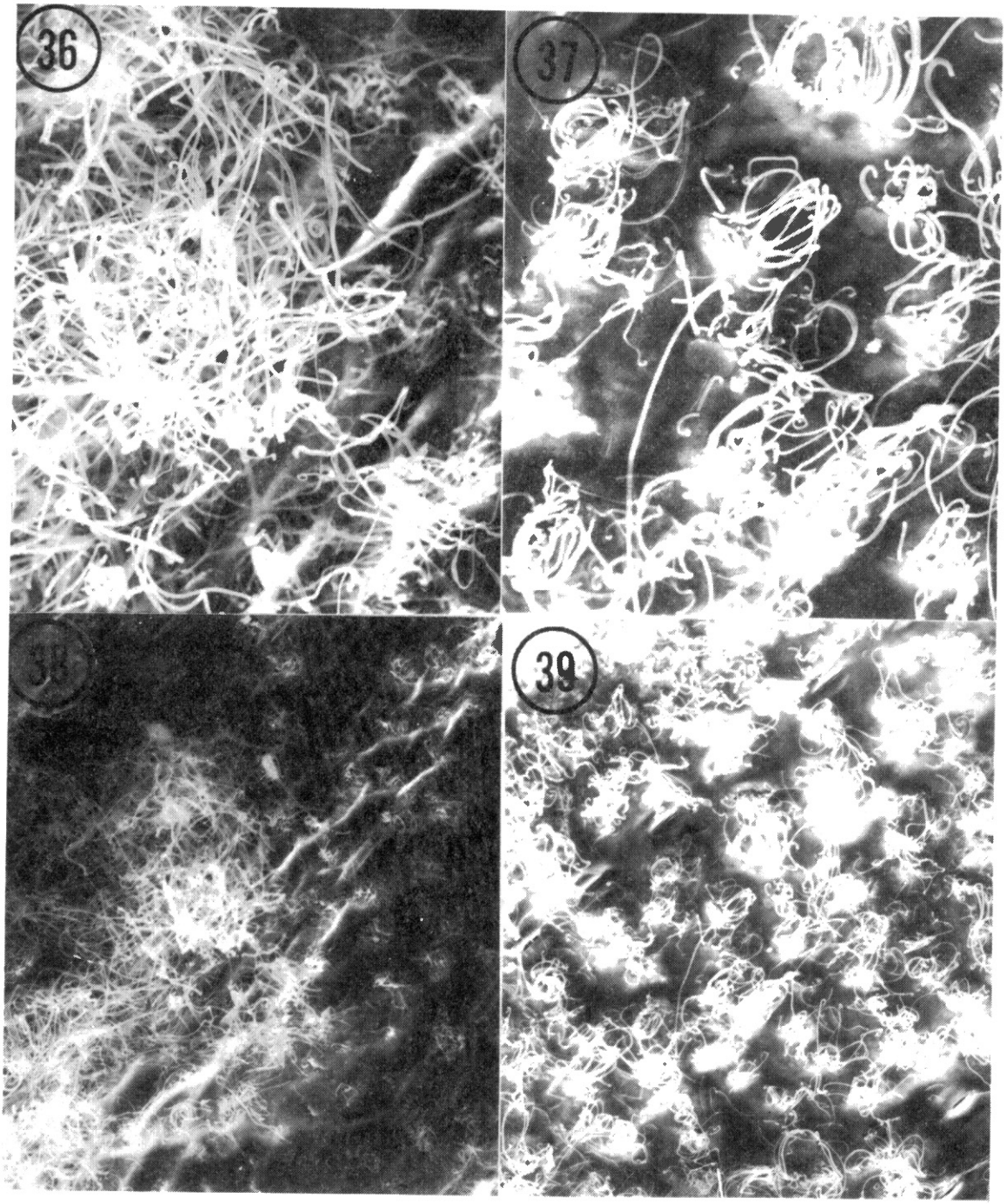
Figures 36-39. Scanning Electron Micrographs Showing Leaf Sheath Structure at Two Different Stages of Growth of R OKY62 BmBm

Figure 36. Leaf Sheath at "Soft Dough" Stage Showing Wax Filaments Over Silica-Cells at x200

Figure 37. Figure 36 at x540

Figure 38. Leaf Sheath at "Half Bloom" Stage Showing Less Wax Deposit x200

Figure 39. Figure 38 Showing Wax Layers x540


36
37
38
39

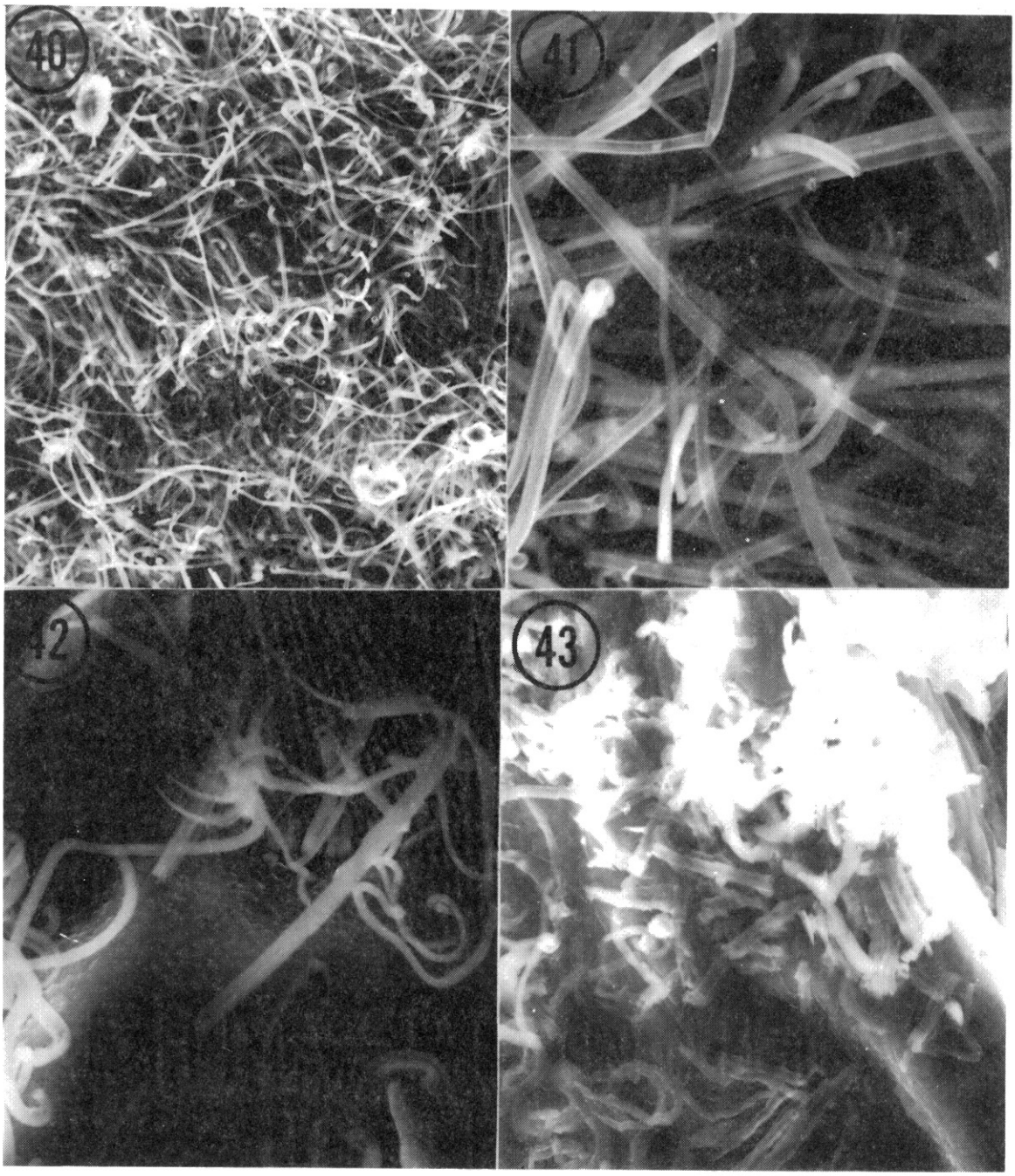
Figures 40-43. Scanning Electron Micrographs Showing Wax Filaments at Different Stages of Plant Growth of R OKY62 BmBm

Figure 40. Waxy Bloom Over Leaf Sheath at "Soft Dough Stage x540

Figure 41. Figure 40 Showing Apparently Hollow Filament With Ending Caps x2000

Figure 42. Leaf Sheath at "Half Bloom" Stage Showing Wax Filaments Over Knobs and Silica-Cells x2000

Figure 43. Leaf Blade at "Half Bloom" Stage Showing Wax Filaments Over Knobs x2000


40
41
42
43

In conclusion epidermal leaf surfaces of bloomless sorghums did not appear to have any waxy bloom when examined with the scanning electron microscope. Major veins were covered with silica celss, cork cells, and silica-cork groups. Stomates and epidermal cells with long walls appeared in interveinal regions. Sparse bloom sorghums had wax in the later stages of development but bloom sorghums showed waxy bloom eary in development. Figures 18 and 42 are preparations in which waxy bloom apparently originated from the epidermal surface. Kraufman (9) observed the occurrence of craterlike pores on the surface of silica cells. Those pores are more clearly seen at higher magnification. They are of different sizes and have uneven striation on their surfaces. Those pores may be analogous to sites where teichode (ectodesmata) traverse the outer walls of silica cells. The origin and function of these pores on the surface of silica cells are unknown. They may be passages for the transport of cutin and waxes to the epidermal surface. Those pores could be opened and closed by biochemical processes to liberate waxes. If wax liberation is related to silica cells it can be understood why there was more accumulation in leaf sheath than leaf blade. Also greenbugs [Schizaphis graminum (Rondani)] might be able to use those pores to feed very easily over the veins into the phloem.

Light Microscopic Observations

Leaf Sheath

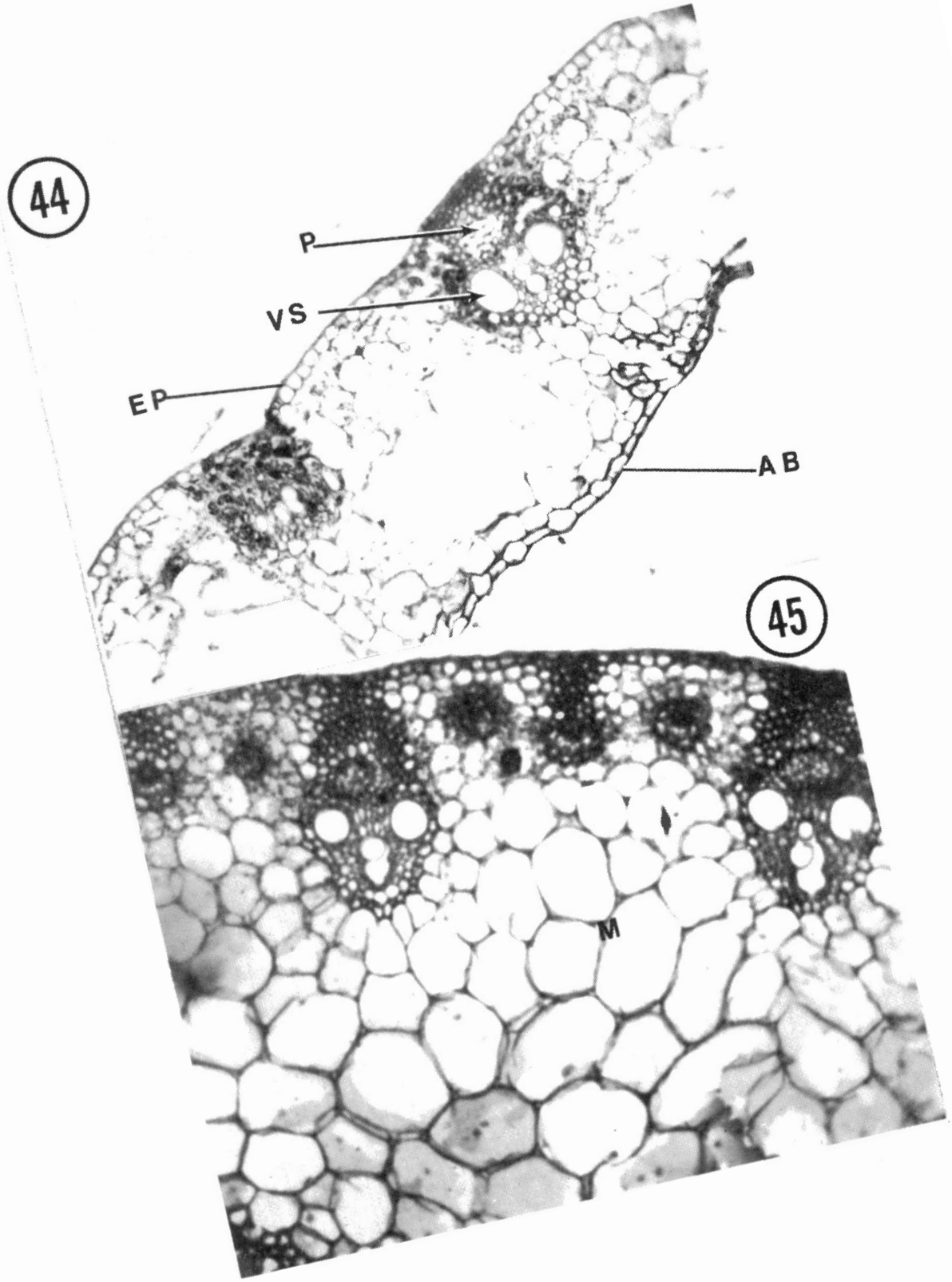
The cross sections of the leaf sheath showed the same structure in all three types of sorghum. The adaxial surface appeared smooth except for slight ribs over larger vascular bundles. The abaxial surface appeared ribbed. The cross section of the leaf sheath showed running vascular bundles parallel to one another. The vascular bundles of different sizes of angular configuration are scarce. In all samples of leaf sheath at "eight leaf" stage (6), small and medium vascular bundles lay close to the epidermal surface. No large vascular bundles were observed at this stage. The vascular bundles lacked protoxylem. Their schlerenchymatous sheaths were narrow at the xylem pole, massive in the phloem region and always confluent with hypodermal sclerenchyma. The adaxial and abaxial epidermal cells were plate shaped (Figure 44). The thickness of the cuticle of the bloomless near-isogenic line R OKY62 bm_1bm_1 was more uniform in adaxial and abaxial epidermis (Figure 44). It had an average thickness of 1.257 $\pm$ 0.064 um as compared to 0.976 $\pm$ 0.0620 um for the sparse-bloom near-isogenic line R OKY62 h_2h_2, and 2.1750 $\pm$ 0.1418 um for the bloom isogenic line R OKY62 BmBm. There were three types of vascular bundles in the "boot", "half-bloom", and "soft dough" stages. The small and medium bundles were like those described earlier, but the larger vascular bundles eventually occupied all the

Figures 44-45. Light Micrographs of Leaf Sheath

VS = vascular bundle
EP = epidermal cells
AB = abaxial epidermis
P = phloem
M = mesophyll

Figure 44. Cross Section of Leaf Sheath of R OKY62 bm_1bm_1 at "Half Bloom" Stage, Showing Medium and Small Vascular Bundles Lying Beneath the Adaxial Epidermis x110

Figure 45. Cross Section of Leaf Sheath of R OKY62 h_2h_2 at "Dough" Stage, Showing Alternating Small and Medium Vascular Bundles and Thick Epidermis x400


44
P
VS
EP
AB
45
M

cross sectional surface of the leaf. They are called girder or I-beam vascular bundles, according to Metcalf (10). The phloem sheath was massive. The schlerenchymatous bundle cap at the phloem side was in contact with the hypodermal schlerenchyma. The area between the radial sheets of sclerenchyma was filled with large colorless cells (Figure 45). There were two small bundles between two large girder shaped vascular bundles. The leaf sheath of all three types was an average of 0.3439 um.

Leaf Blade

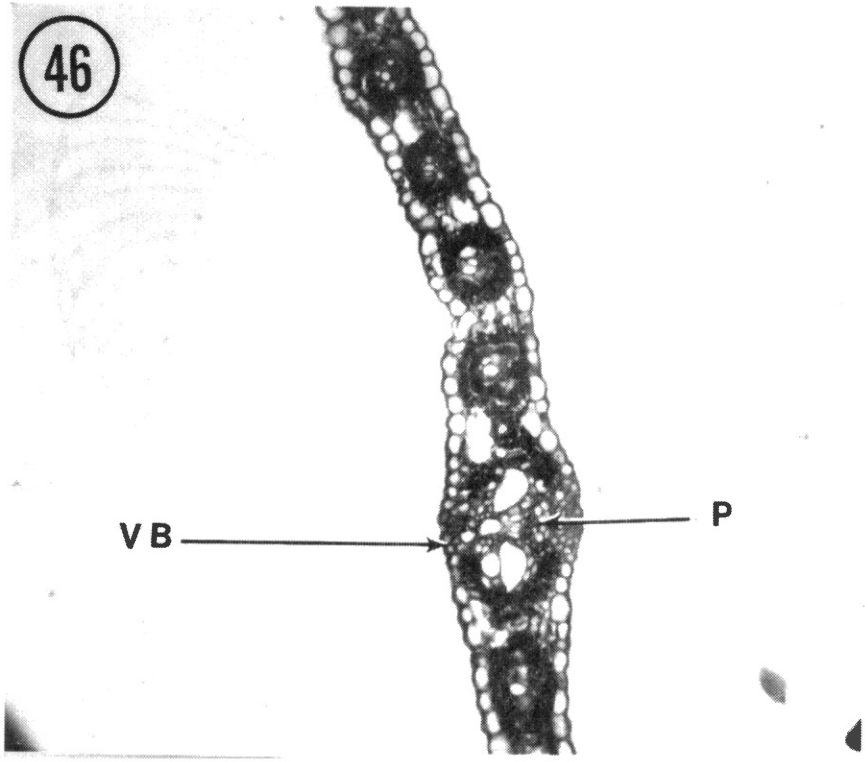
The cross section of leaf blade showed identical structure in all three types of sorghum. The section showed parallel veins like the sheath, but the cross connecting veinlets were not so prominent as in the sheath (Figure 46). The presence of bulliform cells made the difference between adaxial and abaxial epidermal cells (Figure 47). There were two types of vascular bundles. There were small round groups of 12-15 alternating with large oval bundles (Figure 46). The large bundles occupied the entire length of the tissue and represented the principal veins of the leaf. The small bundles round in shape were found deeply embedded in parenchyma in close proximity to the epidermis. Each bundle had a phloem pole directed toward the adaxial epidermis and surrounded by schlerenchyma cells, and a xylem pole separated from the epidermis by several layers of parenchyma cells. The mesophyll of the blade consisted of relatively

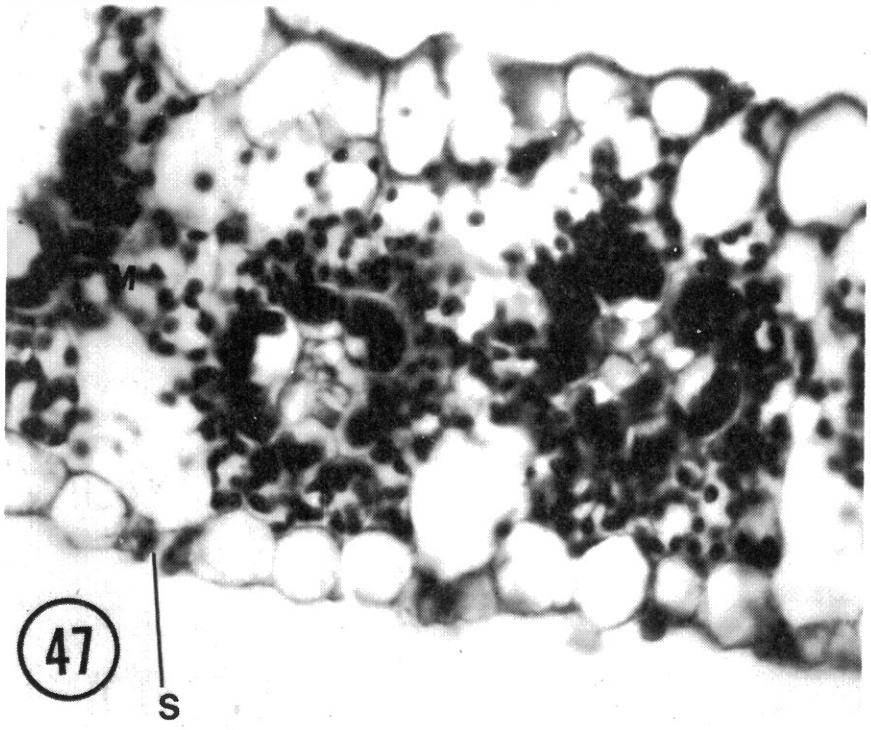
Figures 46-47. Light Micrographs of Leaf Blade

VB = vascular bundle
P = phloem
S = stomate
M = mesophyll cells

Figure 46. Cross Section of Leaf Blade of R OKY62 BmBm at "Eight Leaf" Stage, Showing Thick Cell Wall of Epidermis and Large and Small Vascular Bundles x110

Figure 47. Cross Section of Leaf Blade of R OKY62 $bm_1 bm_1$ at "Half Bloom" Stage, Showing Bundle Sheath, Epidermal Cells and Mesophyll Cells x400


46
VB
P


47
S

compact chlorenchyma with plastids that were smaller and more numerous than those of the sheath. The palisade layer was not well developed.

The thickness of the cuticle layer of both leaf blade and leaf sheath did not show any particular trend (Tables I and II). The means for the thickness of the cuticle (Table III) showed 0.2551 um for bloomless near-isogenic line R OKY62 bm_1bm_1, 0.3341 um for sparse-bloom R OKY62 h_2h_2, and 0.2962 um for bloom R OKY62 BmBm. But the analysis of variance showed no difference in thickness of the cuticle due to the varieties (bloom, sparse-bloom and bloomless). However, the thickness of the cuticle was largely thicker in supplemental irrigation conditions over dryland conditions, 0.4169 um and 0.1734 um, respectively (Table IV). However, the thickness of the epidermis of each variety showed significant differences (Table IV). In conclusion, the three near-isogenic lines did not show any significant differences in thickness. This suggests that the layer measured was the layer of amorphous wax as observed under SEM. The difference in preference by greenbugs [Schizaphis graminum (Rondani)] was due to the amount of wax deposited over the cuticle. There seemed to be two explanations possible. The greenbugs may sense some chemicals in the wax which would indicate food to them. They might also sense the pores opened to extrude wax to reach the phloem of the leaf. Since the bloom has more wax than the two other types of sorghum, greenbugs feed on it more easily. Sparse-bloom,

TABLE I

RANGES, MEANS, STANDARD DEVIATIONS AND STANDARD ERRORS MEASUREMENTS OF CUTICLE THICKNESS OF LEAF SHEATH SAMPLES[a]

Sample Number[b]	Bloomless				Sparse-Bloom				Bloom			
	Range	Mean	Standard Deviation	Standard Error	Range	Mean	Standard Deviation	Standard Error	Range	Mean	Standard Deviation	Standard Error
					Upper Epidermis							
1A	3.0-0.5	1.2570	0.6064	0.07248	2.0-0.05	0.97600	0.0620	0.00653	10.0-0.50	2.1750	1.4181	0.1418
1B	0.5-0.05	0.1830	0.0713	0.00713	0.5-0.05	0.16000	0.0820	0.00820	0.50-0.05	0.1945	0.1010	0.0101
2A	3.0-0.15	0.5083	0.0734	0.00734	2.0-0.05	0.24200	0.3184	0.03184	1.0-0.05	0.1617[c]		
2B	1.0-0.05	0.3000	0.3050	0.04313	0.2-0.05	0.11580	0.0456	0.00580	0.20-0.10	0.1430	0.0685	0.0096
3A	0.25-0.05	0.1274	0.0078	0.00080	0.5-0.1	0.26270	0.1281	0.01650	0.25-0.07	0.1789	0.0770	0.0077
3B	1.0-0.10	0.2510	0.1160	0.01160	2.0-0.5	1.25000	0.5027	0.07100	2.0-0.50	1.3360	0.6120	0.0612
4A	1.0-0.10	0.3800	0.3440	0.03440	3.0-0.15	1.14167	0.9288	0.16960	2.0-1.0	0.3488	0.2583	0.0289
4B	1.0-0.10	0.2900	0.169[c]	0.01690	0.40-0.10	0.20700	0.0527	0.00530	5.0-0.05	0.2099	0.1141	0.0196
					Lower Epidermis							
1A	3.0-0.5	0.7429	0.4720	0.05641	0.10-0.05	0.0594	0.0197	0.00207	3.0-0.50	0.8556	0.5913	0.0613
1B	0.5-0.05	0.1070	0.0074	0.00070	0.5-0.05	0.1945	0.1010	0.01010	.50-0.05	0.1945	0.1010	0.0101
2A	1.75-0.50	0.3586	0.3502	0.03500	0.25-0.05	0.1107	0.0323	0.00320	0.50-0.05	0.1298	0.0840	0.0108
2B	0.25-0.05	0.1000	0.0690	0.00690	0.20-0.05	0.0924	0.0403	0.00520	0.15-0.05	0.0970	0.0970	0.0049
3A	0.25-0.05	0.0838	0.0053	0.00050	0.5-0.05	0.1298	0.0840	0.01800	0.25-0.05	0.1237	0.0645	0.0065
3B	0.25-0.05	0.1230	0.0566	0.00560	0.15-0.10	0.1320	0.2420	0.00340	0.30-0.15	0.2230	0.0560	0.0056
4A	0.25-0.05	0.1570	0.1010	0.01010	0.25-0.05	0.1783	0.0962	0.07756	0.5-0.1	0.2244	0.0661	0.0074
4B	0.25-0.10	0.1710	0.0503	0.00500	0.25-0.05	0.1570	0.0527	0.00530	0.5-0.05	0.1359[c]	0.1014	0.0174

[a] Measurements are in micrometers (um).
[b] 1, 2, 3, and 4 = sample numbers; A = supplemental irrigation, B = dryland.
[c] Means of the total representing missing data.

TABLE II

RANGES, MEANS, STANDARD DEVIATIONS AND STANDARD ERRORS MEASUREMENTS OF CUTICLE THICKNESS OF LEAF BLADE SAMPLES[a]

Sample Number[b]	Bloomless				Sparse-Bloom				Bloom			
	Range	Mean	Standard Deviation	Standard Error	Range	Mean	Standard Deviation	Standard Error	Range	Mean	Standard Deviation	Standard Error
					Upper Epidermis							
1A	2.0-0.25	0.5083	0.3340	0.0352	6.0-0.5	1.7050	1.1593	0.11593	9.0-1.0	3.1750	1.4560	0.1454
1B	2.0-0.05	0.1280	0.0205	0.0205	5.0-0.05	0.2325	0.0703	0.00700	0.15-0.05	0.1150	0.0433	0.0043
2A	0.25-0.05	0.1516	0.0058	0.0006	2.0-0.05	0.2420	0.3184	0.03180	0.1-0.05	0.2627	0.1281	0.0165
2B	0.50-0.05	0.1543	0.0731	0.0103	0.25-0.05	0.1364	0.0499	0.00800	0.25-0.10	0.1930	0.0085	0.0008
3A	0.25-0.05	0.3830	0.3440	0.0344	0.30-0.05	0.1520	0.0487	0.00490	0.25-0.10	0.1675	0.4890	0.0049
3B	0.50-0.05	0.1570	0.1010	0.0101	0.25-0.05	0.1364	0.0499	0.04990	0.5-0.05	0.2100	0.3330	0.0333
4A	0.25-0.1	0.1856	0.0536	0.0060	0.40-0.10	0.3830	0.0306	0.00310	0.5-0.05	0.2441	1.2290	0.0211
4B	0.50-0.05	0.1570[c]	0.1010	0.0101	0.5-0.05	0.3770	0.0030	0.00030	0.5-0.05	0.2410	0.3060	0.0300
					Lower Epidermis							
1A	1.0-0.25	0.4250	0.1473	0.0152	2.0-0.5	0.9100	0.3362	0.0336	3.0-0.5	1.0550	0.5856	0.0586
1B	0.1-0.05	0.0555	0.1570	0.0016	0.75-0.05	0.1521	0.1488	0.0149	0.15-0.05	0.0889	0.0560	0.0056
2A	0.25-0.05	0.1096	0.0066	0.0066	0.20-0.05	0.1158	0.0456	0.0046	0.5-0.05	0.1268	0.0840	0.0108
2B	0.20-0.05	0.0800	0.0365	0.0052	0.20-0.05	0.0924	0.4030	0.0403	0.15-0.05	0.0930	0.0560	0.0056
3A	0.25-0.05	0.3830	0.3340	0.0334	0.25-0.05	0.1150	0.0512	0.0051	0.15-0.10	0.1090	0.336	0.108
3B	0.20-0.05	0.1570	0.1010	0.0101	0.25-0.05	0.0950	0.0405	0.0041	0.5-0.05	0.210	0.333	0.0333
4A	0.5-0.05	0.1440	0.0680	0.0076	0.25-0.05	0.1570	0.0527	0.0053	0.50-0.05	0.1962	0.1400	0.0034
4B	0.10-0.05	0.1570[c]	0.1010	0.0101	0.25-0.05	0.1850	0.0306	0.0031	0.50-0.05	0.2410	0.306	0.030

[a] Measurements are in micrometers (um).
[b] 1, 2, 3, and 4 = sample numbers; A = supplemental irrigation, B = dryland.
[c] Means of the total representing missing data.

TABLE III

MEANS FOR DIFFERENT COMBINATIONS OF VARIETY, LEAF PART AND EPIDERMIS TYPE (IN MICROMETERS)

Environmental Conditions	Bloomless				Sparse Bloom				Bloom				Mean of Irrigation and Dryland
	Leaf Sheath		Leaf Blade		Leaf Sheath		Leaf Blade		Leaf Sheath		Leaf Blade		
	Adaxial Epi.	Abaxial Epi.	Adaxial Epi.	Abaxial Epi.	Adaxial Epi.	Abaxial Epi.	Adaxial Epi.	Abaxial Epi.	Adaxial Epi.	Abaxial Epi.	Adaxial Epi.	Abaxial Epi.	
Irrigation	0.5689	0.3356	0.3070	0.2060	0.7244	0.1046	0.6205	0.3277	0.7114	0.3334	0.5490	0.2109	0.4169
Dryland	0.2560	0.1253	0.1561	0.0831	0.4777	0.1534	0.1656	0.0991	0.2593	0.0768	0.1773	0.0511	0.1734
Means of Combination Irrigation and Dryland	0.4100	0.2300	0.2300	0.1500	0.6000	0.1300	0.3900	0.2100	0.4900	0.2100	0.3600	0.1300	
Means of Variety		0.2551				0.3341				0.2961			
Grand Mean						0.2951							
Means for Leaf Sheath						0.3439							
Means for Leaf Blade						0.2464							
Means for Adaxial Epidermis						0.4144							
Means for Abaxial Epidermis						0.1758							

TABLE IV

ANALYSIS OF VARIANCE OF THE THICKNESS OF CUTICLE BY CONSIDERING VARIETY AS A MAJOR FACTOR AND LEAF PARTS AND EPIDERMIS TYPE AS SECONDARY FACTORS

Source	DF	Sum of Squares	Mean Square	F-Ratio
Mean	1	8.361	8.361	
Blocks	1	1.423	1.423	
Treatments (Variety)	2	0.100	0.050	1.821 NS
Main Plot Error	2	0.055	0.027	
Leaf Part	1	0.228	0.228	5.177*
Epidermis Type	1	1.366	1.366	30.988**
Variety x Leaf Part	2	0.020	0.010	0.228 NS
Variety x Epidermis Type	2	0.151	0.076	1.716 NS
Leaf Part x Epidermis Type	2	0.127	0.127	2.889 NS
Variety x Leaf Part x Epidermis Type	2	0.067	0.033	0.759 NS
Error	9	0.397	0.044	
Sampling	72	9.854	0.137	
Total	96	22.150		

*$F < 0.05$

**$F < 0.005$

NS = non significant

which has a moderate amount of wax, is preferred to bloomless.

CHAPTER V

SUMMARY AND CONCLUSIONS

Three near-isogenic lines of sorghum, bloom R OKY62 BmBm, bloomless R OKY62 bm_1bm_1, and sparse-bloom R OKY62 h_2h_2 were grown in the summer of 1982. The leaf samples were collected to study a possible relationship between the presence of wax on leaf blades and leaf sheaths to the preference of greenbugs [*Schizaphis graminum* (Rondani)]. The plants were grown under two environments, supplemental irrigation and dryland conditions. Leaf samples from leaf sheaths and leaf blades were mounted on aluminum stubs and examined with JOEL JSM 35 SEM. Other samples of each treatment were processed for light microscopy. The thickness of cuticle was measured and statistically analyzed.

The following conclusions could be drawn from the observations:

1. All bloomless samples showed a regular, flat, unbroken epidermal structure under the SEM. The leaf sheath had more prominent veins than the blade. The presence of amorphous wax over the epidermis, which is different from the bloom wax, was observed. As observed by

light microscopy, the cuticle was of equal thickness in both irrigated and dryland conditions.

2. All sparse-bloom samples showed epicuticular bloom wax over the cuticle in the leaf sheath in the form of rod-like structures and coiled fine filaments which seemed to have hollow interiors. The waxy bloom appeared in the leaf blade later than in the leaf sheath. The cuticle layer did not show any significant difference from the two other types. Sparse-bloom tissue had an intermediate amount of bloom wax and probably had intermediate number of pores to extrude wax. This may be related to the intermediate monpreference exhibited by greenbugs to these tissues.

3. All bloom samples had epicuticular wax deposited over the cuticle in the leaf sheath and the leaf blade. The waxy bloom appeared in rod-like coiled filaments as described above. The thickness of the cuticle did not show any significant difference from the two other types. So the greenbugs may use the pores as entries for their stylets to penetrate and feed in the phloem.

SELECTED REFERENCES

1. Amini, Iraj. 1976. A study of greenbug resistance in a progeny from "bloomless" sorghum. Master's Thesis, Oklahoma State University.

2. Artschwager, E. 1948. Anatomy and morphology of the vegetative organs of Sorghum vulgare. USDA Tech. Bull. 957.

3. Ayyangar, G. N. R., V. P. Rao, A. K. Nambiar, and B. W. X. Ponnaiya. 1937. The occurrence and inheritance of waxy bloom on sorghum. Proceedings of the Indian Academy of Science. 5:4-15.

4. Ayyangar, G. N. R., and B. W. X. Ponnaiya. 1941. The occurrence and inheritance of a bloomless sorghum. Curr. Sci. 10:408-409.

5. Blum, A. 1975. Effect of the Bm gene on epicuticular wax deposit and the spectral characteristics of sorghum leaves. SABRAO J. 7:45-52.

6. Cummins, D. G., and J. W. Dobson, Jr. 1972. Digestibility of bloom and bloomless sorghum leaves as determined by a modified in vitro technique. Agron. J. 64:682-683.

7. Hanna, W. W., W. G. Monson, and G. W. Burton. 1974. Leaf surface effects on in vitro digestion and transpiration in isogenic lines of sorghum and pearl millet. Crop Sci. 14:837-838.

8. Krause, C. R. 1982. Identification of salt spray injury to Pinus species with scanning electron microscopy. Phytopathology 72:382-86.

9. Kraufman, P. B., S. L. Soni, W. C. Bigelow, and N. S. Ghosheh. 1973. Scanning electron microscopic analysis of the leaf and internodal epidermis of the rice plant (Oryza sativa). Phytomorphology 23:52-58.

10. Metcalfe, C. R. 1980. Anatomy of the monocotyledons I. Gramineae. Oxford University Press, London.

11. Peiretti, R. A. 1975. Some aspects of greenbug resistance in sorghum as related to "bloomless character". Master's Thesis, Oklahoma State University.

12. Peterson, G. C., Krittika Suksayretrup, and D. E. Weibel. 1982. Inheritance of some bloomless and sparse-bloom mutants in sorghum. Crop Sci. 22:63-64.

13. Sass, J. E. 1975. Botanical Microtechnique. Third Edition. The Iowa State University Press, Ames, Iowa.

14. Starks, K. G., and D. E. Weibel. 1981. Resistance in bloomless and sparse-bloom sorghum to greenbugs. Environ. Entomol. 10:963-965.

15. USDA. 1969. The greenbug situation on surghum. 1968 Crop. Econ. Ins. Rept. 19:63-65.

16. Vanderlip, R. L. 1972. How a Sorghum Plant Develops. Cooperative Extension Service. Kansas State University. Manhattan.

17. Wilkinson, R. E., and D. G. Cummins. 1981. Epicuticular Fatty Acid, Fatty Alcohol and Alkane contents of bloom and bloomless sorghum "Redbine 60" leaves. Crop Sci. 21:397-399.

VITA 2

Issaga Dit Moriba Konate

Candidate for the Degree of

Master of Science

Thesis: STRUCTURAL FEATURES OF LEAVES OF BLOOM, BLOOMLESS, AND SPARSE-BLOOM VARIETIES OF SORGHUM AS RELATES TO DROUGHT TOLERANCE AND GREENBUG RESISTANCE

Major Field: Agronomy

Biographical:

Personal Data: Born in Bafoulabe, Republic of Mali, November 29, 1948, the son of Bakary Konate and Fanta Diarra.

Education: Received the "Baccalaureat" of Mathematics and Physical Science and Chemistry from Lycee Alain L'Aigle (France) and Lycee Aokia Mohamed Bamako (Mali), 1970-71, and the Degree of "Ingenieur des Sciences Appliquees" from Institut Polytechnique Rurale de Mali, 1975; completed requirements for the Master of Science degree at Oklahoma State University in December, 1983.

Professional Experience: Sorghum-millet breeder at SRCVO-SOTUBA Mali from 1976-1980. Trainee in millet breeding at ICRISAT (India) for 10 months in 1977. Attended as representative of Mali in Sorghum Workshop organized by CRID in Bambey (Sengal) February 1979; in regional seminar for identification of general principles of genetic conservation in plant breeding, January 1980; and in sorghum and millet workshop organized by SAFGRAD in Kenya, February 1980. Participated on Malian collection of sorghum, millet and digitaria 1978-1979 and attended USDA program on "Keys to Agricultural Development at the Local Level at University of Wisconson" (Madison), May 1982.

Professional Societies: American Society of Agronomy.