The comparative anatomy of the leaf of Brassica juncea (L.) Coss. and its broadleaved and curled varieties ...

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THE COMPARATIVE ANATOMY OF THE LEAF OF BRASSICA
JUNCEA (L.) COSS. AND ITS BROADLEAVED
AND CURRED VARIETIES

BY

Willa Juanita Ewing

A Thesis Submitted to the Graduate Faculty
for the Degree of
MASTER OF SCIENCE

Major Subject - Horticulture

IOWA STATE
COLLEGE
AMES IA

Signatures have been redacted for privacy
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INTRODUCTION

This study of the leaf of the mustards generally used for potherbs is desirable from the standpoint of comparative anatomy in relation to their suitability for greens. The literature on the descriptive morphology of mustard is very meager, as the plant seems to have attracted very little attention, especially in connection with quality as related to structure.

It is the purpose of this study to present in detail the leaf anatomy of the Chinese mustard (Brassica juncea (L.) Coss.) and two of its varieties, Broadleaf mustard and Southern Curled mustard (Brassica juncea var. crispifolia Bailey) and present the type or types that seem best fitted for greens based upon their structural composition.

In taste these three varieties are alike, having the characteristic flavor of the Cruciferae. They are used for greens and enter into the composition of many salads.

Brassica juncea includes many forms. According to Bailey (1) it is native to tropic and temperate Asia and is widely cultivated in Europe. Professor C. C. Geogeson (2) gives its origin as Africa and China from which it has been introduced. It is cultivated in all parts of India for the seed which is exported as Sarepta seed. The first introduction of Chinese mustard into this country was by
Chinese gardeners to the larger cities in 1893. (2) According to Viehhoever (3), much of it came in as a substitute for *Sinapis alba* L. during the world war. Besides being called Chinese mustard it is also called Indian Mustard, Brown Mustard, and Sarepta Mustard. It is an erect, much branched plant with flat, obovate, lyrate-lobed leaves having a narrow petiole with leafy appendages.

Its broad-leaved form, called Broadleaf mustard, Chinese mustard, Elephant Ear and Spinach mustard, has broad, obovate, lyrate-lobed, glaucous leaves with a wide green midrib, and a winged petiole or long stalk which resembles that of Swiss chard. The plant has a rosette formation, the shortened stem causing the leaves to have the appearance of arising from the roots.

Bailey (1) refers to the curled mustard as *Brassica juncea* var. *crispifolia*. Its leaves are curled out, crisped and are represented in Southern Curled Mustard also called Southern Giant Curled, Fordhook Fancy and California Pepper grass. It was introduced into culture by John Child Lewis (1) in 1890 as California Pepper grass. He received the seed from a customer who recommended the plant as a garnish. It has been sold since as a garden mustard. It is raised extensively in the southern states, principally Texas and Florida, as a winter crop and shipped north for greens.

The growing of mustard for the seed which is used for the manufacture of mustard flour, oil, paste, and spice is practiced
in California, Montana, Kansas, Ohio, Tennessee and Washington. The plant is being used to combat soil erosion in Southern California.
MATERIALS AND METHODS

Plants for this experiment were grown in a greenhouse and in the field at Iowa State College. Ten cultivated mustards were grown, representing three species: Black Mustard (Brassica nigra (L.) Koch), White Mustard (Sinapis alba (L.)) and Chinese Mustard (Brassica juncea (L.) Coss.). Seven horticultural varieties, Spinach Mustard, Elephant Ear, Florida Broadleaf, Fordhook Fancy, Southern Curled Mustard, Southern Giant Curled and Broad Leaf Mustard, of the latter species were grown for comparison and preliminary test.

Each of these mustards fell into one of the following groups according to their leaf characteristics:

Group I.

Leaves light green, glaucous, broadly ovate, hairs scarce, wide ribbed, leaf stalk winged

Broad leaf Mustard
Florida Broadleaf
Spinach Mustard
Elephant Ear

Group II.

Leaves dark green, dull, obovate, noticeably hairy, narrow ribbed, leafy basal appendages
Black Mustard
White Mustard
Chinese Mustard

Group III.

Leaves medium green, dull, rugose, profusely cut, curled and branched

Fordhook Fancy
Southern curled
Southern Giant Curled

Since these characters might affect the suitability of the plant as a potherb, one characteristic variety of each group was selected for the study. It was a mere but interesting coincidence that of the three varieties selected, one was the parent of the other two.

Samples of the leaves were taken serially from the base from the time the plumule appeared to the time the leaves were full grown. The first leaf sample was taken from leaves when they were 2-3 by 3-5 cm. The last sample was from leaves 15 by 33 cm. in the Southern Curled Mustard; 23 by 33 cm. in the Broad Leaf; and 14 by 24 cm. in the Chinese Mustard. Samples were taken between seven and nine in the morning so that the material reached the killing solution in a very turgid condition.

For sampling the leaf was divided into eight parts (Plate II). As venation became more complete and as the leaf enlarged
it was necessary to subdivide the original divisions.

The material was killed and fixed by a chromacetic acid solution of medium strength to which was added five percent formalin per 100 ml. of solution. It was prepared for sectioning by the usual paraffin method. The sections of the young leaf were cut 10 to 12 microns, and those of the mature leaf were 12 to 14 microns thick. They were stained with Mayer's haemalum and a one percent aqueous solution of safranin. After dehydration the material was cleared and sealed with Canada balsam. The fixing and staining gave satisfactory results for the purpose of examining the anatomy of the leaves with which this study is concerned.

For a fermentation test 150 grams of leaves of each variety were placed in 750 ml. beakers with a small amount of water. After two weeks of fermentation more water was added to the beakers and the fermented material, composed chiefly of the parenchymous vein islets, was separated from the heavier fibrovascular tissue.

For a cooking test 250 grams of each of three types of leaves (comparable to the amount which would be used for a family of five) were boiled for three hours. This amount is about what would be used for greens by a family of five. This length of time was necessary to secure a thoroughly cooked compact mass.
PRESENTATION OF DATA

The Young Leaf

The primary leaf emerges above the cotyledons well differentiated. Both the lower and upper epidermis are easily distinguished and the palisade cells are round, tending to oblong and in later stages appear to be elongating.

Cuticle.

The cuticle is thin and is spread evenly over both the upper and lower epidermis. It is twice as thick in the Curled Mustard variety as in the other varieties studied.

The walls of the epidermal cells are, for the most part, straight with a slight thickening and indulation in the lateral walls. (4) The undulated lateral walls were least numerous in the Broadleaf variety. (Plate V)

Epidermis.

Both the lower and upper epidermis of all three varieties are composed of a single layer of cells. The cells are thin-walled and elongated horizontally to the surface. Single cells

...
conical hairs are found on all three varieties. The hairs of
the Broadleaf were much smaller and fewer in number than in the
other two varieties and were confined to the margin of the leaf.
In the case of the Chinese variety the hairs are mostly on the
basal half of the leaf, on the main rib and veins, and on the
leaf margin. In the Southern Curled the hairs are the most
numerous and are uniformly distributed about equally on the two
leaf surfaces.

The stomata are surrounded by two guard cells abundantly
supplied with chloroplasts. The guard cells, in turn, are sur-
rounded by three thin-walled subsidiary cells, one of which is
smaller than the other two. The stomata, averaging 85 per sq.
mm., occur on the midrib in linear groups while those on other
parts of the leaf occur singly and irregularly.

Mesophyll.

The palisade cells are thin-walled, closely compacted, and
with a tendency to roundness. Only one layer of cells is evi-
dent and it occupies about one-half of the total width of the
leaf. The spongy parenchyma is composed of large cells ranging
from spherical to oblong in shape. Intercellular spaces are
evident only in the Broadleaf variety in which case development
has progressed farther and more rapidly than in the other var-
eties. Chloroplasts are abundant in both the palisade and
spongy parenchyma cells and are more prevalent at the cell walls.
The fibro-vascular system is a continuation and expansion of that of the petiole or leaf stalk. The bundles leave the stem and pass out into the leaf blade, branching and re-branching as they approach the margin of the leaf. The finer subdivisions may merge with similar bundles that originated from other stems. The cortex of the leaf blade is much smaller than that of the petiole or leaf stalk. The leaf blade is divided into several lobes, each lobe having its own bundle of vascular tissues. These bundles consist of xylem and phloem, with the xylem being more prominent.

In the young leaf, the vascular bundle surrounding the growing leaf is irregular and is more prominent in the later stages of growth. The xylem and phloem are interwoven, forming a continuous system that extends from the petiole to the apex of the leaf. The xylem is characterized by its large, thick-walled vessels, while the phloem consists of thin-walled sieve tubes and companion cells.

As the leaf matures, the vascular bundle becomes more compact and the xylem vessels become smaller. The leaf blade is divided into several lobes, each lobe having its own bundle of vascular tissues. These bundles consist of xylem and phloem, with the xylem being more prominent.
The Half Crown Leaf

For these studies partially grown leaves were used. They were about one-half the size of the fully grown leaf. The cuticle, epidermis and mesophyll of the leaf in this stage of growth were not significantly different from those in the young leaf. Differences in the fibro-vascular systems, however, had become more pronounced.

In the Broadleaf variety the large midrib divided into eight to 10 small veins each containing six or seven fibro-vascular bundles which extend nearly to the margin of the leaf. In the midrib and in these lateral veins the vascular bundles continually merge and separate as the veins advance towards the margin of the leaf. This causes a tangled network of fibro-vascular tissue within the veins. At about two-thirds of the distance from the main rib to the margin of the leaf the primary veins branch. These secondary veins sub-divide repeatedly and eventually lose their identity being reduced to a single xylem element which is in direct contact with the mesophyll. In addition to the primary lateral veins mentioned above, many small lateral veins arise from the midrib between the primary lateral veins.

A large amount of collenchyma strands which enshakes the vascular tissues is present in the large crescent shaped midrib. This collenchyma extends in similar fashion, but in reduced amounts, into the larger veins. (Plates II, XIII, and V)
In the Chinese variety the veins which go into the basal appendages arise as very small branches of the midrib which enters the leaf blade. The midrib enters the leaf blade and extends about three-fourths of the way to the tip where it loses its identity by branching into several similar sized subdivisions. Other primary veins arise from the midrib between the basal appendages and the point of the final subdivision of the midrib. These primary veins redivide several times until they finally terminate a short distance from the margin of the leaf in a single xylem element.

This variety has a comparatively small amount of collenchyma which is most abundant in the larger veins. (Plates III, V, VI, and Fig. 1).

In the Southern Curled variety the veins branch very profusely without having any noticeably large veins. These veins, however, contain large fibro-vascular strands many of which contain numerous xylem and collenchyma strands which extend to the margin of the leaf. The fine branching is distributed uniformly over the entire leaf, running in all directions and enclosing small irregular shaped vein islets. These islets become curved due to their continued growth after the maturation of the veins. This results in the characteristic rough appearance of this type of mustard. The veins of this variety lie deeply ensheathed in a high midrib of thin-walled parenchyma that in later stages becomes pithy. (Plate III) Heavy walled col- lenchyma encheaths the vascular tissue of all parts of the leaf.
Fully Grown Leaf

Non-skeletal tissue.

For this study fully developed leaves were used. (Plate VII) In comparing the mature leaf with the half-grown one no appreciable or significant changes were noticeable in the cuticle, epidermis or mesophyll of any of the three varieties. No difference is observed as to the relative distance the veins approach the margin.

Skeletal tissues.

The differences observed previously in the fibro-vascular tissues of the three varieties have continued and have become more pronounced especially as regards the skeletal tissue. The crescent-shaped midrib of the Broad Leaf variety has become greatly enlarged. The fibro-vascular bundles are larger and are also more numerous and more branched in this mid-rib. The sheath of collenchymous tissue that surrounds the vascular tissue stains a deep blue with Mayer’s haemalum indicating a cellulose composition of the cell walls. (Plate VI)

The collenchyma strands in this variety are limited chiefly to the midrib, those of the prominent lateral veins being few in number and small in amount.
In a longitudinal section of Broad Leaf the veins are shown arrested before reaching the margin, the rest of the structure from the end of the bundle to the margin being composed of soft leaf parenchyma. (Plate V)

In the Chinese variety the midrib is narrow, and branches into small veins which extend throughout the leafy appendages and major portions of the blade. A cross-section of the rib or small veins shows the smallest amount of collenchyma strands to be found in any of the three types.

The midrib of Southern Curled is narrow, but deep. (Plate III) Numerous veins branch from it and in turn rebranch repeatedly ending abruptly at the edge. A cross-section at the margin of the leaf shows collenchyma strands not only following the bundle ends to the margin but ending in a point beyond them. (Plate V)

Collenchyma strands form one of the chief skeletal and supporting tissues in the mustards studied. Collenchyma is present in all three types studied but in varying amounts. It is most abundant in the midrib of the Broad Leaf variety; moderately abundant and universally distributed in the Southern Curled; and present to a small degree chiefly in the midrib of the Chinese variety.

Fermentation Test

The material or "rag" that remained after the fermentation tests was a mass of long skeletal strands. Under the microscope
this "rag" was revealed to be composed of xylem and thick walled collenchyma strands. (Plate IV)

In order to compare the relative amounts of rag in each type studied the dry weight of a 150 gram sample of fresh leaves representing each type were taken and compared with the dry weight of the "rag" from 150 grams of mustard of each type and the weight recorded.

Table I. Comparative dry weights of the types of leaves and the "rag" of each.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Wet weight</th>
<th>Dry weight of leaf</th>
<th>Dry weight of &quot;rag&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad leaf</td>
<td>150</td>
<td>18.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Chinese Mustard</td>
<td>150</td>
<td>14.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Southern Curled</td>
<td>150</td>
<td>14.9</td>
<td>4.7</td>
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</tbody>
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This test shows Southern Curled Mustard to have more rag per 150 gram weight than any of the other types. It also shows that Chinese Mustard has least amount of rag than either of the other mustards.

Cooking Test

At the end of a three-hour boiling period the wide prominent midrib and larger veins of Broad Leaf were still intact while the surrounding leaf blade was completely packed down to a tender consistency. Brassica juncea had become a more compact, tender consistency than Broad Leaf with a dark liquor surrounding it composed of water and very small pieces of the leaf.
Southern Curled still contained so much skeletal tissue that they still could be lifted from the beaker, whole and intact. The leaves showed the skeletal structure still rigid and even the margin of the leaf still curly and stiff as in the fresh state. (Plate VIII)

In order to supplement and check upon the findings of the fermentation test, the dry weights of 150 grams of mustard of each type were compared with the dry weights of the "rag" and results recorded.

Table II. Comparative dry weights of the three types of mustard and the "rag" of each.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Weight of Mustard</th>
<th>Dry weight of Leaf</th>
<th>Dry weight of &quot;Rag&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad Leaf</td>
<td>150</td>
<td>15.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Chinese Mustard</td>
<td>150</td>
<td>13.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Southern Curled</td>
<td>150</td>
<td>13.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Chinese Mustard here is shown to have less dry weight of "rag" than the other types, Southern Curled having the largest amount. Broad Leaf has also a large amount of rag due to the use of the whole leaf.
SUMMARY

There is no appreciable difference structurally in the young leaf and the half-grown leaf that has a great bearing on their use as potherbs. They each may be used similarly in the young state.

A difference in the amount of skeletal tissue is observed in the latest stages of growth in the young leaf and in the fully grown leaf which affects their use as potherbs.

There is in Broad Leaf a large amount of skeletal tissue in the midrib.

In Chinese Mustard the amount of skeletal tissue is least of the three.

In Southern Curled Mustard is found the largest amount of skeletal tissue.

In the fermentation test Broad Leaf is found to have a large amount of fibrous strands which have been fermented from the vein islets, Chinese Mustard the least and Southern Curled the largest.

The above is confirmed by the cooking test in which the comparative amounts of "rag" are found to be greatest in Southern Curled confined mainly to the midrib of Broad Leaf and giving it the next largest amount of fibrous strands and of smallest significance in Chinese Mustard where the amount is the least.
CONCLUSIONS

1. That type of mustard most satisfactory for greens is that which has the least amount of skeletal tissue.

2. The type of mustard as represented by Broad Leaf is suitable for greens only after the fibrous leaf stalk is removed.

3. The type of mustard as represented by *Brassica juncea* (L.) Coss. (Chinese Mustard) because of its fairly non-fibrous structure should be preferred for use as potherbs.

4. The type of mustard represented by Southern Curled is not suitable for use as potherbs due to its generally strong, fibrous structure. It should be used in small quantities with larger amounts of tender varieties or more suitably as a table garnish.
ACKNOWLEDGMENTS

The author wishes to express her appreciation to Professor B. S. Pickett for placing at hand all facilities necessary to carry out this work and to Professor H. W. Richey and Dr. J. E. Sears for helpful suggestions and criticisms in the organization and completion of the entire experiment.
LITERATURE CITED


EXPLANATION OF PLATES

Plate I.  
A. Young stage of primary leaf of Broad Leaf.  
B. Young stage of half-grown leaf. Broad Leaf.  
C. Young stage of primary leaf of Chinese Mustard.  
D. Young stage of half-grown leaf of Chinese Mustard.  
E. One-celled hair of Chinese Mustard.  
F. Young stage of primary leaf of Southern Curled Mustard.  
G. Young stage of half-grown leaf of Southern Curled.  
H. Cruciferous stomata—guard cells surrounded by three subsidiary cells, one smaller than the other two.  
I. Bundle of mature leaf of Broad Leaf midrib.  
K. Bundle of midrib of Southern Curled Mustard.

Plate II.  
A. Diagrammatic sketches of young, half-grown and mature leaves of Broad Leaf reduced 7X.  
B. Young, half-grown and mature leaves of Chinese Mustard reduced 7X.  
C. Young, half-grown and mature leaves of Southern Curled reduced 7X.  
D. Sections into which leaf was cut for anatomical studies.

Plate III.  
A. Collenchyma strands arrested back of margin of leaf in Broad Leaf.  
B. Collenchyma strands arrested back of margin of leaf in Chinese Mustard.  
C. Collenchyma strands ending at the margin in Southern Curled.
Plate IV. A. Strands and fibers left after fermentation of 15 grams Broad Leaf.

B. Fibrous strands left after fermentation of 150 grams of Chinese Mustard.

C. Fibrous strands left after fermentation of 150 grams in Southern Curled.

Plate V. A. Collenchyma strand terminating short of margin in Broad Leaf.


C. Collenchyma strand terminating in margin.

D. Prominent vein of Broad Leaf showing strong collenchyma strand.

E. Branching of collenchyma strand in Chinese Mustard.

F. Section of leaf near margin of leaf of Southern Curled containing collenchyma strands.

Plate VI. A. Sheath of collenchyma strands in midrib of Broad Leaf.

B. Sheath of collenchyma strands in Chinese Mustard midrib.

C. Sheath of collenchyma strands in tertiary veins.

Plate VII. A. Mature leaf of Southern Curled.

B. Mature leaf of Chinese Mustard.

C. Mature leaf of Broad Leaf.

Plate VIII. A. 150 grams of mature leaf of Southern curled boiled.

B. 150 grams of Chinese Mustard boiled.

C. 150 grams of Broad Leaf boiled.