

Frost-Hardiness in Relation to Leaf Anatomy and Natural Distribution of Several *Solanum* Species¹

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ABSTRACT

The objective of this study was to determine anatomical and morphological differences related to the frost-hardiness of tuber bearing *Solanum* species. Observations were made on about 2-month-old plants of 24 species including seven cultivars of potato (*Solanum tuberosum* L.). These species vary in frost-hardiness from -2.5 to -5.5 C.

No consistent relationship was found between various gross morphological characteristics of the plants and frost-hardiness. However, hardy species (frost killing temperature -4.0 C or colder) usually had short and highly branched stems with smaller and thicker leaves.

Among the various anatomical parameters studied, the number and thickness of palisade parenchyma layers and the stomatal index on the upper leaf surface were closely related to frost-hardiness. All hardy species had two palisade layers and all except three non-hardy accessions (frost killing temperature -3.5 C or warmer) had one palisade layer. On the average, hardy species had a three times greater stomatal index (26.7%) than non-hardy species (8.8%), on the upper surface of the leaf, which is probably due to two palisade layers in hardy species. The palisade parenchyma occupied a larger portion of the leaf cross section in hardy species (62.8%) compared to non-hardy species (51.2%). No significant differences in cell size and intercellular space were detected between these two types of species. Ploidy was not related to leaf anatomy or frost-hardiness. Among the hybrid plants examined, hardy hybrids had two palisade parenchyma layers.

The natural distribution patterns of many wild growing hardy and non-hardy *Solanum* species were analyzed. A difference in the geographical distribution of hardy and non-hardy wild species was found. The hardy species (except *S. commersonii* Dunal ex Poir.) are found in the high Andes of southern Peru, in central and southern Bolivia, northern Chile, and northwestern Argentina. Non-hardy wild species are primarily distributed in Mexico, Guatemala, Venezuela, Colombia, Ecuador, and northern and central coastal Peru. A close relationship was found between the altitude of origin of a species or collection and its frost-hardiness.

It appears that anatomical features of hardy species are the results of their adaptation to environmental stress (primarily low temperature and in some cases together with water stress) under natural selection.

Additional index words: Anatomical and morphological differences, *Solanum tuberosum* L., Frost killing temperature, Stomatal index, Leaf intercellular space, Elevation of natural distribution of potato species and cultivars.

ANATOMICAL and morphological characteristics of plants associated with their freezing stress resistance may be useful criteria for selecting frost-hardy cultivars and may shed light on the mechanism of frost-hardiness. Many early investigators found small cell

size (12, 17), thick cell walls (5), and low stomatal density (8) associated with frost-hardiness. Others found no relationship between cell size and frost-hardiness (1, 10). Levitt (10) concluded that cell size was a minor factor in frost-hardiness. Furthermore, certain plants, when subjected to low temperatures (5 C), increase their first-hardiness in a matter of 2 to 3 weeks without any decrease or shrinkage in cell size (11). These observations have discouraged researchers from looking for anatomical characteristics associated with frost-hardiness. One reason for these negative results is that most workers compared different plant species, whereas very little attention was paid to anatomical differences within species.

Potatoes (tuber bearing *Solanum* spp.) grow well in cool climates. They are commonly grown in temperate regions or at high elevations in the Andes where frost is a chronic problem. The most popular cultivated potato species, *S. tuberosum* L., possesses little frost-hardiness (-2.5 C or warmer) while many wild tuber-bearing *Solanum* species are frost-hardy (-4 C or colder) (13). These wild species have very low yields and poor tuber quality. No "markers" at present have been found that can be used in identifying frost-hardy potato plants.

The present work was undertaken to study several *Solanum* species for anatomical and morphological differences that might be associated with frost-hardiness. Another purpose was to identify leaf anatomical characteristics that might be used in selecting potatoes for frost-hardiness. An attempt was also made to relate the anatomical and frost-hardiness differences to the natural distributions of tuber-bearing *Solanum* species.

MATERIALS AND METHODS

All *Solanum* species, except *S. tuberosum*, (source given in Table 1) were propagated from seeds. Seedlings were transplanted into 15-cm pots and grown in a controlled environment with a 14-hour photoperiod, 450 $\mu\text{E sec}^{-1}\text{m}^{-2}$ radiation and 20/15 C day-night temperature regimes for about 2 months. Seven cultivars of *S. tuberosum* L. ('Russet Burbank', 'Cobbler', 'Norchip', 'Norland', 'Norgold Russet', 'Kennebec', and 'Red Pontiac') were propagated from seed tubers. These cultivars were grown for about 2 months under the same controlled environmental conditions as the other species. Observations were made on at least three different plants of each species.

Frost-hardiness was evaluated by controlled freezing of excised leaflets and the injury to the leaf tissue was evaluated visually as described elsewhere (2).

Qualitative observations were made on growth and branching pattern, leaf color, and relative abundance of hairs on leaflets and stems. Measurements were made on stem thickness, internode length, and the number of leaflets per compound leaf.

An apical leaflet from the third or fourth leaf from the top was excised and infiltrated for 2 min with tap water using a faucet aspirator. Leaf cross sections (75 to 100 μm thick) of fresh material were made perpendicular to the midrib from the central portion of the leaflet with an Oxford Vibratome.

Direct microscopic measurements of the thickness of palisade and spongy-parenchyma layers were made at several locations in

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Table 1. Leaf anatomical characteristics and frost-hardiness of several *Solanum* species.

	Frost killing temperature	Palisade layers	Total cross-section thickness	Epidermal cells/0.1225 mm ² leaf area	Stomatal index (Upper surface)	Avg. diam. palisade cell (Width)	Avg. length palisade cell
	C	No.	μm	No.		μm	
<i>S. tuberosum</i> , Kennebec—2n=48	-2.0	1	290.4	-	-	-	108.9
<i>S. tuberosum</i> , Norchip—2n=48	-2.3	1	200.1	106	0.008	14.1	70.9
<i>S. ambrosinum</i> , PI 365316†—2n=24	-2.5	1	193.6	-	-	13.9	77.4
<i>S. berthaultii</i> , PI 283069—2n=24	-2.5	1	236.0	-	-	15.7	92.0
<i>S. polytrichon</i> , PI 283106—2n=48	-2.5	1	282.4	27.7	0.188	20.8	145.9
<i>S. spagazzinii</i> , PI 310985—2n=24	-2.5	1	245.1	33.5	0.066	18.3	107.3
<i>S. trifidum</i> , PI 255542—2n=24	-2.5	1	210.5	91.7	0.041	17.0	110.6
<i>S. tuberosum</i> , Russet Burbank—2n=48	-2.5	1	205.7	82	0.090	13.4	72.6
<i>S. tuberosum</i> , Cobbler—2n=48	-2.5	2†	229.9	53.7	0.121	12.1	73.8
<i>S. tuberosum</i> , Norgold Russet—2n=48	-2.5	1	187.6	-	-	13.4	69.7
<i>S. tuberosum</i> , Norland—2n=48	-2.5	1	222.6	40.3	0.119	16.0	88.3
<i>S. tuberosum</i> , Red Pontiac—2n=48	-2.5	1	-	76	0.112	-	-
<i>S. bulbocastanum</i> , PI 275198—2n=24	-3.0	1	204.0	-	-	16.0	91.2
<i>S. microdontum</i> , PI 218222—2n=24	-3.0	1	161.4	250	0.025	10.4	50.8
<i>S. mochicense</i> , PI 1365334—2n=24	-3.0	1	278.3	-	-	11.6	163.8
<i>S. mochicense</i> , PI 338616—2n=24	-3.0	1	296	-	0.000	17.1	174.2
<i>S. paucijugum</i> , HGE5941—2n=24	-3.0	1	237.2	102	0.000	15.0	135.5
<i>S. polytrichon</i> , PI 186545—2n=48	-3.0	2†	265.5	-	-	18.3	113.7
<i>S. violaceimarmoratum</i> HHC5040—2n=24	-3.0	1	-	-	0.000	-	-
<i>S. chacoense</i> , PI 275141—2n=24	-3.5	1	256.5	-	-	23.6	150.8
<i>S. morelliforme</i> , PI 275223—2n=24	-3.5	1	254.1	106	0.014	11.1	105.3
<i>S. sucrense</i> , HHC4613—2n=48	-3.5	2†	209.8	-	-	16.8	85.4
<i>S. phureja</i> , PI 225694—2n=24	-3.5	1	208.1	525	0.090	8.6	87.8
<i>S. sparsipilum</i> , PI 275276—2n=24	-3.5	1	89.5	375	0.197	7.8	37.0
<i>S. medians</i> , PI 310994—2n=24	-3.5	1	183.2	-	-	13.7	74.3
<i>S. megistacrolobum</i> , HHC4317—2n=24	-4.0	2	260.6	-	-	13.8	77.4
<i>S. acaule</i> , PI 175396—2n=48	-4.5	2	367.8	121.5	0.274	16.8	113.7
<i>S. multidissectum</i> , PI 210044—2n=24	-4.7	2	258.5	114.5	0.184	17.0	111.3
<i>S. megistacrolobum</i> , OKA3787—2n=24	-5.0	2	304.9	32.8	0.427	19.3	104.1
<i>S. toralapanum</i> , HHC4556—2n=24	-5.0	2	271.0	250	0.254	10.5	81.1
<i>S. toralapanum</i> , HOF1851—2n=24	-5.0	2	332.8	34	0.218	-	108.9
<i>S. verna</i> , PI 320329—2n=24	-5.0	2	173.5	-	-	14.1	67.0
<i>S. commersonii</i> , PI 243503—2n=24	-5.5	2	247.3	69.2	0.247	20.5	114.9
<i>S. sanctae-roxae</i> , PI 230464—2n=24	-5.5	2	312.2	-	-	20.2	116.2

† Identification number at the Potato Introduction Station, Sturgeon Bay, Wis.

‡ Data for these species not used for averaging (see Table 2).

each cross section using an eye-piece micrometer. The average length and width of the palisade cells were calculated by direct microscopic measurements and by counting the number of cells in photomicrographs. The average total volume and lateral surface area of palisade cells was calculated from these measurements, assuming a cylindrical cross section.

Free-hand sections of the upper and lower surface of the excised leaflet were made using a sharp razor blade. The leaflet used was infiltrated with water prior to sectioning as described for cross sections. From the photomicrographs made of the surface (epidermal) layer, the total number of cells and the number of stomata in a given area were recorded. Stomatal index (SI) was calculated as:

$$SI = (\text{no. of stomata} / \text{no. of total cells}) \times 100.$$

Mature leaflets (third or fourth leaf from the top) were excised and weighed (W_1). These leaflets were then vacuum-infiltrated with water, blotted with filter paper to remove surface water, and weighed again (W_2). The weight difference ($W_2 - W_1$) was regarded as the weight of the water added to the intercellular space. The percent intercellular space was estimated from the formula $(W_2 - W_1)/W_2$. This estimation is based on the assumption that the density of the living cells is one.

RESULTS

Frost-hardiness

The frost-hardiness of the species studied varied from -2.5 to -5.5 C (Table 1). For the purpose of this study, the species with hardiness -2.5 to -3.5 C were designated non-hardy and those with hardiness -4.0 to -5.5 C as hardy. The reason for this was that the breeder trying to achieve a frost-hardy potato has usually set -4.0 C as his goal (3). Furthermore, the hardy

and non-hardy species separated out clearly, based on the number of palisade layers.

Morphological Characteristics

Among the 24 tuber bearing *Solanum* species, including seven cultivars of *S. tuberosum*, no consistent relationship was found between various morphological characteristics of plants and frost-hardiness. In general, however, hardy species had shorter and more extensively branched stems with smaller and thicker leaves than did non-hardy species. Furthermore, the leaves of the hardy species had, in general, fewer and thicker hairs. Various types of growth patterns (upright, bushy, viny) were found in hardy and non-hardy species. The internode length varied from 0.5 to 4 cm and number of leaflets in a leaf varied from 1 to 11 in both hardy and non-hardy species.

Leaf Anatomy

Various species studied are arranged in increasing order of hardiness in Table 1. A sharp break was found in terms of the number of palisade parenchyma layers. All *Solanum* species with a first killing temperature of -4.0 C or colder had two very distinct palisade parenchyma layers (Table 1). Of the 24 non-hardy accessions (frost killing temperature -3.5 C or warmer) all except three (*S. tuberosum* cv. Cobbler, *S. polytrichon*, and *S. sucrense*) had one palisade layer.

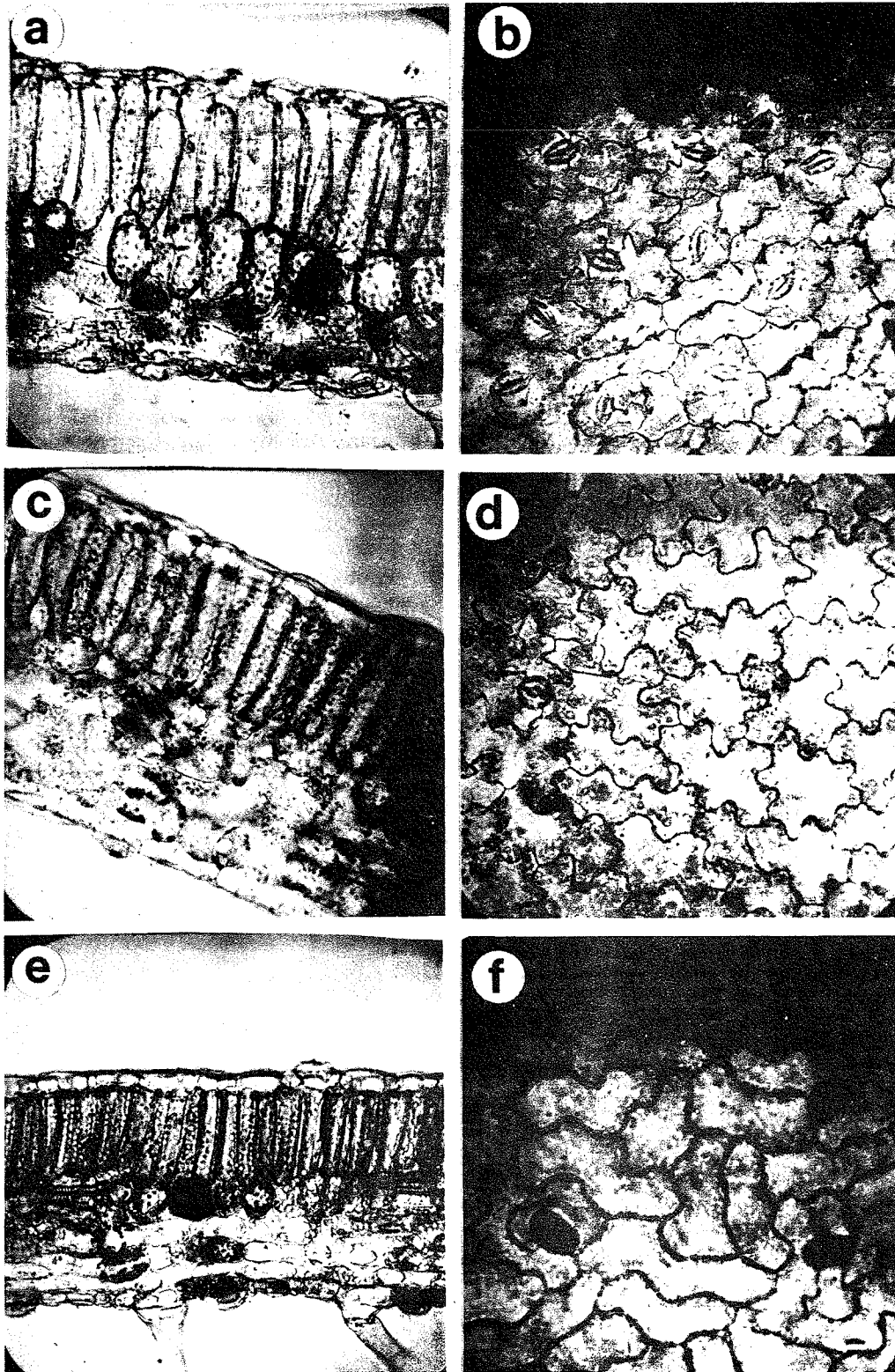


Fig. 1. Photomicrographs of leaf cross-section (a,c,e) and upper leaf surface (b,d,f) of hardy wild (*S. commersonii*; a,b), non-hardy wild (*S. spigazzinii*; c,d), and non-hardy cultivated (*S. tuberosum*, cv Norland; e,f) species.

Table 2. Leaf anatomical differences between hardy and non-hardy *Solanum* species.

	Non-hardy species	Hardy species
Frost killing temperature (C)	-3.5 or warmer	-4.0 or colder
No. of palisade layers	1	2
Palisade layer thickness (%) (palisade/palisade + spongy)	51.2 ± 8.5	62.8 ± 8.9
Leaf thickness (μm)	223.9 ± 50.6	280.9 ± 56.4
Stomatal index, upper surface (%)	8.8 ± 6.2	26.7 ± 8.4
Palisade cell dimensions (μm)		
Width	14.4 ± 3.9	16.5 ± 3.5
Length	101.7 ± 36.6	99.4 ± 18.9
Width/length, ratio	0.16 ± 0.04	0.17 ± 0.03
Epidermal cell (no./0.1,225 mm ² leaf area)	152.3 ± 147.7	103.7 ± 81.1

† The values are averages for the species listed in Table 1 except those marked ‡.
§ Only cells in the top palisade layer were measured.

Photomicrographs of hardy and non-hardy leaf cross sections are shown in Fig. 1. The hardy species (*S. commersonii*) had two distinct palisade layers (Fig. 1a) whereas the non-hardy wild species (*S. spigazzinii*) (Fig. 1c) and commonly cultivated species (*S. tuberosum* cv. Norland) (Fig. 1e) had only one palisade layer. Because of the two palisade layers in hardy species, the palisade cells occupied greater portions of the total leaf cross section than in non-hardy species.

To determine the degree of relationship between frost-hardiness and leaf anatomy, the data presented in Table 1 were averaged for hardy species and non-hardy species (three species with two layers excluded) and are given in Table 2. There was large variability (high standard deviations) within the hardy and non-hardy species for the various parameters studied (Table 2). In spite of the variability some generalizations can be made. For example, the hardy species had thicker leaves, and the palisade parenchyma cells on an average occupied larger portions of the leaf cross section (62.8%) compared to the non-hardy species (51.2%). Palisade cell size was not different in the two types. Data on percent palisade thickness from all the species were plotted as a function of frost-

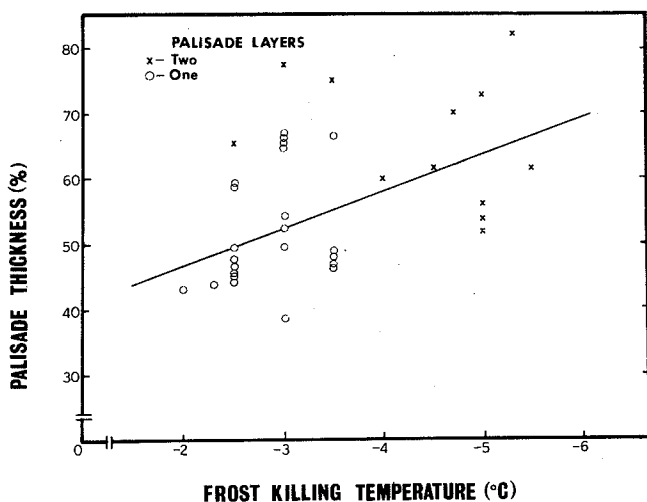


Fig. 2. Relationship between palisade thickness and frost killing temperature of several *Solanum* species (see Table 1 for the list). Palisade layer thickness = Palisade/total thickness excluding upper and lower epidermis. The equation of regression line is $y = 35.20 + 5.65x$ with $r = 0.59^{}$.**

hardiness as shown in Fig. 2. A significant positive correlation was found ($r = 0.59^{**}$).

The heritability of two palisade layers in relation to frost-hardiness was also investigated. For this purpose we checked some hybrids (hardy × non-hardy species) for their frost-hardiness and also examined for palisade layers. The hybrid seed for this purpose was obtained from the International Potato Research Center, Lima, Peru. In three different tests among about 200 plants (for each test) less than 2% of the plants were found to be frost-hardy. All frost-hardy plants had two distinct palisade layers. An example of this is shown in Fig. 3. This plant [seed from crosses of (*S. multidissectum* × *S. stenotomum*) × (*S. phureja*)] survived to -4 C and one of its parents, *multidissectum*, is a two-palisade-layer species (Table 1). Some of the non-hardy hybrid plants were also checked. About 40% of them had a single palisade layer, about 50% had two discontinuous layers (i.e. some places with two layers, but most of the cross sections with only one layer), and very few (about 10%) had two layers. It appears, therefore, that the palisade layer traits may be inherited. Other anatomical characteristics were not studied.

Stomatal Index

Photomicrographs of the upper leaf surface from typical hardy and non-hardy species are shown in Fig. 1. The hardy species had a higher stomatal index and stomatal frequency (Fig. 1b) than did the non-hardy species (Fig. 1d) including the cultivated species (Fig. 1f). Although the epidermal cell size varied greatly among the hardy and the non-hardy species (Table 1), there was a highly significant relationship between the stomatal index of the upper leaf surface and frost-hardiness (Fig. 4, $r = 0.74^{**}$). As the frost killing temperature decreased from -2.0 to -5.5 C, the stomatal index increased from about 2 to 30% (Fig. 4). On

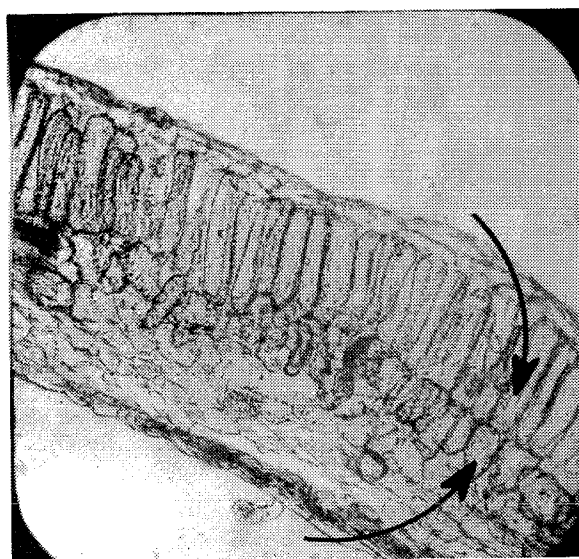


Fig. 3. Photomicrograph of the leaf cross-section of a hardy hybrid potato plant which was obtained by crossing hardy and non-hardy species [(*S. multidissectum* × *S. stenotomum*) × (*S. phureja*)]. About 425x, note two-layered palisade parenchyma (arrow).

the average, the hardy species had three times more stomata per epidermal cell on the upper leaf surface than did the non-hardy species (26.7 and 8.8% respectively, Table 2). The stomatal index for the lower surface of leaves from various hardy and non-hardy species was similar.

Intercellular Space

Measurements of the intercellular space were made at three different times on two hardy and one non-hardy species. Each time four samples were taken (each sample consisted of three leaflets). The average values for intercellular space were $26.4 \pm 1.9\%$ and $30 \pm 2.8\%$ for *S. acaule* and *S. commersonii*, respectively (hardy species). The non-hardy species *S. tuberosum* (cv. Kennebec) had an intercellular space of $25.6 \pm 1.9\%$. These values are averages of 12 separate measurements. Although hardy species had slightly larger intercellular spaces, the differences were not large enough to account for large differences in frost-hardiness.

Ploidy

Most of the species studied were diploid ($2n = 24$) (Table 1). Only three wild species (*S. acule*, *S. polytrichon* and *S. sucrense*) and the cultivated species *S. tuberosum* were tetraploid ($2n = 48$). Ploidy was not found to be related to frost-hardiness. It has been reported that a haploid of the species *S. polytrichon* had smaller stomata than its diploid parent (9). No results were reported on the stomatal index or stomatal frequency. Ploidy was not related to the stomatal index when different species were compared (Table 1).

DISCUSSION

Leaf Anatomy and Frost-Hardiness

Although some work on the relationship of layers of palisade cells to frost-hardiness in *S. acaule* has been reported (7), information in the general area of anatomical characteristics in relation to potato frost-hardiness does not exist. The present study provides some information on leaf anatomy in relation to frost-hardiness.

From this study, it can be concluded that the stomatal index (upper surface), the number of palisade layers, and the percent palisade thickness are associated with frost-hardiness of tuber bearing *Solanum* species. On examination of some hardy \times non-hardy hybrids, we found some evidence of inheritance of palisade layers in relation to frost-hardiness. It, therefore, appears that these leaf anatomical characteristics may have potential as "markers" for identifying frost-hardy potato plants.

Some investigators have found smaller cell size to be associated with frost-hardiness (12, 17). Our data (Table 1) do not substantiate these findings. The results of cell size measurements on *Solanum* species (Table 1) agree with the conclusions of Levitt (10) that cell size is only a secondary factor in frost-hardiness. This was found to be also true for the intercellular space. One must be careful, however, in studying these relationships and interpreting such results, since

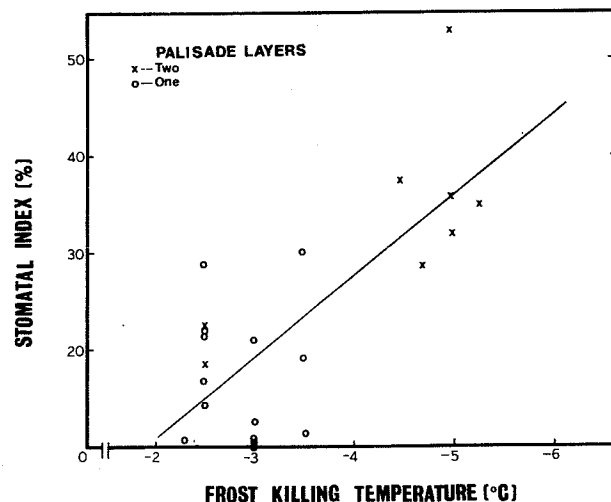


Fig. 4. Relationship between stomatal index and frost killing temperature of several *Solanum* species (see Table 1 for the list). Stomatal index = no. of stomata/total no. of cells in 0.1225 mm^2 area of leaf surface. The equation of the regression line is $y = -15.72 + 8.26x$ with $r = 0.74^{**}$.

comparison among various species may be quite different than comparison among various cultivars of the same species.

Frost-hardy species had a stomatal index three times greater than the non-hardy species (Table 2). This could be due to the presence of two palisade layers in hardy species. Greater number of stomata on the upper leaf surface can possibly provide more efficient diffusion of gases through the palisade layer.

Natural Distribution in Relation to Leaf Anatomy and Frost-Hardiness

In an attempt to find an explanation for the relationship between leaf anatomy and frost-hardiness, we studied the natural distribution patterns of these *Solanum* species. Figure 5 shows such a distribution pattern for all wild potato species studied in this investigation. This distribution map was drawn based on information by Hawkes (6). All hardy wild species (except *S. commersonii*) with two palisade parenchyma layers are native to southern Peru, central and southern Bolivia, northern Chile, and northwestern Argentina. One exception, *S. comersonii*, is found in the coastal regions of southern Brazil, Uruguay, and Argentina (Fig. 5). Non-hardy wild species (one palisade layer), on the other hand, are primarily found in Mexico, Guatemala, Venezuela, Colombia, Ecuador, and northern and central coastal Peru, except for *S. sparsipilum*, *S. sucrense*, and *S. microdontum*. These three species are found in central and southern Bolivia and one of them (*S. microdontum*) also occurs in northwestern Argentina. Apart from these exceptions, there appears to be a distinct geographical separation of hardy and non-hardy species.

It is interesting to note from Fig. 5 that hardy species occur in the high Andes whereas non-hardy species grow mostly at lower altitudes. This contradicts the results of Fibras and Ross (4) who did not find a close relationship between the altitude of origin of a species

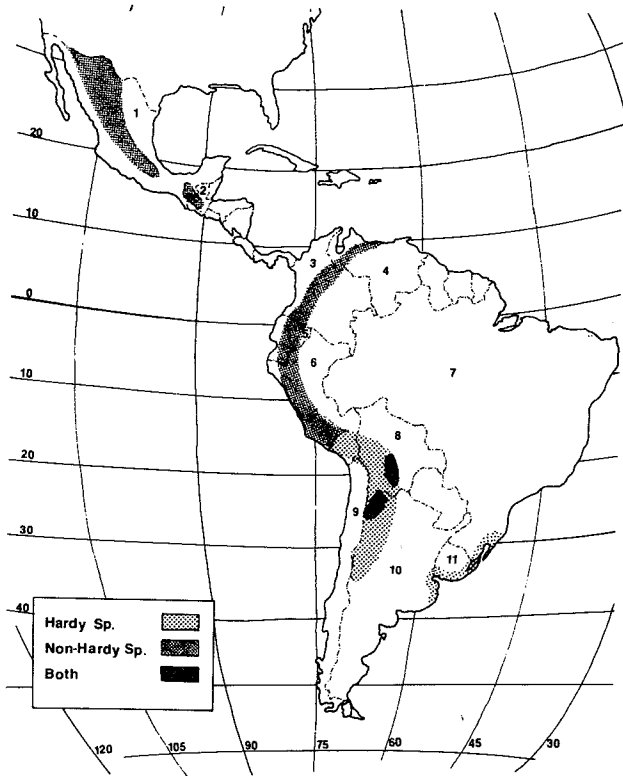


Fig. 5. Natural distribution of the wild *Solanum* species listed in Table 1. Data for geographical distribution obtained from Hawkes (6). 1. Mexico 2. Guatemala 3. Colombia 4. Venezuela 5. Ecuador 6. Peru 7. Brazil 8. Bolivia 9. Chile 10. Argentina 11. Uruguay.

or collection and frost-hardiness. In order to investigate this relationship more closely we plotted the frost killing temperatures against the altitudes of distribution or collection of 54 species (Fig. 6). A relationship between altitude at which a species occurs and frost-hardiness was found ($r = 0.59^{**}$). As the altitude increases from 2,300 to 3,500 m, frost-hardiness of species found there increases from -2.5 to -5.5 C. The species that occurs near the coast (*S. commersonii*) has not been plotted in Fig. 6. This species possesses a high degree of frost-hardiness (-5.5 C), probably due to low temperature exposure during the winter season. Furthermore, some wild species are thought to possess some drought resistance (6), particularly *S. commersonii* that grows in fairly dry areas. Water stress is known to induce thicker and multicellular palisade layers and increased stomatal frequency (14, 15).

While the origin of potatoes is still not resolved, Hawkes (6) has advanced the hypothesis that tuber bearing potato species originated in Mexico and were distributed from there to South America. If this is true, it would appear that as these species spread to the high Andes they acquired frost resistance by natural selection and adaptation to low temperature. In the present study, drought-hardiness of various *Solanum* species was not investigated. However, a direct relationship between frost- and drought-hardiness has been found in other plant species, since freeze-induced dehydration is a primary component of freez-

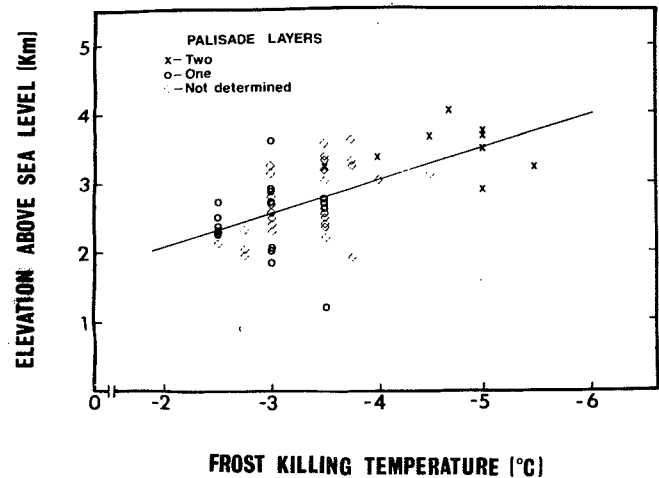


Fig. 6. Relationship between frost killing temperature and elevation of distribution or collection of 54 wild *Solanum* species (22 were the same as listed in Table 1, for a complete list see 13). The equation of the regression line is $y = 1,158 + 467x$, with $r = 0.59^{**}$. Data for elevation of collection or distribution for these species were obtained from the Potato Introduction Station, Sturgeon Bay, Wis. and Hawkes (6). Data for frost killing temperatures (for the species not evaluated in this study) were obtained from Li (13).

ing stress (16). It is likely, that the anatomical features of hardy species are the result of adaptation to environmental stress (primarily low temperature and in some instances water stress) under natural selection. At this stage, it is not possible to completely sort out the influences of these two different stresses on leaf anatomical characteristics. Further work in this area is needed to shed light on the importance of such anatomical characteristics to evolutionary adaptation.

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