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Freezing Tolerance of Primitive Willows Ranging to Subtropics and Tropics[1]

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Abstract Two species of *Salix* subgen. *Protitea*, *S. tetrasperma* from India and Pakistan, and *S. safsaf* from Africa, were mainly used as the experimental material. These willows from different climates were planted in Sapporo.

Freezing tolerance of willows from different climates was intimately related with the winter cold of their native habitats. Twigs of *S. safsaf* and *S. tetrasperma* native to the tropics were hardy to $-10°C$ and the twigs of the same species from the substropics of India and Africa survived freezing below $-90°C$, though the xylem sustained freezing injury between -20 and $-35°C$. However, no low temperature exotherm was associated with xylem injury. Thus, even the twig xylem of tropical willows survived sub-freezing temperatures by extracellular freezing unlike the xylem of many temperate deciduous trees. It was also observed in these willows that development of freezing tolerance seems unlikely to depend on their bud dormancy.

Introduction

Salix is the most widely ranging genus in the world, from Arctic tundra areas to the tropics across the continents of Eurasia and America. Some species further expand southward to the far southern tip of Africa, crossing the African continent (Fig. 3).

Primitive willows, *Protitea* (*Salix* subgenus) mainly range in the mild climates of America (4), Asia and Africa (7). *Protitea* is characterized by its polystamens and bud scale. The ends of the bud scales don't unite in an abdomen-like sack like many other salix species (Fig. 1, B), but they overlap (Fig. 1, A). In this paper, two primitive salix species *Salix tetrasperma* and *S. safsaf* were studied. *Salix tetrasperma* ranges from southern China westwards to Thailand, India and Pakistan (Fig. 2). *Salix*

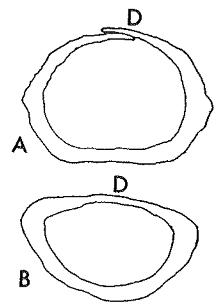

Fig. 1. Comparison of bud scale of willows (4)

A : Primitive willow, *Salix* subgen. *Protitea*. The ends of the bud scale facing twig overlap. B : The other willow. The ends of scale unite.

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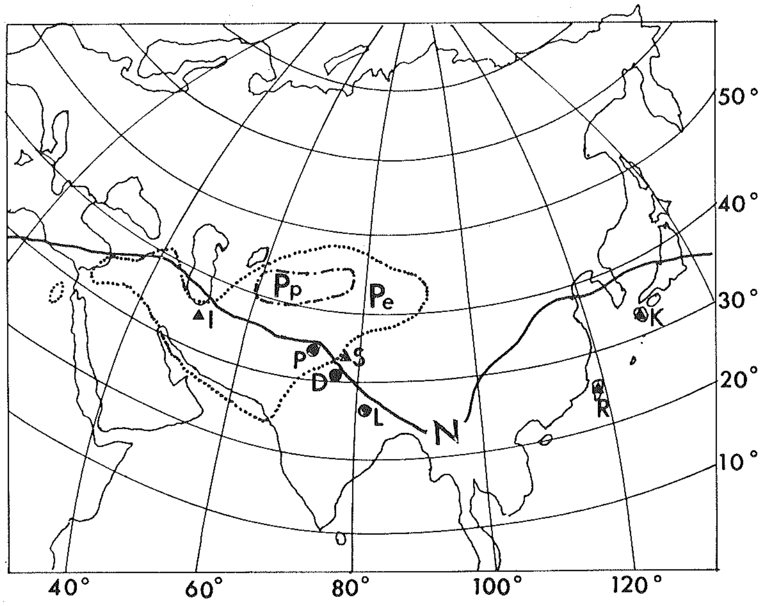

Fig. 2. Distribution of *Salix* subgen. *Protitea* in Asia and Central Asia.

N: Northern limits of natural ranges of *Salix* subgen. *Protitea* (primitive willow). Pe: *Populus euphratica*; Pp: *P. pruinosa*. Both poplars are primitive ones (*Populus* subgen. *Turanga*). Black circles and triangles show the sites of collection of willows. D: Dehra Dun (India); L: Lucknow (India); S: Simla (India); P: Peshawar (Pakistan); I: Karaj (Iran); K: Kumamoto (Japan); R: Mt. Raisan (Taiwan)

Based on private communication from A. Kimura

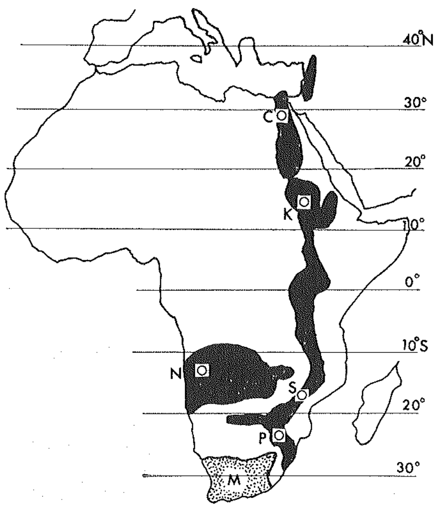

Fig. 3. Distribution of *Salix safsaf* and the site of collection

M: Distribution of *S. mucronata*. C: Cairo; K: Khartoum, S: Salisbury; N: Nova Lisbon; P: Pretoria. Based on private communication from A. Kimura and on Palgrave (7) *safsaf* is widely distributed in Africa, stretching in an unbroken line from the Nile lands of United Arab Republic and Sudan, through Kenya, Uganda, Zaire, Rwande, Zambia and Rhodesia to the Transvaal and Natal of South Africa (Fig. 3). *Salix mucronata,* Cape willow, which is very close to *Salix safsaf* ranges widely in South Africa (Fig. 3), extending to as far south as Cape Town. *Salix tetrasperma* and *Salix safsaf* occur along stream and river banks in mild climates.

There is considerable interest in intraspecific differences in cold hardiness among ecotypes and climatic races of widely ranging species. Many studies on intraspecific differences in cold hardiness have reported that, almost without exception, tree species from colder provenances were hardier than those from warmer ones, and that intraspecific variations in cold hardiness generally appeared to be closely related to the winter minimum of their native habitats (14, 15). These results suggest that winter cold is certainly a prime factor in the natural selection pressures which have led to the evolution of cold hardy climatic races and species.

In this study of *Salix* the following points were investigated: 1) the association between freezing tolerance and the winter cold of their native havitats, 2) the relation between development of freezing tolerance and bud dormancy, 3) the association of xylem injury with deep supercooling (1, 2, 9).

Materials and Methods

Cuttings of *Salix tetrasperma* and *S. safsaf* native to the subtropics and tropics were sent to Sapporo in 1969 and 1970: *S. tetrasperma* from Lucknow (India), Dehra Dun (India) and Peshawar (Pakistan); *S. safsaf* from Cairo (UAR), Khartoum (Sudan), Salisbury (Rhodesia), Nova Lisbon (Angola) and Pretoria (South Afica). Climatic data and the latitude of their native habitats are shown in Table 1. These willows were planted in the nursery of the Institute of Low Temperature Science, Sapporo. Growth cessation of willows in the nursery was defined as the date when winter terminal buds were formed.

Twigs were collected in the nursery in mid December. Five twig pieces, 10 cm long, were cut from each twig sample to be used in hardiness evaluation study. Twigs enclosed in polyethylene bags were subjected to artificial hardening at −3°C for 15 days to induce maximum hardiness. After hardening the twigs, enclosed in polyethylene bags, were frozen at −5°C for 1 hr in the cold room, and then cooled at 2.5°C increments at about 2 hr intervals to successively colder temperatures to −40°C, and then to −70°C at 10°C increments. Twig samples held for 4 hrs or 16 hrs at selected test temperatures then were removed to 0°C. After thawing twigs were placed in water in the greenhouse for 1 month to test the viability of treated twigs and their ability to continue normal development. The viability of willow twigs is easily tested because willow twigs are apt to put forth buds and produce adventitious roots, provided that they are not injured. Browning was also used as a criterion for rating injury. The lowest survival temperature at which no injury was sustained was expressed as the freezing tolerance of bud, cortex and xylem.

The occurrence of exotherms in excised twig pieces (2 cm long) was investigated by differential thermal analysis (DTA). A copper-constantan thermocouple was inserted in the twig pith and another thermocouple connected in series with the first, was inserted into another twig sample which had been previously dried. The twig sample with the inserted thermocouple was placed in a thermos flask, 7.3 cm diameter, 20 cm height, and the flask was directly transferred to the freezer held at about $-40°C$. The temperature of the twig sample was determined by an additional thermocouple and recorded.

Results

The growth cessation of *Salix tetrasperma*, *S. safsaf* and the other *Salix* species which were planted at our nursery was observed once a week from August until late November in 1971. Fortunately, unusually warm weather prevailed throughout the autumn of this year and subtropical and tropical willows were uninjured until late November. As shown in Table 1,

Table 1. Freezing tolerance of winter twigs of willows with reference

Species	Sites of collection	Latitude (Altitude)	Mean air temperature of coldest month (°C)
*Salix safsaf**	Khartoum (Sudan)	15°36′N (380 m)	22.5
"	Salisbury (Rhodesia)	17°55′S (300 m)**	20.0
"	Nova Lisbon (Angola)	12°22′S (1,700 m)	—
"	Pretoria (South Africa)	25°45′S (1,400 m)	10.3
"	Cairo (U.A.R.)	29°52′N (139 m)	12.7
*S. tetrasperma**	Lucknow (India)	26°52′N (111 m)	15.8
"	Dehra Dun (India)	30°20′N (640 m)	10.3
"	Peshawar (Pakistan)	34°01′N (359 m)	10.7
Salix sp.	Simla (India)	36°01′N (2,020 m)	5.2
"	Raisan (Taiwan)	24° N (2,000 m)	8.2
S. agyptica	Karaj (Iran)	35°47′N (1,300 m)	3.0
S. viminalis	"	"	3.0
S. acmophylla	"	"	3.0
S. sieboldiana	Kumamoto (Japan)	32°49′N (39 m)	4.9
S. sachalinensis	Sapporo (Japan)	43°49′N (18 m)	−5.5

* *Salix* subgen. *Protitea* (primitive willow)

** Twigs were collected at the altitude of 30 m, 8 km north of Salisbury.

*** Tissues were uninjured at the lowest test temperatures indicated.

S. tetrasperma from Lucknow and Khartoum and Salisbury did not form their terminal buds until November 28th. The shoot tips were injured for the first time by a severe frost on November 29th. *S. tetrasperma* from Dehra Dun and Peshawar, and *S. safsaf* from Cairo and Pretoria ceased their growth and formed their terminal buds in mid or late October. Potted willows of *S. tetrasperma* and *S. safsaf*, which formed their terminal buds in the nursery in late November, were transferred to a green house in early November. As a result, these willows began bud opening 2 weeks later unlike the *Salix* species from temperate climates. Therefore, the willows from the subtropics seem unlikely to have bud dormancy. It is very interesting that *S. safsaf* from Angola ceased growth in early to mid September and started growth in mid to late October every year.

Twigs of *S. safsaf* native to the tropics of Africa were marginally hardy to −10°C even when well-hardened, and little or no difference was observed in tolerance among the cortex, bud and xylem (Table 1). Twigs of *S. safsaf* from Cairo and *S. tetrasperma* from Dehra Dun and Peshawar where the average air temperature in the coldest month are below 13°C, rooted and the buds put forth new shoots, even when cooled down to −70°C or immersed in liquid nitrogen after prefreezing to −30°C (10), though the xylem tissues all sustained freezing injury between −20 and −35°C (Fig. 4). In both *S.*

to meteorological and geographical conditions of their native habitats

Mean annual temperaturea (°C)	Freezing tolerance (°C)			Time of growth cessation ⊗
	Bud	Cortex	Xylem	
24.8	−10	−10	−10	G
24.8	−10	−10	−10	G
18.8	−17	−17	−17	Mid September
16.7	−30 to −35	−30 to −35	−20 to −25	Mid October
26.5	−90	−90	−25	Late October
25.6	−25	−25	−20	G
25.3	−70 to −90	−90***	−25	Late October
22.7	−90***	−90***	−30	"
13.3	−90	−90	−90***	—
12.5	−90***	−90***	−25	—
16.5	−90***	−90***	−70***	Mid October
16.5	−90***	−90***	−70	"
16.5	−90***	−90***	−70	"
15.9	−90***	−90***	−90***	"
7.6	−90***	−90***	−90***	Mid September

⊗ Time of growth cessation of 2-year-old willows was determined outside in Sapporo in 1971.

G: Growth cessation was not observed until late November.

tetrasperma and *S. safsaf* the least hardy tissue is the xylem, which can not survive freezing below −35°C, unlike the other hardy *Salix* species from temperate climates (Table 1). Also, the freezing tolerance of *S. tetrasperma* and *S. safsaf* did not increase when grown in Sapporo for 7 years. *Salix*

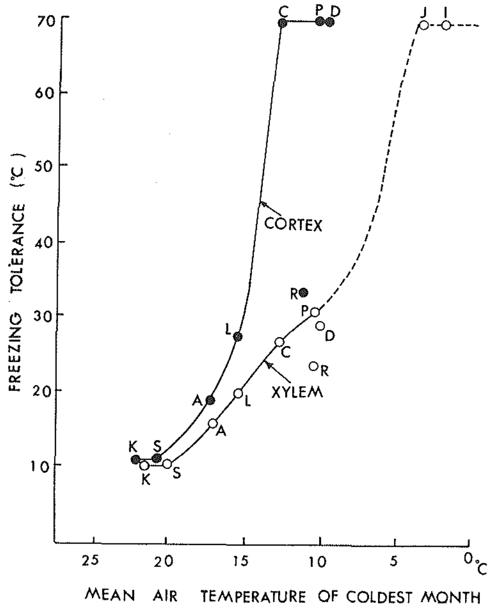

Fig. 4. Freezing tolerance of primitive willow associated with winter cold of their native habitats.

K: Khartoum; S: Salisbury; A: Nova Lisbon (Angola); L: Lucknow; D: Dehra Dun; P: Peshawar; C: Cairo; R: Pretoria

The willows from Karaj, Iran (J) and Simla, India (S) are not primitive one.

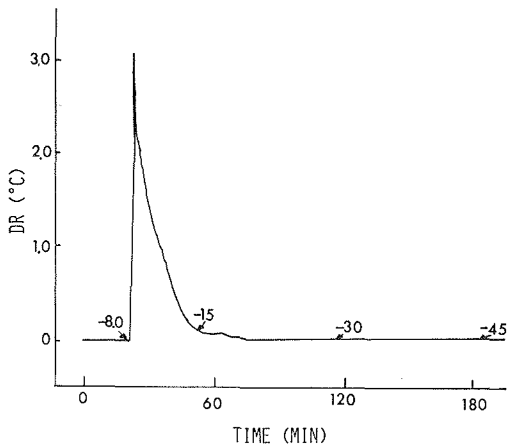

Fig. 5. DTA profile of winter twig piece of *Salix tetrasperma* from Dehra Dun

DR: Differential response. This twig sustained xylem injury around −30°C.

sieboldiana, from a warm temperate climate in southwestern Japan, 4 *Salix* species from Karaj (Iran) and *Salix* species from Simla (India) (Fig. 1), all survived freezing to $-70°C$ or the temperature of liquid nitrogen after pre-freezing to $-30°C$.

It has been reported that low temperature exotherms occurred at nearly the same temperature that resulted in death of the xylem in most hardy deciduous broad-leaved trees because of intracellular freezing in the xylem ray parencyma cells (1, 2, 9). No low temperature exotherm was associated with xylem injury in the twigs of *S. tetrasperma* and *S. safsaf* (Fig. 5), as is true of the hardy *Salix* species native to temperate climates (12).

Discussion

Pauley and Perry (8) reported that the time of growth cessation of *Populus trichocarpa* was inversely correlated with the latitude of origin of each clone. Among clones native to uniform day-length zones, the time at which growth ceased was directly correlated with the length of the frost-free season prevailing in the native habitat of each clone. On the basis of these observations, they concluded that adaptation of *Populus trichocarpa* to various habitats, differing in length of the frost-free season, is effected by a genetic mechanism which controls the duration of their seasonal period of growth. In a uniform environmental study of clones of red-osier dogwood (*Cornus stolonifera*) collected from 21 locations in North America and grown at St. Paul, Minnesota, the timing of cold acclimation differed significantly among clones ; northern clones hardened earlier than those from southern or coastal regions, which sustained winter injury to branch tips in late fall and early winter, although all clones withstood $-90°C$ by early December (15). In many northern tree species, decreasing photoperiods cause growth cessation of plants by triggering the onset of bud dormancy, and then hardiness increases from fall to winter. However, Irving and Lanphear (3) clearly demonstrated that low temperatures (at 5°C for 8 hrs), given during the dark period with long days under controlled conditions, induced hardiness to a level not significantly different from that of short days, without developing bud dormancy. This fact indicates that bud dormancy and cold hardiness development are distinctly separate and independent. In Sapporo, decreasing temperature in fall causes growth cessation in most subtropical willows in late october, though these buds do not enter dormancy. Usually the shoots of subtropical and tropical willows sustained frost injury in early or mid November in Sapporo. However, the middle and basal parts of shoots generally remained uninjured and their hardiness increased until mid December depending upon the genetic ability of the willow concerned. *Salix tetrasperma* from Lucknow (India) did not cease growth even in mid November but twigs of this willow became hardy to $-25°C$ in mid December. These facts support the consideration of Irving and Lanphear (3).

As reported in a previous paper (11), the twigs of subtropical willows above the snow level sustained winter desiccation by late March in Sapporo, through little or no winter injury was observed in the willows collected from warm-temperate climates in the same locality.

George and Burke (2) performed DTA experiments on twigs collected in midwinter from trees over the natural range of red oak, yellow birch, black walnut and wild cherry. The trees were grown from seeds collected from native stands, and grown on a single site. For the four species tested low temperature exotherms were formed in all trees and the exotherm temperatures were generally close to $-40°C$. From these results they considered that woody species with low temperature exotherms are not evolving away from supercooling as a freezing resistance mechanism and are therefore physically limited in their migration northward. In contrast, in most of the willows native to the tropics and subtropics, the cortex survived freezing between -20 and $-70°C$ depending upon their native regions and the xylem sustained injury between -20 and $-35°C$. However, little or no low temperature exotherm associated with xylem injury was detected. The same result was obtained in the less hardy twigs, both in fall and spring, of very hardy willow, *S. sachalinensis*, native to a cool-temperate climate in Japan (12). These results suggest that willow twigs all survive sub-freezing by tolerating extracellular freezing, but not by freezing avoidance. The question still remains as to why the genus *Salix* could not develop the ability to avoid freezing to low temperatures by supercooling, like most temperate deciduous hardwood species.

It is presumed that the genera of *Populus* and *Salix* were differentiated from primitive poplars (*Populus* subgen. *Turanga* (6)). At present *Populus* subgen. *Turanga*, which is divided into two species, *P. euphratica* and *P. pruinosa*, occurs in the Near East and Central Asia. Although considerable uncertainty exists as to the origin and the original distribution of *Salix*, its ancestral species is presumed to have been distributed in the Near East in the Mesozoic era. In this era the climates in the Near East, India and northern Africa are known to have been cold, because the South Pole is presumed to have been located in the Indian ocean and the equator passed by way of Celebes Island, South China, northern Kazakh of the USSR and France. In the beginning of early Tertiary time, the equator began to move toward the present equator and finally settled about 40 million years ago. As a result of the move, the climates in the Near East, India and northern Africa became hot. Thus, some species of *Salix* extended northward from their original areas into more severe cold northern regions. However, some *Salix* species have remained in their original areas and adapted to tropical climates. At present, *Salix* occurs in a wide range from the tropics to the Arctic circle in the North Hemisphere. The species native to tropical and subtropical areas are the primitive form of *Salix*, whereas the species native to northern areas show higher levels of evolution. The willows native to the tropics still retain the ability to tolerate freezing to $-10°C$. And most willows native to subtropical areas of Africa and India have a high degree of freezing tolerance, unlike other plant species occurring in the same localities as the willows, although they have not been exposed to low temperatures for countless generation.

Literature Cited

1. George, M. F., M. J. Burke, H. M. Pellet and A. G. Johnson. 1974 Low temperature exotherms and woody plant distribution. HortScience, **9**: 519-522.
2. ——— and ———. 1977 Cold hardiness and deep super-cooling in xylem of Shagbark hickory. Plant Physiol., **59**: 319-325.
3. Irving, R. and F. O. Lanphear. 1967 Environmental control of cold hardiness in woody plants. Plant Physiol., **42**: 1191-1196.
4.* Kimura, A. 1936 Primitive willows in America. Shokubutsu and Dobutsu, **4**: 143-146.
5. ———. 1938 Symbolae Iteologicae VI. Science Report Tōhoku Imp. Univ. Ser. 4 Biology, **13**: 381-394.
6. ———. 1939 Symbolae Iteologicae VII. Science Report. Tōhoku Imp. Univ. Ser. 4 Biology, **14**: 191-193.
7. Palgrave, K. C. 1977 Trees of Southern Africa. C. Struik Publishers, Cape Town, 959 pp.
8. Pauley, S. S. and T. O. Perry 1954 Ecotypic variation of the photoperiodic response in populus. J. Arnold Arboretum, **35**: 167-188.
9. Qumme, H. C., C. Stuschnoff and C. J. Weiser 1972 The mechanism of freezing injury in xylem of winter apple twigs. Plant Physiol., **51** : 273-277.
10. Sakai, A. 1960 Survival of the twigs of woody plants at $-196°C$. Nature, **185**: 393-394.
11. ———. 1970 Freezing resistance in willows from different climates. Ecology, **51** : 485-491.
12. ———. 1978 Freezing tolerance of evergreen and deciduous broad-leaved trees in Japan with reference to tree regions. Low Temp. Sci., Ser B: **35**: 1-19.
13. ———. 1978 Low temperature exotherms of winter buds of hardy conifers. Plant and Cell Physiol., **19**: 1439-1446.
14. ——— and C. J. Weiser. 1973 Freezing resistance of trees in north America with reference to tree regions. Ecology, **54**: 118-126.
15. Smithberg, M. H. and C. J. Weiser. 1968 Patterns of variation among climatic races of redosier dogwood. Ecology, **49**: 495-504.

* In Japanese with English summary.