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Physiological and Ecological Studies in Environmental Adaptation of Plants
II. Freezing Resistance of Weed Seedlings Influenced by Difference in the Altitude of the Habitats of Parent Plants

By OSAMU SHIBATA

Department of Biology, Faculty of Science,
Shinshu University
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Summary

Seeds of *Pennisetum alopecuroides*, *Plantago asiatica*, *Setaria viridis*, *Setaria glauca*, and *Achyranthes Fauriei* were collected from altitudinally different habitats, and, after germination on a constant temperature, freezing resistance in these seedlings was investigated.

Freezing resistance, varying in degree according to each species, was intensified with the increasing elevation of the seed origin. In most species tested, freezing resistance was more intensitive on the seedlings derived from the Pacific Ocean side than Japan Sea side although the altitude of the seed origin was the same (about 30m) in both cases. The freezing resistance of *P. alopecuroides* seedlings was intensified by decreasing the incubation temperature for seed germination, but the difference in freezing resistance according to the altitude of the seed origin was never removed.

The degree of freezing resistance in seedlings seems to vary according to the temperature level at the germination period, although the degree of resistance is determined by the environmental temperature of the original habitat. The varying level of freezing resistance seems to be a factor in permitting species-distribution into a new habitat, especially a higher or colder one.

Introduction

The vertical distribution of plants has been considered to result mostly from the fact that every species of plant requires a particular temperature level for adequate growth. In practice, however, their habitats are never limited to a single vegetation zone, because most plants have a physiological plasticity as regards temperature, varying in degree according to species. Therefore, it is usual that

there is a considerable temperature difference between the upper limit area and the lower limit area of habitats for a given species. For plants in such environmental conditions, the possibility that a large difference in the habitat's temperature can cause an essential change in the temperature characteristics of plants has been reported by some workers (1, 3, 9, 10).

SHIBATA (11) has reported that, in the same species, the seeds of some plants growing at high altitudes could germinate at a lower temperature than those of plants growing at low altitudes. These results may suggest that the temperature level for seed germination is essentially different in the two kinds of seeds.

When daytime temperatures at high altitudes are relatively mild and suitable for germination, night temperatures reach low lovels, often going below freezing point, especially in the late spring and early autumn. Therefore, although seeds originating at low altitudes or in a warm area could germinate at high altitudes or in a cool area, such seedlings would find it very difficult to survive in a habitat with such intense cold at night, when they have no tolerance to freezing.

This paper will report on the freezing resistance of weed seedlings varying according to the altitude of the haditat in which the parent plants grew.

Materials and Methods

Weed seeds of various species (*Pennisetum alopecuroides*, *Plantago asiatica*, *Setaria viridis*, *Setaria glauca*, and *Achyranthes Fauriei*) were collected from several sites altitudinally different yet abot equal in latitude 36 16'N (11), and, for freezing, seedlings of about 5 days old from these weed seeds were used. The incubation temperature for seed germination and growth was usually 25°C, and in some cases 15° or 35°C.

A procedure to test the freezing resistance of woody plants has been established by SAKAI (6), and SAKAI and OTSUKA (7), and the procedure has also been applied to adult herbs and ferns (5, 8). In the present case, however, the freezing technique for herb seedlings was modified in some aspects from that of SAKAI and OTSUKA's (7) because, in a preliminary experiment, it was ascertained that the temperature lavel which causes freezing injury to the herb seedlings was considerably higher than in the case of the mature plants. About 10 seedlings packed in Saran Wrap sheet were used for each freezing experiment. After the seedlings were kept at 5°C for 1 hr, they were exposed to 0°C for 2 hrs. The cooled seedlings were transferred at hourly intervals to successively colder temperatures varying by 1°C until the desired temperatures were attained. After 1 hr exposure at the desired temperature, the frozen seedlings were thawed in the air at 0°C for 1 hr. To determine the survival rate, they were kept for 7 days at room temperature in the same Saran Wrap sheet packs, and the surviving seedlings were then counted. The lowest

temperature at which they could survive was expressed as the degree of freezing resistance of the seedlings according to SAKAI and OTSUKA (7).

Results

The results obtained with *Pennisetum alopecuroides* were shown in Table 1. Freezing resistance in the seedlings germinated and grown at 25 °C gradually increased with the altitudinal increase of the seed collection sites. The seedlings derived from seeds at 30m altitude on the Japan Sea side and on the Pacific Ocean side, or from those collected at 300m and 600m altitudes, showed the same degree of freezing resistance at −1°C, but the survival rate at a lower temperature was higher on the Pasific Ocean side or at the higher altitudes. In *Pennisetum*, the maximum level of resistance appeared at −4°C in seedlings derived from seeds at 900m altitude which is the highest among the habitats.

With *Plantago asiatica* (Table 2), a clear relation between habitat altitude and survival rate was not found. The freezing resistance in seedlings derived from seeds at 1500m altitude, however was remarkably higher than those derived from

Table 1. Freezing temperature and survival rate of *Pennisetum alopecuroides* seedlings derived from various altitudes of seed origin. Seed germination and seedling growth were incubated at 25°C. J : Japan Sea side, P : Pacific Ocean side.

Altitude of seed origin (m) / Freezing temp. (°C)	30J	30P	300	600	900
−1	100	100	100	100	100
−2	13	73	100	100	100
−3	0	0	45	90	100
−4	0	0	0	0	10
−5	0	0	0	0	0

Table 2. Freezing resistance and survival rate of *Plantago asiatica* seedlings derived from various altitudes of seed origin. Seed germination and seedling growth were incubated at 25°C. J : Japan Sea side, P : Pacific Ocean side.

Altitude of seed origin (m) / Freezing temp. (°C)	30J	30P	300	600	900	1500
−2	100	100	100	100	100	100
−3	100	100	100	71	100	100
−4	0	86	57	45	86	100
−5	0	14	30	27	0	100
−6	0	0	0	0	0	57
−7	0	0	0	0	0	71

Table 3. Freezing resistance and survival rate of *Setaria viridis* seedlings derived from various altitudes of seed roigin. Seed germination and seedling growth were incubated at 25°C. J : Japan Sea side, P : Pacific Ocean side.

Altitude of seed origin (m) / Freezing temp. (°C)	30J	30P	300	600	900
−1	100	100	100	100	100
−2	100	100	100	100	100
−3	75	67	100	60	75
−4	10	0	0	0	0
−5	0	0	0	0	0

Table 4. Freezing resistance and survival rate of *Setaria glauca* seedlings derived from various altitudes of seed origin. Seed germination and seedling growth were incubated at 25°C. J : Japan Sea side, P : Pacific Ocean side.

Altitude of seed origin (m) / Freezing temp. (°C)	30J	30P	300	600	900	1500
−1	100	100	100	100	100	100
−2	100	100	100	100	100	100
−3	0	100	20	75	100	100
−4	0	75	10	63	30	38
−5	0	0	0	0	0	0

seeds at habitats below 900m altitude, and the survival rate of the former was still about 70% at −7°C.

The results in the case of *Setaria viridis* and *S. glauca* were shown in Table 3 and 4. The lowest degree of freezing resistance in *S. viridis* seedings derived from seed at 30m altitude on the Japan Sea side was −4°C, being higher than the degree in seedlings derived from seeds from other habitats, but this difference appeared to be negligible because the survival rate of the former was very low. Therefore, the degree of freezing resistance for the *S. viridis* was suggested to be about the same in all the habitats as between −3° and −4°C. The degree of freezing resistance in *S. glauca* seedlings derived from seeds at 30m altitude on the Japan Sea side and from seeds at the other sites, however, was clearly lower in the former case than in the latter.

In order to investigate whether the incubation temperature for seed germination and growth influences the degree of freezing resistance or not, the freezing resistance in the seedlings germinated and grown at, variously, 15°, 25°, or 35°C was examined by using *P. alopecuroides* which showed a clear relation between its seed origin and the degree of freezing resistance. The relation between the freezing

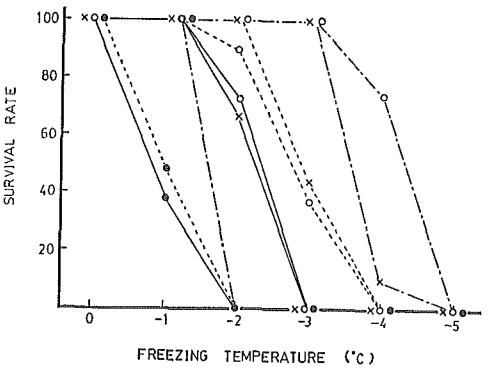

Fig. 1. The relation between freezing resistance in *Pennisetum alopecuroides* seedlings deriving from various altitudes of seed origin and the incubation temperature for seed germination and seedling growth. Incubation temperature : 15°C ; ○, 25°C ; ×, 35°C ; ●, seed origin : 30 (J)m ; ——, 300m ; ……, 900m ; —·—.

temperature and the seedlings' survival rate, varying according to incubation temperature, was shown in Fig. 1. With seedlings derived from the plants having their habitats at 30m on the Japan Sea side or at 300m altitude, those incubated at 35°C were about the same in survival rate, but with seedlings derived from the other habitats, the survival rates increased in proportion to the increasing altitude of their seed origin and to the lowering of the incudation temperatures.

A similar result was also obtained on *A. Fauriei* seedlings

Table 5. Freezing resistance and survival rate of *Achyranthes Fauriei* seedlings derived from various altitudes of seed origin. J : Japan Sea side, P : Pacific Ocean side.

Freezing temp. (°C)	Incubation temp. (°C)	Altitude of seed origin (m)				
		30J	30P	300	600	900
−2	15	100	100	100	100	100
	25	100	100	100	100	100
−3	15	100	100	100	100	100
	25	50	100	100	100	100
−4	15	100	100	100	100	100
	25	13	20	57	60	100
−5	15	0	0	50	100	100
	25	0	0	14	20	25
−6	15	0	0	13	57	100
	25	0	0	0	0	0

(Table 5). In the case of *A. Fauriei* seedlings from 900m altitude, the freezing resistance greatly increased in those incubated at 15°C rather than at 25°C

Discussion

Studies on the freezing resistance of plants have generally been carried out with woody plants, and relatively rarely with weeds (5, 8). In addition, the specimens used for these studies were, in most cases, mature plants. Because freezing

injury in weed seedlings results from higher temperatures than in mature plants, a study of freezing resistance in seedlings seems to be more important than in mature plants in discussing plant distribution. The reason is that the temperatures at which weed seedlings of some species are damaged by freezing, but at which the mature plants can survive, are often observed during spring and autumn nights in the field.

P. alopecuroides and *S. viridis*, in general, possessed a lower degree of freezing resistance than the other species, and the result seems to be in agreement with a field observation that the upper elevation of vertical distribution of the two species is relatively low (11). Except for *S. viridis*, the species used in the research had a higher degree of freezing resistance on the Pacific Ocean side than on the Japan Sea side, although the seeds were collected at about the same height of altitude. In addition, a difference between the two sides has been found with regard to the germination rate of seeds of some species (11). These physiological differences may be factors in the characteristics of Japan Sea elements (2, 4).

It is well known that *P. asiatica* has a wide distribution range vertically as well as horizontally. The ability to expand the distribution range was verified by the high degree of freezing resistance of such seedlings, and the fact that the survival rate of seedlings derived from 1500m altitude was still high even at −7°C suggests that this species has a possibility of being distributed at yet higher altitudes. In fact, *P. asiatica* has been found at a maximum altitude of around 2300m in the Northern Japan Alps (11). In *P. asiatica* at 1500m altitude, the leaves are pubescent in most plants, but the pubescent character seems to be weakened by transplantation to a lower elevation (10). It is not clear at present whether this pubescent character has a relation to the high degree of freezing resistance or not.

As well as *P. asiatica*, *A. Fauriei* seedlings germinated at 15°C showed a very high degree of freezing resistance. It is suggested that the intense freezing resistance related to the high altitude of the seed origin and to the germination at low temperatures to some process of metabolic change, though at present the physiological process for it is not clear in detail.

The fact that some species used in the present research raised their degree of freezing resistance with the lowering of the incubation temperature, suggests that these species may be able to expand their distribution range to far higher altitudes or to much cooler areas. On the other hand, relatively warm temperatures at ground level in spring may be effective in weakening their freezing resistance, although the species seedlings possessed sufficient ability to resist cold temperatures. The balance between the two possibilities suggested above, which counteract each other, seems to be a factor in determing whether the seedlings are able to

gain a new habitat or not.

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