Factors Modifying Frost Tolerance of Legume Species

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ABSTRACT

Variability in seedling death of legumes because of spring frost in the USA and Canada is associated with several factors. Experiments were conducted to evaluate factors that modify frost tolerance of seedling legumes. Experiment 1 was comprised of two hardenings, three freezing temperatures, eight legume species, and four growth stages. Experiment 2 had three temperatures, four durations of temperature, and four legume species. Experiment 3 included three soil types, two soil water levels, and two legume species. At each growth stage, legume seedlings were placed in a programmable freezing chamber at 3°C and temperature decreased/increased 1°C h^{-1} to and from a minimum freezing temperature. Duration of minimum freezing temperature was 1 h for Experiments 1 and 3 and varied according to the treatment in Experiment 2. Hardening increased seedling survival up to 40% over unhardened seedlings across growth stages and species. Forage legumes were more frost tolerant than soybean [*Glycine max* (L.) Merr.] and field pea (*Pisum sativum* L.) at all temperatures. Increase in duration of freezing temperature decreased the frost tolerance of all species when freezing temperature was near the LT_{50} (temperature that kills 50% of seedlings). Seedling survival of both alfalfa (*Medicago sativa* L.) and soybean was greater in light-textured soil than the heavy-textured soil with soil water at field capacity. However, one-third of field capacity soil water allowed greater seedling survival in the heavy-textured than the light-textured soil. The results suggest that the factors studied should be considered to assess the frost tolerance of legume seedlings.

FROST is the temperature that causes freezing. Frost tolerance is the ability of a plant to resist freezing temperature. Freezing temperature is the temperature below the freezing point (0°C). Hardening is a physiological change of a plant with cold temperature treatment (commonly termed as vernalization).

Establishment of fall- and spring-seeded legumes occasionally ends in failure because of climatic hazards. Spring-seeded grain and forage legumes, and legume-grass mixtures cover a significant hectarage in the northern states of the USA. One hazard of these crops is the loss of seedling stand because of freezing temperature, which may occur from early April through the first part of June in the northern Great Plains. Seedling stand loss for both grain and forage legumes has been reported in cooler areas of the USA and Canada (Brown and Blackburn, 1987; Annu. Res. Report No. 16, 1998). In spite of this hazard, few studies have been conducted on frost tolerance of grain and forage legumes under conditions more typical of the field. Most of the previous research evaluated factors related to over wintering of forage legumes (Megee, 1935; Steinmetz, 1926; Jung and Smith, 1961; Calder et al., 1966).

Variability in factors associated with the effect of temperature makes it more difficult to decide how much damage occurs from low temperature alone. Factors such as crop species, growth stage, duration of freezing temperature, soil moisture, soil type, hardening, freezing and thawing sequences, occurrence of pathogens, and insect pests contribute to a highly complex pattern that determines frost tolerance of a particular species (Brandsaeter et al., 2000). Hume and Jackson (1981) evaluated 30 genotypes of soybean from different sources and maturity groups at −2, −2.5, and −3°C at the cotyledon, unifoliolate, and first trifoliolate leaf stages in New Zealand. They found that most death occurred at −3°C when grown in 25/19°C (day/night) temperature compared with those at 15/9 and 20/14°C. This indicated that exposure to low temperature before freezing increased the freezing tolerance of soybean seedlings. Calder et al. (1965) studied both greenhouse and field-grown alfalfa seedlings. They hardened seedlings at 2°C for 48 h and found that unhardened alfalfa was killed at −4.5°C and hardened alfalfa survived. However, at −3.5°C both hardened and unhardened plants survived and hardened plants grew an average of 50 mm more than unhardened alfalfa during the 2 wk of recovery. The 2-d hardening period significantly increased tolerance of alfalfa seedlings to freezing temperature.

Peltier and Tisdal (1932) reported that survival percent for 35-d-old alfalfa seedlings submitted to −13.6°C temperature increased from 0 to 93% when the hardening period increased from 4 to 14 d. However, 9- to 14-d-old alfalfa and red clover (*Trifolium pratense* L.) seedlings had 100% survival at −4.1°C temperature when hardened compared with 39 and 30% survival, respectively, without hardening (Tisdal and Pieters, 1934).

Growth stage was found to be very important for tolerance to low temperature. Hume and Jackson (1981) reported that the cotyledonary and first trifoliolate leaf stages of soybean were more tolerant to freezing temperature (−3.8°C) than the unifoliolate leaf stage. However, Hicks (1978) indicated that the soybean plant tolerated freezing temperature more at the unifoliolate leaf stage than at the third trifoliolate leaf stage.

Calder et al. (1965) reported that legume seedlings were very susceptible to freezing temperature in the vegetative stage (up to 48 d after seeding) compared with later stages. However, Peltier and Tisdal (1932) and Tisdal and Pieters (1934) reported that seedlings 2 wk or more after emergence were more tolerant to low temperature. None of these investigators reported a uniform survival rate of the seedlings at a particular temperature. As a result, the data could not be reproduced by other researchers.

Arakeri and Schmid (1949) grew alfalfa, sweetclover (*Melilotus officinalis* Lam.), red clover, alsike clover

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Abbreviations: LT_{50}, temperature that kills 50% of seedlings.

(*T. hybridium* L.), and white clover (*T. repens* L.) in the greenhouse at 20°C. Seedlings of the desired stage were hardened at 4°C for 15 d and then at 10°C for 2 d in a growth chamber. These were then placed in a freezing chamber at −10°C for 8 h and then transferred back to the greenhouse at 20°C for 2 wk to obtain survival counts. They found that seedlings grown for 1, 2, and 3 wk in the greenhouse before hardening had less than 20% survival rate across all legume species. Alfalfa had greater survival (23%) by the fourth week than later stages, but alsike, red, and white clovers had more survival after 5 wk of growth. Sweetclover could not survive until 9 wk of growth. This result may not represent seedling survival under field conditions where hardening temperature typically prevails only a short time period in the spring (0–3 d common) and only rarely does a minimum temperature hold for extended periods in the northern Great Plains.

Meyer and Badaruddin (2001) conducted a series of experiments with 10 legume species at four growth stages and at −2, −4, −6, and −8°C temperature. Legume seedlings were hardened for 3 d and frozen for 1 h at the desired minimum temperature in a freezing chamber where temperature decrease/increase was 1°C h^{-1} to/from a minimum temperature. The LT_{50} temperature of alfalfa, red clover, sweetclover, alsike clover, white clover, sainfoin (*Onobrichis viciifolia* Scop), soybean, field pea, and navy and pinto (*Phaseolus vulgaris* L.) beans were −7.0, −6.3, −6.8, −7.4, −7.1, −7.3, −4.6, −5.6, −3.5, and −3.3°C, respectively. They indicated that the LT_{50} was the most critical temperature below which seedling death occurred fast for a particular legume species.

Seedlings from an old stand of alfalfa survived more in a light-textured, well-drained soil than poorly drained, heavy-textured soil (Russell et al., 1978). Peltier and Tisdal (1932) and Calder et al. (1965) indicated that seedling survival rate of alfalfa increased with increase in soil moisture from 25% field capacity to saturated condition. The interaction between legume seedling growth stage and soil water content was not significant.

Delayed planting of warm-season dry beans may reduce the risk of spring frost, but the risk of a killing fall frost is increased by delayed planting in short growing-season environments (Halvorson et al., 1995). Delayed planting causes economic losses through the reduction of yield and quality of soybean (Helms et al., 1990) and dry beans (Blaylock, 1995). Sims et al. (1989) reported that warm-season dry beans should be seeded as early in May as machinery can safely be used in the field. They also suggested that, for dry beans, seeding should not be delayed beyond May or first week of June in order for the crop to mature prior to the first killing frost in fall.

Previous studies have evaluated several factors associated with the temperature tolerance of seedling legumes, but most studies were conducted under conditions not commonly prevailing in the northern Great Plains. Therefore, the objective of this study was to determine how legume species, hardening, freezing temperature, duration of freezing temperature, soil type, and soil moisture modify the frost tolerance of legume seedlings under conditions more commonly prevailing in the northern Great Plains.

MATERIALS AND METHODS

General Procedure

Experiments were conducted in the greenhouse and growth chamber. Pot size was 100 by 100 by 90 mm. A greenhouse mixture (1:1) of Fargo clay (fine, montmorillonitic, frigid Vertic Haplaqoull) and Sunshine mixture (Sun Gro Horticulture Canada Ltd., Bellevue, WA) was used as the rooting media. Ten to 12 seeds for forage legumes (alfalfa, red clover, sweetclover, alsike clover, white clover, and sainfoin), and three to four seeds for grain legumes (soybean and field pea) were seeded at 15- to 20-mm and 20- to 25-mm depths, respectively. Seedlings were hand thinned to six plants per pot for forage legumes and two plants per pot for grain legumes. The greenhouse was maintained at 25/19°C day/night temperature. Sodium vapor lights were used to supplement light intensity from about 2 m above the plant canopy during daylight (about 14 h). Average light intensity was 1392 μmol m^{-2} s^{-1}. The supplemental light helped maintain good and uniform growth and development for the legume species.

Plants at the appropriate growth stage were hardened in a vernalization chamber maintained at 3.5 to 4°C temperatures for 3 d. After hardening, the plants were put in a growth chamber at 15/5°C day/night temperature for 2 d. The plants were then placed in a programmable freezing chamber at 3°C and the temperature decreased 1°C h^{-1} until the desired minimum temperature was reached. The temperature was increased from the minimum temperature at 1°C h^{-1} until 3°C was reached.

After freezing, the plants were transferred to a growth chamber (same as before) for 1 d before they were transferred to the greenhouse (environmental conditions same as before). Unfrozen control pots for each species were maintained in the growth chamber while freezing occurred. In the greenhouse, all the plants were observed for 2 wk.

Number of dead plants, plant height (measured from ground to the tip of the youngest leaf), above-ground dry biomass, regrowth potential, and leaf chlorosis (% of leaf area) were determined at 10 to 14 d after freezing. Biomass was dried for 72 h at 60 to 65°C. Regrowth potential was recorded on a 0-to-4 scale, where 4 was considered equal to the nonfrozen control plants and 0 equal to no regrowth. Initiation of a new leaf was considered start of regrowth within 2 wk after freezing. Leaf chlorosis was recorded by visual estimate on total leaf area in each pot. Biomass yields and plant heights were converted to percentage of the control plants.

A factorial randomized complete-block design with four replicates was used for all experiments. Each experiment had two runs in the freezing chamber. Data were analyzed by PROC GLM in SAS (SAS Institute, 1990). Run in the freezing chamber was considered a random factor, and all other treatments were considered fixed effects.

Specific Procedures

Experiment 1

Seedling growth stages of 1, 2, 3, and 4 wk were subjected to −4, −6, and −8°C freezing temperatures with and without hardening. Legume species evaluated were ‘Vernal’ alfalfa, ‘Arlington’ red clover, yellow-flowered sweetclover, ‘Aurora’ alsike clover, white clover, ‘Eski’ sainfoin, ‘Trail’ soybean, and ‘Trapper’ field pea. In general, legume species (except field pea) had hypocotyl arc, unifoliolate leaf, first trifoliolate leaf, and second trifoliolate leaf stages corresponding to 1, 2, 3, and 4 wk of age. Field pea had 1, 2, 3, and 4 true leaves during 1, 2, 3, and 4 wk of age, respectively.

Unhardened plants were transferred directly from the greenhouse to the freezing chamber and from the freezing chamber to the greenhouse after being frozen. Separate runs were conducted for each hardening process by growth stage by freezing temperature combination. Plants were kept 1 h at the minimum temperature. A total of 48 runs of the programmable chamber (2 hardening processes by 4 seedling ages by 3 temperature by 2 runs) were conducted. Correlation coefficients were determined for regrowth potential and percent leaf chlorosis (independent variable) with percent seedling survival (dependant variable) averaged across the legume species at −6°C.

Experiment 2

Freezing temperature was −4, −5, and −6°C, duration of freezing temperature was 0.5, 1, 2, and 4 h, and legume species were Vernal alfalfa, Arlington red clover, Trail soybean, and Trapper field pea. Legume seedlings were kept at the minimum temperature according to prescribed treatment. Separate runs were conducted in the freezing chamber for each freezing temperature by duration of freezing temperature combination. Two-week-old seedlings were used for this experiment.

Experiment 3

This experiment evaluated the effects of soil type and soil water on seedling survival of alfalfa and soybean separately near its specific LT_{50} (Meyer and Badaruddin, 2001). Soil types were Fargo clay, Heimdal loam (Heimdal-Emrick loam, Heimdal-coarse-loamy, mixed Udic Haplaborolls, Emrick-coarse-loamy, mixed Pachic Udic Haplaborolls), and a greenhouse mixture (1:1 Fargo clay and sunshine mix). Soil water levels were raised to field capacity and one-third field capacity before transferring the pots to the freezing chamber. Field capacities for Fargo clay and Heimdal loam were 0.3448 and 0.2217 g of water/g of soil, respectively, determined by Joel Bell, Soil Physics Research Specialist, Department of Soil Science, North Dakota State Univ., Fargo, ND. The greenhouse mixture was watered with a volume corresponding to the volume required for the Heimdal loam for different treatments. Two-week-old Vernal alfalfa and Trail soybean were used as test species for this experiment. Minimum freezing temperature for alfalfa and soybean were −7 and −5°C, respectively, which was near the respective LT_{50} (Meyer and Badaruddin, 1999). In the freezing chamber, separate runs were conducted for each species. The duration of minimum temperature was 1 h. Since the minimum freezing temperatures were different for two species, data were analyzed and presented separately.

RESULTS

Experiment 1

Freezing temperature ($P < 0.001$), growth stage ($P < 0.05$), legume species ($P < 0.01$), and temperature by species interaction ($P < 0.05$) were significant for percent seedling survival. The hardening process, temperature, and legume species interactions ($P < 0.05$) were significant also for seedling survival.

When averaged across legume species, unhardened seedling survival was from 2 to 40% less than that of hardened seedlings as temperature decreased from −4 to −8°C (Table 1). Hardening increased seedling survival of field pea by 33% over unhardened at −4°C, but hardening had no effect on the small-seeded legumes, sainfoin, and soybean at this temperature. Seedling survival of small-seeded legumes and sainfoin was increased by 7 to 32% with hardening at −6°C, with alfalfa an exception. Hardened soybean and field pea seedlings had significantly higher survival (113% for soybean and 50% for field pea) compared with those of unhardened seedlings at −6°C. Hardening had no effect on survival of soybean and field pea at −8°C temperature because all seedlings (hardened and unhardened) were killed. However, seedling survival responses of forage legumes to hardening were 10 to 250% greater than unhardened seedlings at −8°C.

Regrowth potential and leaf chlorosis were significantly different for the legume species studied and were highly correlated (r = 0.84*** and −0.80***, respectively) with seedling survival (Table 2). Leaf chlorosis was greater for both soybean and field pea than forage legumes. Sweetclover and alsike clover had less leaf chlorosis than the other forage legumes. The regrowth potential of forage legumes were equal (except white clover), and greater than regrowth potential of soybean and field pea. Dry matter and plant height were not significant.

Table 1. Percent of legume seedling survival for two hardenings, three temperatures, and eight legume species across four growth stages.

	Unvernalized			Vernalized		
	Temperature, °C					
Legume species	−4	−6	−8	−4	−6	−8
	% seedling survival					
Alfalfa	100.0	77.2	18.0	100.0	77.6	26.3
Red clover	88.0	60.3	4.5	92.8	67.2	15.3
Sweetclover	100.0	68.3	10.2	99.0	88.1	16.3
Alsike clover	100.0	78.3	27.0	99.5	89.1	40.7
White clover	100.0	79.3	18.1	99.0	93.3	20.0
Sainfoin	100.0	68.7	21.7	99.5	90.0	20.1
Soybean	65.6	12.5	0.0	60.9	26.6	0.0
Field pea	46.9	21.9	0.0	62.5	32.8	0.0
LSD(0.05)			9.8			

Experiment 2

Temperature ($P < 0.01$), duration of minimum freezing temperature ($P < 0.05$), legume species ($P < 0.01$),

Table 2. Dry matter yield, leaf chlorosis, regrowth potential, and plant height of eight legume species across four growth stages at −6°C.

Legume species	Dry matter yield	Leaf chlorosis	Regrowth potential	Plant height
	% of control			
Alfalfa	57.8	33.5	71.4	72.0
Red clover	59.6	37.3	71.3	78.8
Sweetclover	62.7	18.0	76.8	71.7
Alsike clover	56.5	17.8	78.2	70.5
Sainfoin	63.5	30.5	77.7	77.9
White clover	57.1	20.8	78.5	71.8
Soybean	68.5	89.6	11.3	78.2
Field pea	59.6	77.7	27.3	70.1
LSD(0.05)	NS	12.6	11.2	NS

NS = Not significant at 0.05 probability level.

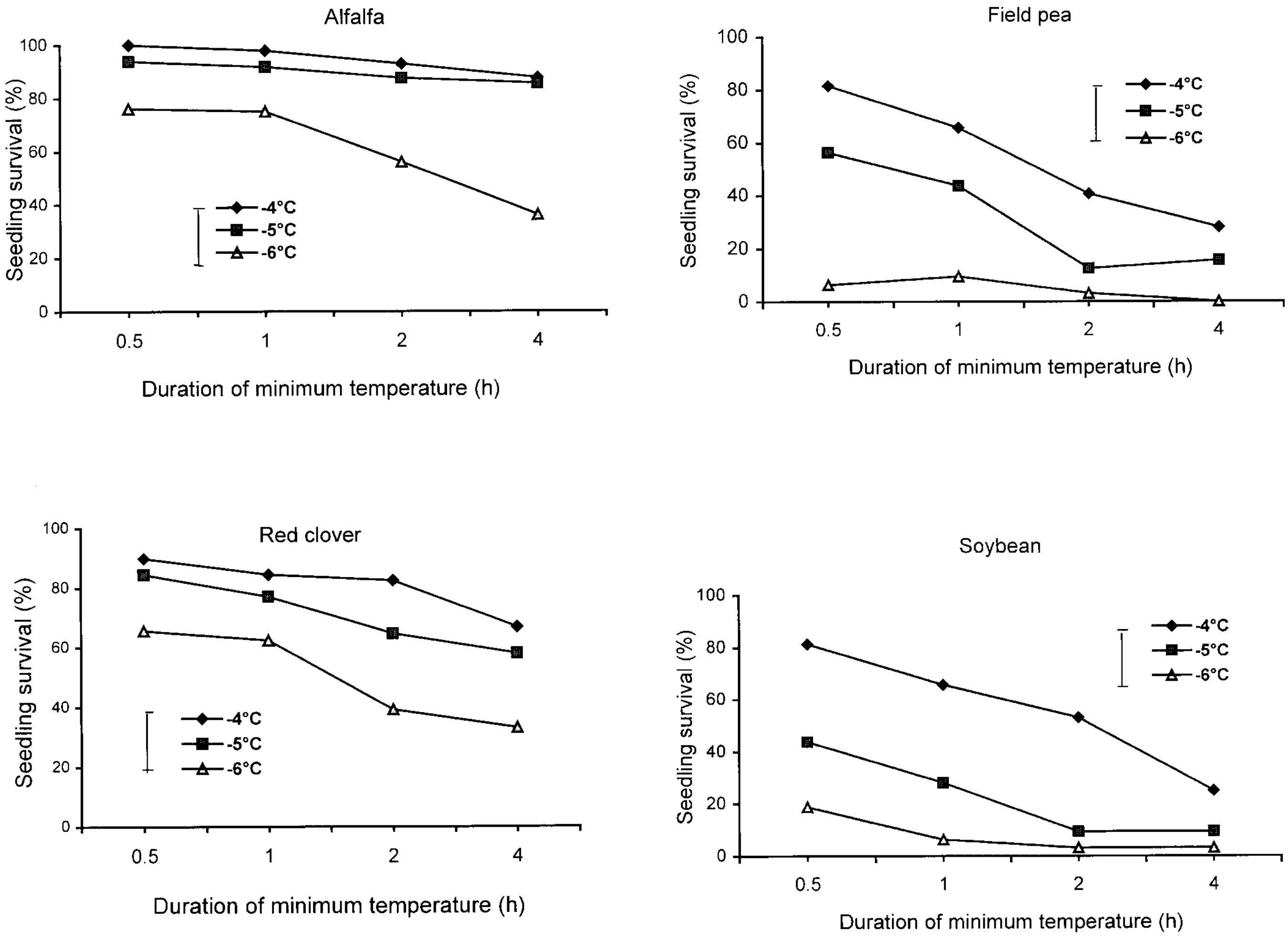

Fig. 1. Interaction effects of three temperatures and four durations of minimum temperature on percent seedling survival of alfalfa, red clover, field pea, and soybean. The vertical bar is the LSD(0.05).

and their interactions ($P < 0.05$) on seedling survival were significant. Alfalfa seedling survival did not vary with duration of temperature at −4 and −5°C (Fig. 1). In contrast seedling survival of red clover at −4 and −5°C was reduced significantly at 4-h duration compared with 0.5 to 2 h. At −6°C, seedling survival of both alfalfa and red clover was significantly lower at 2- to 4-h duration than 0.5 to 1 h.

Field pea and soybean seedling survival was reduced significantly at 2- and 4-h duration compared with 0.5 and 1 h at −4 and −5°C temperatures (Fig. 2). Percent seedling survival for both these species was too low at −6°C to evaluate duration effects.

Experiment 3

Soil type and soil water interactions were significant for seedling survival of both alfalfa and soybean (Fig. 3). Seedling survival for both species followed a similar response at field capacity in light-textured greenhouse mixture and Heimdal loam but greater than heavy-textured Fargo clay soil. However, seedling survivals of alfalfa and soybean were more in Fargo clay than light-textured greenhouse mixture and Heimdal loam at one-third field capacity.

DISCUSSION

Experiment 1

Hardening of seedlings had substantial positive effects (up to 40%) on seedling survival over the unhardened seedlings at a particular temperature across legume species (Table 1). The effects of hardening were specific to a species and temperature but were not specific to a growth stage. Legume species response to hardening was greater when temperature slightly exceeded the critical killing temperature (LT_{50}) for a particular species (Meyer and Badaruddin, 2001). For example, seedling survival of field pea was increased 33% by hardening at −4°C (slightly greater than the LT_{50} of field pea) and up to 50% at −6°C (lower than LT_{50}). Likewise, seedling survival of forage legumes with hardening was up to 32% greater at −6°C (temperature $< LT_{50}$) and up to 240% at −8°C (temperature $> LT_{50}$) compared with unhardened seedlings of these species. Our results suggest that when freezing temperature exceeds the LT_{50} (lower than LT_{50}) of a particular species, the percent seedling death will be dependent more on hardening than other factors. This means that the greater the speed that temperature drops to a killing temperature, the greater the seedling death that will occur.

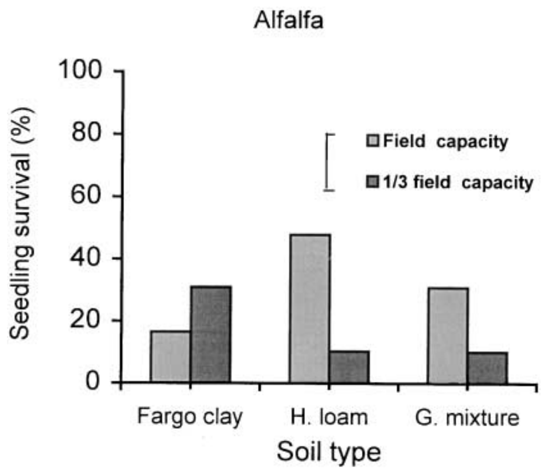

Fig. 2. Interaction effects of soil type [Fargo clay, Heimdahl (H) loam, and greenhouse (G) mixture] and soil water (field capacity and one-third field capacity) on alfalfa. The vertical bar is the LSD(0.05).

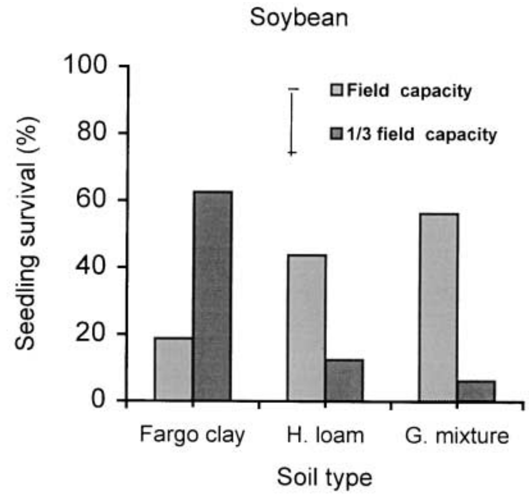

Fig. 3. Interaction effects of soil type [Fargo clay, Heimdahl (H) loam, and greenhouse (G) mixture and soil water (field capacity and one-third field capacity) on soybean. The vertical bar is the LSD(0.05).

Tisdal and Pieters (1934) reported that prehardening of alfalfa seedlings increased percent survival from 0 to 60% when frozen at −4.1°C. However, prehardening increased seedling survival of alfalfa from 0 to 93% over unhardened seedlings when frozen at −13.6°C (Peltier and Tisdal, 1932). The difference in survival was more in 1932 because the freezing temperature was much lower than that in 1934. Our results corroborated those reported by both Tisdal and Pieters (1934) and Peltiers and Tisdal (1932).

Experiment 2

Increasing duration of minimum temperature significantly affected the frost tolerance of the legume species. Temperature duration did not affect the seedling survival of alfalfa at 4 and −5°C, but 2- to 4-h duration significantly reduced the tolerance at −6°C compared with shorter durations. Temperature of −6°C was near the LT_{50} of alfalfa. Seedling survival of red clover at 4 h of duration at −4°C was equivalent to survival at 0.5-h duration at −6°C. Duration had greater effects on survival of soybean and field pea than red clover and alfalfa at all three temperatures. The three freezing temperatures utilized in this experiment were closer to the LT_{50} temperature of soybean and field pea than those of red clover and alfalfa (Meyer and Badaruddin, 2001). This may explain the greater effect of duration of minimum temperature on frost tolerance of soybean and field pea than red clover and alfalfa. The results suggest that, when a minimum freezing temperature is close to the LT_{50} of a legume species, duration of freezing temperature significantly changes the LT_{50} of the species. This also indicates that freezing temperature and the modifying factors studied have less of an effect on a legume species until the minimum freezing temperature approaches the LT_{50}.

Experiment 3

The effect of soil type and soil water on modifying frost tolerance of alfalfa and soybean was tested near their LT_{50}. The greenhouse mixture and Heimdal loam were well drained and coarse textured, and the Fargo clay was poorly drained and heavy textured. Both species showed greater frost tolerance at field capacity when grown on coarse-textured, well-drained soil than heavy-textured, poorly drained soil. However, the percent survival at one-third field capacity was greater on the heavy-textured Fargo soil. Russell et al. (1978) reported that alfalfa seedlings had greater winter losses from frost heaving on poorly drained soil than moderately and well-drained, light-textured soil. Peltier and Tisdal (1932) reported that alfalfa seedling survival was increased from 28 to 33% when soil water content increased from 17 to 33%. However, Calder et al. (1965) reported that saturated soil had an extremely adverse effect on cold hardiness of alfalfa compared with effects at field capacity and 25% field capacity. There was no difference in seedling survival between field capacity and 25% field capacity soil water when averaged across soil types.

CONCLUSION

The factors evaluated in these studies showed that the frost tolerance of legume species could be modified significantly by changing the duration of minimum temperature, hardening prior to freezing, soil type, and soil water. However, the extent to which these factors modify the effect of freezing temperature appears dependent on how close the freezing temperature is to the critical killing temperature (LT_{50}) of each species.

Freezing temperature under field conditions is beyond the control of producers. However, results of this study will give a better understanding in improving management practices of legume crop production in several ways. Depending on soil type and soil moisture at planting, species (genotypes) selection would be a major production decision. If there is a limitation of changing crop species in a particular situation, changing date of planting for species that are vulnerable to a short spring freezing temperature would be an option.

These results have an impact on the hybridization and selection programs of frost-tolerant legume species. This result will help in selecting ideal temperature, length of freezing temperature, soil type, and moisture conditions for testing genotypes of a particular legume species. The results also suggest the importance of further detailed studies on why the species behavior are different with changed conditions while the genetic background is the same.

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