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Evaluation of Freezing Tolerance of Two Fennel *(Foeniculum vulgar* L.) Ecotypes Under Controlled Conditions

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*The planting of fennel (*Foeniculum vulgare *L.) in the fall is important in temperate areas where this leads to more efficient use of limited available water, although winter mortality is a serious barrier for employing a fall planting system for this plant. Improved cold tolerance through selection of frost-resistant ecotypes could reduce winter losses or acclimate the plants to cold temperatures. To determine whether cold acclimation (AC) could reveal potential frost tolerance, two local ecotypes of fennel (Gonabad and Kerman) were exposed to low temperatures under controlled conditions to reveal any potential frost tolerance. Compared with non-acclimated control plants, AC increased survival at a lethal temperature (LT_{50}), the temperature at which plants showed 50% less dry matter (DMT_{50}) and the number of nodes per main stem in both ecotypes at 3 weeks after applying a freezing treatment, whereas plant height and plant dry weight were less in AC treatment compared with non-acclimated (NAC) plants. Percentage of survival and the number of nodes per main stem did not show significant reduction up to −6°C but at a lower temperature was reduced significantly ($p \leq 0.01$). Increasing freezing intensity beyond −3°C reduced the plant height as compared with controls, and plant DMT production was also less in all treatments as compared with 0°C. Plant height, number of nodes per main stem, and plant DMT in the Gonabad ecotype were 73%, 68%, and 146%, respectively, more*

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than the Kerman ecotype at −9°C. The percentage of survival in the Gonabad ecotype at 9°C under AC conditions was 33% more than NAC plants, whereas at the same temperature in the Kerman ecotype, a 94% increase over NAC was seen. Imposing −9°C as compared with 0°C temperature level under NAC conditions caused 52%, and 95% reductions of both Gonabad and Kerman ecotypes, respectively.

KEYWORDS *acclimation, culinary herb,* DMT_{50}*, frost resistance,* LT_{50}*, winter hardening*

INTRODUCTION

An approach to hostile growth environments for major agronomic crops is switching to locally adapted medicinal plants as cash-crops for farmers (2). For many years, fennel (*Foeniculum vulgar* L.) has been used for traditional medical purposes. Though different organs of fennel plants contain chemicals with medical value, most interest is in fennel fruit (commonly known as seeds) from which an essential oil is extracted. Fennel seeds are used as an appetizer and stomach stimulator (24) but also have applications in the cosmetic industry (13), and food technology (12).

In farming systems, spring planting of fennel competes with that of other crops and frequently results in the plant's flowering during periods of high temperatures and poor water supply. Fall planting of fennel could increase the efficiency of winter and spring precipitation and result in higher biomass and subsequently possible higher yield due to a longer growth period. Higher yield and production of crops, such as annual forage legumes (19), lentils (6) and chickpeas (20), have been obtained through fall planting rather than spring planting. Yet, freezing temperatures are a major environmental constraint limiting growth, development, and distribution of fall-seeded plants. An earlier experiment (15) with early November and early December plantings of fennel resulted in 95% and 75% mortality, respectively, owing to winter frost (up to −12°C). These results contrast with no mortality being observed in fennel planting at mid-March and mid-April, indicating that fall planting can be possible only if the plants are winter hardy (3).

An increase in freezing tolerance in fennel, similar to that observe in other crops after exposure to a period of low temperature (7,10), could be assumed to increase winter survival of the plant. This cold acclimation (8,14) in plants is initiated when frost is preceded by a period of relatively low temperatures (3), although the threshold temperatures below which the hardening occurs differ among plant species and cultivars (5). For example, AC has been observed for pea plants after exposure to 2 days at 2 °C (22), whereas arabidopsis requires exposure to only 4°C (17).

No investigations on the response of fennel plants to AC are apparent, yet survival of fall planting of fennel in temperate regions with freezing winter temperatures requires understanding the possibility of acclimation. This study evaluated the effect of acclimation on the freezing tolerance of two fennel ecotypes by monitoring survival, DMT production, and morphological traits.

MATERIALS AND METHODS

Plant Material

Fennel, *Foeniculum vulgare* Mill (family Umbelliferae) was used in this study. Seeds (fruit) of fennel ecotypes Kerman and Gonabad were sown in containers (15-cm diameter) filled with a sand:plant debris:field soil mixture (1:1:1, v:v:v:) and transferred to glasshouse (natural photoperiod, 19°C to 25°C). At the two- to three-leaf stage after emergence, the plants were thinned to five plants per container and maintained under the same environmental conditions. At the three- to five-leaf growth stage, plants were acclimated for 3 weeks under the following conditions:

Week 1: 11 h, 8°C day,13 h, 5°C night
Week 2: 10 h, 6°C day, 14 h, 4°C night
Week 3: 9 h, 5°C day, 15 h, 3°C night

At 24 hours before the end of the acclimation period, the plants were irrigated and transferred in their containers to a thermogradient freezer with 5°C for subjection to freezing temperatures (0, −3, −6, −9, −12, and −15°C) for acclimation. The temperature was reduced at the rate of −2°C per h^{-1}, and the plants were sprayed with ice nucleation active bacteria to induce freezing. After 1 hour at exposure to the freezing temperature, the containers with plants were immediately transferred to growth chamber at 4 ± 1°C for 24 hours to reduce the speed of ice melting and then returned to the glass house for 3 weeks (Figure 1). The acclimation temperatures were chosen to cover the most frequent AC temperature range of 0°C to 10°C (9) and based on the environmental patterns that naturally occur in the study location. None of the plants was flowering, ensuring the plants were not exposed to freezing temperatures during the sensitive growth stage.

Survival counts, taken at 3 weeks after return of the plants to the glasshouse, were determined by counting the number of live plants remaining within each container. At the end of flowering, plant height, number of nodes per main stem, and dry weight of the survived plants were measured. The lethal temperature (LT_{50}: 50% of plants dying) and temperature at

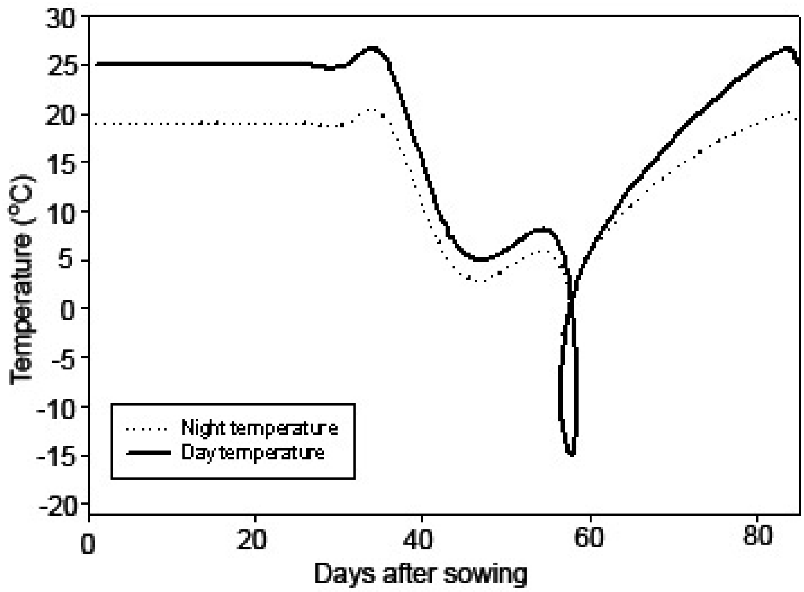

FIGURE 1 Day and night temperatures conditions from sowing, in the two experiments with freezing temperatures.

which had 50% less DMT_{50} were determined from a linear regression line fitted within the linear relationship between the freezing temperature and survival and DMT production for each ecotype.

Statistical Analysis

This experimental results were analyzed by analysis of variance based on factorial split plot with completely randomized arrangement with six replications. A least significant difference (LSD) test was used to separate means. Acclimation and non-acclimation were accommodated as main plots and combination of ecotype and temperature were allocated as subplots.

RESULTS AND DISCUSSION

Plant Survival

Averaged across both ecotypes, acclimation resulted in 17.5% survival enhancement (Table 1) as compared with NAC plants. Freezing temperatures had a negative relationship with survival as the percent survival decreased with lower freezing temperatures. Within the fennel ecotypes, survival was significantly different ($p < 0.05$), with the Gonabad ecotype having 60% survival at −12°C, whereas none of the Kerman ecotype plants

TABLE 1 The Effect of Ecotype and Acclimation on Fennel

	Treatment		
	Survival*	LT_{50}	DMT_{50}
Variable	(%)	(°C)	(°C)
Ecotype			
Gonabad	68.4	−11.0	−9.2
Kerman	57.4	−8.9	−7.6
LSD (0.05)	3.6	0.8	1.6
Acclimation condition			
Acclimated	71.7	−11.4	−10.0
Non-acclimated	54.2	−8.5	−6.8
LSD (0.01)	5.2	1.2	2.1

LT = lethal temperature for 50% plant kill; DMT = temperature for 50% reduction in dry weight.
*Plants surviving at 3 weeks after exposure to freezing temperatures.

survived this temperature. This difference between the ecotypes indicates existing genotype differences for winter hardening among fennel ecotypes.

The interaction of ecotype and freezing temperature on survival was significant ($p < 0.05$), although by reducing the temperature, survival of both ecotypes was reduced. At −9°C, the Gonabad ecotype had 30% more survival than the Kerman ecotype. The plants of the Kerman ecotype were completely lost at −12°C, whereas the Gonabad ecotype had 30% survival at this temperature level. A similar response has been reported for peas (*Pisum sativum*) wherein a freezing treatment on two winter genotypes and one spring genotype showed that 4 weeks after freezing at −6°C, the spring genotype had 3% survival and winter genotypes between 69% to 83% survival (21). The higher survival of the Gonabad ecotype may be due to this plant's originating from colder regions than the Kerman ecotype. This possibility was obvious under NAC conditions wherein at −9°C, the Gonabad ecotype showed 67% survival whereas the Kerman ecotype survival was only 6%.

The LT_{50} was significantly different between two ecotypes ($p \leq 0.05$), being −11°C for the Gonabad ecotype and −8.9°C for the Kerman ecotype (see Table 1). Acclimation treatment improved LT_{50} to −3°C of both ecotypes as compared with control plants ($p \leq 0.01$). The response of ecotypes to acclimation, however, was different. Acclimation improved the LT_{50} of Gonabad and Kerman ecotypes at −2.8°C and −3.2°C, respectively.

DMT_{50} of Gonabad ecotype was 1.6°C lower than that of the Kerman ecotype. Acclimation improved the DMT_{50}, averaging −6.8°C under NAC conditions for both ecotypes and −3.2°C under acclimated conditions. The DMT_{50} of the Gonabad ecotype was 1.8°C less than the Kernman ecotype under NAC and 1.5°C less under acclimated conditions. This indicated better regrowth of the Gonabad ecotype after subjection to the acclimation treatment. Azizi (1) also reported that a wheat variety with DMT_{50} of −14.2°C had a better regrowth as compared with other study wheat cultivars.

Plant Height

To attain required frost resistance, plants should cease their growth during the cold acclimation (16). In such conditions, plants form a compact morphology, and winter types of plants usually have a leaf rosette. Freezing significantly ($p < 0.05$) reduced plant height of both fennel ecotypes at the end of flowering (Figure 2a). Increasing the freezing temperature showed a negative relationship with plant height. At temperature of −12°C, plant height was 15% of 0°C temperature. Azizi (1) recorded the tallest wheat plants occurred in plants not subjected to freezing and, by lowering the temperature to −16°C, plant height was reduced to 16% of control plants. The reduction in height of two ecotypes in the current study differed in response to freezing. Lowering the temperature to −9°C resulted in 58% and 26% reduction of plant height in Kerman and Gonabad ecotypes, respectively, as compared with NAC controls, and represented a significant difference between the ecotypes ($p < 0.05$).

In comparison of NAC and AC plants, at the end of flowering, NAC plants height was 43% more than that of AC plants. NAC plants were taller to a temperature of −6°C as compared to AC plants, but the result was reversed at lower temperatures so that at −9°C, the AC plants were 44% taller than the NAC plants. This difference indicates that freezing temperatures to −6°C are not enough to induce any effect on plant height. No significant differences ($p > 0.05$) between the two ecotypes of acclimated plants height in response to freezing temperature levels occurred. A significant difference ($p < 0.01$) between the two study ecotypes, however, was observed among NAC plants for plant height.

Nodes Per Main Stem

Freezing temperature levels to −6°C did not induce any significant difference ($p > 0.05$) on the number of nodes on the main stem of the acclimated plants, but with acclimation at −9°C temperature and −12°C temperature, the number of nodes decreased by 30% and 81%, respectively. The significant difference ($p < 0.05$) between AC and NAC plants (see Figure 2b) was apparent as freezing temperatures decreased to −9°C, wherein the number of nodes in AC plants was 2.1 times more than that of NAC plants, and at −12°C, only three nodes were in AC plants, whereas all NAC plants were destroyed at this freezing temperature.

Despite the lack of differences in number of nodes per main stem between Gonabad and Kerman ecotypes at mild freezing (−3°C and −6°C), at −9°C, the number of nodes per main stem was 68% more in the Gonabad

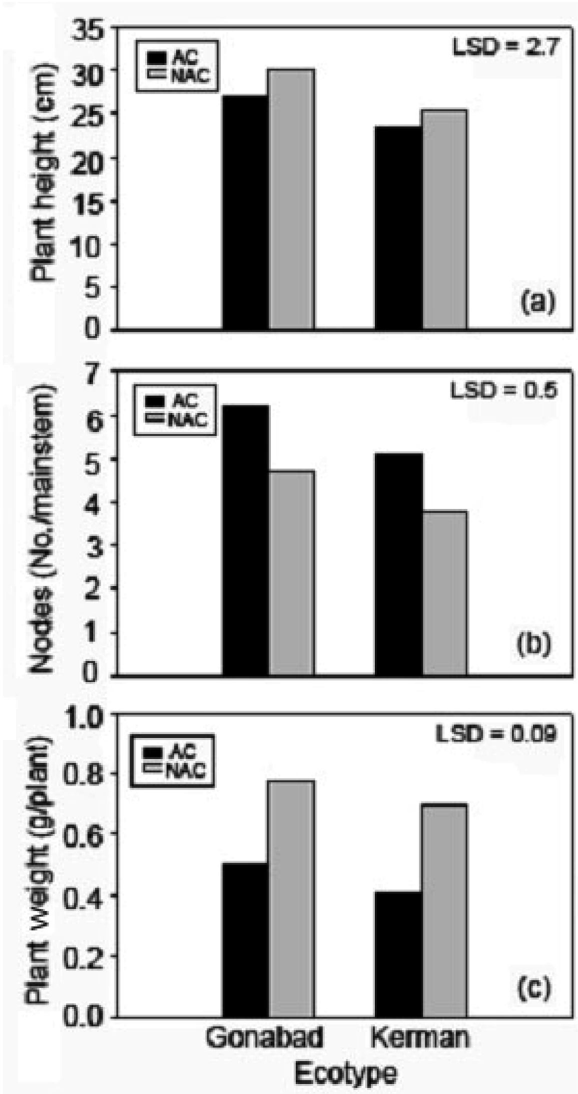

FIGURE 2 The effect of acclimation on plant height (a), number of nodes on main stem (b), and plant weight (c).

ecotype. At −12°C, only three nodes were observed in the Gonabad ecotype whereas no plants of the Kerman ecotype survived.

Dry Weight

Generally, AC plants had less dry weight as compared with NAC plants (see Figure 2c). Both ecotypes of fennel, as compared with control the respective control plants, showed a significant reduction (p < 0.01) in dry weight across all freezing temperatures. At –9°C and –12°C, the dry weight of AC plants was 36% and 7% of control plants, respectively. Dry weight reduction in AC plants at –9°C as compared with 0°C treatments was 40% but was 75% in NAC plants at the same temperature. Hekneby et al. (11) studied freezing hardiness of several annual legumes and observed that plant dry weight of alfalfa under NAC at –1°C and –4°C was greater than that in AC treatments, but at –7°C, the AC plants had higher dry weight than NAC plants.

Under NAC conditions, freezing treatments had less effect on dry weight of the Gonabad ecotype than on the Kerman ecotype. For instance, at –9°C, the reduction of dry weight of the Kerman ecotype was about 80% and of the Gonabad ecotype was 47% as compared with 0°C. Under acclimated conditions, dry weight of the Gonabad ecotype at –9°C temperature was 35% of plants at 0°C as compared with the 46% for the Kerman ecotype. At –12°C, the dry weight of the acclimated Gonabad ecotype was 42% at 0°C, but none of the Kerman ecotype survived at this temperature. Chen et al. (4) observed 60% and 80% reduction of wheat regrowth using freezing treatments of –15°C and –20°C, respectively.

CONCLUSION

Our study indicates winter survival potential, although to a different extent within the two studied ecotypes of fennel. The freezing tolerance in the Gonabad ecotype was higher than that in the Kerman ecotype, probably owing to adaptation of this ecotype to cool conditions in which the Gonabad ecotype grows. Acclimation improved LT_{50} and DMT_{50} of both ecotypes, but a better regrowth was observed in the Gonabad ecotype after severe freezing temperature (–9°C and –12°C).

REFERENCES

1. Azizi, H. 2005. Evaluation of cold tolerance in wheat under field and controlled conditions. MSc thesis, College of Agriculture, Ferdowsi University of Mashhad, Iran. (In Persian).
2. Bannayan, M., F. Nadjafi, M. Azizi, L. Tabrizi, and M. Rastgoo. 2008. Yield and seed quality of Plantago ovata and Nigella sativa under different irrigation treatments. *Ind. Crops Prod.* *27*(1):11–16.
3. Bourion, V., I. Lejeune-Henaut, N. Munier-Jolain, and C. Salon. 2003. Cold acclimation of winter and spring peas: carbon partitioning as affected by light intensity. *Eur. J. Agron.* *19*(4):535–548.
4. Chen, T.H., L.V. Gusta, and D.B. Fowler. 1983. Freezing injury and root development in winter cereals. *Plant Physiol.* *73*(3):773–777.

5. Dereuddre, J., and C. Gazeau. 1992. Les végétaux et les très basses températures. *In* D. Côme, ed. *Les végétaux et le froid*, Hermann, Häfelin, Stuttgart, Germany. pp. 107–175.
6. Erskine, W., and R. S. Malhotra. 1996. Progress in breeding, selecting and delivering production packages for winter sowing chickpea and lentil. *In Problems and Prospects for Winter Sowing of Grain Legumes in Europe*. AEP Workshop, European Association for Grain Legume Research, Paris. p.118.
7. Gomez-Galindo, F., L. Elias, V. Gekas, W. B. Herppich, M. Smallwood, M. Sommarin, et al. 2005. On the induction of cold acclimation in carrots (*Daucus carota* L.) and its influence on storage performance. *Food Res. Inter. 38*(1):29–36.
8. Graham, D., and B. D. Patterson. 1982. Responses of plants to low, nonfreezing temperatures: proteins, metabolism, and acclimation. *Ann. Rev. Plant Physiol. 33*:347–372.
9. Gusta, L.V., D. B. Fowler, and N. J. Tyler. 1982. Factors influencing hardening and survival in winter wheat. *In* P. H. Li and A. Sakai, eds. *Plant cold hardiness and freezing stress, mechanisms and crop implications*. Vol. 2. Academic Press, London. pp. 23–40
10. Hasdai, M., B. Weiss, A. Levi, A. Samach, and R. Porat. 2006. Differential responses of Arabidopsis ecotypes to cold, chilling and freezing temperatures. *Ann. App. Biol. 14*(2)8:113–120.
11. Hekneby, M., M. C. Antolin, and M. Sanchez-Diaz. 2006. Frost resistance and biochemical changes during cold acclimation in different annual legumes. *Environ. Exp. Bot. 55*(3):305–314.
12. Hornok, L. 1992. *Cultivation and Processing of Medicinal Plants*. Akademiai Kiado, Budapest. 338 pp.
13. Javidtash, A. 1989. From planting to harvesting fennel. A publication of the Fars Research Center of Agricultural and Natural Resources. No. 41/47. Center of Excellence for Special Crops, Ferdowsi University of Mashad, Mashad, Iran. (In Persian).
14. Levitt, J. 1980. Chilling, freezing, and high temperature stresses. *In Responses of Plants to Environmental Stresses*, Vol. 1.2nd ed. Academic Press, New York, pp. 67–346.
15. Rashed-Mohassel, M. H., and A. Nezami. 1998. The effects of planting date and population density on growth and yield of fennel. Final report, Ferdowsi University of Mashhad. Mashad, Iran. (In Persian).
16. Rapacz, M. 1999. Frost resistance and cold acclimation abilities of spring-type oilseed rape. *Plant Sci. 147*(1):55–64.
17. Ristic, Z., and E. N. Ashworth. 1993. Changes in leaf ultrastructure and carbohydrates in *Arabidopsis thaliana* L. (Heyn) cv. Columbia during rapid cold acclimation. *Protoplasma 172*(2–4):111–123.
18. Salehi, P., B. Asghari, and F. Mohammadi. 2007. Hydrodistillation–headspace solvent microextraction: An efficient method for analysis of the essential oil from the seeds of Foeniculum vulgare Mill. *Chromatographia 65*(1–2):119–122.
19. Shrestha, A., O. B. Hesterman, J. M. Squire, J. W. Fisk, and C. C. Sheaffer. 1998. Annual medics and berseem clover as emergency forage. *Agron. J. 90*(2):197–201.

20. Singh, K. B., R. S. Malhotra, M. H. Halila, E. J. Knights, and M. M. Verma. 1994. Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica 73*(1–2):137–149.
21. Swensen, J. B., and G. A. Murray 1983. Cold acclimation of field peas in a controlled environment. *Crop Sci. Soc. Am. 23*(1):27–30.
22. Weiser, R. L., S. J. Wallner, and J. W. Waddell. 1990. Cell wall and extension mRNA changes during cold acclimation of pea seedlings. *Plant Physiol. 93*(3):1021–1026.
23. Yamini, Y., F. Sefidkon, and S. M. Pourmortazavi. 2002. Comparison of essential oil composition of Iranian fennel (*Foeniculum vulgare*) obtained by supercritical carbon dioxide extraction and hydrodistillation methods. *Flavour Fragr. J. 17*(5):345–348
24. Zargari, A. 1988. *Medicinal Plants*, Vol. 2. Tehran University Press, Tehran, Iran. (In Persian).