International Journal of Plant Production 2 (2), April 2008
ISSN: 1735-6814 (Print), 1735-8043 (Online)
This is a refereed journal and all articles are professionally screened and reviewed.
GUASNR
www.ijpp.info

Quantifying the threshold frost hardiness for over-wintering survival of wheat in Iran, using simulation

M. Gholipoor

Department of Agronomy and Plant Breeding, Shahrood University of Technology, Shahrood, Iran
*Corresponding authors. E-mail: manouchehrg@excite.com

Accepted 26 Jan. 2008; Published online 10 Feb. 2008

Abstract

The value of frost tolerance in wheat is increased with decreasing the temperature in late autumn and/or early winter (phase I, acclimation), then shows plateau state for a period with the coldest temperature (II), finally appears to decrease with warming the temperature (III, de-acclimation). This study was aimed to determine the threshold frost hardiness in wheat for avoiding the winter-kill events, and estimating the probability of occurrence of winter-killing across the each phase in five locations of Iran. The model of wheat acclimation/de-acclimation to temperature was recoded in QBASIC programming and run for long-term (39-44 years) weather data, the nonlinear functions were used for describing the changes in frost tolerance across the late autumn to early spring (say, for distinguishing the three phases). Results indicated that the threshold frost hardiness for acceptable [probability (P) = 95%] over-wintering survival is -10 ^{o}C for Isfahan, -8.5 ^{o}C for Shiraz, -16 ^{o}C for Kermanshah, -18.5 ^{o}C for Tabriz, and -14.5 ^{o}C for Mashhad, for fully (P=100%) avoiding the winter-kill events, the wheat should be contained frost hardiness -12, -8.5, -16.5, -21, and -16.5 ^{o}C for named locations, respectively. The probability of occurrence of named events over phase III for wheat contained frost hardiness lower than the threshold value (P=10% for experiencing the death) was 3% in Tabriz, but zero in other locations, the order of locations for magnitude of this probability over phase II tended to be as Kermanshah (79%)> Isfahan (73%)> Tabriz (56%)> Mashhad (50%)> Shiraz (40%), the rest probabilities appeared to devote to the phase I.

***Keywords**:* Wheat; Acclimation; De-acclimation; Freezing; Simulation

Introduction

Wheat is one of the basic staple foods in Iran. According to report of FAO (www.fao.org), in Iran about 10.27 million hectares are under annual crops, 7.011 million hectares equal to 68.27% is under cereals, and wheat is grown on 72.75% of cereal farms. The cultivation area for winter wheat is considerably higher than spring wheat, because it has more advantages, including about 15-25% higher yield, and possibility of using the late-winter and/or early spring rainfalls, which could be important for arid and semi-arid regions with winter-dominant precipitations, like Iran.

Winter wheat should have sufficient frost tolerance to survive unfavorable winter temperatures. The value of frost tolerance of this crop after exposure to low temperatures, i.e. cold-acclimated wheat, is considerably higher, as compared to non-acclimated wheat. Acclimation results in producing myriad of measurable changes in biochemical and physiological characters which often correlate with frost tolerance (Howarth and Ougham, 1993; Levitt, 1980; Mahfoozi et al., 2001a,b), for example, it has been reported that the lipid composition of the plasma membrane and chloroplast envelopes change during cold acclimation in a way that the threshold temperature of membrane damage is lowered compared to non-acclimated plants (Uemura and Steponkus, 1999), this is due to the increasing fluidity of the cold acclimated membranes, which results from a change in lipid composition towards an increase in desaturated fatty acids (Vézina et al., 1997), because of the alterations in lipid components of membranes, the protein fraction in them also changes, during cold hardening the protein ratio of thylakoid membranes (Uemura and Steponkus, 1999), and the activity of plasma membrane H^+-ATPase (Hellergren et al., 1983) increase, and tonoplast enzymes are changed (Yoshida et al., 1999).

It is generally accepted that the value of frost tolerance in wheat is increased with decreasing the temperature in late autumn and/or early winter (phase I, acclimation), then shows plateau state for a period with the coldest temperature (II), finally appears to decrease with warming the temperature (III, de-acclimation). But it should be noted that across the each phase, air (crown) temperature, and consequently the frost tolerance may fluctuate (Pomeroy et al., 1975; Gusta and Fowler, 1976), temporary exposure to warm temperatures during the acclimation period causes rapid de-acclimation, the greater the loss of cold tolerance during the warm period, the longer it takes for the plants to re-acclimate. Based on these issues, it could be said that the winter-kill events may occur for wheat contained frost hardiness lower than threshold value across the each phase.

Fowler et al. (1999) developed a framework using the known low temperature responses of wheat, then it was used to improve a functional model that provides more flexible winter-kill routines for the CERES-Wheat model (Ritchie, 1991). This framework has successfully facilitated the investigation of production risks, cause-and-effect processes, and the evaluation of genetic theories [see Fowler et al. (1999) for more detail].

The value of absolute minimum mean temperature considerably differs across the Iran, therefore the necessary threshold frost hardiness may be not the same for different locations. This study was aimed to determine the threshold frost hardiness in wheat for avoiding the winter-kill events, and estimating the probability of occurrence of winter-killing across the each phase in five locations of Iran. The result of this study may be useful for breeding programs, and for choosing the appropriate variety for cultivation.

Materials and methods

Five locations with reliable daily weather data, including Isfahan (32.67 ^{o}N, 51.87 ^{o}E and 1600 m asl), Shiraz (29.55 ^{o}N, 52.60 ^{o}E and 1488 m asl), Kermanshah (34.32 ^{o}N, 47.12 ^{o}E and 1322 m asl), Tabriz (38.13 ^{o}N, 46.28 ^{o}E and 1364 m asl), and Mashhad (36.27 ^{o}N, 59.63 ^{o}E and 990 m asl) were selected for this study. These locations represent a large geographical area and several climatic zones in Iran [see Figure 1, and related descriptions in report of Soltani and Gholipoor (2006)], and are of major Agricultural importance in the

Iran. For each location, 39 (1966-2004 for Tabriz) to 44 years (1961-2004) of daily data for rainfall and maximum and minimum temperatures and sunshine hours were available.

In this study, the approach of Fowler et al. (1999) was used to estimate the daily value of frost tolerance in winter-wheat across the phases I, II and III. Briefly, in this approach, the LT50 (temperature at which 50% of the population is killed in a controlled freeze test) is used as an index for level of cold hardiness. The threshold temperature (average daily crown temperature) for the initiation of cold acclimation is equal to 10 °C, daily rate of hardening is estimated using the assumption that acclimation rates at non-lethal below freezing temperatures are similar to those at 0 °C. Daily calculation of de-hardening (as daily change in LT50) is based on this assumption that temperatures above 10 °C result in a loss of cold hardiness at all stages of acclimation while partially acclimated plants de-acclimate at temperatures above 8 °C, it is considered that the de-acclimation rates at temperatures above 18 °C are similar to those at 18 °C, when the daily mean crown

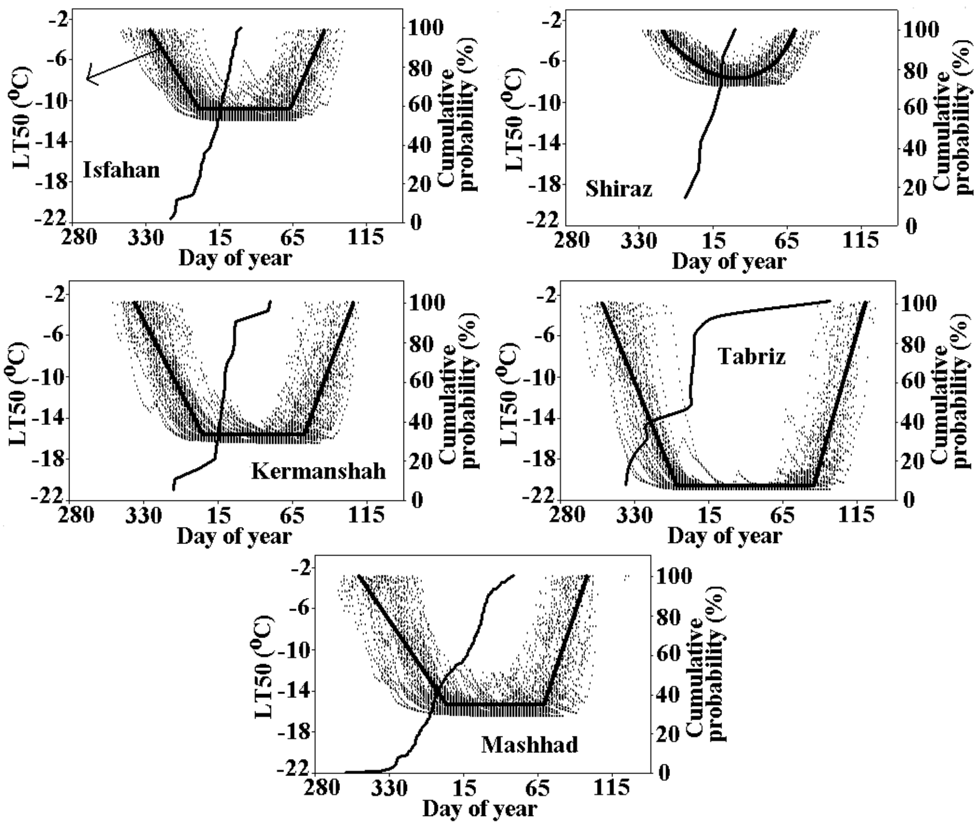

Figure 1. Acclimation/de-acclimation-resulted changes in LT50 (temperature at which 50% of the population is killed), say in "frost tolerance", of winter-wheat across days of year for 5 locations of Iran (the line with symbols). Cumulative probability of day of year with winter-kill events for winter-wheat contained potential frost tolerance lower than threshold value (probability of occurrence of winter-killing= 10%) in five locations of Iran (the line without any symbol).

temperature is above -4 ^{o}C, the daily change in cultivar low temperature tolerance (rate of de-hardening) after transition from the vegetative to the reproductive growth stage is calculated using an appropriate equation. When the daily mean crown temperature is colder than -3 ^{o}C and the difference between the minimum LT50 attained during low temperature acclimation and the crown temperature is greater than -12 ^{o}C, the daily change in low temperature tolerance is calculated using a series of equations. Death of the plant is assumed to occur if the average daily soil temperature at crown depth falls below the daily adjusted LT50. In this approach, LT50 estimates are updated daily, thereby providing a current record of the plant's ability to tolerate the low temperatures.

As mentioned, instead of air temperature, the mean daily crown temperature is used for calculating the rate of acclimation/de-acclimation to temperature. When mean air temperatures are less than 0 ^{o}C for long period, i.e. frozen soils, the plant crown temperature is higher than the air temperature due to heat retained in the soil, the insulating effect of snow at air temperatures less than 0 ^{o}C raises the temperature of the plant crown higher than the air temperature, and magnitude depends on snow depth (Ritchie, 1991). A sub-model was made using QBASIC programming for calculating the snow cover and snow melting as report of Ritchie (1991), this procedure has been used by many researchers including Soltani et al. (2006), in this sub-model the value of snow is calculated based on maximum temperature, this temperature is also used for daily calculating the amount of snow melting. The crown mean temperature was calculated for either snow-covered or no-snow-covered frozen soil, as it has been implemented in CERES-Wheat model (Ritchie, 1991).

The coldest temperature which the fully-acclimated winter-wheat can tolerate is called potential frost tolerance, hereafter, the term "frost tolerance" means frost tolerance at a given time of the acclimation and/or de-acclimation phase, and its value is lower than the value of potential frost tolerance, it is obvious that across the period with the coldest temperature the value of frost tolerance is similar to that of potential frost tolerance. The probability of the occurrence of winter-kill events for different values of potential frost tolerance in wheat (-3 to -21^{o}C with interval of -0.5 ^{o}C) was calculated using the normalizing for potential frost tolerance -3 ^{o}C [see Figure 1 in Fowler et al. (1999)]. The normalizing procedure has been used by many researches, including Soltani et al. (2006), for different goals.

The changes in value of LT50 (which was calculated by running the model for weather data) across the late autumn to early spring was described as:

$$\mathrm{LT50} = \mathrm{PFT} \times \; f\,(\mathrm{DOY}) \tag{1}$$

Where PFT is the potential frost tolerance [here, it was considered as the threshold frost hardiness for fully (probability = 100%) avoiding the winter-kill events], DOY the day of year, f (DOY) the DOY function, and LT50 is equivalent to the frost tolerance. In this study, three functions [i.e., f (DOY)] were selected due to their simplicity as follows:

- Segmented function

$$f(DOY) = \frac{(DOY - DOY_b)}{(DOY_p - DOY_b)} \qquad if \;\; DOY_b < DOY \leq DOY_p \tag{2}$$

$$f(DOY) = \frac{(DOY_c - DOY)}{(DOY_c - DOY_p)} \qquad if \;\; DOY_p < DOY < DOY_c$$

$$f(DOY) = 0 \qquad if \;\; DOY_c \leq DOY \leq DOY_b$$

- Beta function

$$f(DOY) = \left[\left(\frac{DOY - DOY_b}{DOY_p - DOY_b} \times \frac{DOY_c - DOY}{DOY_c - DOY_p} \right)^{\left(\frac{DOY_c - DOY_p}{DOY_p - DOY_b}\right)} \right]^{\alpha} \qquad if \;\; DOY_b < DOY < DOY_c \tag{3}$$

$$f(DOY) = 0 \qquad if \;\; DOY_b \geq DOY \geq DOY_c$$

- Dent-like function

$$f(DOY) = \frac{(DOY - DOY_b)}{(DOY_{p1} - DOY_b)} \qquad if \;\; DOY_b < DOY < DOY_{p1} \tag{4}$$

$$f(DOY) = \frac{(DOY_c - DOY)}{(DOY_c - DOY_{p2})} \qquad if \;\; DOY_{p2} < DOY < DOY_c$$

$$f(DOY) = 1 \qquad if \;\; DOY_{p1} \leq DOY \leq DOY_{p2}$$

$$f(DOY) = 0 \qquad if \;\; DOY_c \leq DOY \leq DOY_b$$

Where DOY_b is the DOY for which the frost tolerance initiates to increase (beginning of the phase I), DOY_p the potential frost tolerance for segmented function, DOY_{p1} the lower potential frost tolerance for dent-like function (beginning of the phase II), DOY_{p2} the upper potential frost tolerance for dent-like function (end of the phase II), DOYc the frost tolerance reaches to minimum value (end of the phase III), and a the shape parameter for beta function that determines the curvature of the function. The parameters of these functions were calculated using the NLIN procedure of SAS software (SAS, Institute, 1989). Some additional attributes, including ratio of snow to rainfall and period with snow-covered-soil were also calculated for interpreting the short-term fluctuations in LT50 across the winter.

Results and discussions

The mean annual temperature is 16.4 °C for Isfahan, 17.8 °C for Shiraz, 14.4 °C for Kermanshah, 12.6 °C for Tabriz, and 14.3 °C for Mashhad. The number of days with the mean temperatures lower than 10 °C [The temperatures which may potentially result in cold acclimation of winter wheat (Fowler et al., 1999)] over a period from 22 September to 11 May (and the absolute lowest mean temperature, probability of occurrence of the mean temperatures lower than 0 °C over 6 November till 4 February) is 112 (-13.1 °C, 8.01%) for Isfahan, 85 (-5.5 °C, 0.83%) for Shiraz, 132 (-17 °C, 13.20%) for Kermanshah, 153 (-17 °C, 35.93%) for Tabriz, and 128 (-17.5 °C, 18.91%) for Mashhad.

The mean number of days per year with winter-kill events, and probability of occurrence of winter-killing for wheat contained different values of potential frost tolerance were given in Table 1. For the low value of potential frost tolerance (-3 °C), number of days with winter-kill events was 5.12 in Isfahan, 0.59 in Shiraz, 12.07 in Kermanshah, 38.66 in Tabriz, and 18.53 in Mashhad. With increasing the value of potential frost tolerance from -3 to -3.5 °C, the probability of winter-kill occurring was decreased for Isfahan and Shiraz (by 2.9%, and by 11.1%, respectively), but not changed for other locations. For wheat contained potential frost tolerance -5 °C, the probability of occurrence of winter-kill events was 73.5% in Isfahan, 77.8% in Shiraz, 97.4% in Kermanshah, 100% in Tabriz, and 97.6% in Mashhad, number of days with named events ranged from 0.51 (in Shiraz) to 21.74 (in Tabriz), which is lower than that for wheat contained potential frost tolerance -3 °C. For Shiraz, when potential frost tolerance of wheat was increased from -7 to -7.5 °C, the value regarding number of days with freezing injury (winter-killing), and that regarding the named probability appeared to be constant on 0.16 and 33.3%, respectively, for potential frost tolerance -8.5 °C, the named values tended to be zero. On the other hand, the value of probability of experiencing (and value regarding the number of days with winter-kill events) for this potential frost tolerance was 8.8% (0.37) in Isfahan, 46.2% (2.93) in Kermanshah, 71.1% (7.21) in Tabriz and 56.1% (3.28) in Mashhad. In Isfahan, with changing the potential frost tolerance from -9 to -9.5 °C, both named values showed no change, over the potential frost tolerances -10 to -11.5 °C, the probability of occurrence appeared to be constant on 2.9%, but the value regarding number of days with freezing-injury tended to vary between 0.21 and 0.14. For potential frost tolerance -12 °C, the probability of successful over-wintering was 100% in Isfahan, but 17.1-26.3% in other three locations (i.e. the rest locations minus Shiraz). Kermanshah and Mashhad tended to have the same values of probability of winter-kill happening (0%) for potential frost tolerance -16.5 °C, but different values (2.6% versus 2.4%, respectively) for -16 °C, similar situation was also true for number of days with winter-kill events. In Tabriz, the probability of avoidance from winter-killing appeared to be 94.7% for potential frost tolerances -16 to -18 °C, 97.4% for -18.5 to -20.5 °C, and 100% for -21 °C.

The root mean square of deviations (RMSD), and linear regression statistics [intercept (a), slope (b) and correlation (r)] for predicted, versus, say, observed LT50 were presented in Table 2. RMSD ranged from 1.000 to 4.902 °C (i.e. 8.34 to 40.01% of the mean).

Table 1. Number of days per year with winter-kill events (NO.), and probability of winter-kill occurring (*P*) for winter-wheat contained potential frost tolerance (PFT) -3 to -21ºC in five locations of Iran.

PFT	Isfahan		Shiraz		Kermanshah		Tabriz		Mashhad	
	No.	*P* (%)	No.	*P* (%)	NO.	*P* (%)	No.	*P* (%)	No.	*P* (%)
-3.0	5.12	100	0.59	100	12.07	100	38.66	100	18.53	100
-3.5	5.01	97.1	0.53	88.9	11.28	100	30.55	100	14.77	100
-4.0	3.53	94.1	0.51	77.8	9.91	100	23.47	100	10.74	100
-4.5	3.37	85.3	0.51	77.8	9.63	97.4	22.89	100	10.37	100
-5.0	3.05	73.5	0.51	77.8	9.26	97.4	21.74	100	9.93	97.6
-5.5	2.47	67.6	0.42	66.7	8.02	89.7	19.05	94.7	9.12	87.8
-6.0	1.88	47.1	0.33	55.6	7.00	84.6	17.29	94.7	8.72	82.9
-6.5	1.23	26.5	0.26	44.4	6.26	79.5	14.26	89.5	8.05	82.9
-7.0	1.19	23.5	0.16	33.3	5.86	74.4	11.92	89.5	6.56	75.6
-7.5	1.12	17.6	0.16	33.3	4.63	71.8	9.37	84.2	5.51	75.6
-8.0	0.65	14.7	0.05	11.1	3.47	56.4	7.84	78.9	4.51	65.9
-8.5	0.37	8.8	0	0	2.93	46.2	7.21	71.1	3.28	56.1
-9.0	0.23	5.9	0	0	2.63	41.0	5.53	60.5	2.98	51.2
-9.5	0.23	5.9	0	0	2.21	35.9	3.24	55.3	1.79	36.6
-10.0	0.21	2.9	0	0	1.16	28.2	1.89	47.4	1.49	29.3
-10.5	0.19	2.9	0	0	0.88	25.6	1.29	42.1	1.21	29.3
-11.0	0.16	2.9	0	0	0.70	20.5	1.21	34.2	0.95	24.4
-11.5	0.14	2.9	0	0	0.67	20.5	0.82	31.6	0.81	24.4
-12.0	0	0	0	0	0.63	20.5	0.45	26.3	0.72	17.1
-12.5	0	0	0	0	0.54	20.5	0.32	21.1	0.67	14.6
-13.0	0	0	0	0	0.51	15.4	0.26	18.4	0.65	14.6
-13.5	0	0	0	0	0.16	10.3	0.24	15.8	0.6	12.2
-14.0	0	0	0	0	0.14	10.3	0.18	13.2	0.47	7.3
-14.5	0	0	0	0	0.12	10.3	0.16	10.5	0.21	4.9
-15.0	0	0	0	0	0.09	7.7	0.16	10.5	0.21	4.9
-15.5	0	0	0	0	0.05	5.1	0.13	7.9	0.16	2.4
-16.0	0	0	0	0	0.02	2.6	0.11	5.3	0.16	2.4
-16.5	0	0	0	0	0	0	0.11	5.3	0	0
-17.0	0	0	0	0	0	0	0.11	5.3	0	0
-17.5	0	0	0	0	0	0	0.11	5.3	0	0
-18.0	0	0	0	0	0	0	0.11	5.3	0	0
-18.5	0	0	0	0	0	0	0.05	2.6	0	0
-19.0	0	0	0	0	0	0	0.05	2.6	0	0
-19.5	0	0	0	0	0	0	0.05	2.6	0	0
-20.0	0	0	0	0	0	0	0.05	2.6	0	0
-20.5	0	0	0	0	0	0	0.05	2.6	0	0
-21.0	0	0	0	0	0	0	0	0	0	0

The value of statistic a, which more approaches to zero, that of statistic b which more approaches to 1, and lower RMSD, but higher r could be considered as relatively higher value of adequacy of fit for each function, accordingly, the beta and dent-like functions appeared to be more appropriate for describing the changes in frost tolerance of wheat across the late autumn to early spring (say, for distinguishing the three phases) in Shiraz, and in other locations, respectively.

As it is seen in Figure 1, the phases I (decrease in LT50, say increase in frost tolerance), II and III (decreasing the frost tolerance) are clearly distinguishable.

Table 2. Root mean square of deviations (RMSD, °C) and linear regression statistics [intercept (a), slope (b), and correlation coefficient (r)] for predicted vs. say "observed" frost tolerance in wheat for five locations.

Function	Location	RMSD	a ± S.E.	b ± S.E.	r
Dent-like					
	Isfahan	1.775	-1.094 ± 0.096	0.920 ± 0.010	0.777
	Shiraz	1.234	-3.725 ± 0.204	0.513 ± 0.026	0.593
	Kermanshah	2.360	-2.017 ± 0.107	0.818 ± 0.008	0.813
	Tabriz	2.919	-1.582 ± 0.140	0.809 ± 0.008	0.835
	Mashhad	2.678	-1.216 ± 0.119	0.799 ± 0.009	0.783
Segmented					
	Isfahan	1.976	-2.277 ± 0.075	0.844 ± 0.011	0.703
	Shiraz	0.976	-1.977 ± 0.075	0.644 ± 0.013	0.743
	Kermanshah	2.775	3.095 ± 0.129	0.882 ± 0.009	0.781
	Tabriz	4.902	3.505 ± 0.233	1.113 ± 0.013	0.756
	Mashhad	3.010	-4.351 ± 0.141	0.856 ± 0.010	0.747
Beta					
	Isfahan	1.507	-3.292 ± 0.68	0.649 ± 0.007	0.705
	Shiraz	1.000	-0.723 ± 0.044	0.886 ± 0.006	0.848
	Kermanshah	2.139	-4.093 ± 0.085	0.689 ± 0.008	0.629
	Tabriz	2.562	-5.166 ± 0.111	0.611 ± 0.008	0.642
	Mashhad	2.418	-5.183 ± 0.091	0.604 ± 0.007	0.776

It should be mentioned that in each location, the threshold value of potential frost tolerance (probability of avoiding the winter-kill events equal to 100%) (i.e., -12, -8.5, -16.5, -21, and -16.5 °C for Isfahan, Shiraz, Kermanshah, Tabriz and Mashhad, respectively) was used as model input for calculating the daily LT50 (the value of symbols in Figure 1).

As it was previously mentioned, across the each phase, wheat experiences either acclimation or de-acclimation, due to fluctuation of air (crown) temperature, but the ratio of mean acclimation rate to mean de-acclimation rate tends to be different for phases (Fowler et al., 1999). In Isfahan, the averaged acclimation rate over phase I (DOYs 320 to 2, for this case and other below cases see Figure 1) was 0.23 $^{o}Cd^{-1}$, on the other hand, the mean rate of de-acclimation tended to be 0.06 $^{o}Cd^{-1}$, the frost tolerance showed no change (i.e. no acclimation and/or de-acclimation were done) only for 0.5% of the DOYs. In Shiraz, the first phase started about 26 days later, as compared to Isfahan, the length of the phase I appeared to be shorter in Shiraz than in Isfahan (35 versus 47 d, respectively), the mean value of acclimation rate, of de-acclimation rate, and probability of no-change occurring in frost tolerance was 0.17 °C, 0.08 °C and 1.1%, respectively, the net rate of increase in frost tolerance (i.e. mean acclimation rate minus mean de-acclimation rate) was lower for Shiraz, as compared to Isfahan. The difference between Kermanshah and Mashhad for the beginning, and for the end of first phase was sensible (17 and 10 d, respectively), the mean rate of acclimation (and the probability of occurrence of acclimation rates equal to and/or greater than 1 $^{o}Cd^{-1}$) were 0.26 $^{o}Cd^{-1}$ (4.4%) for Kermanshah, which are lower than those for Mashhad [0.31 $^{o}Cd^{-1}$ (7.8%)], the mean de-acclimation rate (and the probability of occurrence of de-acclimation rates equal to and/or greater than 1 $^{o}Cd^{-1}$) also tended to have less magnitude for Kermanshah than for Mashhad [0.085 $^{o}Cd^{-1}$ (3%), versus, 0.13 $^{o}Cd^{-1}$ (4%), respectively], these issues suggest that the fluctuation of frost tolerance was more considerable in Mashhad than in Kermanshah, this is also confirmed by the data regarding probability of no-change occurring in frost tolerance, and by the coefficient of variation of LT50 (57% versus 50%, respectively). In Tabriz, the named phase appeared to be characterized over period 300 to 358, the mean value of acclimation rate tended to be sensibly higher in Tabriz, as compared to Mashhad (0.33 versus 0.31 $^oCd^{-1}$, respectively), but the probability of occurrence of acclimation rates equal to and/or greater than 3 $^oCd^{-1}$ was sensibly lower in Tabriz than in Mashhad (0.12% versus 0.16%, respectively), this was also true for that of occurrence of de-acclimation rates equal to and/or greater than 2 $^oCd^{-1}$.

Across the phase II, the averaged rate of acclimation was nearly equal to that of de-acclimation, resulted in no change occurring in overall frost tolerance. The estimated length of this phase was 61 and about 27 d for Isfahan and Shiraz, respectively, the probability of occurrence of acclimation rates equal to and/or greater than 0.2 $^oCd^{-1}$ was 2.1% for Isfahan which is considerably lower than that for Shiraz (10.8%), Isfahan also tended to have the lower value for probability of occurrence of de-acclimation rates equal to and/or greater than 0.2 $^oCd^{-1}$, as compared to Shiraz, therefore the fluctuation in frost tolerance appears to be higher for shiraz, when compared with Isfahan. The probability of occurrence of acclimation to low temperature (and of occurrence of de-acclimation, of no change occurring in frost tolerance) was 87% (12%, 1%) for Mashhad, and 90% (7%, 3%) for Kermanshah, based on these values, and on the fact that overall frost tolerance appears to show plateau state, it is cleared that in response to temporary little warming the temperature, the frost tolerance drastically decreases, but slowly shows increasing trend with decreasing the temperature, this is also confirmed by the probability of occurrence of acclimation and de-acclimation rates lower than 0.08 $^oCd^{-1}$, which tended to be higher for acclimation than for de-acclimation. This difference between acclimation and de-acclimation was also true in Tabriz [70% (7%, 23%), respectively], among the locations, the probability of no change occurring in frost tolerance was the highest for Tabriz, this may be due to higher snow falling [ratio of snow to rainfall: 30% versus 2-14%, respectively, the ratio of period with snow-coverage on soil to length of the phase (i.e. SCE: snow cover extend): 32% versus 7-16%, respectively] and consequently more considerable stability of temperature around the crown in Tabriz, as compared to other locations (coefficient of variations for mean temperature: 17% versus 19-25%, respectively).

Similar to the phases I and II, the length of phase III tended to be shorter in Shiraz, as compared to Isfahan (29 versus 34 d, respectively), the probability of occurrence of acclimation rates higher than 1.5 $^oCd^{-1}$ (and averaged acclimation rate), and that of occurrence of de-acclimation rates higher than 1.5 $^oCd^{-1}$ (and averaged deacclimation rate) were 0.04% (0.16 $^oCd^{-1}$) and 0.05% (0.37 $^oCd^{-1}$), respectively, for Shiraz, which are lower than those obtained for Isfahan [0.5% (0.23 $^oCd^{-1}$) and 3% (0.60 $^oCd^{-1}$), respectively], the difference between these locations for the value regarding the no change occurring in frost tolerance was also sensible (1.7% versus 2.1%). The probability of no change occurring in frost tolerance was nearly zero for Kermanshah and Mashhad, the averaged net rate of decrease in frost tolerance appeared to be lower for Kermanshah, when compared with that for Mashhad (0.17 versus 0.24, respectively), because of this difference, and of the issue that these locations tended to have the same threshold frost tolerance for over-wintering survival of wheat (P=100%), the length of phase III expectedly appeared to be longer for Kermanshah than for Mashhad (39 versus 35 d, respectively). The averaged acclimation and de-acclimation rates tended to be the highest for Tabriz, this is also true for averaged net rate of decrease in frost tolerance, and for the length of the phase III (39 d, like that of the phase III for Kermanshah).

Figure 1 also shows the cumulative probability of day of year with winter-kill events for wheat contained potential frost tolerance below the threshold value (the probability of winter-kill occurring equal to 10%). In Shiraz, it was evident that the named events may be happened over the period starts from the DOY 361 (15 d after initiation of the phase I) and ends to the DOY 30, the probability of occurrence of winter-killing at the DOY 361, and at the DOY 30 was equal to 14 and 14%, respectively, the median of the DOYs across the named period was 15 (with a mean of 13.29), the probability of experiencing the winter-kill events by wheat over the phases I, II and III was about 60, 40 and 0%, respectively. The length of the period over which the freezing-injury may happen was 48 d for Isfahan, which is longer than that obtained for Shiraz (34 d), similar to Shiraz, the 1st part of the phase I tended to have no winter-kill events for Isfahan, the probability of happening the freezing-injury over phase I [in fact over 2nd part (42%) of the phase I] was 27% which is considerably lower than that for Shiraz, for phase III, this probability appeared to be zero. The median of DOYs with freezing-injury (and the mean value) was 18 (18.4) for Kermanshah and 4 (4.9) for Mashhad, the probability of occurring the winter-kill events over the phase II tended to be higher for Kermanshah than for Mashhad (79 versus 50%, respectively), in both locations, the rest probability appeared to devote to the phase I. In Tabriz, the length of the period with winter-killing was 65 d (the DOYs 350 to 50), among these DOYs, the DOYs 1 to 6 tended to have considerable value of named probability (39%), in other words, these DOYs may be relatively more dangerous for over-wintering survival of wheat, the probability of experiencing the freezing-injury by wheat over the phase III appeared to be about 3% for Tabriz, but, as mentioned, zero for other locations, the value of named probability was higher for phase II, when compared with that for phase I (56% versus 41%, respectively).

Conclusion

Generally, the results indicated that the threshold frost hardiness for the acceptable [probability (P)= 95%] over-wintering survival of wheat is -10 ^{o}C for Isfahan, -8.5 ^{o}C for Shiraz, -16 ^{o}C for Kermanshah, -18.5 ^{o}C for Tabriz, and -14.5 ^{o}C for Mashhad, for fully (P=100%) avoiding the winter-kill events, the wheat should be contained potential frost tolerance -12, -8.5, -16.5, -21, and -16.5 ^{o}C for named locations, respectively. The probability of occurrence of these events over phase III for wheat contained potential frost hardiness lower than the threshold value (P=10% for experiencing the death) was 3% in Tabriz, but zero in other locations, the order of locations for magnitude of this probability over phase II tended to be as Kermanshah (79%)> Isfahan (73%)> Tabriz (56%)> Mashhad (50%)> Shiraz (about 40%), the rest probabilities appeared to devote to the phase I.

The short-term fluctuation of the crown-temperature and consequently variability of frost tolerance was relatively lower for location with higher snow coverage on the soil (i.e. Tabriz), generally, the snow coverage on the soil has the insulating effect and tends to minimize the risk of winter-kill events by decreasing the fluctuation of crown temperature (Ritchie, 1991). Unfortunately, reports indicating that the snow falling has shown decreasing trend across the few past decades, for example, satellite records indicate that the

Northern Hemisphere annual SCE has decreased by about 10% since 1966 (Robinson, 1997), similar to the results in North America, in Eurasia, April SCE has significantly decreased (Brown, 2000), over the Canada, there has been a general decrease in snow depth since 1946, especially during spring, in agreement with decreases in SCE (Brown and Braaten, 1998), Dore (2005) stated that reduction in snow cover during the mid- to late 1980s has been strongly related to temperature increase in snow-covered areas, in Iran, although it was found no published report regarding the decrease in snow-falling, and in SCE, the warming of surface air temperature has been evident [e.g. Gholipoor (2008), Gholipoor et al. (2006), Ghorbani and Soltani, (2002)] which may be indicator of the decrease in SCE. Based on these issues, and on the issue regarding the probable continuing decrease in snow falling and SCE over the future, it seems that the threshold frost hardiness for fully (P=100%), rather than acceptable (P=95%), avoidance of the winter-kill events should be considered by breeders (for breeding programs) and agronomists (for choosing the appropriate variety for cultivation).

Here, it was focused on the minimum frost tolerance for avoiding the frost injury. It is obvious that, after choosing appropriate frost-tolerated variety, applying some treatments may increase over-wintering survival of plant, for example, leaving the stubble on soil surface may decrease the ice-encasement-resulted anaerobic stress by cracking the ice sheet, exogenous (as foliar) application of betaine can increase the frost tolerance of wheat (Allard et al., 1998), it is also effective to increase the freezing tolerance in alfalfa (Zaho et al., 1992), Arabidopsis (Alia et al., 1998), and to enhance the chilling tolerance in tomato (Park et al., 2006) and maize (Chen et al., 2000).

References

Alia, H.H., Chen, T.H.H., Murata, N., 1998. Transformation with a gene for choline oxidase enhances the cold tolerance of Arabidopsis during germination and early growth. Plant Cell Environ. 21, 232–239.

Allard, F., Houde, M., Krol, M., Ivanov, A., Huner, N.P.A., Sarhan, F., 1998. Betaine improves freezing tolerance in wheat. Plant Cell Physiol. 39, 1194–1202.

Brown, R.D. 2000. Northern Hemisphere snow cover variability and change, 1915– 1997. J. Clim. 13, 2339–2355.

Brown, R.D., Braaten, R.O., 1998. Spatial and temporal variability of Canadian monthly snow depths, 1946–1995. Atmos–Ocean 36, 37- 45.

Chen, W.P., Li, P.H., Chen, T.H.H., 2000. Glycinebetaine increases chilling tolerance and reduces chilling-induced lipid peroxidation in *Zea mays* L.Plant Cell Environ. 23, 609–618.

Dore, M.H.I., 2005. Climate change and changes in global precipitation patterns: What do we know? Environment Int. 31, 1167–1181.

Fowler, D.B., Limin, A.E., Ritchie, J.T., 1999. Low-temperature tolerance in cereals: Model and genetic interpretation. Crop Sci. 39, 626–633.

Gholipoor, M., Soltani, A., Shekari, F., Shekari, F.B., Karimi, S., 2006. Quantitative evaluation of climate change during past 44 years of Kermanshah and its effect on chickpea phenology, using simulation. (Abstract in persian). Proceedings of 9th Iranian Crop Science Congress, Aug. 27-29, Aboureyhan Campus- University of Tehran, Tehran, Iran.

Gholipoor, M., 2008. Evaluating past surface air temperature change in Tabriz, Iran, using hourly-based analyzing. Int. J. Agric. Res. 3, 131-139.

Ghorbani, M.H., Soltani, A., 2002. Evaluating climate change in Gorgan for past 40 years (Text in persian, abstract in english). J. Agric. Sci. Natural Resources, 9: 3-13.

Gusta, L.V., Fowler, D.B., 1976. Effects of temperature on dehardening and rehardening of winter cereals. Can. J. Plant Sci. 56, 673–678.

Hellergren, J., Widell, S., Lundborg, T., Kylin, A., 1983. Frost hardiness development in *Pinus sylvestris*: The involvement of a K^+-stimulated Mg^{2+}-dependent ATPase from purified plasma membranes of pine. Physiol. Plant. 58, 7-12.

Howarth, C.J., Ougham, H.J., 1993. Gene expression under temperature stress. New Phytol. 125, 1-26.

Levitt, J., 1980. Response of plants to environmental stresses. 2nd ed. Academic Press, New York.

Mahfoozi, S., Limin, A.E., Fowler, D.B., 2001a. Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. Crop Sci. 41, 1006–1011.

Mahfoozi, S., Limin, A.E., Fowler, D.B., 2001b. Developmental regulation of low-temperature tolerance in winter wheat. Annals of Bot. 87, 751–757.

Park, E.J., Zoran Jeknic, Z., Chen, T.H.H., 2006. Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. Plant Cell Physiol. 47, 706–714.

Pomeroy, M.K., Andrews, C.J., Fedak, G., 1975. Cold hardening and dehardening responses in winter wheat and winter barley. Can. J. Plant Sci. 55, 529–535.

Ritchie, J.T., 1991. Wheat phasic development. p. 31–54. In Hanks, R.J., Ritchie, J.T. (Eds.) Modeling plant and soil systems. Agron. Monogr. 31. ASA, CSSA, and SSSA, Madison, WI.

Robinson, D.A., 1997. Hemispheric snow cover and surface albedo for model validation. Ann. Glaciol. 25, 241–245.

SAS Institute, 1989. SAS/STAT Users Guide, Version 6, fourth ed. SAS Institute, Cary, NC.

Soltani, A., Gholipoor, M., 2006. Teleconnections between EL Nino/Southern Oscillation and rainfall and temperature in Iran. Int. J. Agric. Res. 1, 603-608.

Soltani, A., Robertson, M.J., Torabi, B., Yousefi-Daz, M., Sarparast, R., 2006. Modelling seedling emergence in chickpea as influenced by temperature and sowing depth. Agric. For. Meteorol. 138, 156-167.

Uemura, M., Steponkus, P.L., 1999. Cold acclimation in plants: Relationship between the lipid composition and the cryostability of the plasma membrane. J. Plant Res. 112, 245-254.

Vézina, L.P., Ferullo, J.M., Laliberté, G., Laberge, S., Willemot, C., 1997. Chilling and freezing. In: Prasad, M.N.V. (Ed.) Plant Ecophysiology. John Wiley and Sons, Inc., NewYork, p 61-100.

Yoshida, S., Hotsubo, K., Kawamura, Y., Murai, M., Arakawa, K., Takezawa, D., 1999. Alterations of intracellular pH in response to low temperature stresses. J. Plant Res. 112, 225-236.

Zhao, Y., Aspinal, D., Paleg, L.G., 1992. Protection of membrane integrity in *Medicago sativa* L. by glycinebetaine against the effects of freezing. J. Plant Physiol. 140, 541–543