

Intraspecific variability in cold hardiness of Taurus Cedar (*Cedrus libani* A. Rich.) in Türkiye

Bora İmal

bimal@karatekin.edu.tr

Çankırı Karatekin University

Akın Semerci

Gaziosmanpaşa University

Carlos A. Gonzalez-Benecke

Oregon State University

Research Article

Keywords: Cold hardiness, Lebanon cedar, visual scoring, chlorophyll fluorescence, provenance trial

Posted Date: June 16th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3026172/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at European Journal of Forest Research on January 20th, 2024. See the published version at <https://doi.org/10.1007/s10342-023-01648-8>.

Abstract

To better guide *Cedrus libani* provenances movement to northerly and higher altitudes sites for afforestation, the limits of hardiness to cold should be better known. In this study, we quantified the cold hardiness of seven *C. libani* provenances sampled from a provenance trial in Ankara, Türkiye, a site located outside the natural range of distribution of the species. Visual damage observation and chlorophyll fluorometry screening methods were used to assess variation in cold hardiness (LT50, the temperature estimated at which 50% of the needle tissue is damaged) among populations. Overall, *C. libani* can tolerate winter temperatures up to -21°C. Even though there were significant differences in cold hardiness among populations, the maximum difference was only 1.9°C between the most and the least cold-resistant provenances. Cold tolerance was generally greater for provenances that experienced colder temperatures in March and lower levels of total precipitation in their native ranges. We also conclude that the fluorometry method provides a more efficient and stable comparison of cold hardiness than visual observation for *C. libani*. The results of this study may be useful for assisted migration and breeding programs, as well as for developing guidelines for genetic materials transfer, to increase productivity or adaptability of *C. libani*.

1. Introduction

While global warming causes milder winter temperatures on average, it is also expected to increase temperature variance and the probability of extreme weather events (Vavrus, Walsh et al. 2006; Francis and Skific 2015). The intensity and duration of cold extremes are expected to persist in Europe (Vavrus et al. 2006; Kodra et al. 2011). Consequently, frost damage for trees will likely not disappear in a globally warmer climate and may become even more problematic. Therefore, tree species adapted to the future climate of Central Europe must be tolerant to increased summer drought stress and tolerant to present and future low air and soil temperatures in winter (Bachofen et al. 2016).

Populations or species that originated in Mediterranean climates are expected to be better adapted to the future climate of Central Europe than temperate-originated ones (Bachofen et al. 2016). Furthermore, when growing under drought conditions, *Pinus sylvestris* seedlings from Spanish and Turkish populations have been reported to perform better than seedlings from Central or Northern European populations (Taeger et al. 2013; Matías and Jump 2014; Semerci et al. 2017). Such results support expectations of a northwards shift of Mediterranean tree species to Western and Central Europe. Even though drought tolerance is an important factor affecting trees survival under future climatic conditions in Central Europe, Mediterranean species might be vulnerable to other factors when they are exposed to more northerly climates, where frost might constrain the northerly expansion of Mediterranean populations to Central Europe, where winter temperatures are considerably lower than in the Mediterranean region.

The distribution range of Taurus cedar, *Cedrus libani* A. (Rich.), was wider than today, but forests have changed since the Late Glacial era (Hajar et al. 2010). Currently, it exists only about 2000 and 400 ha in Lebanon and Syria, respectively (Khuri et al. 2000; Haroutunian, Chojnacky et al. 2017). Although the same disturbances have continued in Anatolia (Asia Minor), only small populations have survived in a well-preserved state in disjoint areas, particularly the almost inaccessible topography of the Taurus Mountains has prevented *C. libani* from extinction (Boydak 2003; Boydak and Çalikoğlu 2008). Some scattered populations can be found in other parts of Anatolia. This disjoint distribution together with site conditions (i.e., elevation, microclimate, absence or rareness of associating tree species) leads to morphological, physiological, phenotypical and molecular differences between distinct provenances of *C. libani* (Dirik 2000; Kayihan et al. 2006; Boydak and Çalikoğlu 2008; Fady et al. 2008).

C. libani typically grows between 800 and 2100 m. a.s.l., smaller stands may occur in lower (500–600 m a.s.l.) or higher (up to 2400 m a.s.l.) elevations. Mean yearly precipitation varies between 600 and 1200 mm (Boydak 2003). Prolonged summer droughts are common in its habitat, and they can withstand frost at about -20 °C and -25 °C in arboreturns in Massachusetts, USA, and Bayreuth, Germany (Goor and Barney 1976; Messinger, Güney et al. 2015). Extremely cold winter temperatures of -35 °C may occur on the top of the Taurus Mountains in its natural distribution in Türkiye (Boydak 2003).

Because of its plasticity towards climate extremes, high survival rate and unique wood properties, large afforestation/reforestation efforts with *C. libani* are being undertaken outside its natural range in Türkiye and other countries such as Italy, Iran and Bulgaria. Moreover, it has also been planted in parks and gardens in many European and South American countries (Boydak and Çalikoğlu 2008). Messinger et al. (2015) indicated that *C. libani* has a high adaption to current and future climate conditions in Central Europe, highlighting its promising potential for establishing stable and productive forest stands in Central Europe. Similarly, Frischbier et al. (2019) reported that *C. libani* has performed well in sites established on clear-cuts in Germany, Austria and Switzerland, which represent a broad range of geological and climatic conditions typical for Central Europe.

C. libani has been widely used in afforestation/reforestation activities in Türkiye and Lebanon, as a major species for ecosystem restoration and increasing forest cover during the last three decades. As a result, many cedar stands were established, mainly through planting, in the Aegean, Marmara, Black Sea and Inner and Eastern Anatolian regions of Türkiye, doubling the planted area to about 47000 ha. Additionally, there are 11000 ha of pure natural stands of the species in Türkiye (Boydak 2020). Simulations of *C. libani* future geographical distribution for the year 2100 on the entire Eastern Mediterranean region show that only a few areas in Syrian and Lebanon may allow its survival of the species in its native range (Hajar et al. 2010). Under future climate scenarios (i.e. RCP 4.5 and RCP 8.5) the area suitable for *C. libani* would increase outside its natural range, especially in Central-Northern Turkish, while, on the other hand, Southern Turkish ranges will lose potential distribution at lower elevations, pushing the species to higher elevation sites (López-Tirado et al. 2021). Climate change would be a driver for assisted migration for the species, extending the distribution to a larger area than the present distribution.

Cold hardiness is an important trait reflecting adaptation to climate. It can be assessed by evaluating the freezing damage after natural frost events in field trials, but this method possesses limitations related to uncontrolled conditions. A better solution is to subject samples to different freezing temperatures under controlled conditions and to evaluate the freezing damage in those samples (Burr et al. 2001). Cold stress results in various observable or measurable symptoms of injury including the death of whole plants, visible necrosis of specific tissues and organs; or less obvious cellular symptoms that can be detected by vital staining, osmotic responsiveness, chlorophyll fluorescence, or by measuring relative electrolyte leakage in the affected tissues. Visual observation (VO) and chlorophyll fluorometry (CF) are commonly used methods for screening cold hardiness (Burr et al. 2001; Prada et al. 2014).

Due to climate change, assisted migration of select populations of *Cedrus libani* may be required to preserve the species. However, it is unknown if these populations possess the necessary cold hardiness to survive at higher latitudes and/or higher elevations where the minimum temperatures are colder than the populations currently experience. Therefore, the objectives of this study were to: (1) determine if there is frost hardiness difference among provenances (2) compare two screening methods (VO and CF) commonly used to assess frost hardiness (3) determine relations between cold hardiness of provenances and the climate of their native ranges.

2. Materials and methods

2.1. Provenances

This study was based on the *C. libani* provenance trial that was established in 1990 in Ankara (outside of natural distribution area), Türkiye. The provenance test includes 35 provenances, which represent the entire range of the species. A randomized complete block design with three replications was applied in the field. Each provenance was represented by 16 trees in each block (Gökdemir et al. 2012).

For this study, we selected 7 provenances for cold hardiness assessment: Arslanköy (ARS), Çığlıkara (CIG), Ermenek (ERM), Göksun (GOK), Gölhisar (GOL), Niksar (NKS) and Sultandağı (SUL) (Fig. 1). The 7 provenances were selected using survival data from the provenance trial carried out at age 20 years. Across all 35 provenances at age 20 years, mean survival was 88%, while survival of 7 sampled provenances spanned between 56 and 94% (Table 1).

Table 1 Characteristics of <i>Cedrus libani</i> provenance origin and study sites, including survival (%) at age 20 years, Emberger bioclimatic classification and weather indexes for the period 1960–2019.														
Label	Provenance	Lat.	Lon.	Elevation	Survival	Bioclimatic zone	Subzone	Q	S	Abs Tmin	Rain	Tmax	Tmin	Rain _s
				(m.a.s.l.)	(%)			(mm/°C)		(°C)	(mm)	(°C)	(°C)	(mm)
ARS	Arslanköy	37° 00'	34° 13'	1800	71	Rainy	Very cold	109.6	4.8	-12.7	991.8	28.1	-3.6	134.1
CIG	Çığlıkara	36° 33'	29° 48'	1550	92	Semi-arid	Cold	51.0	2.5	-18.6	500.0	32.0	-2.0	81.0
ERM	Ermenek	36° 30'	32° 44'	1710	94	Semi-arid	Cold	50.8	2.6	-14	473.8	30.6	-1.8	79.6
GOL	Gölhisar	36° 55'	29° 29'	1650	90	Semi-arid	Ext. cold	57.1	2.6	-33.5	621.4	29.8	-8.5	78.4
GOK	Göksun	38° 08'	36° 46'	1730	92	Semi-arid	Cold	43.8	2.3	-19.7	448.0	32.7	-2.8	73.7
NKS	Niksar-Akıncı	40° 47'	36° 34'	1100	56	Semi-arid	Cold	48.6	2.6	-23.4	436.7	29.7	-1.6	76.7
SUL	Sultandağı	38° 32'	31° 09'	1400	83	Semi-arid	Very cold	46.9	2.5	-27	443.5	29.5	-3.5	72.9
Provenance Trial Site														
ANK		40° 03'	32° 28'	1010	88	Semi-arid	Very cold	36.6	2.1	-24.9	367.3	30.8	-4.2	63.2

Abs Tmin: absolute minimum temperature (°C); Rain: annual rainfall (mm); Tmax: mean maximum temperature in summer (°C); Tmin: mean minimum temperature in winter (°C); Rain_s: mean total rainfall during summer (mm); Q: mean rainfall-temperature coefficient (mm/°C); S: Summer drought index.

Meteorological data from provenances origin and study sites were obtained from the Turkish Meteorology Service using data from the nearest weather station (about 10 km) available (Türkiye Meteorological Service 2020). According to Emberger Bioclimatic Classification (Baylan and Ustaoglu 2020), Mediterranean bioclimate conditions characterized by summer drought index (S) are dominant at the provenances and study sites (i.e. S < 5).

Nevertheless, the rainfall to temperature coefficient (Q) of ARS provenance (rainy) was higher than the rest (semi-arid). Subzones classification indicates that winters of the provenances sites span between cold and extremely cold, while winters of the test site (ANK) are classified at an intermediate position, as very cold (Table 1).

2.2. Branch sampling and cold hardiness tests

The whole-plant freeze testing method was used to estimate frost damage (Burr et al. 2001). Distal parts of the branches of approximately 20 cm length were frozen at different temperatures in a programmable freezer. At the study site, branchlets (~ 20 cm in length) were collected from the southern middle part of the crown (fully exposed to the sun) from 10 trees per provenance (70 samples total). Dominant trees distributed over all three blocks were chosen. Sample collection was carried out using a ladder and a telescopic tree pruner in the middle of February 2019. Immediately after branchlets excision, samples were placed in plastic bags and sprayed with distilled water while kept in the dark in a cooler and transported to the laboratory where they were kept moist at a refrigerator at + 4°C for one day until measurements started.

The day after sample excision the whole branchlets were inserted in a low-temperature freezer equipped with a programmable controller (VötschVT3 4034). The control group was kept at + 4°C. The freezing rate was kept to 5°C/h until each target temperature (-15, -20, -22.5 -25 and - 30 °C) was reached (Burr et al. 2001). Each target temperature was achieved independently to keep each treatment group at the target temperature for 5 h. After that, thawing was done by increasing temperature by 5°C per hour until they reached room temperature.

2.2.1. Visual observation

After thawing, the remaining twigs of each freezing treatment were left for two weeks at a greenhouse temperature (+ 20°C) to allow visible signs of freezing damage to develop. Freeze-injured tissue typically develops a brown or yellowish color from the oxidation of polyphenols (Lindén 2002). To improve the visibility of symptoms, water was sprayed on samples every three days. The cold injury was assessed visually by recording the percentage of discolored needles on branches two weeks after freezing tests (Burr et al. 1990; Prada et al. 2014). Visual observation (VO) scoring in needles was done by using a scale of 0 to 4 depending on the percentage of the foliar area damaged (0 = 0–20%; 1 = 21–40%; 2 = 41–60%; 3 = 61–80%; 4 = 81–100% of the foliar area damaged). For statistical analyses, the class value was used on each category (10, 30, 50, 70 and 90%, respectively).

2.2.2. Chlorophyll Fluorometry

Chlorophyll fluorescence (CF) measurements provide information about the overall photosynthetic potential of the plant and its responses to stress or disturbances (Mohammed et al. 1995). The ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence (F_v / F_m) is linearly correlated with the quantum yield of net photosynthesis. The parameter F_v / F_m was used for CF as it is highly correlated with low-temperature tolerance (Strand and Öquist 1985; Rose and Haase 2002). High values of F_v / F_m reveal undamaged tissue, while low values are indicative of freezing damage. Measurements of F_v / F_m were performed using a Handheld chlorophyll fluorometer (Opti-Sciences OS30p). After thawing the twigs at each freezing treatment, needles were acclimated to the dark for at least 30 minutes before they were measured. From here and after we will refer to F_v / F_m as CF.

2.3. Statistical analysis

A non-linear model reflecting the relationship between temperature (expressed as positive values) and tissue damage (VO or CF) was fitted for each sample. After testing several sigmoidal curves, the model proposed by Kreyling et al. (2012) was selected:

$$Y_T = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + e^{k \cdot (T_m - T)}} \quad \text{Eq. 1}$$

where Y_T is the tissue damage (VO or CF) at temperature T , Y_{min} is the lower horizontal asymptotic value of the response variable, Y_{max} is the upper horizontal asymptotic value of the response variable, k represents the steepness of the response curve, and T_m is the inflection point of the curve, which corresponds to LT50, the temperature at which 50% pf damage was observed (Pinheiro and Bates 2000).

The Statistical Analysis Software version 9.4 (SAS Institute Inc. Cary, NC) was used for all statistical analyses. To determine the relationships between LT50 and environmental variables (elevation, latitude and minimum temperatures of each winter month, January to March, including the average across all months), simple correlation analysis was performed for each environmental variable and LT50. Additionally, multiple linear regression was performed for modeling LT50 as a function of environmental variables. The model with significant parameter estimates with the lowest AIC was finally selected. In order to detect multicollinearity among explanatory variables, the variance inflation factor (VIF) was monitored, discarding variables included in the model with a VIF larger than 5 (Neter et al. 1996). Model parameters were selected using a Stepwise selection procedure. Residuals were examined to ensure that statistical assumptions of normality and homoscedasticity were met. Non-linear model fitting (PROC NLIN) was used to determine LT50 (relationship between temperature and tissue damage) and to model the relationship between LT50 and environmental variables. Analysis of variance, including Tukey multiple comparisons tests (PROC MIXED), was used to test the effects of provenances on LT50. All figures were produced using SigmaPlot version 14 (Systat Software, Inc. San Jose, CA).

3. Results

Long-term minimum monthly temperature (percentile 5 of minimum monthly temperatures for 27–55 years of observations) of provenance origin and study sites are shown in Fig. 2. During winter, provenance origin sites ARS and ERM reached a minimum temperature above – 15°C, while provenance origin sites CIG, GOL and NKS, as well as the study site ANK, showed minimum temperatures ranging between – 15 and – 25°C. Provenance origin sites SUL and GOK had the lowest temperatures, reaching below – 25°C during January and February.

3.1. Cold hardiness variations among provenances

Overall, the two methods used to assess frost damage on *C. libani* showed similar results, indicating that lethal damage in needles started below – 20°C. There was a sharp increase in damage between – 20 and – 25°C (Fig. 3).

Figure 3. Relationships between temperature and a) chlorophyll fluorescence (CF, Fv/Fm) and b) visual observation of percent damage after two weeks (VO, %) of *Cedrus libani* provenances. Symbols represent mean values (n = 10).

There were significant differences in LT50 among *C. libani* provenances ($P < 0.0001$), and the values of LT50 differed among screening methods ($P = 0.0072$). Across provenances, LT50 of *C. libani* ranged between – 23.4 and – 21.5°C, when evaluated with the VO method, and between – 23.8 and – 22.4°C, when evaluated with the CF method. Independent of the screening method, the most and least frost-resistant provenances were NKS and ARS, respectively (Table 3).

Table 3
Average LT50 (°C), determined with visual observation of percent damage after 2 weeks (VO) and chlorophyll fluorescence (CF) for *Cedrus libani*. Values in parenthesis indicate standard error. Values with the different letter indicate difference at $\alpha = 0.05$

Provenance	VO		CF	
ARS	-21.5 (0.55)	c	-22.4 (0.54)	b
CIG	-23.3 (0.21)	a	-23.6 (0.34)	ab
ERM	-21.9 (0.31)	c	-23.0 (0.27)	ab
GOK	-22.3 (0.32)	abc	-23.6 (0.37)	ab
GOL	-22.5 (0.42)	abc	-23.4 (0.16)	ab
NKS	-23.4 (0.18)	a	-23.8 (0.21)	a
SUL	-23.1 (0.17)	ab	-23.6 (0.27)	ab

Cold hardiness was related to the climate within the native ranges of the provenances. There was no correlation between LT50 and latitude of provenance origin, independent of the screening method ($P = 0.218$ and $P = 0.198$, for VO and CF, respectively). On the other hand, there was a significant non-linear relationship between LT50 and elevation of provenance origin ($P < 0.0001$), where LT50 declined sharply as elevation of origin site larger than 1600 m.a.s.l. Provenances originated on sites with elevations lower than 1600 m.a.s.l. displayed relatively constant and higher cold hardiness (Fig. 4).

There was a correlation between LT50 and the minimum temperature of March at the provenance origin ($P < 0.0001$), and that relationship was non-linear, decreasing frost hardiness as the minimum temperature of March was higher than – 10°C. Correlation between LT50 and the minimum temperature of other winter months (Dec, Jan and Feb, as well as the average of all winter months) was also tested, but minimum temperature of March showed the largest correlation (data not shown). Furthermore, LT50 was also correlated with total annual rainfall at the provenance origin ($P < 0.0001$), where cold hardiness was generally greater for provenances that experienced lower levels of precipitation in their native ranges (Fig. 5).

Using data from CF determination method, a multiple non-linear regression was fitted to describe the general effect of both, total annual rainfall (62.6% of the explained variance) and minimum temperature of March (29.5% of the explained variance) of the origin site on LT50 ($P < 0.0001$; $R^2 = 0.92$). Parameter estimates and fit statistics of the models that depict the relationships between LT50 (determined using CF), and environmental characteristics of provenance origin sites (elevation, total rainfall and minimum temperature of March), as well as an equation to estimate LT50 from the combined effect of those environmental variables, are shown in Table 4.

Table 4

Parameter estimates and fit statistics for the models that depict the relationships between LT50 (determined using CF) and elevation (m.a.s.l.), minimum temperature of March annual rainfall of *Cedrus libani* provenances origin sites. All parameter estimates were significant at $\alpha = 0.05$.

Environmental Variable	Model	Parameter	Parameter Estimate	Standard Error
Elevation	$LT50 = a + b \left(\frac{c}{elevation} \right)$	a	-23.6780	0.1745
		b	2040.6	140.5
		c	23.118	12.4437
Rainfall	$LT50 = a + b \bullet Rainfall$	a	-24.441	0.399
		b	0.00196	0.00067687
Tmin3	$LT50 = a \bullet (1 + e^{-b \bullet Tmin3})$	a	-23.659	0.0753
		b	0.5356	0.0230
Rainfall and Tmin3*	$LT50 = a \bullet (Rainfall)^b \bullet (Tmin3)^c$	a	-28.1166	1.3161
		b	-0.0389	0.00690
		c	0.0233	0.00330

Elevation: provenance origin elevation (m.a.s.l.); Rainfall: provenance origin total annual rainfall (mm); Tmin3: provenance origin minimum temperature of March (°C); LT50: temperature at which 50% of the needle tissue is damaged.

*: Tmin3 is included as positive number

When all environmental variables were included into the model, elevation was not selected for the final linear model. Overall, cold hardiness increased (decreasing LT50) for provenances origin sites with a lower minimum temperature of March and with a lower total annual rainfall. The combined effect of both climatic variables is shown in Fig. 6.

3.2. Comparison of screening methods

Overall, mean LT50 assessed by VO and CF were -22.6 and -23.3°C , a difference of about 0.7°C between screening methods, implying that, in practice, both methods gave similar results for *C. libani*. Furthermore, there was a strong correlation between the two methods used to determine frost tolerance across provenances, ($P < 0.001$; $R^2 = 0.79$; Fig. 7).

Figure 7. Relationship between LT50 determined using visual observation (VO) and chlorophyll fluorescence (CF). The dashed line represents a 1:1 relationship. b) Coefficient of variation for LT50 determined using visual observation of damage (VO) and chlorophyll fluorescence (CF). Error bars represent standard error ($n = 10$).

4. Discussion

4.1. Cold hardiness variations among provenances

Species from cold climates are likely to have high resistance to frost than those from warmer climates. Consequently, a classification of climate that takes minimum temperatures into account is most likely to be correlated with the cold hardiness of species. The U.S. Department of Agriculture (USDA) refined the method and adopted 11 cold hardiness zones. Plants have been ascribed hardiness ratings that are numerically equivalent to the lowest climatic zone in which they have been observed to survive. According to hardiness zones developed by USDA, *C. libani* is classified in Zone 7, having a minimum temperature of -17.7°C as its zonal limit. However, practically all species have measured cold hardiness that exceed the limits defined by their particular cold hardiness zones. The discrepancy is about 6.9°C for Zone 7. Therefore, we can extend the minimum temperature of zone 7 to -24.6°C (Bannister and Neuner 2001). In our experiment, the frost hardiness of *C. libani* ranged from -23.8 (with CF) to -21.5°C (with VO). Hence, our determinations of cold hardiness of *C. libani* are consistent with the cold hardiness zones made by the USDA. Our findings are also consistent with a previous study of Kreyling et al. (2015) who observed visual signs of natural frost damage on *C. libani* at the Ecological Botanical Garden of the University of Bayreuth, Germany when temperature decrease to -23.7°C in mid-winter. The authors also measured cold tolerance (LT50 obtained by relative electrolyte leakage method) of *C. libani* between -18.7 and -23.3°C in March. According to our results and that previous work on the cold hardiness of *C. libani*, we may advise practitioners that the species should not be planted in areas where the coldest temperature is lower than -21°C .

In this study, we found that old hardiness patterns could largely be explained by the climate of the provenance origins indicates that cold hardiness was genetically differentiated among populations. Subsequently, there is a potential for the selection of more frost tolerant *C. libani* populations, which is an important advantage for a species that have to survive in harsh environments, especially in continental areas or at high altitudes. Nevertheless, the magnitude of provenance variation in mid-winter cold tolerance was relatively small (1.9°C between the most tolerant and the least tolerant provenances), when compared with other conifer species. For example, the range in average midwinter cold hardiness among 12 red spruce provenances was 8 to 10°C and 14 to 18°C among balsam fir provenances (DeHayes et al. 2001). Similarly, cold hardiness in winter differed by about 10°C between provenances of Black pine (Kreyling et al. 2012). The reason for the small variation in cold hardiness observed among provenance of *C. libani* may be low variation in geographic and climatic conditions across the range of distribution of the species.

Our results indicate that, independent of the screening method employed, NKS (Niksar-Çatalan) was the hardest, and ARS (Mersin-Arslanköy) was the least hardy provenance to cold. However, according to the RAPD analysis of 14 Turkish populations by Kayihan et al. (2006), the parent population of NKS could be the Mediterranean population of ARS. In this study, the authors did not find a clear pattern for geography, as the two distantly located seed stands NKS and ARS (see Fig. 1) were genetically similar, suggesting that the northern population NKS may have been introduced from the southern ARS population in the past. According to historical records, the NKS population might be introduced to its current area of distribution around the second century B.C., being isolated from core populations in Türkiye (Fady et al. 2008). Nevertheless, the authors did not find genetic similarities between the NKS and ARS provenances.

Cold hardiness is generally greater at higher latitudes and altitudes, reflecting the decreasing temperature as latitude and altitude increase. This relationship has often been interpreted but is less frequently quantified (Bannister and Neuner 2001; Bannister 2007). Our results, however, showed no relationship between the cold hardiness and latitude of provenances origin of *C. libani*. Moreover, contradicting the general idea, provenances originated from lower altitudes generally displayed higher cold hardiness. Consistent with our result, other studies also did not find a relationship between cold hardiness and latitude of provenances origin in *P. albicaulis* (Bower and Aitken 2006), *P. pinaster* (Prada et al. 2016) and *brutia* (Semerci et al. 2021).

Our results indicate that cold hardiness increased (decreased LT50) with decreasing annual rainfall (62.6% of the explained variance) and with decreasing minimum temperature of March (29.5% of the explained variance), suggesting that the selection of frost-tolerant provenances could be based on the current climatic conditions within the species ranges. Furthermore, the fact that cold hardiness patterns could be explained by the climate of the provenance origins indicates that cold hardiness was genetically differentiated among populations. A negative correlation between annual rainfall and cold hardiness in *C. libani* may indicate evidence that cold hardiness is physiologically related to drought tolerance in conifers. Drought and frost, for instance, lead to similar physiological responses in a coniferous forest tree aiming to prevent cellular dehydration. Thus, decreasing latitudes associated with increasing temperatures and decreasing rainfall may be increased both drought and cold hardiness in *C. libani*. This result is consistent with the study of Kreyling et al. (2015) who quantified cold tolerance (LT50 obtained by relative electrolyte leakage method) of 27 native and exotic (Northern Hemisphere) tree species. The authors concluded that, in general, cold tolerance increased with decreasing precipitation over the species' ranges. Therefore, as suggested by Kreyling et al. (2015), we may imply that drought-tolerant Mediterranean species (or populations) might be pre-adapted to frost.

Cold tolerance of tree species or provenances is related to the extreme minimum temperatures occurring within the species' ranges (Bannister 2007). Consistent with this, cold tolerance was generally greater for provenances that experienced colder March temperatures at the provenances' origin in our study. Similarly, Kreyling et al. (2012) found significant correlations ($R^2 = 0.77$ in the first winter, and $R^2 = 0.80$ in the second winter) between cold hardiness and mean minimum winter temperature of *P. nigra* provenances origins.

4.2. Comparison of screening methods

In the literature, the visual observation method generally is strongly correlated with the Chlorophyll fluorometry method. Although, in general, visual scoring was less precise than the fluorometry method (Binder and Fielder 1996; L'Hirondelle et al. 2006; Peguero-Pina et al. 2008; Prada et al. 2014; Semerci et al. 2021). In our study, the two cold hardiness screening methods (VO and CF) compared in this study were well correlated and both methods led to similar LT50 values for each provenance. Visual scores estimated only a bit lower (0.7°C) the level of damage compared to Chlorophyll fluorometry, implying that both methods provide similar accuracy, being comparable and useful tools for cold damage assessment. On the other hand, we measured frost damage 4 hours after the freeze-thaw treatment using fluorometry and two weeks after the freeze-thaw treatment using visual scoring. Thus, the major advantages of Chlorophyll fluorescence over visual scoring are the shorter time to completion and the elimination of observer bias in visual scoring.

Conclusions

According to the results using the CF screening method, the most sensitive population can withstand up to -22.4°C and the most tolerant population can tolerate a temperature of -23.8°C. Our results imply that the cold hardiness of the needles is adaptive to long-term climatic conditions at the seed origin (genetic heritage). According to our results, CF is the most recommendable method for assessing cold hardiness since it was faster and more objective than the visual observation. From a practical standpoint, we recommend that *C. libani* should not be transferred to areas where winter temperature decreases to -21°C and below, suggesting that a northward migration of Mediterranean *C. libani* to Central and Western Europe is not limited by frost damage as the minimum temperature of that region rarely decreases to -20°C. However, under global warming conditions, drought may

be a limiting factor in this region. Analyzing frost tolerance at a single point in time may not be enough to guide assisted migration efforts. Hence, our results should be supported by new studies which contain spring and autumn frost tolerance assessments.

Declarations

Contribution of the co-authors

Bora İmal and Akkın Semerci designed and conducted the field experiment, Carlos A. Gonzalez-Benecke ran the data analysis, led discussion, all authors wrote the paper and discussed the results.

Acknowledgments

We thank the General Directorate of Forestry and all the individuals linked to the establishment and maintenance of the provenance trials in Türkiye.

References

1. Bachofen C, Wohlgemuth T, Ghazoul J, Moser B (2016) Cold temperature extremes during spring do not limit the range shift of Mediterranean pines into regions with intermittent frost. *Functional Ecology* 30: 856–865. <https://doi.org/10.1111/1365-2435.12581>
2. Bannister P (2007) A touch of frost? Cold hardiness of plants in the Southern Hemisphere. *New Zealand Journal of Botany*, 45 (1): 1-33. <https://doi.org/10.1080/00288250709509700>
3. Bannister P, Neuner G (2001) Frost resistance and the distribution of conifers. *New For* 30: 167-184.
4. Baylan KA, Ustaoglu, B (2020). Distribution of Types and Subtypes of The Mediterranean Bioclimatic Zones According to Emberger Bioclimatic Classification in Turkey. *Ulusal Çevre Bilimleri Araştırma Dergisi*, Sayı 3: 158-174. (in Turkish with an abstract in English)
5. Binder WD, Fielder P (1996) Chlorophyll fluorescence as an indicator of frost hardiness in white spruce seedlings from different latitudes. *New Forests* 11: 233-253.
6. Bower AD, Aitken SN (2006) Geographic and seasonal variation in cold hardiness of whitebark pine. *Can J For Res* 36: 1842-1850.
7. Boydak M (2003) Regeneration of Lebanon cedar (*Cedrus libani* A. Rich.) on karstic lands in Turkey. *For Ecol Manag* 178: 231–243.
8. Boydak (2020) Sedinin (*Cedrus libani* A. Rich.) Tahribi Sonucu Toroslarda Oluşan Çıplak Karstik Alanların Ekim Yöntemiyle Yeniden Sedin Ormanlarına Dönüştürülmesinin Öyküsü, *Orman ve Av Dergisi*, 5: 20-30.
9. Boydak M, Çalıkoğlu M (2008) Biology and Silviculture of Lebanon Cedar (*Cedrus libani* A. Rich.) Ogem-Vak Press, Ankara
10. Burr KE, Hawkings CDB, L'Hirondelle SJ, Binder W D, George MF, Repo T (2001) Methods for measuring cold hardiness of conifers. In: Bigras FJ, Colombo SJ (eds) *Conifer cold hardiness*. Kluwer Academic, Dordrecht, pp 369-401
11. DeHayes DH, Schaberg PG, Strimbeck GR (2001) Red Spruce (*Picea rubens* Sarg.) cold hardiness and freezing injury susceptibility. In: Bigras FJ, Colombo SJ (eds) *Conifer cold hardiness*, Kluwer Academic Publishers, Dordrecht, USA, pp 495-529
12. Dirik, H (2000) Effet du stress hydrique osmotique sur la germination des graines chez les provenances de *Cedrus libani* (A. Rich.) d'origine Turque. *Ann For Sci* 57: 361–367
13. Fady B, Lefevre F, Vendramin GG, Ambert A, Regnier C, Bariteau M (2008) Genetic consequences of past climate and human impact on eastern Mediterranean *Cedrus libani* forests, implications for their conservation. *Conserv Genet* 9: 85-95
14. Francis J, Skific N (2015) Evidence linking rapid Arctic warming to mid-latitude weather patterns. *Phil Trans R Soc A* 373: 20140170. <http://dx.doi.org/10.1098/rsta.2014.0170>
15. Frischbier N, Nikolova PS, Brang P, Klumpp R, Aas G, Binder F (2019). Climate change adaptation with non-native tree species in Central European forests: early tree survival in a multi-site field trial. *European Journal of Forest Research* 138: 1015-1032. <http://dx.doi.org/10.1007/s10342-019-01222-1>
16. Goor AY, Barney CW (1976) *Forest Tree Planting in Arid Zones*. The Ronald Press Company, New York.
17. Gökdemir Ş, Örtel E, Çetinaş Ş, Altun ZG, Karatay H, Boza A (2012) Results of Provenance trials of Taurus Cedar (*Cedrus libani* A. Rich.) at the 20 years in Turkey. Central Anatolia Forestry Research Institute. Technical Bulletin No: 298 Ankara, Turkey.
18. Hajar L, Francois L, Khater C, Jomaa I, Deque M, Cheddadi R (2010) *Cedrus libani* (A. Rich.) distribution in Lebanon: past, present and future. *Comptes Rendus Biologies* 333: 622-630. doi:10.1016/j.crv.2010.05.003
19. Haroutunian G, Chojnacki D C, El Riachy R, Chojnacki C C (2017) Reducing reforestation costs in Lebanon: adaptive field trials. *Forests* 8: 169. doi:10.3390/f8050169
20. Kayihan GC, Kaya Z, Kandemir G, Önde S (2006) The genetic structure of *Cedrus libani* (A. Rich.) seed stands determined by random amplified polymorphic DNA markers. *Forest Genetics* 12(3): 181-190

21. Khuri S, Shmoury MR, Baalbaki R, Maunder M, Talhouk SN (2000) Conservation of the *Cedrus libani* populations in Lebanon: history, current status and experimental application of somatic embryogenesis. *Biodiversity & Conservation* 9(9): 1261-1273
22. Kodra E, Steinhäuser K, Ganguly AR (2011). Persisting cold extremes under 21st-century warming scenarios. *Geophysical Research Letters*, 38: L08705. doi:10.1029/2011GL047103.
23. Kreyling J, Wiesenberger GLB, Thiel D, Wohlfart C, Huber G, Walter J, Jentsch A, Konnert M, Beierkuhnlein C (2012) Cold hardiness of *Pinus nigra* Arnold as influenced by geographic origin, warming, and extreme summer drought. *Environmental and Experimental Botany* 78:99-108. <https://doi.org/10.1016/j.envexpbot.2011.12.026> \h.
24. Kreyling J, Schmid S, Aas G (2015) Cold tolerance of tree species is related to the climate of their native ranges. *Journal of Biogeography* 42: 156–166
25. Lindén L (2002) Measuring cold hardiness in woody plants. Dissertation, University of Helsinki
26. L'Hirondelle SJ, Simpson DG, Binder WD (2006) Overwinter storability of conifer planting stock: operational testing of fall frost hardiness. *New For* 32: 307–321
27. López-Tirado J, Vessella F, Stephan J, Ayan S, Schirone B, Hidalgo PJ (2020). Effect of climate change on potential distribution of *Cedrus libani* A. Rich in the twenty-first century: an ecological niche modeling assessment. *New Forests* <https://doi.org/10.1007/s11056-020-09798-y>
28. Matias L, Jump AS (2014). Impacts of predicted climate change on recruitment at the geographical limits of Scots pine. *Journal of Experimental Botany* 65: 299-310.
29. Messinger J, Güney A, Zimmermann R, Ganser B, Bachmann M, Remmele S, Aas G (2015) *Cedrus libani*: a promising tree species for Central European forestry facing climate change? *Eur J For Res* 134: 1005-1017 doi: 10.1007/s10342-015-0905-z
30. Millar CI, Stephenson NJ, Stephens SL (2007) Climate change and forests of the future: managing in the face of uncertainty. *Ecol. Appl.* 17: 2145-2151.
31. Mohammed GH, Binder WD, Gillies SL (1995) Chlorophyll fluorescence: A review of its practical forestry applications and instrumentation. *Scan. J. For. Res.* 10: 383-410.
32. Neter, J., Kutner, M. H., Nachtsheim, C. J., & Wasserman, W. (1996). *Applied linear statistical models* (4th editio). Irwin.
33. Prada E, Alía R, Climent J, Raquel D (2014) Seasonal cold hardiness in maritime pine assessed by different methods. *Tree Genetics & Genomes* 10: 689-701 <https://doi.org/10.1007/s11295-014-0714-4>
34. Prada E, Climent J, Alía R, Díaz R (2016) Life-history correlations with seasonal cold hardiness in maritime pine. *American Journal of Botany* 103 (12): 1-10.
35. Peguero-Pina JJ, Morales F, Gil-Pelegrin E (2008) Frost damage in *Pinus sylvestris* L. stems assessed by chlorophyll fluorescence in cortical bark chlorenchyma. *Ann. For. Sci.* 65: 813
36. Pinheiro JC, Bates DM (2000) *Mixed-effects models in S and S-plus*. Springer Series in Statistics and Computing. Springer-Verlag, New York.
37. Rose R, Haase D (2002) Chlorophyll fluorescence and variations in tissue cold hardiness in response to freezing stress in Douglas-fir seedlings. *New For* 23: 81-96
38. Semerci A, İmal B, Gonzalez-Benecke CA (2020) Intraspecific variability in cold tolerance in *Pinus brutia* sampled from two contrasting provenance trials. *New For* 52: 621-637 <https://doi.org/10.1007/s11056-020-09815-0>
39. Semerci A, Semerci H, Çalışkan B, Çiçek N, Ekmekçi Y, Mencuccini M (2017) Morphological and physiological responses to drought stress of European provenances of Scots pine. *European Journal of Forest Research* 136: 91-104. doi: 10.1007/s10342-016-1011-6.
40. Strand M, Öquist G (1985) Inhibition of photosynthesis by freezing temperatures and high light levels in cold-acclimated seedlings of Scots pine (*Pinus sylvestris*). I. Effects on the light-limited and light-saturated rates of CO assimilation. *Physiol. Plant.* 64: 425-430
41. Taeger S, Fussi B, Konnert M, Menzel A (2013) Large-scale genetic structure and drought-induced effects on European Scots pine (*Pinus sylvestris* L.) seedlings. *European Journal of Forest Research* 132: 481-496
42. Türkiye Meteorological Service (2020). General Directorate of Türkiye Meteorology Affairs.
43. Vavrus S, Walsh JE, Chapman WL, Portis D (2006) The behavior of extreme cold air outbreaks under greenhouse warming. *Int J Climatol* 26: 1133-1147

Figures

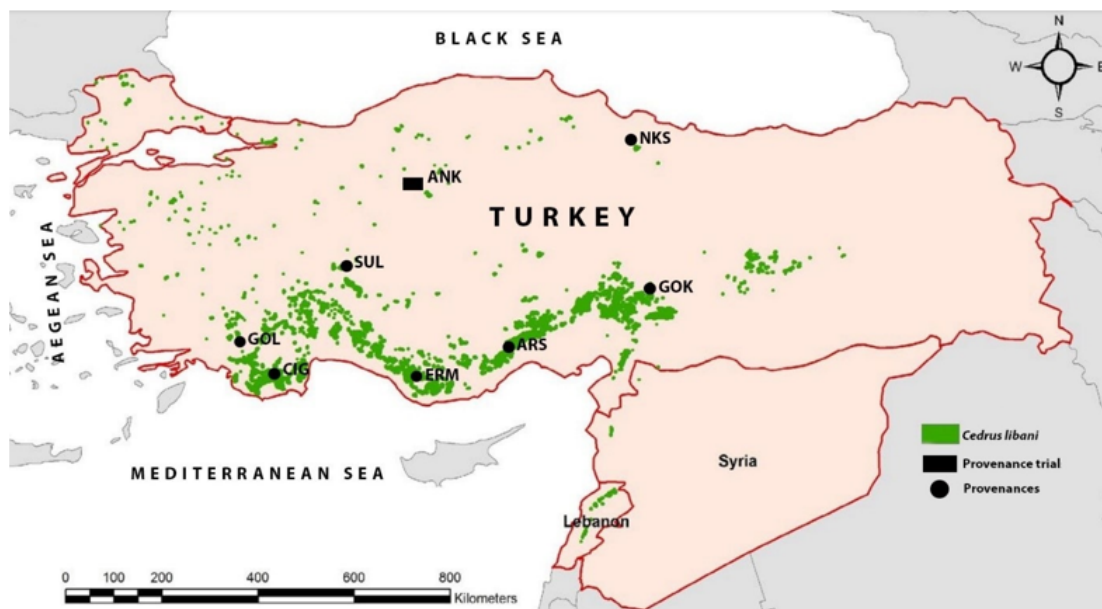


Figure 1

Geographic distribution of natural and planted stands of *C. libani* (Adapted from (López-Tirado et al. 2021)), including the location of provenance trial (black square, ANK) and provenances (black circle) locations.

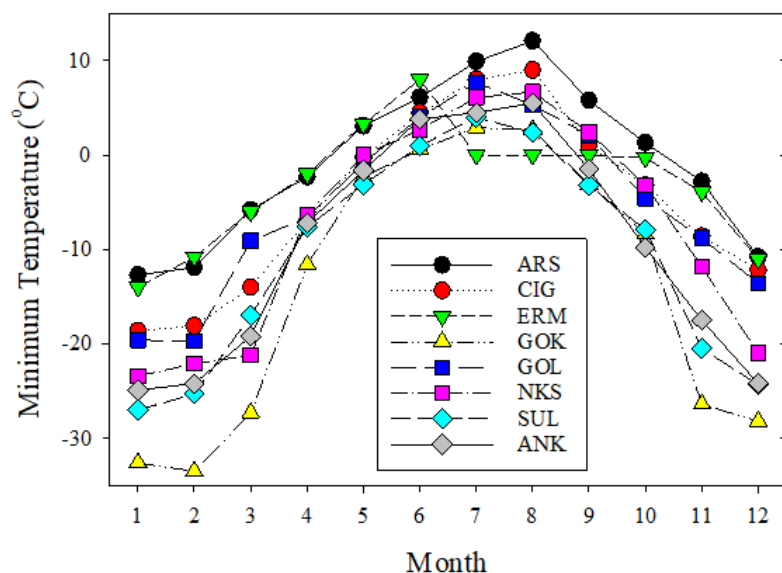


Figure 2

Minimum monthly temperature for provenance origin (ARS, CIG, ERM, GOK, GOL, NKS, SUL) and study (ANK) sites. Each symbol represents the percentile 5 of minimum monthly temperatures of 27-55 years of observations.

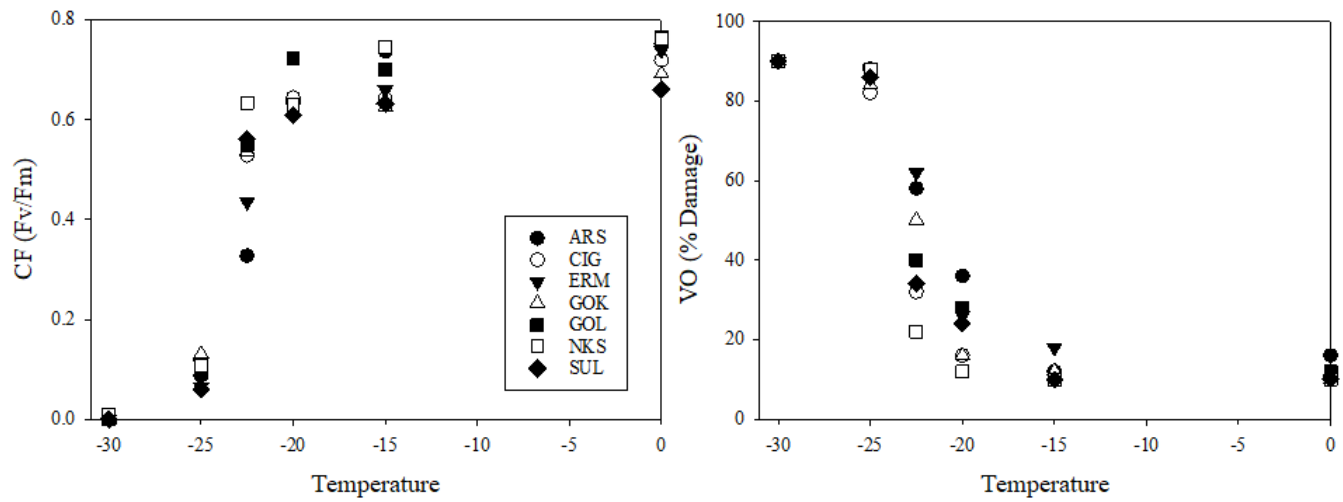


Figure 3
Relationships between temperature and a) chlorophyll fluorescence (CF, Fv/Fm) and b) visual observation of percent damage after two weeks (VO, %) of *Cedrus libani* provenances. Symbols represent mean values (n=10).

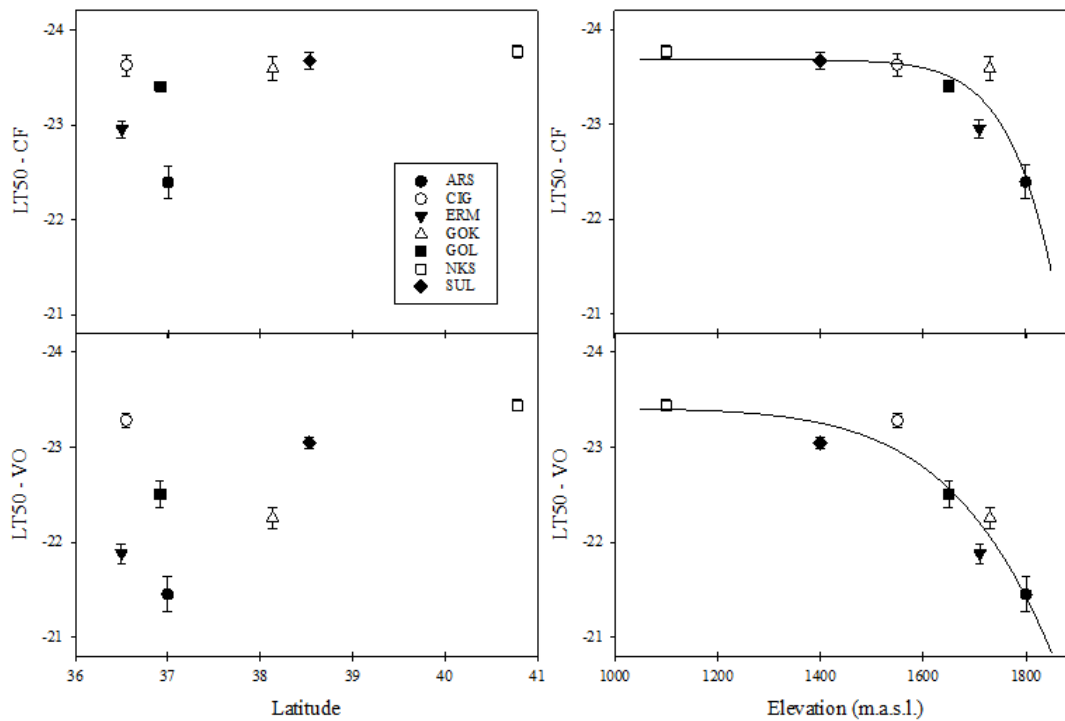


Figure 4
Relationship between LT50 determined using chlorophyll fluorescence (CF, upper panel) and visual observation of percent damage after 2 weeks (VO, bottom panel) methods and latitude (left) and elevation (right) of *Cedrus libani* provenances. Error bars represent standard error (n=10).

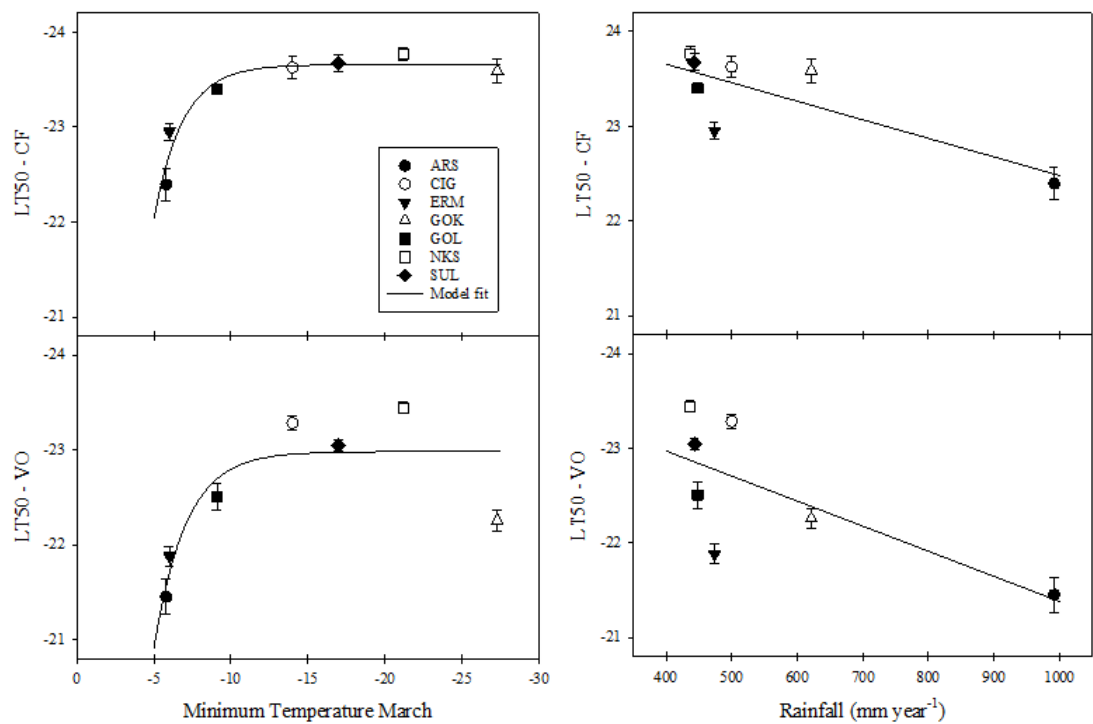


Figure 5

Relationship between LT50 determined using chlorophyll fluorescence (CF, upper panel) and visual observation of percent damage after 2 weeks (VO, bottom panel) methods and minimum temperature of March (left) and annual rainfall (right) of *Cedrus libani* provenances. Error bars represent standard error (n=10).

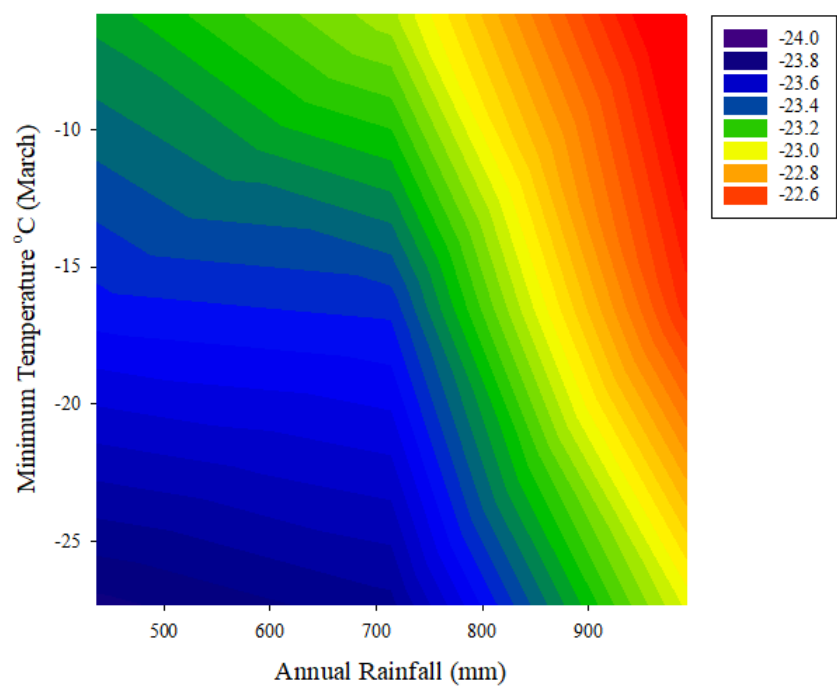


Figure 6

Combined effect of annual rainfall (mm) and minimum temperature of March (°C) on cold hardiness of *Cedrus libani*. LT50 was determined using chlorophyll fluorescence.

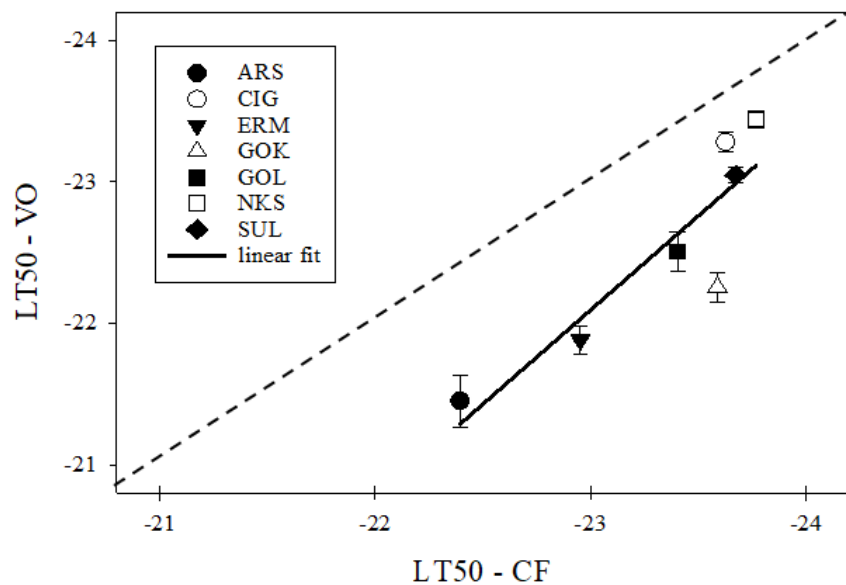


Figure 7

Relationship between LT50 determined using visual observation (VO) and chlorophyll fluorescence (CF). The dashed line represents a 1:1 relationship.
b) Coefficient of variation for LT50 determined using visual observation of damage (VO) and chlorophyll fluorescence (CF). Error bars represent standard error (n=10).