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FROST RESISTANCE IN INDIGENOUS BARLEY (*Hordeum vulgare* L.) LANDRACES CULTIVATED AT DIFFERENT ALTITUDINAL RANGES IN ETHIOPIA

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Abstract

Physiological investigations were made on 25 barley (*Hordeum vulgare* L.) landraces and accessions to screen for frost resistance. Samples were collected from three sites, in Amhara administrative region, north Wolo and Tigrai administrative region, south and east Tigrai (north Ethiopia) in November 1999. Experimental work was conducted from January to March after the seedlings have been grown for about a month in an open field at the Science Campus, Addis Ababa University. Accessions from IBCR were included for altitude ranges from which field collections were not made. Ethnobotanical study was also conducted in order to get information on the indigenous practices that may be enabling farmers to continue barley cultivation in frost prevailing areas.

In the surveyed areas, at least half of the farmlands of the peasant farmers were prepared for barley cultivation during the main rainy season, the rest being for legumes as well as wheat. The interviewees indicated environmental stresses such as insufficient rainfall, low soil fertility and frost effect as main limiting factors of barley production. They also responded that none of them had ever practiced selection of barley for resistance to frost. However, early maturing barley types are cultivated in higher altitudes when there is scarcity of rainfall, which also escape severe frost. The late barley landraces were neglected by the farmers due to the unreliable rainfall. The ethnobotanical investigation indicated that barley is used as a major food grain alone or in combination with other cereals utilized in different forms of foodstuffs. Moreover, it has both religious and cultural values.

A total of 25 samples were evaluated using conductivity and chlorophyll a fluorescence methods. Based on membrane stability and fluorescence studies, the potential survival temperature of the

samples ranged from –6.48 °C to –8.64 °C. Barley landraces from Abune-Yosef, Smp3 (Tedowasha) and Smp13 (Awarye), which were collected from altitudes of 3270 and 3300m asl. as well as accessions Acc5 (*Kokufa*) and Acc2 (*Chenka*) from Bale altitude of 2430m and 1650m asl., respectively were found to be relatively the most frost resistant in both methods. Samples Smp21 (*Saeda-Shewa*) from Tsibet-Embahasti altitude of 3100m asl. as well as Acc13 from Arsi altitude of 2800m asl. were found to be relatively the most susceptible in conductivity and fluorescence analysis methods, respectively. The study showed that –5 °C (using conductivity method) and –4 °C (using fluorescence analysis) were the highest sub-zero temperatures at which the samples could be differentiated easily. Altitude of site of sample collection had a slight positive correlation with frost resistance. The two methods had consistency and showed variations among the samples and are thus recommended for screening barley landraces and accessions. Fluorescence analysis was found to be a more sensitive method.

1 INTRODUCTION

1.1 Scope of the problem

Barley is a cereal crop that belongs to the grass family (Poaceae), tribe Hordeae (Triticeae), genus *Hordeum* and Section Cerealia Andes. The genus *Hordeum* includes both the cultivated and wild types. All the cultivated forms are included in *Hordeum vulgare* L. in recent classification.

The idea about origin and evolution of cultivated barley is controversial. Barley is domesticated from wild races (Zohary, 1969; Qiquan, 1981). Zohary (1969) suggested that the cultivated barley was derived from *H. spontaneum* (progenitor), while *H. agriocrithon* is a hybrid of the progenitor with the cultivated barley. On the other hand, Qiquan (1981) hypothesized that the six-rowed wild barley (*H. agriocrithon*) is the second stage from which the cultivated *H. vulgare* is derived. According to Purseglove (1972) barley, together with emmer wheat, was the first cereal to be domesticated in the Middle East before 9,000 years. He described it to be the most important of the early cereals. Bothmer and Jacobson (1985) assumed the Fertile Crescent in the Near East to be the original area of cultivation of barley. Orlov (1928) also described Ethiopia as an important geographical center for the origin and variations of many important forms of *Hordeum sativum* (Jessen), now called *Hordeum vulgare* L. Moreover, Vavilov (1951) after his expedition to Ethiopia in the 1920s established that Ethiopia is a center of origin of barley although later he revised Ethiopia to be secondary center of diversity, for the existence of the wild type is not yet confirmed. Based on studies on some authors (example, Endashaw Bekele, 1983 and Mulugeta Negassa, 1985) genetic diversity of barley put Ethiopia as a possible center for the origin of barley. Moreover, the existence of unique endemics such as the deficiens and labile (irregular) types as well as the abundant forms with features that are considered primitive in barley have been cited as evidence for the possible origin of barley in Ethiopia (Zemede Asfaw, 2000).

Barley is the fourth most important cereal crop, in production, next to wheat, maize and rice in the world. The range of cultivation of barley extends to latitudes as high as 64 °N, 67 °N, 70 °N in Alaska, Finland and Norway, where other crops do not grow, as well as to high altitudes in mountain slopes in Tibet and Ethiopia, for example, above other cereals (Poehlman, 1985). The major barley production areas of the world include most of Europe, the Mediterranean fringe of North Africa, Ethiopia, the Near East, USSR, China, India, Canada and USA (Harlan, 1976). In Ethiopia, barley (*Hordeum Vulgare* L.) is cultivated in a wide altitudinal range (1450 - 4000 m asl). and the diversity is higher in the highlands (2500 - 3000m asl.) Abebe Demissie and Bjornstad, 1997). It is cultivated in all regions and the most barley producing ancient provinces are Shewa, Arsi, Gojam, Gonder, Welo, Bale and Tigrai (Zemede Asfaw, 1988; Berhane Lakew *et al.*, 1996). Barley is a fast maturing crop that ripens earlier than other cereals, which indicates that it requires fewer heat units to reach physiological maturity (Poehlman, 1985). As a result, farmers are able to harvest twice a year in seasons locally called *kiremt*, long rainy season, and *belg*, short rainy season, which extend from June to September and from February to April, respectively. *Kiremt* and *belg* account for 85 % and 15 % of the total barley production, respectively (Fekadu Alemayehu and Hailu Gebre, 1987). Welo, Shewa and Bale are the main *belg* barley producing regions.

Barley is a traditional crop in the highlands of Ethiopia that has been cultivated at least for about 5000 years (Purseglove, 1972). It is the most important crop after maize, teff and sorghum (Berhane Lakew *et al.*, 1996). Consumer preference in the rest of the world has been shifted towards other cereal grains such as wheat. However, barley is now found to have the desirable nutritional composition and medicinal values. Researchers are motivated to improve it using genetic engineering and they are intending for the formation of barley ice cream and other products (Zemede Asfaw, 2000). In the highlands of Ethiopia it is the major staple food grain utilized in various forms of foodstuffs, which accounts for about 60 % of the populations total plant food (Zemede Asfaw, 1990). It is consumed in the form of bread, roasted grains, porridge, alcoholic and non-alcoholic beverages. Besides, barley straws are used for thatching roofs and making mattresses. Moreover, agronomic trials of malt barley have been practiced since late 1960's at different centers of the Institute of Agricultural Research (IAR) and on farms of the Ministry of State Farms Development (MSFD). Nevertheless, the share of malting barley in the barley production had been very low (about 2-3%) as compared to food barley and cultivation of malting barley is confined to Arsi and Bale regions (Amsal Tarekegn *et al.*, 1996).

Barley (*Hordeum vulgare* L.) despite its adaptability to wide ranges of ecological situations, is known to be susceptible to extreme environmental stresses (Stanca *et al.*, 1996). Environmental stresses such as water, salt and temperature stresses affect the productivity of the crop (Weltzien and Stivastava, 1981). The degree of susceptibility to moisture stress exists at all stages of the life cycle of the plant. However, the time of floral initiation and anthesis appear to be the two critical stages where moisture stress greatly affects the productivity of barley.

Cold and frost that occur seasonally or episodically are the limiting factors of crop production in major parts of the world (Malhotra and Saxena, 1993). The tolerable temperature.for terrestrial vascular plants ranges from -5 °C to about + 55 °C (Larcher, 1980). The mean temperature of the earth's land mass is, however, below 0 °C in which one third of the earth's surface is expected to be under severe frost, and only one fourth is absolutely safe from frost. Hence, the threat of frost effect is serious in wider area of the world (Larcher, 1980). At any time of the year frost occurs in high altitudes of the Arctic and Antarctic. In European Alps for nearly half of the year (from end of October to the end of April) the daily mean temperature remains at or below 0 °C. More than 75% of the precipitation falls as snow (Sakai and Larcher, 1987).

In mountains and highlands of equatorial zone annual temperature variation is not remarkable, but the diurnal (day/night) variations are very marked. As Hedberg (1952) cited in Hedberg (1995) described the area with the feature of "summer every day and winter every night" whereby episodic frost occurs most nights of the year alternating with high day temperatures. According to Weltzien and Stivastava (1981), within the International Center for Agricultural Research in the Dry areas (ICARDA), in higher altitudes temperature of below freezing point "winter kill" occurs commonly and only "winter hardy " barley genotypes survive whereas growth may be retarded at lower altitudes with temperature below 5 °C. In temperate zone barley varieties are reported to have freezing tolerance in the range of -16 °C to -20 °C, depending on provenance and variety (Sakai and Larcher, 1987).

In Ethiopia unreliable rainfall (water stress), poor soil fertility due to soil erosion, frost, water logging, pests such as field rats, cut worm, shoot fly, aphids and ball worm are the main constraints of barley production (Berhane Lakew *et al.*, 1995). The highlands of Ethiopia harbor about 23 fungi, 2 bacteria, 2 viruses and 9 nematodes which attack barley (see Yitbarek Semeane *et al.*, 1996). However, there are landraces with high level of tolerance or resistance to powdery mildew, loose smut, leaf virus, net blotch, Septoria blotch, scald, yellow dwarf virus and strip mosaic viruses and some lines are found to have multiple resistance or resistance to all races of a disease (Harlan, 1976). Moreover, the Ethiopian barley germplasm is of considerable importance to barley breeding worldwide having characteristics.such as distinct grain colour (Qualset, 1975), high lysine content (Harlan, 1973), high tillering capacity (Berhane Lakew *et al.*, 1995), and fragile rachis (Hailu Gebre and Fekadu Alemayehu, 1987) reviewed in Berhane Lakew *et al.* (1995). However, there is little report on frost effects on barley in Ethiopia apart from a few survey reports. Field observations in different highlands of Ethiopia showed that frost effect is one of the major constraints of barley production in long rainy season. In North Gonder, complete crop failure and in North Welo, frost effect was more severe above 2800 m (Asmare Yallew *et al.*, 1998a and b) and in North West Shewa (Chilot Yirga *et al.*, 1998) barley was seen highly affected than wheat and fababean. In Dinsho highlands of Bale, the temperature falls below 0 °C in October and November causing frost damage of crops including barley (Bekele Hundie *et al.*, 1998). Though barley has a long history of cultivation and wide range of uses, its yield in Ethiopia is very low (1.01 t/ha) (Berhane Lakew *et al.*, 1996). Since 1955 when barley research in Ethiopia was started, the major evaluation criterion has been biological yield such as improving grain yield, nutritional quality, resistance to lodging and foliar diseases (Berhane Lakew *et al.*, 1996). Thus, the identification of frost resistant landraces has been largely ignored and the amount of annual production los. due to frost effect had been neglected. Accompanied by a study on the ethnobotany of barley, the present work is an attempt to provide an evaluation of the frost resistance in barley landraces collected from different sites.

1.2 Objectives

1.2.1 General objectives

To investigate the frost resistance potential of upland barley landraces collected and accessions from IBCR (Institute of Biodiversity Conservation and Research)

To document the indigenous knowledge of the farming community in some frost prone areas concerning frost and barley cultivation.

1.2.2 Specific objectives

-To screen barley landraces and accessions based on frost resistance.

-To identify the general survival potential (lethal sub-temperatures) of the samples.

-To assesses the altitude-frost resistance relationships.

-To document the indigenous practices of the farming community that may help to reduce the effect of frost on barley.

-To document the uses of barley in the study area.

2 MATERIALS AND METHODS

2.1 Field survey and collection of barley landraces.

Field survey was carried out in November 1999 in northern Ethiopia, specifically in the Amhara Regional State; North Welo in Bugna wereda, Abune-Yosef high lands (12° 11'N 39°14'E) and Tigrai Regional State: Southern part, Maichew, Enda-Mekoni wereda, Embahasti highlands (12°13'N 39°31'E) and Eastern part, Ganta-Afeshum wereda, Mitsnah (Mugulat) mid-highlands (12°20'N 39°29'E), which are all known barley growing areas.

Ethnobotanical data was collected in these survey areas through interview (appendix 2), through discussions with groups of farmers and agricultural development representatives and field observations. In each area interview was administered on a total of thirty farmers who were selected randomly in the presence of the local peasant association leaders. About 28 barely landraces were collected from the farmlands of the target areas. The farmers' variety names, areas of collection, altitude, topography, source of collection, and type of collected material were registered in collection record sheets prepared ahead of time.

Sampling of the varieties of a landrace in a given farmland was made at random. Sampling was carried out walking along contour plough as well as moving side ways up and down (in a zigzag form) of a farmland taking a spike in each footstep. About 50 samples were taken from a farmland that confirms the probability of 95 % sampling (Marshall and Brown, 1975).

2.2 Experimental study

2.2.1. Selection of landraces and accessions

In the field, landraces which were extensively cultivated in the given survey areas, were recorded through observation and discussions with farmers and finally collected. Moreover, the spikes that were sampled the highest in a given farmland for each population were chosen for experimental purposes. The nature of spikes and seeds were used for distinguishing the frequency of the components of a population. Samples were also selected to represent the altitudinal ranges designed. Samples from the field were collected between 2900m and 3500m altitudinal ranges except one landrace from 3630m asl. Accessions that grow in altitudes that were not included in the surveyed areas (below 2900m and above 3500m) were obtained from the Institute of Biodiversity Conservation and Research (IBCR). These were selected randomly from passport data of barley collections after categorizing the landraces based on the altitudinal range from which they were collected. The 25 samples taken for the controlled experiment are Smp30, Smp34, Smp35, Smp28, Smp18, Smp19, Smp21, Smp25, Smp23, Smp13, Smp3, Smp12, Acc1, Acc2, Acc3, Acc4, Acc5, Acc6, Acc7, Acc8, Acc9, Acc10, Acc11, Acc12 and Acc13. The twelve landraces with *Smp* and thirteen with *Acc* at the start of the experimental code represent those collected from the field and those obtained from IBCR, respectively. The landraces that have been used for the controlled experiment and others collected in the field are described in the Results section (Table 3) whereas descriptions of the accessions are given in appendix 1.

2.2.2 Growth condition

The experimental work was conducted between January and March 2000. Barley seeds threshed from single spike of each selected landrace and seeds from the accessions (totally twenty five landraces and accessions) were germinated first in separate Petridishes with moistened filter paper (12.5 cm diameter) and allowed to grow for about 9 days in the Ecophysiology laboratory, Biology Department, Science Faculty, Addis Ababa. On January 1st, 2000, seedlings about 5cm long were transplanted to cylindrical polyethylene plant pots size (30 x 40cm) filled with a 2.5 kg soil mixture of sand, manure and clay in 2:1:1 v/v. For each test, four seedlings 20cm far apart from each, arranged in squares, were planted in duplicate polyethylene pots. They were sufficiently watered daily with tap water and allowed to grow in the open field, Science Campus, Addis Ababa. The daily average maximum-minimum temperatures were 27 °C ± 2 and 22 °C ± 3, respectively and the average minimum night temperature was 4.2 °C ± 1.

2.2.3 Freezing treatment and data collection

2.2.3.1 Conductivity method

Leaves were taken as test tissues following Palta *et al.* (1977a and b) and Pearce and Willison (1985). Fully expanded younger leaves were cut, tagged and immediately put in conical rubber tubes in which they were washed and transported to the ecophysiology laboratory where freezing treatment was carried out. Cutting of sample leaves was confined to the time between 9:00 and 10:00 a.m. in order to avoid variation of the plant response to the daytime temperature differences.

Freezing treatment was carried out using leaf discs about 6 mm in diameter punctured from each sample leaves using a cork borer No. 3 following Pearce and Willison (1985). The leaf discs were cut at the base of the leaves, leaving the differently light green colored part. The leaf discs were individually put in separate centrifuge test tubes (1.3 x 9.5cm) with two drops of distilled water (0.8 – 1micro Simmons (μs)) to keep the moisture of the leaf discs. The capped test tubes were immersed into an ethanol bath in Flow-through-cooler chamber (Model Ministat, Huber 76 Offenburg-Elgers Weier, W. Germany). For each test samples, capped test tubes with leaf disks moistened with two drops of water were left at room temperature (19-20°C) for control. Freezing treatment was done twice to minimize errors in calibrating the test temperature scale and in the first test two leaf samples were used for each target temperature. In total three leaf samples were used for each test temperature following (Pearce and Willison, 1985). Always the level of ethanol in the chamber was adjusted to be above the tissue under treatment. Cooling was carried out continuously from room temperature to the test temperature, and each test temperature treatment was carried out after the completion of the former. The temperature was monitored by two thermometers, one in the ethanol bath and another in a test tube with leaf disk other than the samples under treatment. Thus, temperature adjustment was first done by the knob of the apparatus followed by checking the temperature reading of the ethanol bath. Duration of test temperature is shown in Table 1. Except for zero test temperature freezing was initiated by inoculating fine ice between 0 °C and –1 °C, which was equilibrated for about 30 minutes following Pearce and Willison (1985).

Table 1. Test temperatures and duration of treatment for electrolyte leakage evaluation.

Treatment no.	Temperature (°C)	Duration
	20 up to 10	1hr (10min / 2 °C)
	8 up to 2	2hrs (15min / 2 °C)
1	0	1.5hrs
2	-1	1.5hrs
3	-2	1.5hrs
4	-3	1.5hrs
5	-4	1.5hrs
6	-5	1.5hrs
7	-6	1.5hrs
8	-7	1.5hrs
9	-8	1.5hrs
10	-9	1.5hrs

As shown in Table 1 each temperature scale starting from zero took one and half hours, unless it was a test temperature where an extra half hour was added for further equilibration. Hence each scale took two hours when it was test temperature. Tissues equilibrated for about 2 hours at test temperature were allowed to thaw by warming up to the room temperature manually equilibrated to the duration of five minutes per 2 °C. Then the test tubes were removed from the flow-through-cooler followed by equilibration at room temperature (19 °C to 20 °C) for 30 minutes.

Later on, 3 ml of distilled water (0.8 - 1µs) that was kept at room temperature was added to both treated and untreated test tubes and all were put in a shaking water bath (Model 50 precision scientific company, USA). It was adjusted at 120 cycles per minute at a temperature of 19 °C to 20 °C. Conductivity measurement of the bathing solution was taken after 24 hrs for both treated and control samples using a conductivity meter (Model C- 523, Instruments, S.A, ESPANA). After the first reading of conductivity of the bathing solution of both treated and control samples, test tubes were put in an autoclave (Portaclave 50L, Astell scientific Ltd., U.K) to kill the tissues and measure the maximum conductivity (expected 100 % leakage). The autoclave was adjusted at 121°C with 1.037 bars for about 30 minutes. The samples were removed quickly when the temperature reached nearly below 90 °C at which all the pressure was exhausted (0 bar) to avoid excess evaporation of the sample solution. The test tubes were then cooled at room temperature and put in the shaking water bath for about 24 hours, and the conductivity reading was recorded again. Percent leached as a result of treatment was calculated from the conductivity measurement of the bathing solution of the samples after treatment before autoclaving as a percent of that after autoclaving, following Pearce (1980), Pearce and Willison (1985) and Zhang and Willison (1987). Electrolyte leakage in to a bathing solution is a conventional method of assessing freezing injury, which primarily reflects changes in plasma membrane integrity due to freezing (Boorse et al., 1998). It estimates freezing injury quantitatively by measuring the electrolyte leakage which includes K^+, Ca^{++}, H^+, and Cl^- (Palta *et al.*, 1997a) from the tissue after thawing. The percent damage was calculated from the relationship given below.

$$\% \text{ Damage} = \frac{\% \text{ leached}_T - \% \text{ leached}_C}{100 - \% \text{ leached}_C} . 100$$

Where, T is the sample treated to frost

C is the sample at room temperature (control)

The percent leached and percent damage values in the result section are the means of three treatments and the vertical bars indicate the standard errors of the means of the three treatments.

2.2.3.2 Chlorophyll a fluorescence

Chlorophyll fluorescence has been widely used as a method of evaluation to determine the status of plants using intact or excised leaves (Schreiber *et al.,* 1995; Lazar and Naus, 1998; Fracheboud *et al.,* 1999). In this work, measurements of photosynthetic efficiency of excised leaves were taken at room temperature (19 to 20 °C) using a Plant Efficiency Analyzer (PEA) (Hansatech Instruments Ltd, King's Lynn, Nortolk PE 32 15h, England). Measurement was done after the leaves were adapted in dark using plastic leaf clips on which the sensor head was put for the source of illumination from light emitting diodes. The light emitting diodes bombard red light at a peak wavelength of 650 nm, which is easily absorbed by green leaves, and the Fluorometer calculates Fv, Fv/Fm after measurement of the Fo, and Fm from the fluorescence emitted. Fo, dark fluorescence, is the measure of the initial fluorescence when all the PSII reaction centers are open whereas Fm, maximum fluorescence, is the peak fluorescence measured in presence of saturation light intensity when all the Q_A (Quinon A) of PSII are reduced. Fv is the difference of Fm and Fo. The Fv/Fm is the potential quantum yield. The same excised leaves were used for conductivity test and fluorescence measurements for each test temperature. However, the leaf part and size used were different. The leaf parts just next to the region from which leaf discs have been punctured were used for fluorescence measurements. Moreover, leaf tissues used were larger (about 20 mm long) so that handling could be easy during fluorescence measurement. Three fluorescence measurements from three different leaf samples were taken for each test temperature. Fluorescence readings from each excised leaf (sample) were also measured three times: 1st – Just soon after the leaf is excised, before treatment, control.

2nd – After 30 minute of each test temperature treatment.

3rd- After 24 hours of each test temperature treatment.

All these measurements were taken after the excised leaf was adapted in the dark for 30 min. Furthermore, measurements were taken on adaxial side fairly on the same spot near to the central part of the leaf blade, just next to the bored region where the mid rib was adjusted to be at the center of the leaf clip hole. Always the leaf clip hole was fully covered by leaf tissue during the measurement and Fo, Fm, Fv, Fv/Fm and Tm (time when Fm had taken) were recorded from the liquid crystal display (LCD) of the PEA. The treatment was the same as for that of conductivity measurement but fluorescence measurement was taken after the leaves were thawed at room temperature.

Percentiles for the curves were computed as

(a) Percent of Fv/Fm value from the control = $\frac{Fv/Fm_T}{Fv/Fm_C} . 100$

(b) Percent Damage PSII = $\frac{Fv/Fm_C - Fv/Fm_T}{Fv/Fm_C} . 100$

Where, Fv/Fm_C = potential quantum yield un treated samples

Fv/Fm_T = potential quantum yield of the treated samples at 0 °C to - 8 °C

The Fv/Fm and Fo values in the result section are the means of three treatments and the vertical bars indicate the standard errors of the means of the three treatments.

3 DATA ANALYSIS

One way ANOVA following Fisher's pair wise comparisons least significance test at $p < 0.05$ (through MINITAB 10.5 window) was applied to analyze variations of the mean % damage from the electrolyte conductivity and Fv/Fm ratio from chlorophyll fluorescence evaluation at each test temperature, and to see the significance level of the LT_{50} values of the landraces.

T-test at $p < 0.05$ was applied to confirm where the average percent leached value obtained from treated samples was significantly above the value of that at room temperature (control). The temperature at which 50 % lethality or inactivation of cells occurs (LT_{50}) was calculated from the equations of the curves best fitted to the values of % damage against the test temperature using simple linear regression (ignoring the values of % damage below –4 °C test temperatures) following Boorse *et al.* (1998). Moreover, slopes of the curves were determined, which were also used by Cardona *et al.* (1997). Regression lines were also used to show the relationship of LT_{50} (frost tolerance) and slopes of the curves as well as the association with altitude. Simple correlations were used to determine the strength of association between altitude and LT_{50} as well as between LT_{50} and slopes of the curves.

Moreover, the descriptive statistics (% value) was also used to evaluate the decrease in Fv/Fm ratio among the landraces and accessions at different test temperatures, as used in high temperature stress evaluation in legumes by Srinivasan *et al.* (1996).

4 RESULTS

4.1 Barley ethnobotany in northern Ethiopia

4.1.2 Barley cultivation

The survey was conducted in the northern highlands of Ethiopia in the altitudinal range of 2500-3800 m above sea level. The areas have a bimodal rainfall except Embahasti (eastern Tigrai) study site. In the study area farmers cultivate different crops. Limited number of crops largely dominated by barley and legumes were cultivated during the field survey. The dominant crops are shown in order of priority in Table 2.

Table 2. Crops cultivated in order of production priority in the survey area.

Crops	Number of respondents			Total percentage
	Abune-Yosef	Tsibet-Embahasti	Mitsnah	
Barley, wheat, oat, lentil	0	0	3	10
Barley, pea, oat	1	4	4	30
Barley, fababean, wheat	7	0	3	33.33
Barley, bean (fababean), wheat, vegetables	2	6	0	26.67

The interviewees indicated that their farmlands were small and they thus could not use fallow system. Nevertheless, farmers of both Abune-Yosef and Tsibet-Embahasti highlands used more than half of their farmlands for barley cultivation both in short and long rainy seasons. As shown in Table 4, about 90 % of the respondents (27 out of a total of 30) indicated that there is no crop in the study area that can substitute barley. According to the respondents, barley is cultivated for a long period of time and they have many landraces. Farmers could enumerate the barley landraces that are cultivated in all these areas up to 43 in total of which about 28 were collected as shown in Table 3. However, barley production is declining due to the major constraints such as insufficient rainfall, poor soil fertility, frost, improper use of farmlands and problem of pests. Although frost is one of the major constraints of barley, selection of landraces specifically for frost is not common in the farming community (Table 4).

Table 3. Local name, area of collection and altitude of the landraces collected from field.

Experime-ntal number	Collection number	Local variety name	Area of collection			Altitude (m)
Smp30	30	*Zewtat* (T)	E. Tigrai	Ganta-Afeshum	Mitsnah	2900
Smp34	34	*Netselasigem Saesaa)* (T)	"	"	"	2900
Smp35	35	*Gunazasigem* (T)	"	"	"	2990
Smp28	28	*Tsaedaatena* (T)	"	"	"	3000
Smp18	18	*Keyihshewa* (T)	S. Tigrai	Enda-Mekoni	Tsibet-Embahasti	3000
Smp19	19	*Haftusene* (T)	S. Tigrai	Enda-Mekoni	Tsibet-Embahasti	3000
Smp21	21	*Tsaedashewa* (T)	S. Tigrai	Enda-Mekoni	Tsibet-Embahasti	3100

... continued Table3

Experime-ntal number	Collection number	Local variety name	Area of collection			Altitude (m)
Smp25	25	*Tselimsigem* (T)	S. Tigrai	Enda-Mekoni	Tsibet-Embahasti	3130
Smp23	23	*Tselimdemhay* (T)	S. Tigrai	Enda-Mekoni	Tsibit-Embahasti	3150
Smp13	13	*Sekutere* (*Awariye*) (A)	N. Welo	Bugna	Abune-Yosef	3270
Smp3	3	*Sekutere* (*Tedowasha*) (A)	"	"	"	3300
Smp12	12A	*Nechita* (A)	"	"	"	3630
	4	*Tikurawariye* (A)	"	"	"	3300
	5	*Nechawariye* (A)	"	"	"	"
	6	*Keygebse* (A)	"	"	"	"
	7	*Tikurtemej* (A)	"	"	"	"
	8	*Mehoni* (A)	"	"	"	"
	9	*Nechtemej* (A)	"	"	"	"
	10	*Keyawariye* (A)	"	"	"	"
	11	*Abejira* (A)	"	"	"	"

...continued Table3

Experimental number	Collection number	Local variety name	Area of collection			Altitude (m)
	16	*Himbilil* (T)	S. Tigrai	Enda-Mekoni	Tsibit-Embahasti	2950
	17	*Saesaa* (T)	"	"	"	2950
	20	*Kinchibe* (T)	"	"	"	3020
	24	*Gidme* (T)	"	"	"	3135
	26	*Abejira* (T)	"	"	Adi-Atsgeba	2915
	27	*Tselimo* (T)	E. Tigrai	Ganta-Afeshum	Mitsnah	3000
	31	*Gunazatselimo* (T)	"	"	"	2990
	32	*Netselad*emhai (T)	"	"	"	2970
	33	*Gunazademhai* (T)	"	"	"	2970

(A) = Amharic language, (T) = Tigrigna language, (A+T) = spoken in both

The farmers primarily use presence or absence of hull for classifying the landraces. Secondly, the number of rows was very important factor for simple folk classification. Based on these the landraces that were collected from the field are classified as given in Figure 1. The major barley landraces cultivated by the farmers were the hulled and six-rowed types with 82 % and 82 % from the collected samples, respectively.

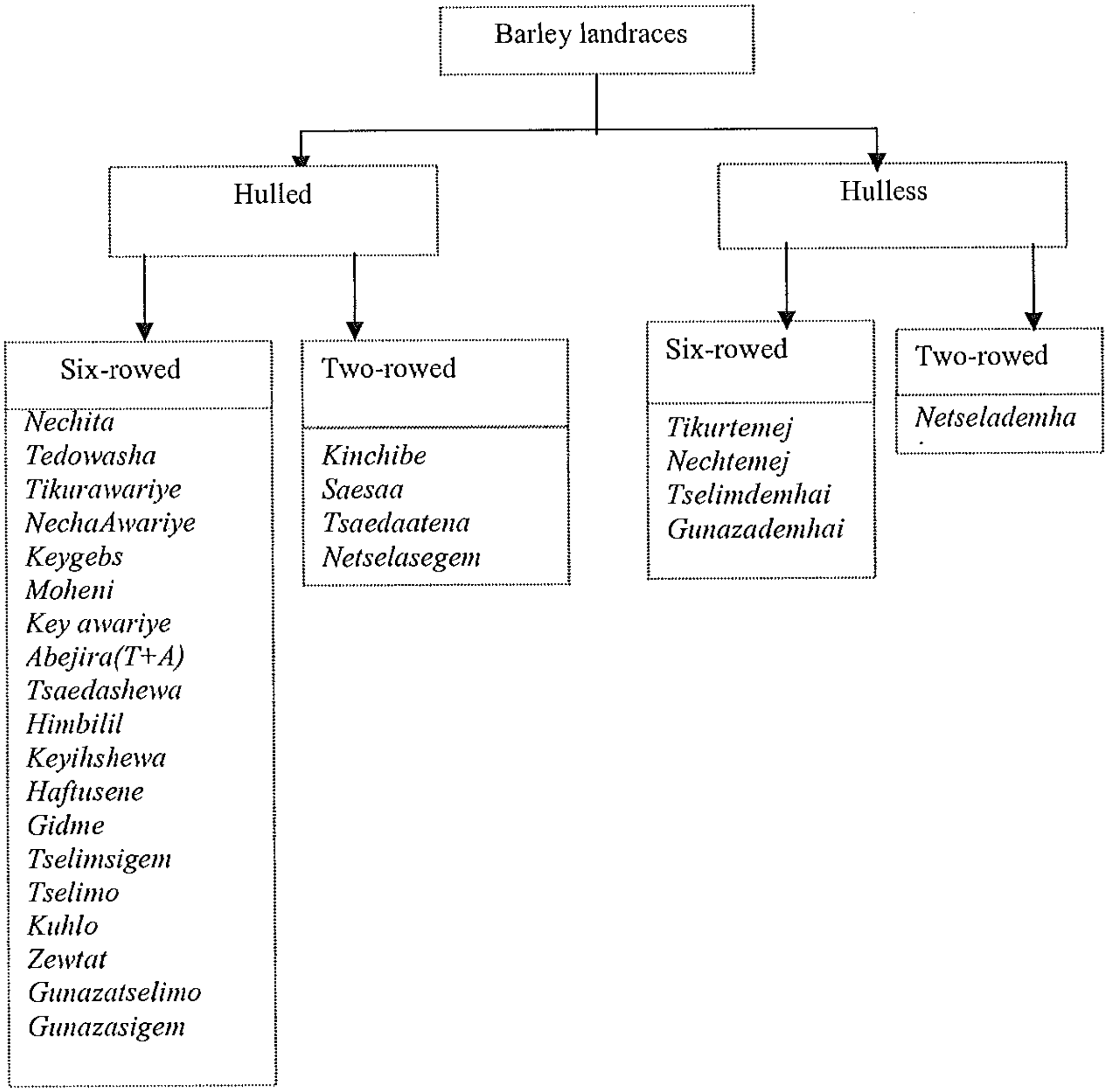

Fig.1. Barley landrace classification based on grain and spike morphology

Table 4. Responses given by farmers regarding barley cultivation

Attributes of barley cultivation	Alternatives given	Number of respondents	Percentage of total respondents	Study site (s) where the response was obtained
Proportion of farmlands used for barley cultivation	Greater than half of the cultivated area owned	20	66.67	Abune-Yosef and Tsibet-Embahasti
	About half the cultivated area owned	10	33.33	Mitsnah
	Less than half the cultivated area owned	0	0	
The crop that can be a substitute for barley	Wheat	3	10	Mitsnah
	Others	0	0	
	No crop	27	90	Abune-Yosef, Tsibet-mbahasti, partly Mitsnah
Trend of barley production and/ or cultivation	Declining	30	100	In all sites
	Not declining	0	0	

...continued Table4

Reasons for declining barley production in order of decreasing severity	Low rainfall, poor soil fertility, frost, aphids	10	33.33	Abune-Yosef
	Low rain fall, poor soil fertility, improper use of the land, frost, weeds	10	33.33	Tsibet-Embahasti
	Low rain fall, poor soil fertility, poor land management, windy air	10	33.33	Mitsnah
Whether or not farmers selection landraces for frost tolerance	Farmers do select landraces for frost tolerance	0	0	
	Farmers do not select landraces for frost tolerance	30	100	In all sites
Seasons used for barley cultivation	Only long rainy season	10	33.33	Mitsnah
	Long and short rainy seasons	20	66.67	Both Abune-Yosef and Tsibet-Embahti

Unlike for the frost resistance, the respondents were able to identify the late from the early barley and vise versa (Table 5).

Table 5. Late and early barley landraces commonly cultivated in the study area.

Locality	Late barley	Early barley
Abune-Yosef	*Nechita*	*Abejira, awariye, tedowasha, Mehoni*
Tsibet-Embahasti	*Rie, kinchibe*	*Saesaa, shiwa, haftusene, himbilil*
Mitsnah	*Atsa, atena*	*Netselasegem (saesaa), demhai, gunaza*

The late barley landraces (require high moisture) are cultivated in higher altitudes during long rainy season and when there is early and sufficient rainfall. The early barley landraces are cultivated in both lower and higher altitudes in both seasons. Moreover, the latter types are also used for cultivation under irrigation in Abune-Yosef.

From personal observation and the respondents the topography of the farmlands used for barley cultivation is undulating hills. The implements they use also confirm this; hoes are used even in farmlands that are plowed using oxen due to the presence of inaccessible spots of land. Lands that retain moisture are also preferred. Unreliable rainfall was described as the main constraint by the respondents. They indicated that provided there is sufficient rainfall, fertilizer application, using fallow system and repeated plowing and early planting or sowing were among the ways that could be used to increase production. In addition, they indicated that late barley landraces were preferred both for their high yields and frost resistance in cases when there is early and sufficient rainfall. In Abune-Yosef and Tsibet-Embahasti study sites frost effect was described to be serious when there is insufficient rainfall.

4.1.2 Barley utilization

According to the respondents barley was the major staple food grain utilized in the study area. The major foodstuffs prepared in all the study sites are *enjera* (thin leavened bread, which is most favorite foodstuff of Ethiopians), *besso* (roasted barley flour), *kolo* (roasted barley grain), as well as both alcoholic and non-alcoholic drinks. Detailed description of the food items made of barley is given in Table 6. In Mitsnah fresh cold dough (*tihlo*) is prepared from *besso*, which is eaten with meat sauce having a diluent soup-like (*hilbet*) at the center. Besides, *besso* has religious value in this area. Moistened *besso* with blessed water after the name of Saint Abune Aregawi in Abune Aregawi church is dried and then distributed to the followers of the church. In all the study area barley straw was the main animal feed.

Table 6. Foodstuffs prepared from barley in the northern highlands.

Local name	Description	Kinds of material	Preferential grain quality	Utilization rate	Site specificity
enjera (A+T)	Thin, leavened bread (loaf)	Fine flour, thin dough	Hull-less, large grains (late barley)	Most of the time	In all sites
Besso (A+T) or *Tihni* (T)	Utilized as moistened powder (*firaro* (T))	Grains roasted and ground to fine or coarse flour	Any type,	Most of the time	In all sites
	Utilized as thick porridge (*genfo)* (A), *geat* (T)	Grains roasted and ground to fine or coarse flour	any type , white grains	Some times	In all sites
	Utilized as cold fresh dough (*tihlo* (T))	Grains roasted and ground to coarse flour	White grains	Most of the time	Eastern Tigrai
•	Utilized as boiled soup (*atmit (A), sibko*(T))	Grains roasted and ground to fine flour	Any type	Some times	In all sites
kolo (A+T)	Roasted grains	Grains roasted and pounded	Large grains	Some times	In all sites
kita (A) or *kicha* (T)	Pisa-like un spiced	Fine flour, thick dough	Any type	Somc times	In all sites

...continued Table6

Local name of drinks	Description	Kinds of material	Preferential grain quality	Utilization rate	Site specificity
Besso bitbit (*zurbegone*) (A)	Fresh besso mixed or diluted with water	Grains roasted and ground to fine or coarse flour	Any type, white grains	Some times	In all sites
Bordae (A)	Thick, little fermented	*besso* and malt	Any type	Some times	Not in Eastern Tigrai
Tela (A) or *siwa* (T)	Fermented but undistilled	Coarse roasted flour and malt	Black barley	Some times	In all sites
Areki (A+T)	Fermented and distilled	Coarse roasted flour and malt	Black barley	Rare in villages	In all sites

(A) = Amharic language, (T) = Tigrigna language, (A+T) = spoken in both

The preference of landraces for foodstuff preparation is developed in the farming community but this is done when there are alternatives. The choice of landraces for alcohol preparation is stricter in that in the absence of the preferable landraces exchange with neighborhoods is carried out. The late barley landraces are found to be the most important types that are required for the preparation of most foodstuffs. Table 7 shows the landraces preferred for the preparation of some foodstuffs.

Table 7 The preferred barley landraces for some foodstuffs.

Selected foodstuffs	Preferred landraces for the preparation		
	Abune-Yosef	Tsibet-Embahasti	Mitsnah
Enjera	*Sekutere(Awariye), Nechita, Mehoni, Abejira,*	*Tsaedashewa, keyihshewa, saesaa,rie*	*Atena, atsa, Demhai*
Besso	*Nechita, sekutere*	*Saedashewa, saesaa*	*Atena, atsa, gunazasigem, netselasigem, kuhilo*
kolo	*Mehoni, nechita, temej*	*Tsaedashewa, keyihshewa, rie*	*Atena, kuhilo, demhai*
For alcoholic drinks and malt preparation	*Tikur awariye, keygebs*	*Tselimsigem abejira, kinchibe*	*Tselimo, Gunazatselimo*

4.2 Physiology of frost resistance

4.2.1 Electrolyte conductivity method

4.2.1.1 Percent leakage

Percent leakage was calculated from the bathing solution of the treated samples before autoclaving as a percentage of that after autoclaving as described in the Materials and Methods. The percent leakage of the control ranged from 14.56 % to 21.4 % with an average of 17.68 %. The percent leakage of the treated samples was significantly different from that of the control at all test temperatures except at –1 ^{0}C and –3 °C, while the increase in percent leakage was highly significant in test temperatures –4 ^{0}C to –9 ^{0}C (P=0) (Table 7 and Fig.4).

Table 8. Values of t-test analyses between % leakage of control and the treated samples at test temperatures 0 ^{0}C to –9 ^{0}C.

Test temperature (^{0}C)	0	-1	-2	-3	-4	-5	-6	-7	-8	-9
t value*	1.38	-2.18	-2.81	-1.85	-9	-10	-7.81	-21	-25	-37
P value	0.17	0.03	0.01	0.06	0.00	0.00	0.00	0.00	0.00	0.00

*Degree of freedom for all cases is 48.

4.2.1.2 Percent leakage versus percent damage

The concentration of effusate, measured as percent leakage, as a result of freezing treatment increased with decreasing test temperature up to –9 ^{0}C in all landraces and accessions under treatment. The percent damage, with relatively lower value, increased with increasing percent leakage where they tended to be equalized at -9 °C (Fig.2).

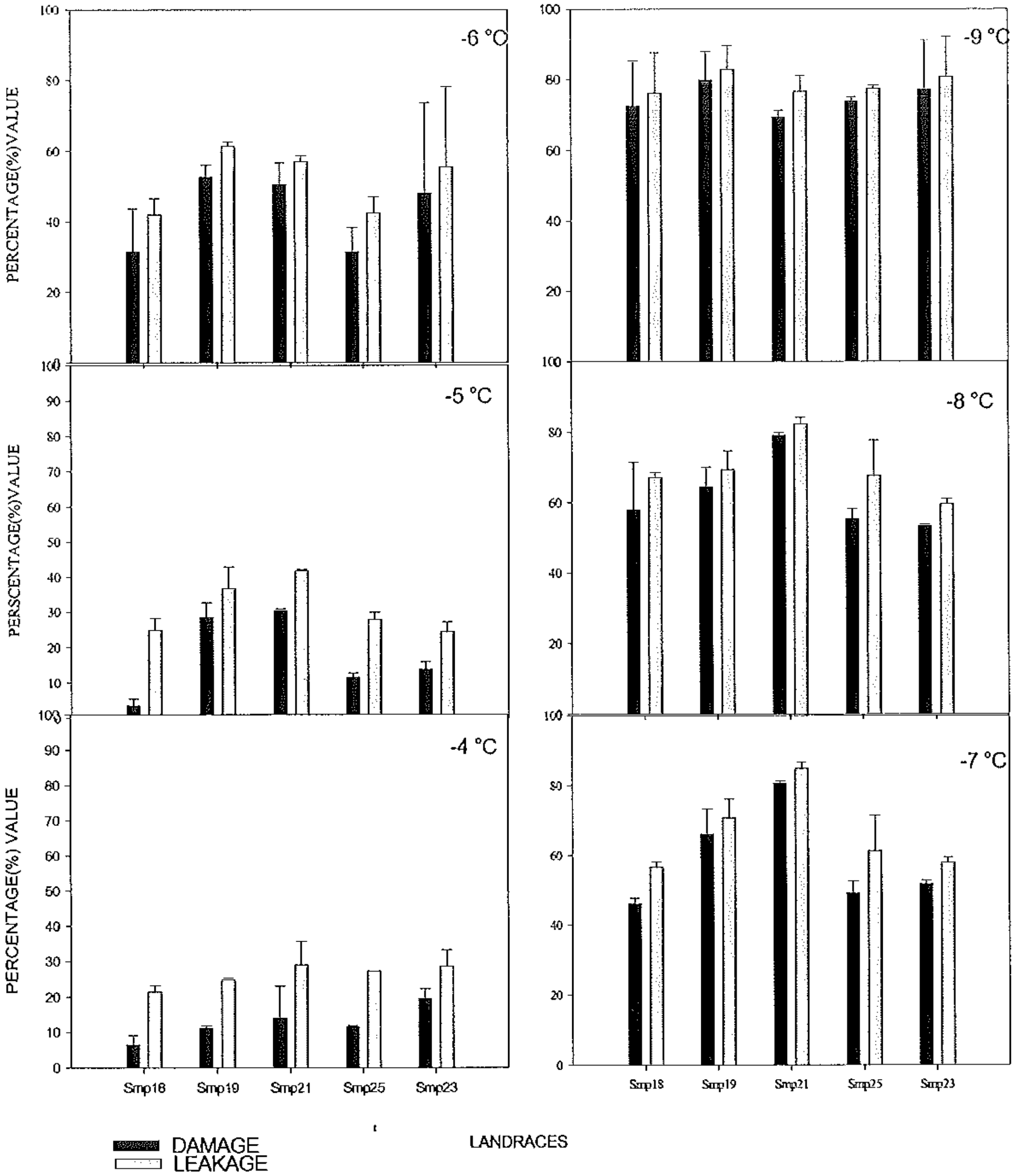

Fig.2. The relationships of % leakage and % damage at -4 °C, -5 °C, -6 °C, -7 °C, -8 °C and -9 °C test temperatures for samples from the same locality.

The analysis of variance of the means of % damage of landrace varieties collected from the same locality (Tsibet-Embahasti highland) with altitudinal range of 3000m-3150m showed no significant difference in membrane stability.

4.2.1.3 Critical test temperature for screening the samples

Analysis of variance of percent damage following Fisher's pair wise comparison at test P<0.05 showed no significant difference among the samples at all test temperature except at –5 °C (Table 8). At –9 °C the means of the samples showed little variation (P=0.96).

Table 9. Means of variance of the percent damage of 25 samples from Fisher's pair wise comparison at test temperatures from 0 ^{0}C to – 9 ^{0}C.

Test-temperature (^{0}C)	0	-1	-2	-3	-4	-5	-6	-7	-8	-9
P value*	0.94	0.69	0.98	0.82	0.72	0.001	0.43	0.32	0.65	0.96

Table 10. Matrix showing landraces and accessions, which have shown significantly different response to test temperature –5 ^{0}C (×).

	Acc1	Acc2	Acc3	Acc4	Acc5	Acc6	Acc7	Acc13	Acc8	Smp30	Smp34	Smp35	Smp28	Smp18	Smp19	Smp21	Smp25	Smp23	Smp13	Smp3	Acc9	Acc10	Acc11	Acc12	Smp12
Acc 1																									
Acc 2																									
Acc 3																									
Acc 4																									
Acc 5																									
Acc 6		×																							
Acc 7																									
Acc13	×		×	×	×	×	×																		
Acc 8	×			×		×																			
Smp30								×																	
Smp34																									
Smp35	×			×	×	×																			
Smp28								×																	
Smp18		×					×	×	×		×	×													
Smp19	×		×	×	×	×	×			×	×		×	×											
Smp21	×		×	×	×	×	×			×	×		×	×											
Smp25								×							×	×									
Smp23								×							×	×									
Smp13								×	×			×			×	×									
Smp3								×				×			×	×									
Acc9								×							×	×									
Acc10														×		×									
Acc 11	×			×	×	×								×			×		×	×					
Acc 12	×		×	×	×	×	×			×			×	×			×	×	×	×	×				
Smp12														×		×									

The response at –5 °C treatment was less than 10 % damage for samples Smp18, Acc5, Acc1, Smp13, Acc11 and Smp3, which had all relatively high membrane stability, whereas Acc2, Acc8, Smp34, Smp12, Acc13, Smp19 and Smp21 had relatively low membrane stability (above 20 % damage) (Fig.3).

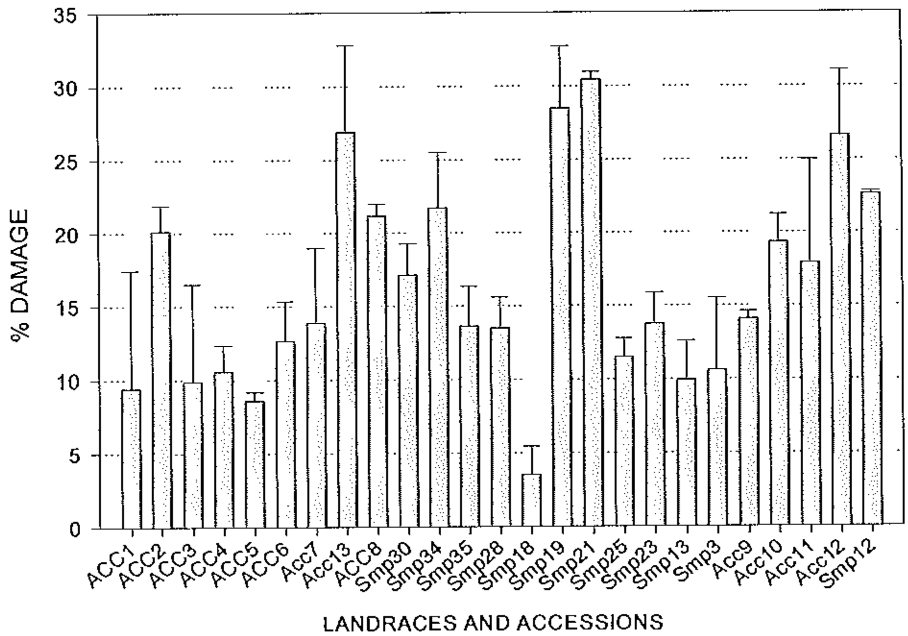

Fig.3. Responses in membrane stability of the samples at -5 °C.

4.2.1.4 Assessment of frost tolerance

Non-linear line graphs of the % damage of the landrace varieties were fitted against the test temperatures selecting samples with higher and lower slopes in each altitudinal range. All the curves tended to attain a sigmoidal function, but lacked the upper asymptote (Fig.4).

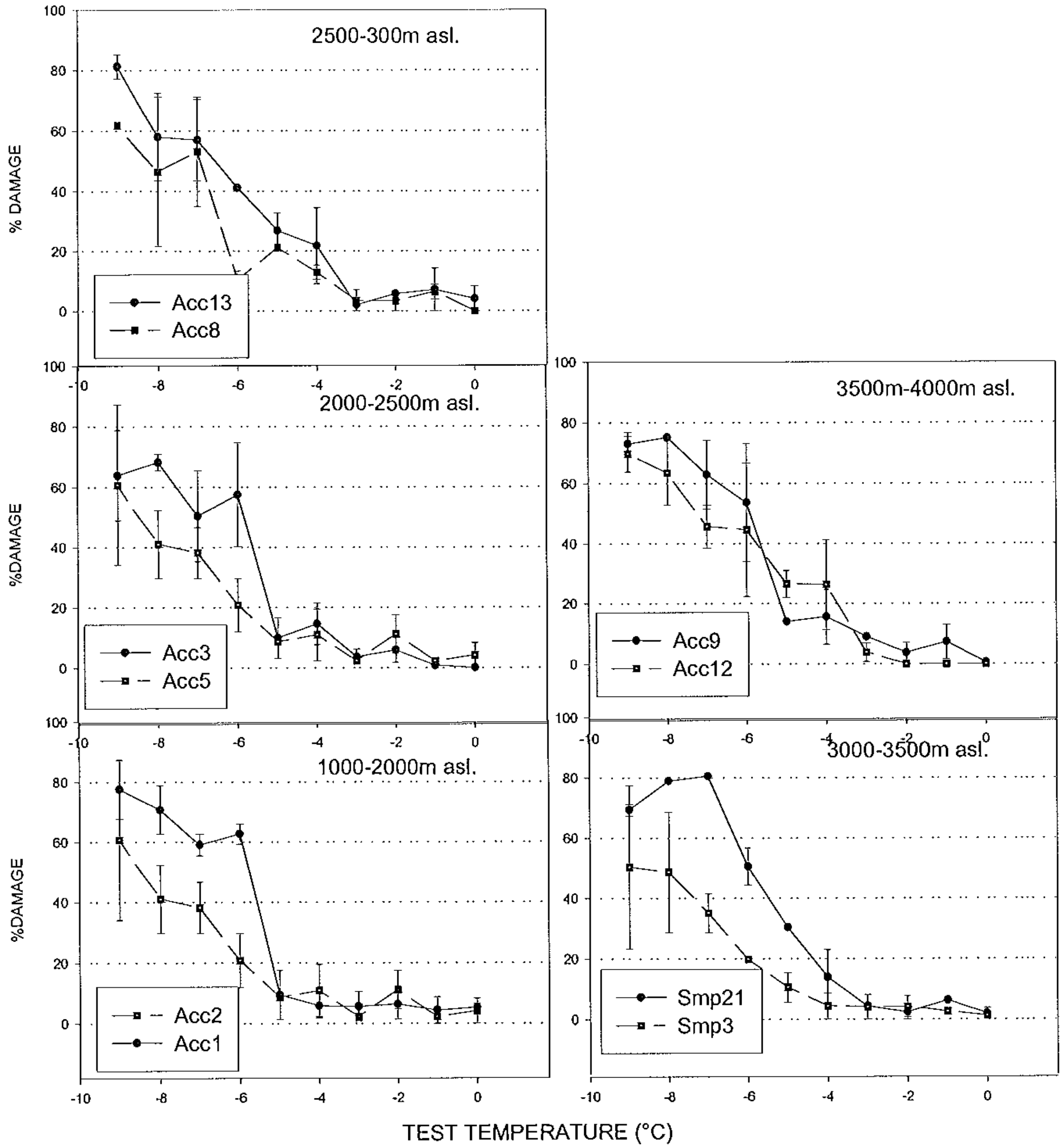

Fig.4. The % damage of samples with relatively high (---) and low (——) membrane stability from each altitudinal range.

Regression lines of the graphs of percent damage and test temperatures were used to calculate the freezing temperature at which 50 % of the tissues were inactivated or killed (LT_{50}). The slopes of the linear portion of the graphs were also used to evaluate and compare the frost tolerance of the samples, where values were found inversely related to tolerance (Table 10).

Based on LT_{50} and slope values the landraces and accessions had the following order of decreasing frost tolerance: Smp3 > Acc5 > Acc2 > Smp13 > Acc8 > Acc4 > Smp35 > Smp18 > Smp23 > Acc7 > Smp30 >Acc6 >Smp34 >Smp25>Acc3> Smp28 > Acc12 > Acc10 > Acc11 > Acc13 > Acc1 > Smp12 >Acc9 > Smp19 > Smp21. However, the analysis of variance of the means of LT_{50} indicated that they were not significantly different (n=24, p=0.267).

Table 11. Ranks of landraces and accessions based on frost tolerance.

Rank	Landraces and Accessions	LT_{50}	|Slope|
1	Smp3	-8.64	4.560
2	Acc5	-8.46	4.837
3	Acc2	-8.28	5.141
4	Smp13	-8.28	5.184
5	Acc8	-8.10	5.400
6	Acc4	-7.92	5.508
7	Smp35	-7.92	5.661
8	Smp18	-7.56	5.940
9	Smp23	-7.56	6.165
10	Acc7	-7.47	6.240
11	Smp30	-7.38	6.670
12	Acc6	-7.2	6.731
13	Smp34	-7.2	6.790
14	Smp25	-7.2	6.822
15	Acc3	-7.2	6.837
16	Smp28	-7.2	6.895
17	Acc12	-7.2	6.921
18	Acc10	-7.02	7.061
19	Acc11	-7.02	7.060
20	Acc13	-7.02	7.089
21	Acc1	-6.84	7.300
22	Smp12	-6.84	7.575
23	Acc9	-6.75	7.699
24	Smp19	-6.66	7.704
25	Smp21	-6.48	7.795

The regression line (Fig.5) depicts the relationship between the LT_{50} and the slope values of the linear portion of the graphs and which showed strong correlation ($r = 0.962$, $p=0$). Samples such as Smp3, Acc5 with the highest LT_{50} (- value) had the least slope and the reverse was true to samples such as Smp21and Smp19.

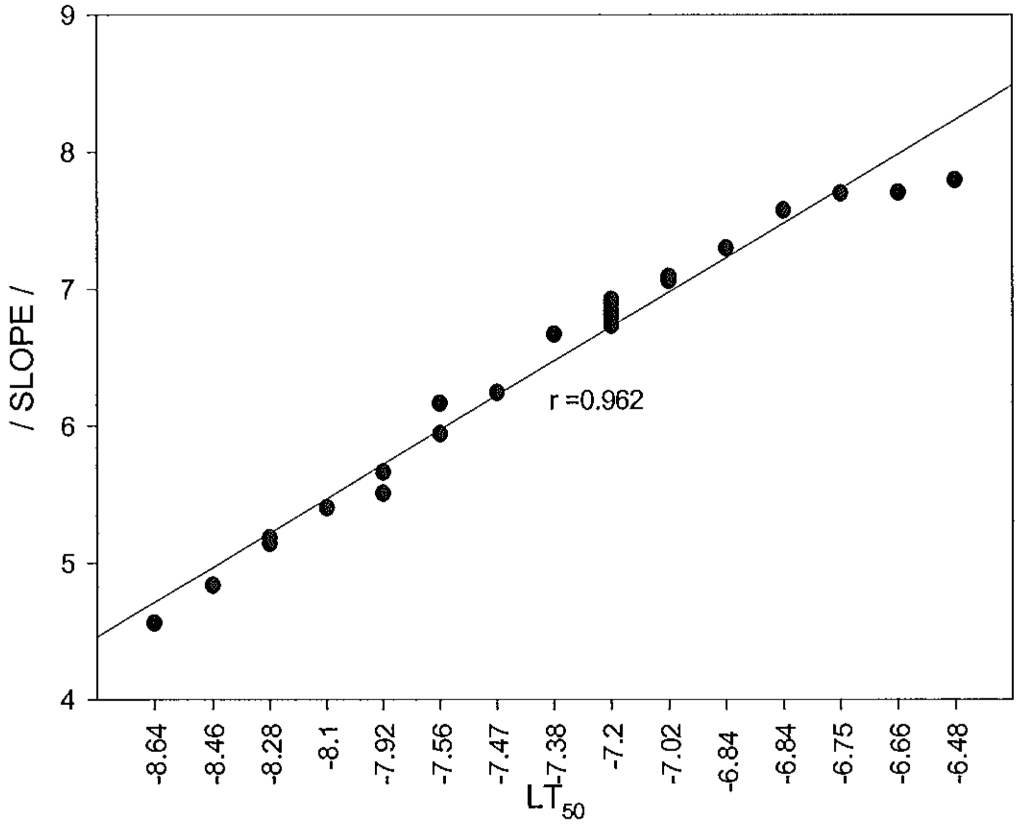

Fig.5. Relationships of the LT50 and slopes of the linear portion of the graphs of test temperatures vurses percentage damage.

4.2.1.5 Frost resistance-altitude relationship

The LT_{50} values of the landraces collected from different altitudes were fitted to their corresponding altitudes from which the samples had been collected (Fig.6).

The frost resistance character of the collected samples was found to be positively correlated with altitude; however, the relationship was weak (r=0.25, P=0).

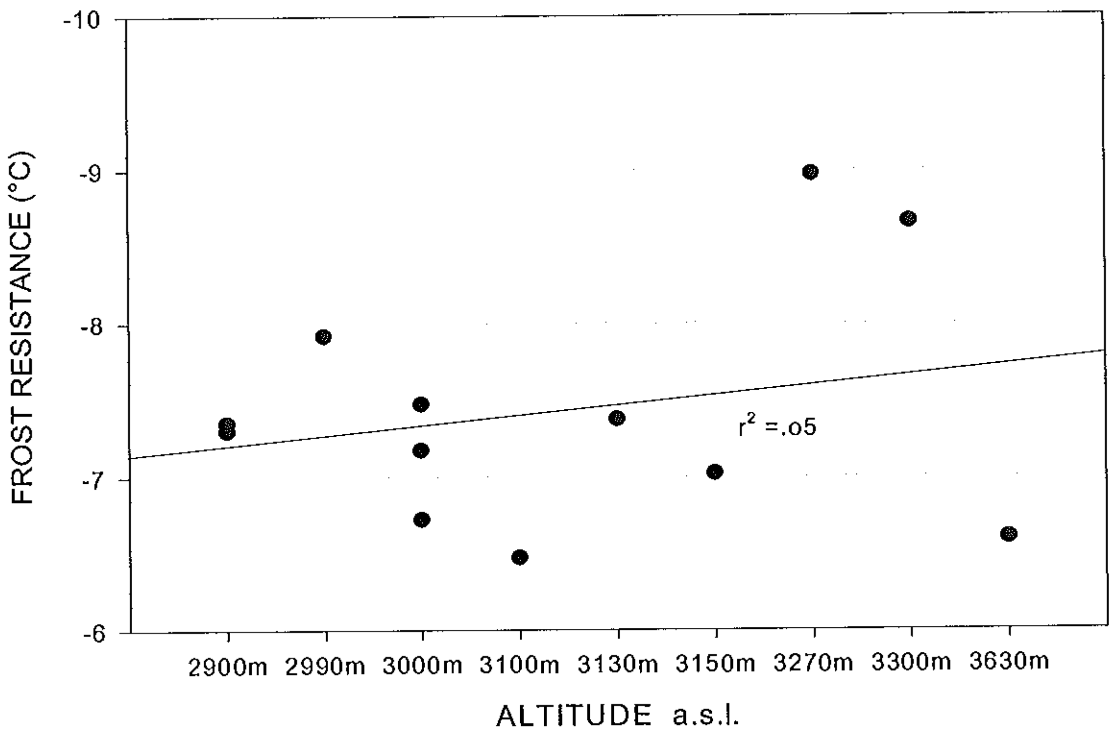

Fig.6.The frost tolerance and altitude interrelationship of landraces collected from field.

4.2.2 Chlorophyll a fluorescence evaluation method

4.2.2.1 Potential quantum yield (Fv/Fm)

The potential quantum yield measured after a leaf sample has been adapted in the dark was used to estimate the maximum photosynthetic efficiency of PSII of the plant. According to Bjorkman and Demmig (1987), an Fv/Fm value of 0.80 can be considered as an average reference point for the optimal quantum efficiency of unstressed leaves of higher plants.

The Fv/Fm values remained above 0.8 up to –4°C for about 90 % of the total samples and sharply declined as the test temperatures were decreased. Values for Acc13 and Acc1 declined down to about 70 % of the control much lower than the others. The potential quantum yield of the samples was measured after 24 hours and the PSII function was not recovered, while the untreated samples under the same temperature showed no change within that given time range. The line graphs of Fv/Fm as measured using PEA after treatment and the percent of Fv/Fm value of the treated from that of control samples are given in Fig.7 to Fig.15.

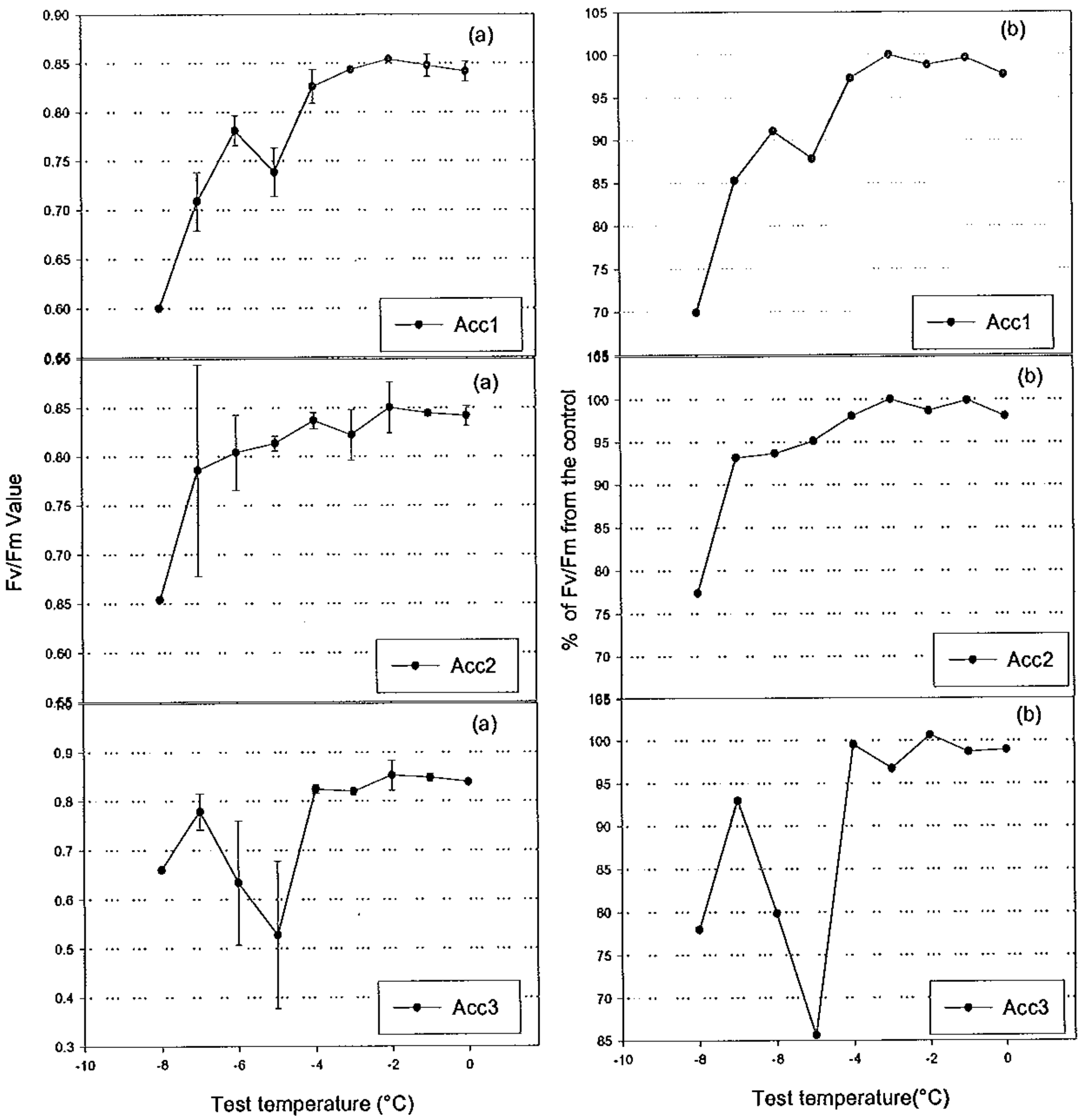

Fig.7. Potential quantum yield after treatment(a) and the corresponding perccentage of potential quantum yield from the control (b) of Acc1, acc2 and Acc3.

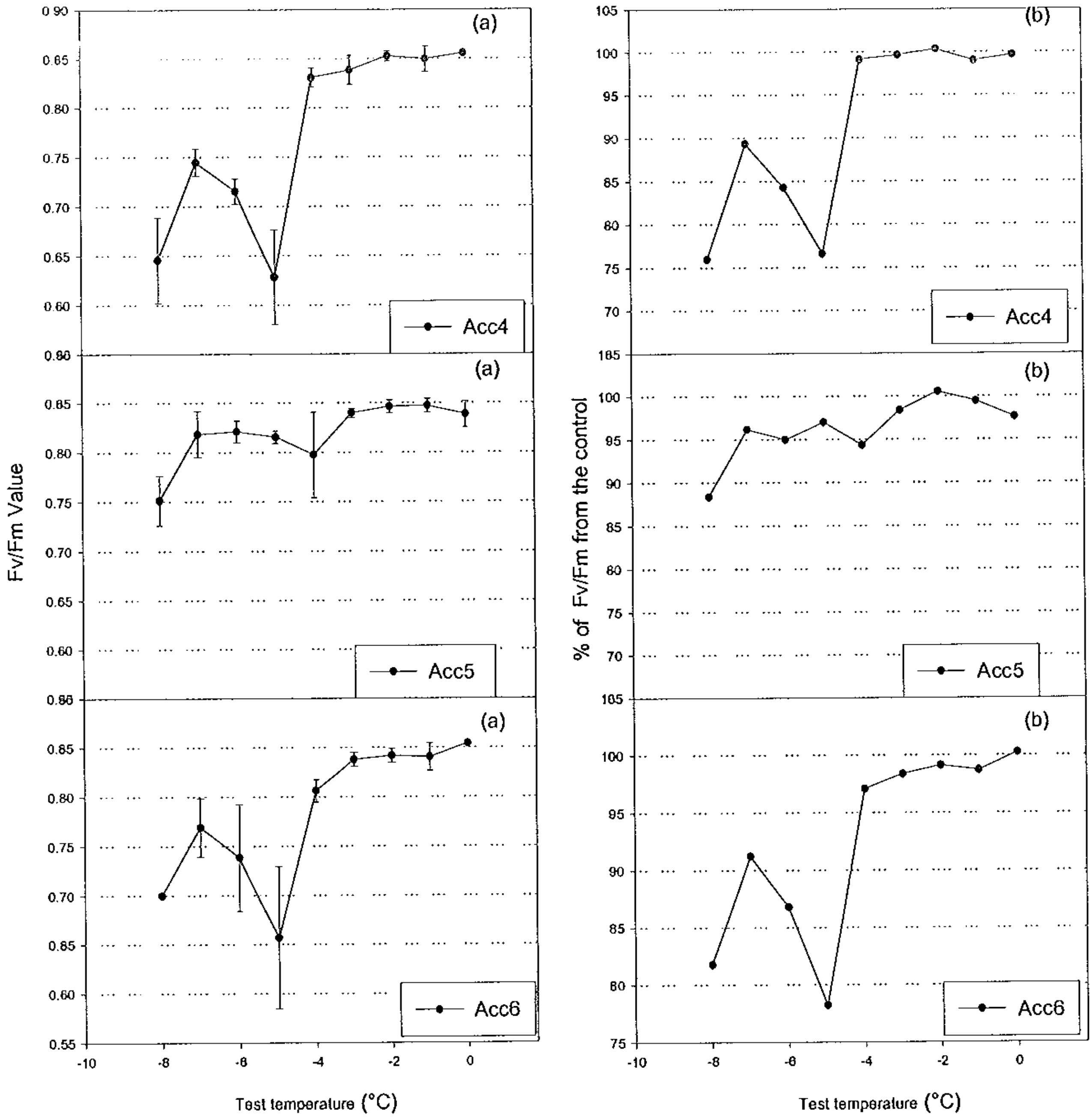

Fig.8. Potential quaantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Acc4, Acc5, and Acc6.

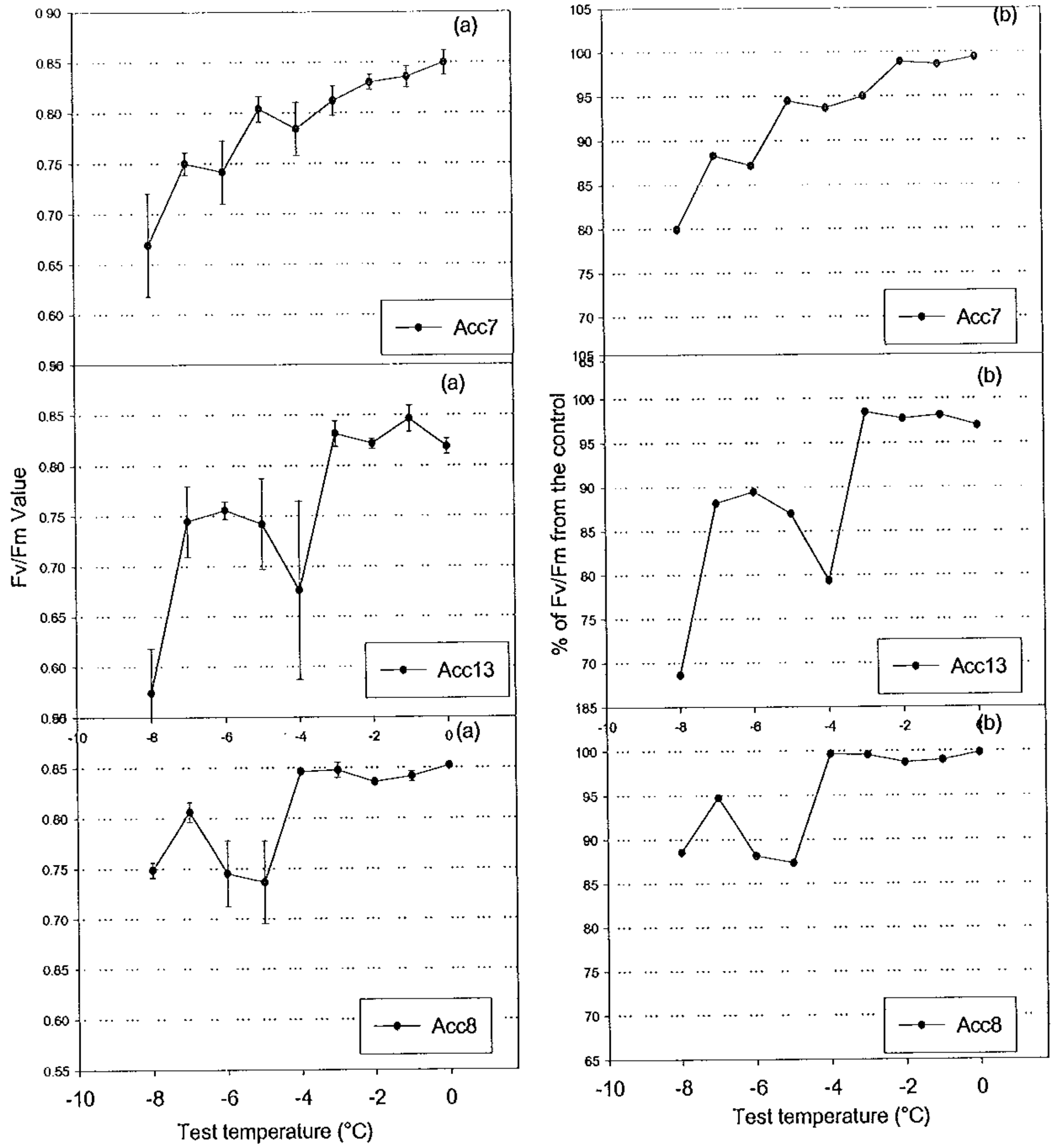

Fig.9. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Acc7, Acc13 and Acc8.

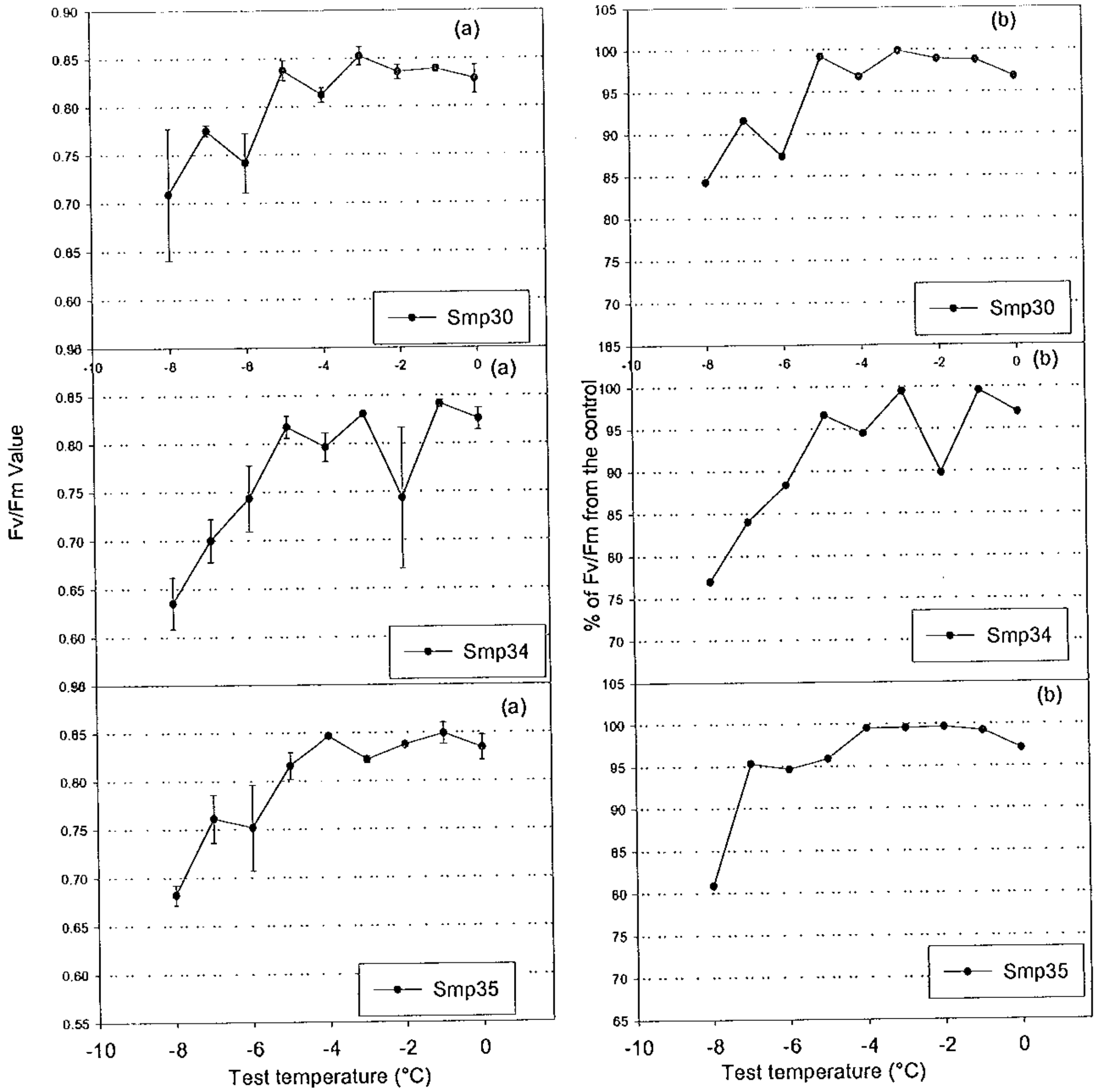

Fig.10. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Smp30, Smp34 and Smp35.

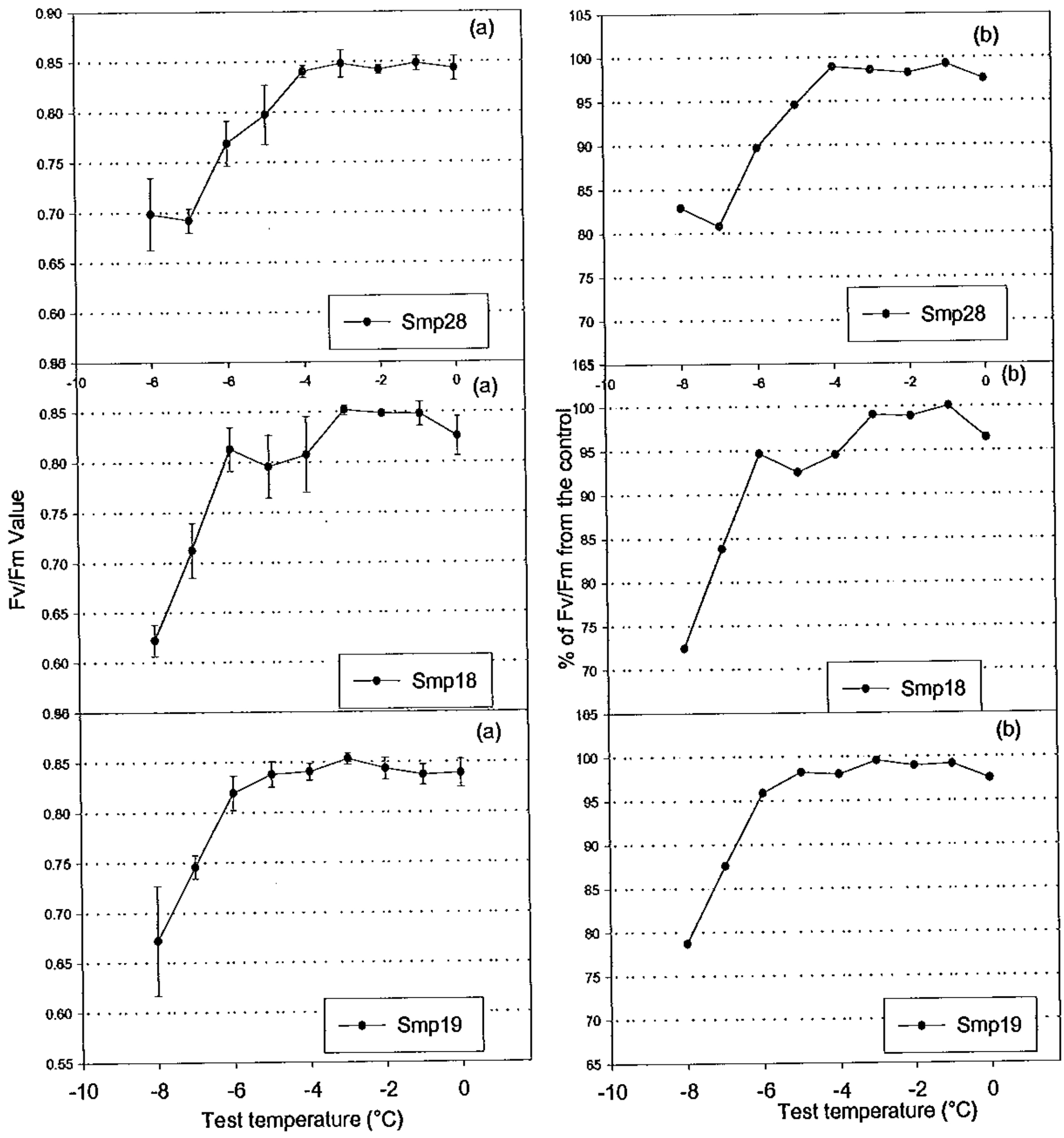

Fig.11. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Smp28, Smp18 and Smp19.

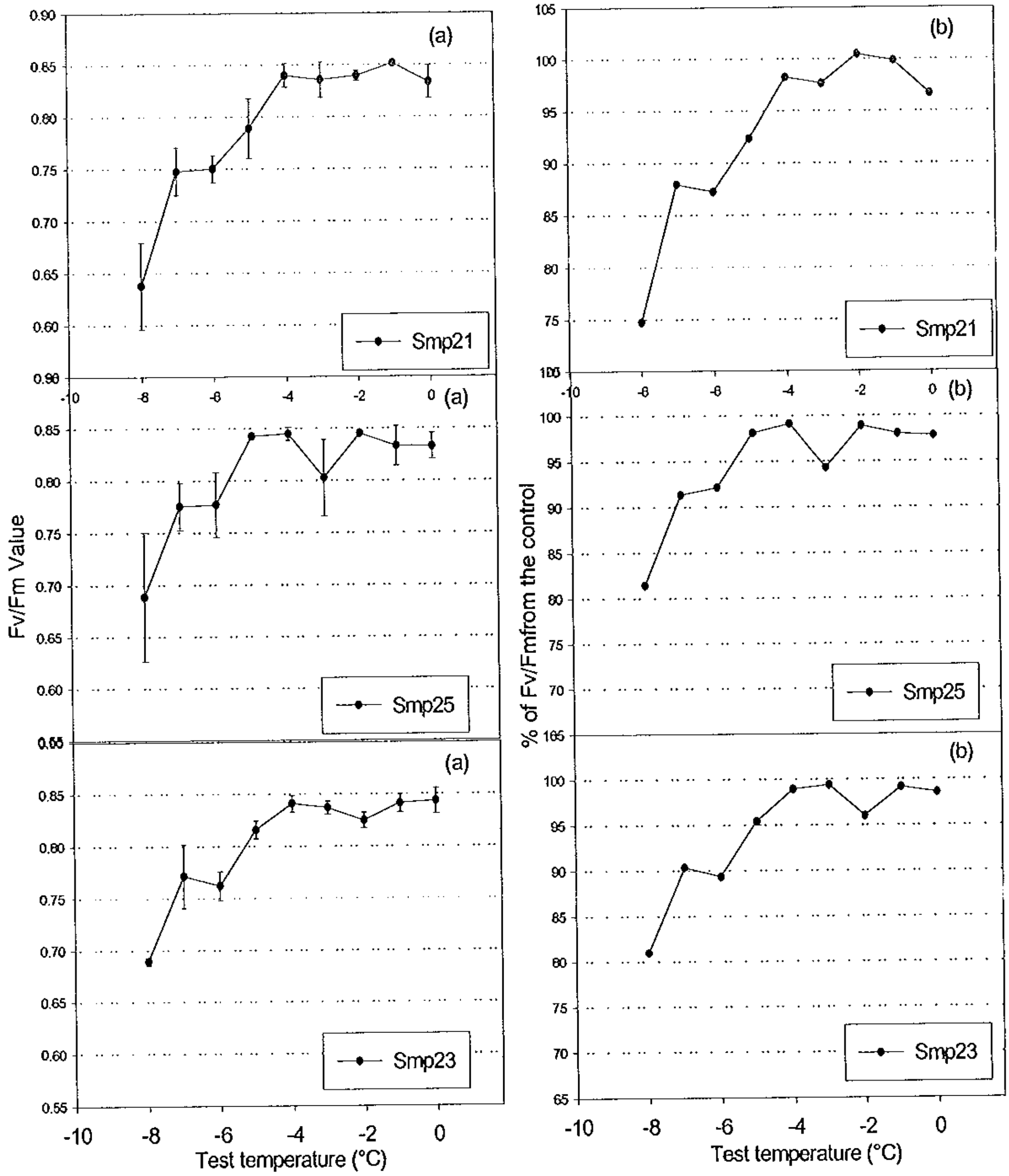

Fig.12. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Smp21, Smp25 and Smp23.

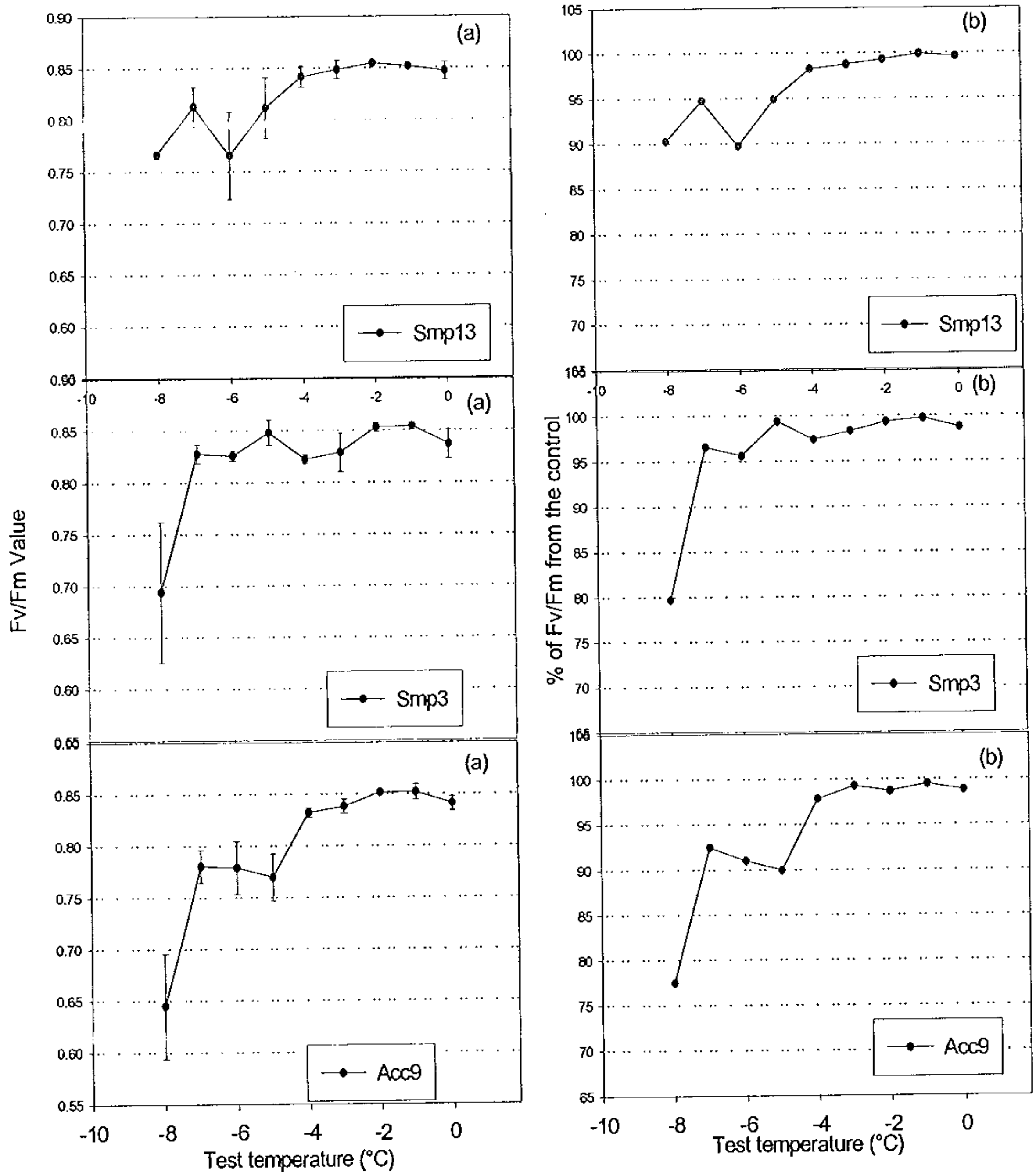

Fig.13. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Smp13, Smp3 and Acc9.

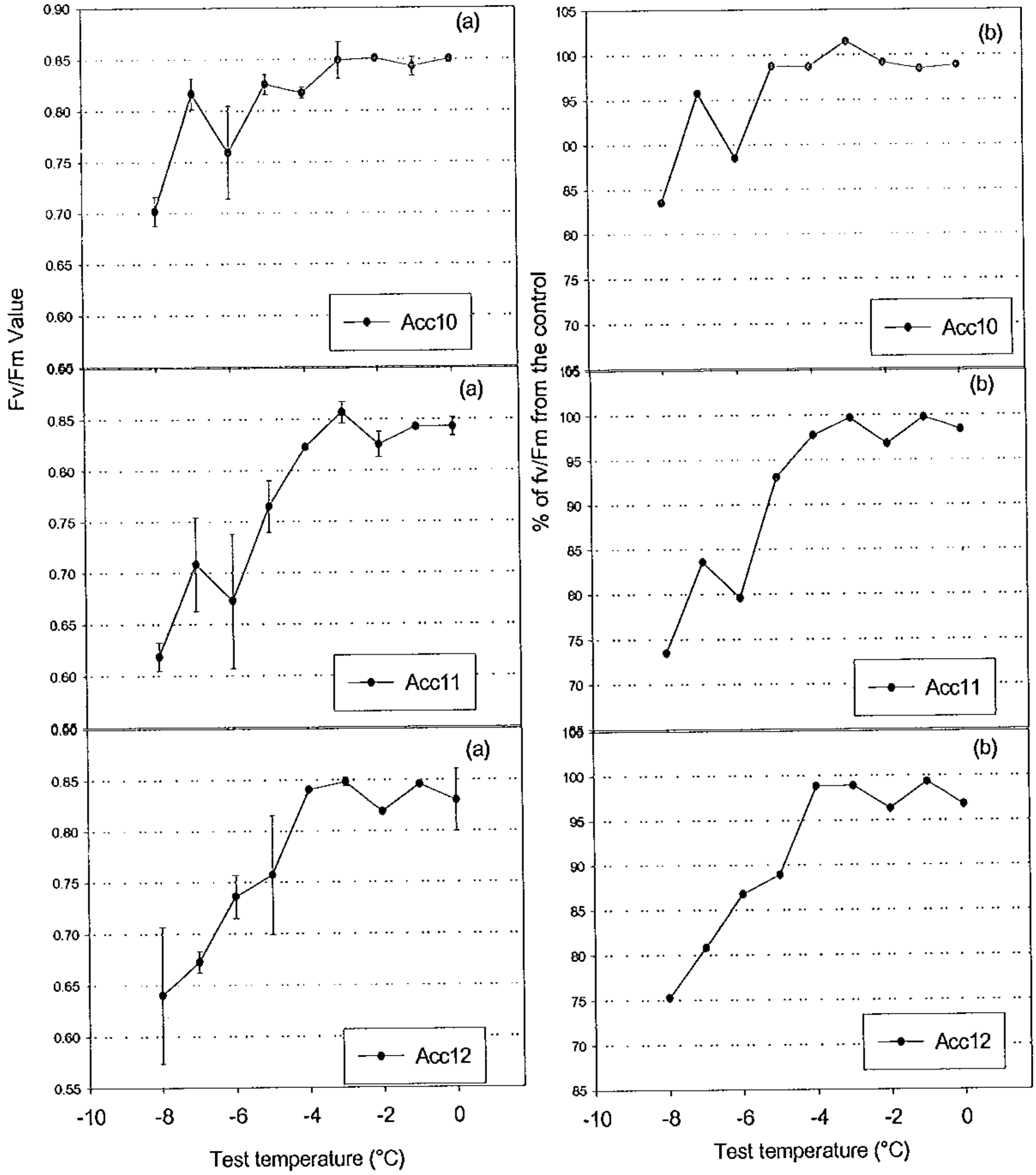

Fig.14. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Acc10, Acc11 and Acc12.

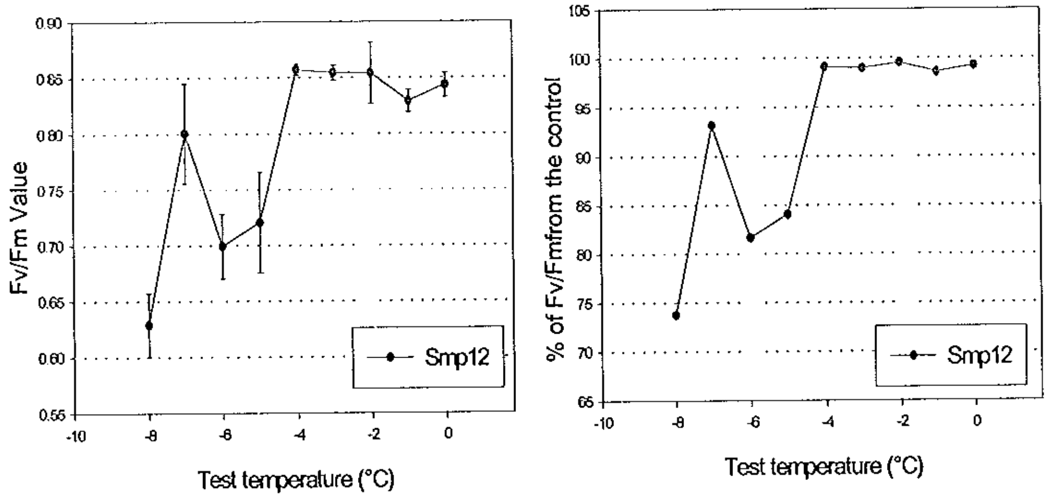

Fig.15. Potential quantum yield after treatment (a) and the corresponding percentage value of Fv/Fm from the control (b) of Smp12.

The regression lines of the Fv/Fm percent declined from control as a result of freezing treatment are shown in Fig.16 to19.

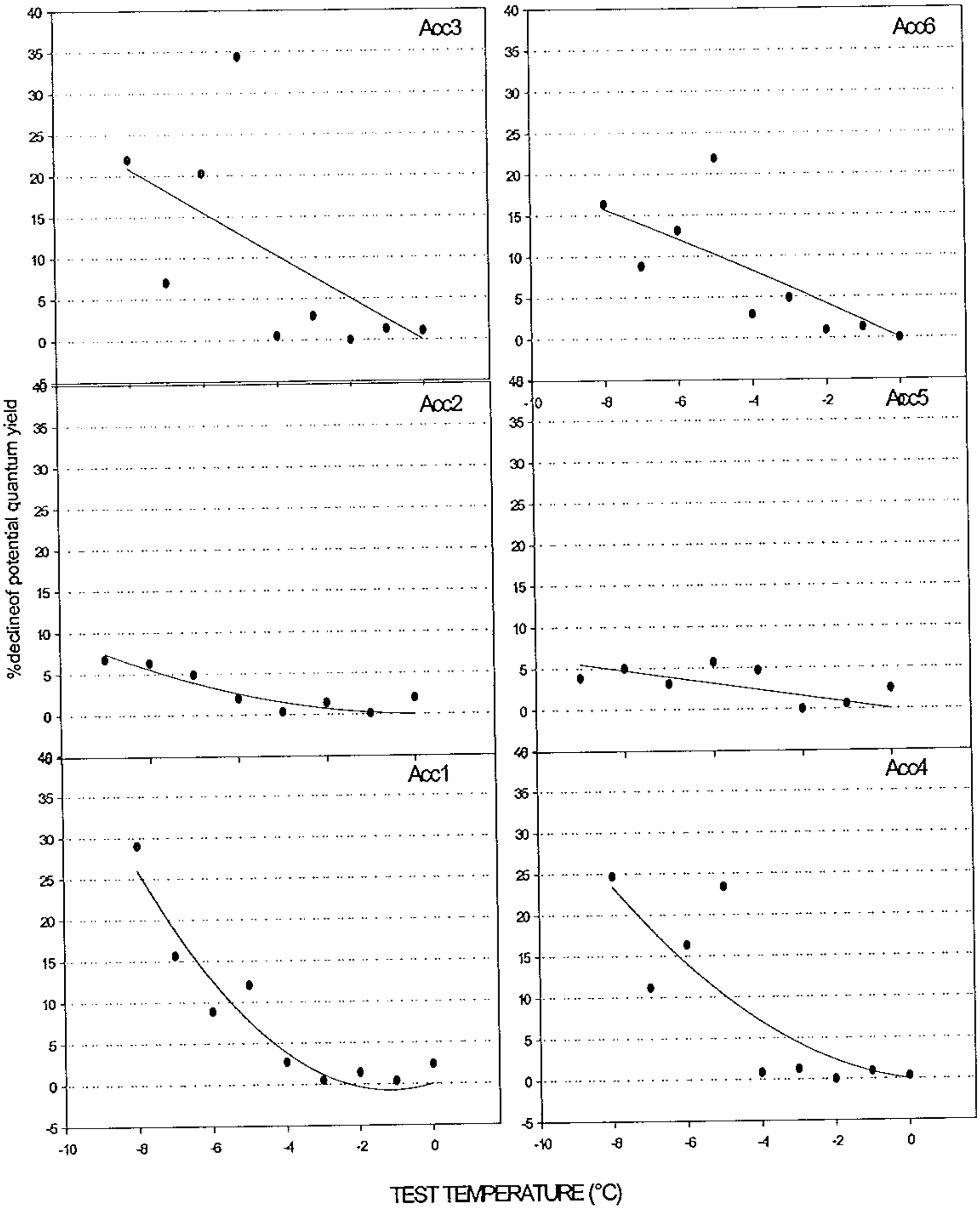

Fig.16 Percent decline of the Fv/Fm in samples Acc1, Acc2, Acc3, Acc4, Acc5 and Acc6 after treatment in the test temperatures 0 °C to -8 °C.

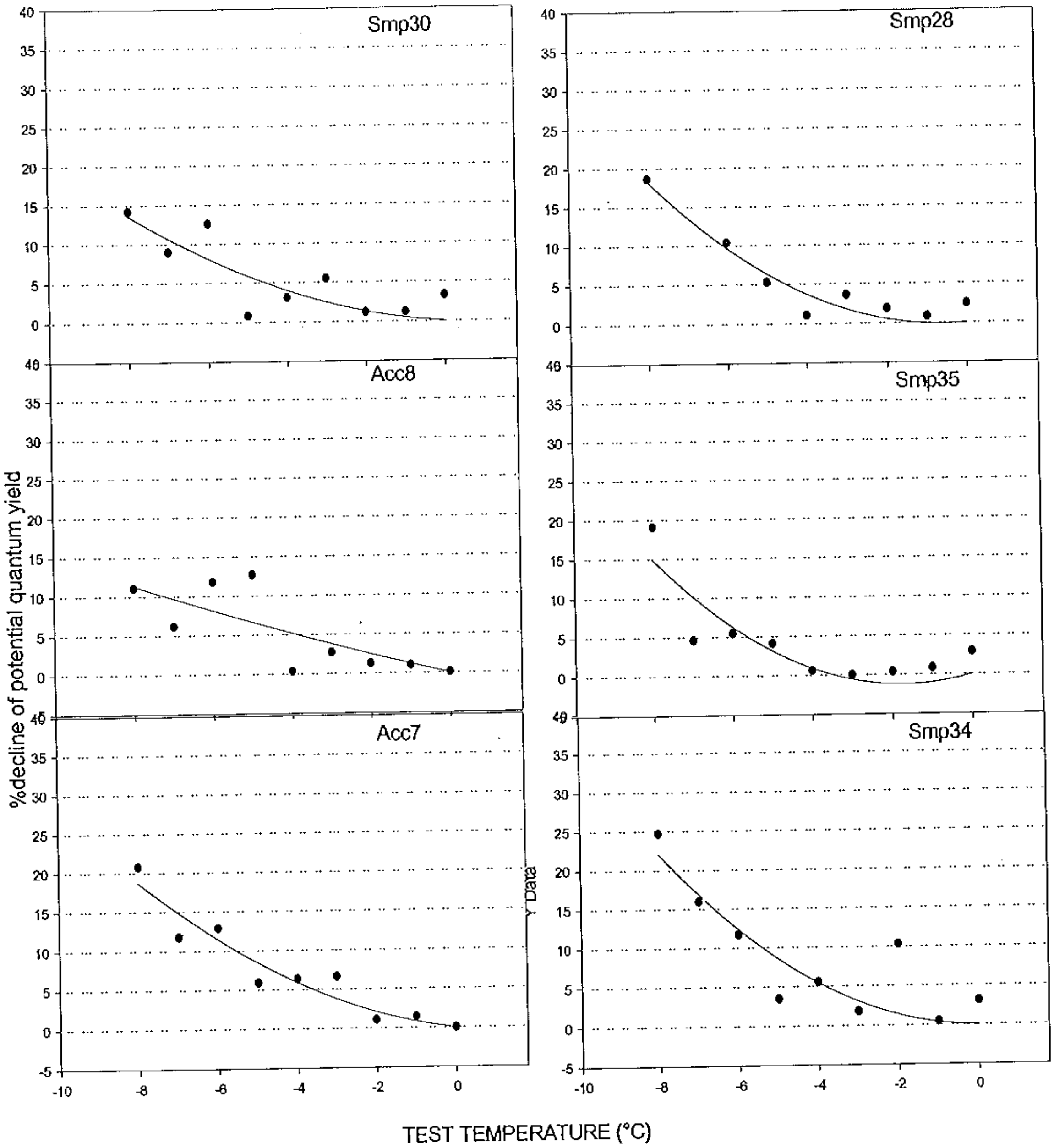

Fig.17 Percent decline of the Fv/Fm in samples Acc7, Acc8, Smp30, Smp34, Smp35 and Smp28 after treatment in the test temperatures 0 °C - -8 °C.

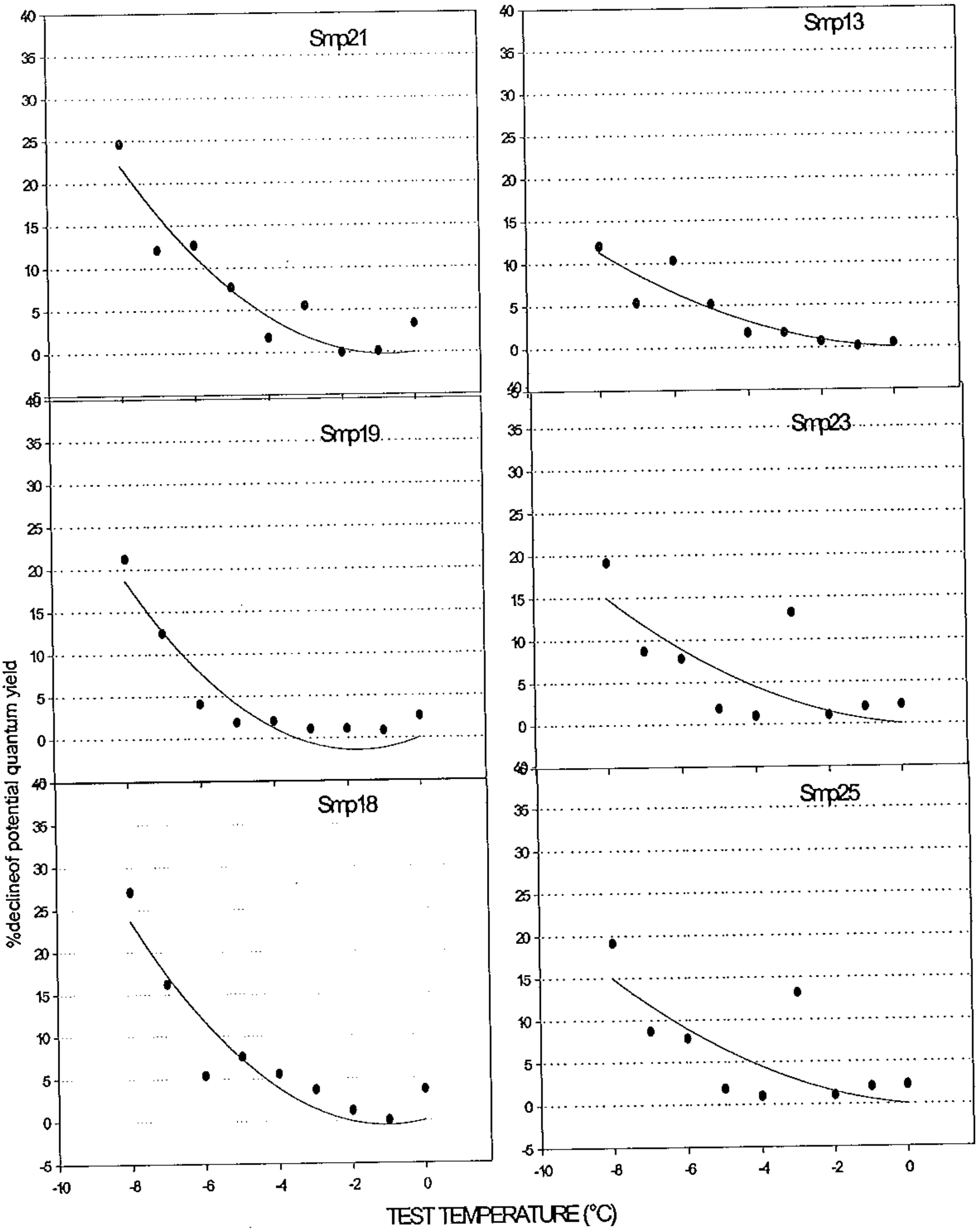

Fig.18. Percent decline of the Fv/Fm in samples Acc18, Acc19, Smp21, Smp25, Smp23 and Smp13 after treatment in the test temperatures 0 °C - -8 °C.

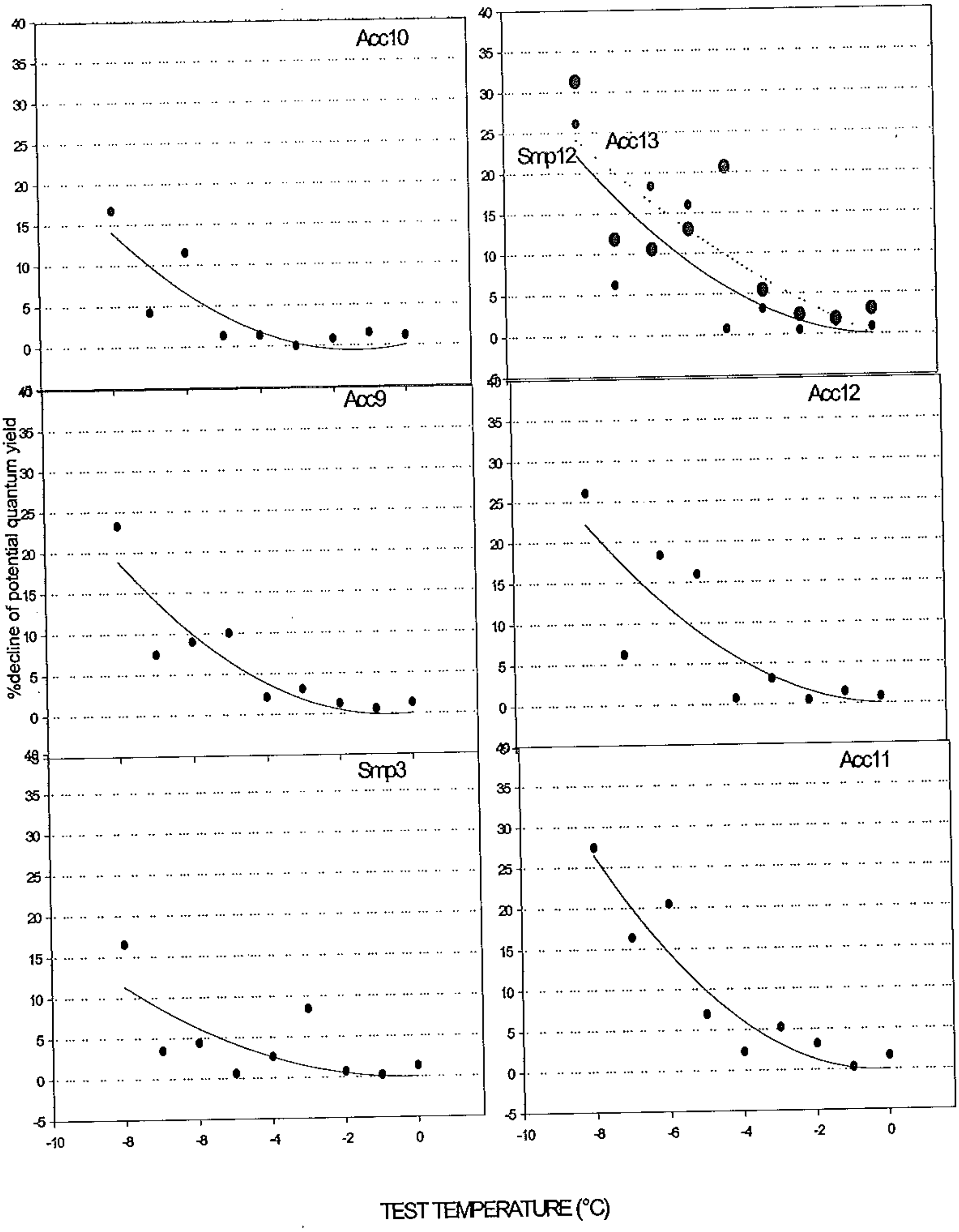

Fig.19. Percent decline of the Fv/Fm in samples Smp3, Acc9, Acc10, Acc11, Acc12, Smp12 and Acc13 after treatment in the test temperatures 0 °C - -8°C.

The percent decline of Fv/Fm from the original control as a result of freezing indicated that Smp3, Acc2, Acc5, Smp13, Acc8, and Smp35 were relatively the least affected whereas Acc13, Acc1, Smp34, Acc3, Acc4, Acc11, Acc12, Smp21 and Smp12 were the most affected samples. The analysis of variance of the Fv/Fm ratio of the landrace varieties and accessions indicated significant variation above –3 °C and the highest significant difference was observed at –4 °C (Table 12).

Table 12. Values of means of variance of the Fv/Fm ratio of 25 samples from Fisher's pair wise comparison at test temperatures from 0 ^{0}C to – 8 ^{0}C.

Test-temperature (°C)	0	-1	-2	-3	-4	-5	-6	-7	-8
P value*	0.69	0.98	0.06	0.76	0.01	0.02	0.09	0.02	0.04

5.2.2. The initial fluorescence (Fo)

The Fo value of the samples after treatment decreased from test temperature 0 °C to –7 °C and started increasing at –8 °C except for Acc13, which showed an increase in the value of Fo at –7 °C prior to others. The value of Fo after 24 hours increased in most of samples (Fig. 21).

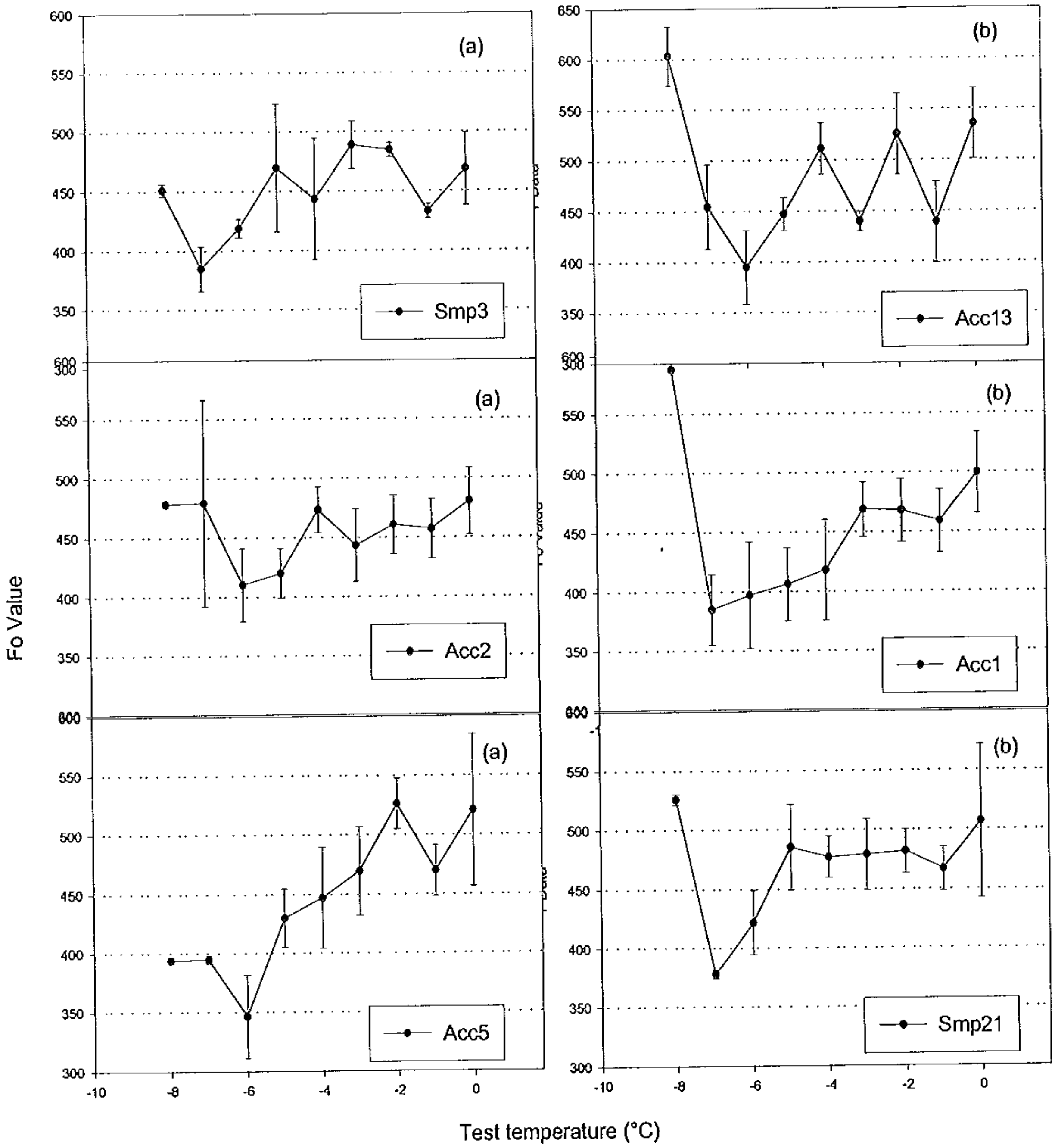

Fig.20. Response of PSII in initial fluorescence of the samples after treatment in some selected samples.
*vertical bars indicate standard errors of the means of three samples.

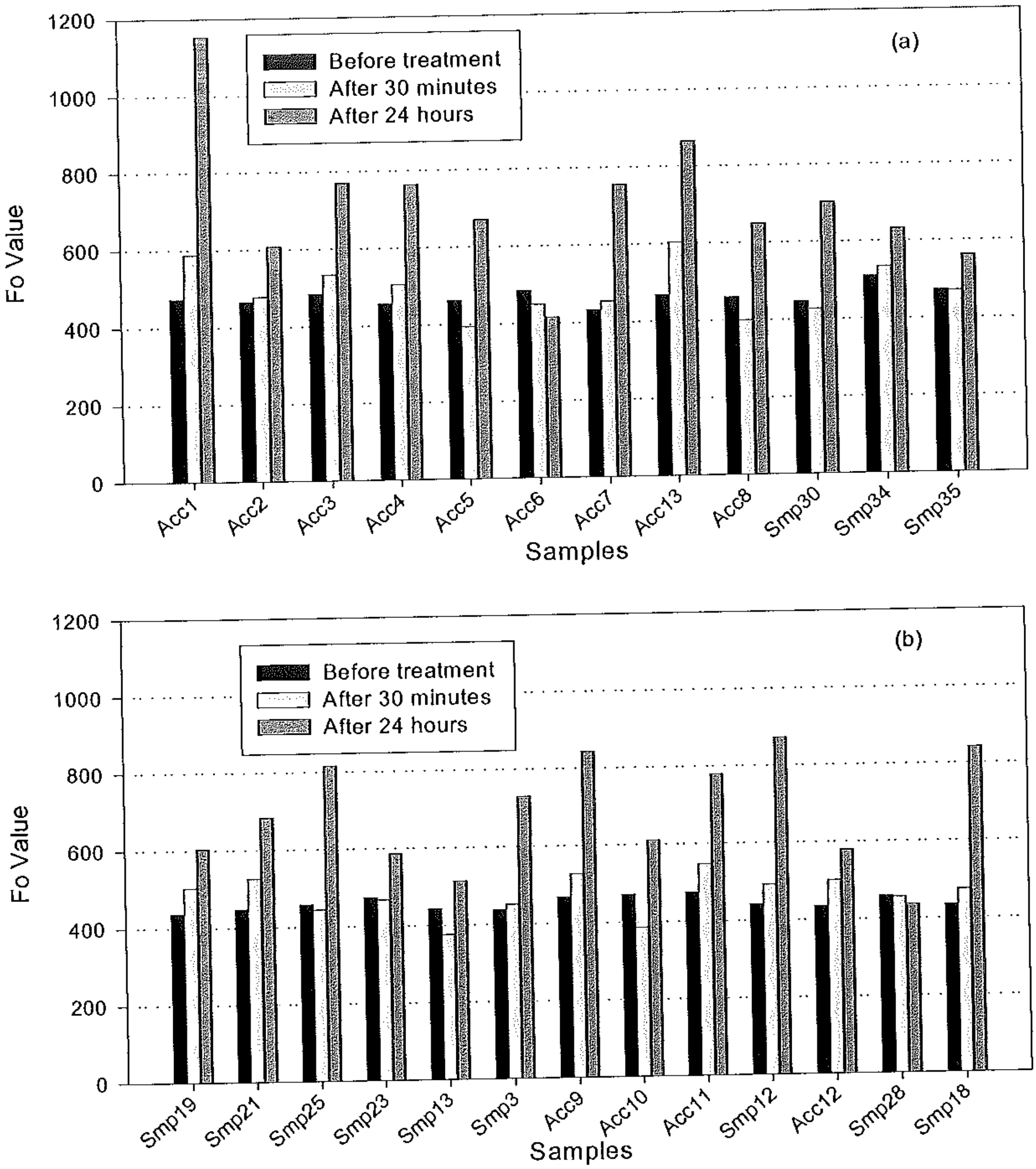

Fig.21. Fo values of the samples(in a and b) measured before treatment and after treatment (at 30 minutes and 24 hours) at-8 °C.

5 DISCUSSION

5.1 The ethnobotany of barley in the northern part of Ethiopia

5.1.1 Barley cultivation

Barley cultivation in most of the surveyed areas is practiced in undulating hills. Barley grows well in sites that can retain moisture and are selected by the farmers, which indicates low water stress is the primary production constraint as indicated in Table 3. During the field survey, most early barley landraces that were planted in June were mature and ready for harvest whereas the late barley landraces in the higher altitudes were at early heading stage. According to the farmers, usually April and May are the convenient planting months and December and January are the harvesting months for late barley while June is the appropriate sowing month and November to December is the harvesting time for early barley. In the study sites barley is extensively cultivated. According to the farmers, greater than half of the farmlands in Abune-Yosef and Tsibet-Embahasti sites and about half of their farmlands in Mitsinah are used for barley cultivation. Farmers use both *maresha* (sharpened iron sheet pulled by oxen so as to plow the farmland) and hoes equally for barley cultivation because even the lands that are plowed using oxen are with inaccessible spots. Farmers could enumerate the barley landraces that are cultivated in all these areas up to 43 in total of which about 28 were collected. The farmers mainly use morphologies of the spikes and kernel for identifying the barley landraces. Naming of the landraces is also used after the morphologies of the spikes and kernel. They primarily use presence or absence of hull followed by the number of rows for categorizing the major barley landraces, this agrees with the result of Zemede Asfaw (2000), who reported farmers and communities more frequently use barley groups based on grain morphology. Our result also shows that kernel colors are found to be used for distinguishing landraces, for instance there are three types of *awariye*: *tikurawariye* (black), *keyawariye* (red) and *nechawariye* (white) as well as two types of shewa: *keyihshewa* (red) and *tsaedashewa* (white).

Legumes such as fababean, pea and lentil are also cultivated with less proportion to barley in higher altitudes and wheat is usually cultivated below but near 3000m asl. However, barley cultivation and production are declining in the surveyed areas, mainly due to insufficient rainfall, poor soil fertility and poor farming management as a result of shortage of farmlands, i.e., application of fallow system is unaffordable to the farmers, crop rotation is limited and the yearly and frequent plowing aggravates soil erosion. In Abune-Yosef frost effect was indicated as the third factor. In Tsibet-Embahasti frost effect was indicated as the fourth factor, but it is not common in Mitsinah. In Mitsinah, when there is insufficient rainfall, high wind speed that may facilitate transpiration during flowering stage causes failure in seeding. According to the farmers and personal field observation, frost effect in Abune-Yosef was one of the major constraints in barley production in the long rainy season. During the field survey, frost effect on barley was severe as the entire crop was affected (stunted and decolorized). In some farmlands cattle were let to graze on the standing crop of barley due to complete loss of seeding. According to the respondents, effect of frost becomes more severe when there is insufficient rainfall and in that year, indeed, rain was not sufficient in the whole country, and it started late in July. However, farmers interviewed in Abune-Yosef and Embahasti sites indicated that the only means that they can minimize decline in barley production due to frost effect are by using those landraces that mature early and by early sowing of late barley landraces when there is early and sufficient rainfall. For instance, landraces like *sekutere (abejira, awariye, tedowasha*) and *mehoni* in Bugna wereda; *saesaa, shiwa, haftusene* and *himblil* in Tsibet-Embahasti and *netselasigem (saesaa*), *demhai* and *gunazasigem* in Mitsnah are cultivated during insufficient rainfall. Moreover, landraces described in the first two sites are also cultivated during the short rainy season and using irrigation. The late maturing barley landraces *nechita* in Abune-Yosef; *rie* and *kinchibe* in Tsibet-Embahasti and *atsa* and *atena* in Mitsnah are cultivated in the long rainy seasons with sufficient rainfall. In spite of the longer maturation period, respondents pointed out that these late barley landraces are chosen, as they relatively provide high yields, with heavy seeds that provide more flour per number of seeds (which they say the flour takes high amount of water during kneading). However, these landraces are, nowadays, less cultivated and in the first two sites are confined to higher altitudes (up to 3630m in Abune-Yosef and 3300m in Tsibet-Embahasti) and almost rarely cultivated in Mitsnah. Unless the rainfall condition changes farmers in the study area now tend to abandon cultivation of the late barley landraces and the majorities were not capable of storing seeds due to the insufficient production. Hence, this necessitates the application of *ex-situ* conservation of the late barley. Moreover, community seed banks have to be established in barley cultivating sites, for this is the best mechanism that keeps the dynamic conservation of landraces at their native locality (*in situ* conservation). In addition it keeps in progress the indigenous knowledge of the farming community, which is acquired in the millennia. This program has been applied in some parts of Tigrai by the initiative of Relief Society of Tigrai (REST) since 1991(Berg, 1992 cited in Boef *et al.*, 1996) and the concerned governmental and non-governmental organizations should establish and expand the community seed banks in wider area of Ethiopia.

In Abune-Yosef, it is indicated that exchange of barley landraces between relatively higher and lower altitudes, for instance from 3000m to around 3600m above sea level, is applied. However, the interviewees suggested that barley landraces from lower altitudes are harvested with lower or unsatisfactory yield but mature faster in the higher altitudes whereas late barley landraces from higher altitudes, according to them, are rarely cultivated in lower altitudes because they need high moisture to mature.

5.1.2 Barley utilization

According to the respondents barley is used mainly for food. All the interviewees prepare *enjera*, *besso, kollo* and *tella* from barley, and selection of landraces for each foodstuff is common as also reported by Zemede Asfaw (1990) for Gibat and Mecha, in the central highlands of Ethiopia. *Besso* has different forms of utility such as cold soup, porridge, moistened flour (*fraro*) and *tihlo*. *Tihlo*, thick dough, which is made by kneading using cold water, is very popular, in Mitsnah (Eastern Tigrai as a whole). *Tihlo* is a special delicious food that is served in cultural and religious ceremonies and other special occasions throughout the tradition of the society. It is eaten freshly with sauce usually meat *wot* mixed with soup like diluent locally called *hazo*. The best *besso*, which is used for *tihlo*, in this area is relatively white and well roasted or cooked flour nearly in its natural colors. But to have both features they use special roasting technique, which is different from other areas described such as in NorthWest Ethiopia (Asmare Yallew *et al.*, 1998a). Barley landraces called *netselasigem* or *saesaa* is preferably chosen for its nature of relatively white flour. After the barley seeds are soaked in water, pounded and again dried, roasting follows. Unlike in other areas roasting is not carried out on iron pan rather on clay pan locally called *Gundo*, specifically prepared for this purpose. Moreover, clean uniform sized sand particles are first put on the heated clay pan and then barley seeds are mixed over and roasted together. According to the interviewees, this process has dual purposes: the clay pan roasts relatively slower and the sands roast the seeds in every corner at a time, speeds up roasting and also prevents the direct contact of the seeds to the pan. This in turn helps minimize the characteristic color change as a result of roasting. Roasted barley seeds are then separated from the sand particles on the clay pan where the sand particles are reused.

On the other hand, *besso* has religious value in this area. *Besso* is moistened with blessed water (after the name of Saint Abune Aregawi in Abune Aregawi Church). After it is dried in grain-textured way, it is then ready for distribution to the followers of the church. It is believed that taking this orally, anointing with dissolved in water or having it makes blessed. The high-energy source nature of barley is also well understood in this area, cattle that have lost their weight are also made to recover by feeding with barley seeds, as also documented by Zemede Asfaw (1990) for central highlands. On the other hand, eating darkly roasted barley (*kolo*) and smelling the smoke soon after roasting forms obligate part of a cultural coffee ceremony in Abune-Yosef.

Selections of the foodstuffs prepared from barley for the family member is not common but usually porridge and soup are given to mothers after delivery. In all the survey areas about 90 % of the interviewees claimed that there is no crop that can substitute barley, which is cultivated in higher altitudes. The farmers in Abune-Yosef indicated that the absence of a crop that can substitute barley is one of the major problems in crop rotation application, only barley- legumes is possible, in that the latter are usually used for sauce. It is unaffordable for the farmers to cultivate legumes in fields as wide as that for barley.

Barley straw is the most preferred animal feed in these areas during the dry season. For families without cattle it is an important source of income or means for borrowing oxen. Like in other areas described by other authors, the farming community also uses barley straw in house construction, for instance in thatching roofs in Abune-Yosef and Tsibet-Embahasti highlands and as component of roof strata of houses locally called *Hidmo,* common in Mitsnah.

5.2 Physiology of frost resistance

5.2.1 Electrolyte conductivity method

5.2.1.1 Percent leakage

In the present study percent leakage, calculated as percent damage, was used to evaluate the frost resistance of barley landraces. The average percent leakage of the control samples in our work ranged from 14.56 % to 21.4 % with an average of 17.68 %. This can be due to the effect of the cork borer on the marginal cells of the disk, the effect of environmental factors and the distilled water is also found to cause damage. The difference among the controls may also be ascribed to the extent of tissue succulence as described by Calkins and Swanson (1990). The loss of membrane stability as a result of freezing damage initiates leakage of readily diffusible materials from the cell (Palta *et al.,* 1977 a; Calkins and Swanson, 1990). Palta *et al.* (1977 a) analyzed effusate to include sugars, K^+, Ca^{++}, H^+ and Cl^- from onion bulb cells.

Significant increase in percent leakage compared with the controls was obtained starting from the test temperature –1 °C. Thus, significant frost damage on cell membrane started at this test temperature, which occurred after the initiation of ice nucleation (between 0 °C and –1 °C). Naturally plants are prone to ice nucleation through frost reaching the leaves and the frozen soil in contact with the roots (Burke *et al.*, 1976). Freezing points of leaves of afro-alpine giant rosette plants *in vivo* were found to be in the range between 0 °C and –0.5 °C (Beck *et al.,* 1984). In the present study severe freezing effect had occurred starting from –4 °C where strikingly significant increase in % leakage was observed, the barley laminae (excised leaf samples) under these treatments turned to be soft and looked water soaked. This result is in consistence with the observation of Wanner and Junttila (1999) using Arabidopsis at test temperatures –4 °C to –14 °C as well as with the results of Hansen and Beck (1987) for winter barley. Such treated leaves were quickly dried when they were put at room temperature for dark adaptation. This may be due to the failure in reabsorption of the water in the intercellular space and the subsequent evaporation.

5.2.1.2 Percent damage

The most important effect of freezing is formation of ice and the subsequent physical damage (Levitt, 1980). The percent damage of the samples in the present work increased with increasing percent leakage and as the test temperature decreased the difference between them minimized. The value of the percent damage is commonly taken as indicator of the freezing effect on living tissue because it is computed relative to potential percent leakage of the untreated tissue of the plant (control). In this work percent damage increased with decreasing test temperatures. This agrees with the works of Pearce and Willison (1985) who reported the damage in wheat laminae increased from 2 % to 49 % when the test temperature was decreased from –2 °C to –8 °C. The highest percent damage was recorded in the present work at the lowest test temperature used (-9 °C), where little variations were observed among the samples ($p=0.96$). This result is in agreement with the work of Dexter *et al.* (1932) who found less marked difference between winter and spring varieties of rye, oat, and barley at –10 °C.

The analysis of variance of the means of the percent damage showed significant variation among landraces and accessions only at –5 °C. Hence, this temperature can be taken as critical test temperature or higher sub-zero temperature at which the samples can be separated easily. This also agrees with the finding of Dexter *et al.* (1932) who reported that –5 °C separated the winter and spring varieties of rye, oat and barley. The response of the samples based on membrane stability can be put in order of decreasing as follows: Smp18, Acc5, Acc1, Acc3, Smp13, Acc4, Smp3, Smp25, Acc6, Smp28, Smp35, Smp23, Acc7, Acc9, Smp30, Acc11, Acc10, Acc2, Acc8, Smp34, Smp12, Acc12, Acc13, Smp19 and Smp21. Thus while Smp18 had relatively least affected cell membranes, Smp21, Smp19, Acc13 and Acc12 had relatively susceptible cell membrane at this test temperature, where the more stable membranes of cells of a sample, relatively the more tolerant the plant is. Many researchers agree that the primary target of freezing injury to be the cell membrane as a result of dehydration (Sakai and Larcher, 1987). However, the physiological effect of freezing in living cells is far from understood but it is known that it has many effects that collectively result in cell death (Levitt, 1980). According to Levitt (1980), the most important effect of freezing is formation of ice and the subsequent physical damage. On the other hand, Palta *et al.* (1977a) assumed that the intrinsic membrane proteins controlling K^+ and sugar transport (active transport systems) were damaged in freeze killed cells, for they had got large ion and sugar efflux with almost unchanged water permeability of onion bulb scale tissue. Freeze induced injury was also proposed due to SS bonding between molecules containing SH-group in the cell membrane which was followed by aggregation, which up on thawing causes protein degradation (Levitt, 1962 cited in Levitt, 1980). Freezing was also supposed to cause phase transition of lipid bilayer (inverted hexagonal (H_{II}) or gel phase), which were expected to be lethal because such defect were found to allow solute efflux and penetration of ice nuclei into the cytosol (McKersie and Leshem, 1994).

5.2.1.3 Assessment of frost resistance

The electrolyte conductivity measurement of leakage indicates the extent of injury at a given test temperature.According to Zhang and Willson (1987) this method becomes promising and most important next to viability test when it is expressed in LT_{50}. The LT_{50} can be determined from the middle point of the steeper portion of the curves (Cardona *et al.*, 1997). In our study, however, this couldn't be demonstrated on the curve because the upper asymptote was not determined due to the limitation of the freezing apparatus, which did not freeze beyond –9 °C. The relative frost tolerance of the 25 samples ranged from –8.64 °C to –6.48 °C, they were found to be moderately hardy plants based on the six resistance adaptations proposed by Levitt (1980). Similarly under controlled environment, Anderson *et al.* (1993) also found –9.6 °C to –7.7 °C to be the tolerance for six types of Bermuda grasses. The lower the sub-zero temperature at which 50 % cells of a given sample die, the more resistant the plant is. Though the samples were not significantly different in freezing resistance, the numerical ranking of our result revealed that landraces from Abune-Yosef: Smp3 (*Tedowasha*) and Smp13 (*Awariye*) were found to be relatively the highest frost resistant from the field collected samples. Whereas landrace Smp12 (*nechita*), which is collected as high as 3630m from the same area was found to be the least resistant next to landraces Smp21 (*tsaedashewa*) and Smp19 (*haftusene*) that were collected from Tsibet-embahasti (Southern Tigrai) altitude of 3100m and 3000m asl., respectively. This work showed that accessions from IBCR Acc5 (*chenka*) and Acc2 (*kokufa*) had relatively higher membrane stability next to Smp3. Both Acc5 and Acc2 were collected from Bale region at altitudes of 2430m and 1650m asl., respectively. On the other hand, accessions Acc13 and Acc1 were relatively least frost resistant. They were collected from Arsi at 2800m and Bale at 1630m asl., respectively. Samples with greater LT_{50} (-ve values) had flatter curves with lower slopes while steep slopes were associated with lower LT_{50} (-ve values). This is in agreement with the work of Cardona *et al.* (1997) who assessed low temperature tolerance of three ecotypes of seashore Paspalum (*Paspalum vaginatum* S.)

5.2.2 Chlorophyll a fluorescence

5.2.2.1 Potential quantum yield (Fv/Fm)

In general, the potential quantum yield measurement of the samples after treatment have shown significant decrease from the control starting from the first test temperature, which indicated PSII was affected even before sub-zero test temperature and before ice nucleation was initiated. The result also indicated that Fv/Fm values measured after 24 hours after treatment were significantly lower than the values measured after 30 minutes. The Fv/Fm values measured from untreated samples in the same time interval, however, showed little difference (result not shown). This indicates that the effect of freezing on PSII was not reversible.

Generally, at temperatures above –4 °C more than 80 % of the samples had Fv/Fm values similar to the control. Moreover, two samples (accessions Acc1 and Acc13) had Fv/Fm value below 70 % of the control that is they showed a decrease of photosynthetic efficiency by about 30 % at –8 °C. The analysis of variance of the means of Fv/Fm values showed significant variation starting from –4 °C and unlike that of the conductivity method, -4 °C was found to be the highest sub-zero temperature at which the samples could be separated. The Fv/Fm response of each sample after treatment decreased gradually at different rates. The slopes of the regression lines, fitted from the percent decline of the potential quantum yield from the controls, were used to compare the samples' resistance to freezing. In decreasing order of frost resistance the samples could be analyzed as follows: Acc5, Acc2, Smp3, Smp13, Smp35, Acc10, Acc8, Smp30, Smp19, Smp23, Smp25, Smp28, Acc9, Acc7, Acc6, Smp21, Smp34, Smp18, Acc12, Acc1, Smp12, Acc4, Acc11, Acc3 and Acc13. This result is in agreement with the conductivity method in that the first three (highly resistant types) were also those recorded in the conductivity method, thus indicating that the results could be relied upon.

5.2.2.2 The initial fluorescence (Fo)

The average initial fluorescence value of the control for all samples at all test temperatures ranged from 448.7 to 497.2 with mean of 469.6. Following treatment the Fo started to decline gradually to an average value of 392 at –7 °C and then increased sharply above 500 in those which were susceptible whereas in those which were identified as resistant showed only a little increase or not at all. The Fo of Acc13, which was relatively the most susceptible, however, started to sharply increase at –7 °C. It reached up to 600 at –8 °C. The fluorescence properties suggest that the gradual decline of the Fo may be ascribed to the photoinhibitory and energy dependent non-radiative energy dissipation (regulatory mechanisms) (Krause and Weis, 1991; Masresha Fetene *et al.*, 1997). The increase in Fo, however, shows the physical dissociation of the PSII reaction center from the light harvesting system (antennae). Similar result was also found by Srinivasan *et al.* (1996) in groundnut with heat stress. However, unlike the report of Demmig and Bjorkman (1987) the Fo measured after 24 hours at test temperatures 0 °C to –6 °C (where photoinhibitory related decline in Fo occurred) showed no recovery, it was irreversible as in the test temperatures where an increase in Fo occurred. The result showed that relatively frost resistant types could be found both from upland and low land barley landraces and accessions in a narrow range. Similar results were reported by Stanca *et al.* (1996) for most barley cultivars tested in the field. Although the frost resistance was found positively correlated with the altitude of site of sample collection the association was weak. This is probably due to the inadequate sampling of the late barley landraces from higher altitudes since they were immature during the time of sample collection. Moreover, most of the upland barley landraces were found to be relatively susceptible perhaps due to the relatively fleshy nature of the leaves, which may release more electrolytes during freezing as observed by Calkins and Swanson (1990) who criticized the method. Other possible reason could be a shift in the location of the original growing area by man, i.e., the relatively susceptible barley from higher altitudes might have been brought from lower altitude and the relatively resistant types from lower altitude might have been brought from higher altitude. The variation of the samples in frost resistance in the field may also be ascribed most probably to age (maturation period) differences as most of the upland barley landraces are late maturing. Flowering and fruiting carried out just after the plant has been exposed to cold temperatures for longer time may help the plant prepare itself in tolerance or avoidance of the severe stress of frost (acclimate). On the other hand, when early barley landraces are cultivated at higher altitudes during insufficient and/ or late rainfall, flowering could start before the onset of frost, as this part is the most susceptible (Poehlman, 1985), hence the crop could escape from frost stress.

6 SUMMARY AND RECOMMENDATIONS

Our work was intended to screen barley landraces and accessions based on frost resistance using controlled experiments and to document the indigenous knowledge of the farmers about the cultivation and utilization value of barley (*Hordeum vulgare* L). The result from the field survey showed that more than half of the farmlands of the respondents were used for barley cultivation and the harvested barley grain was used all in all for food, while the straw is used for animal feed and as an income source. The interviewees indicated environmental stresses such as insufficient rainfall, low soil fertility, and frost effect as the main limiting factors of barley production. They also responded that none of them had ever selected barley landraces, which are resistant to frost except by using early barley (age-based) and by sowing early when there is sufficient rainfall. The farmers, nowadays, prefer the early landraces due to the unreliable nature of rainfall. This may necessitate an ex-situ conservation of the late barley landraces before they are lost.

The result of the experimental work, on the other hand, demonstrated that based on membrane stability and fluorescence studies landraces Smp3 and Smp13 as well as accessions Acc5 and Acc2 were found to be relatively frost resistant. The membrane stability of the samples had no significant difference among each other except at test temperature –5 °C. Chlorophyll a fluorescence among the samples was not significantly different up to –3 °C and the lowest test temperature where significant change occurred was –4 °C. Thus, both –5 °C and –4 °C can be assumed as potential test temperatures by which the samples could be differentiated.

The altitude and frost resistance of the samples collected from the surveyed areas was found to be positively correlated, but it was weak. However, including the accessions the frost resistance was found negatively correlated. Further study with test temperatures greater than –9 °C and field trial is recommended to evaluate further the findings before definite advice could be offered to farmers. Both methods used have shown differences among the samples. Thus, the methods, which are relatively easy, could be used for further screening and validation of large number of landraces. The fluorescence analysis method could also be used in the field with quick results.

8 REFERENCES

Abebe Demissie and Bjornstad, A. (1997). Geographical, altitude and agro-ecological differentiation of isozyme and hordein genotypes of landrace barleys from Ethiopia: implications to germplasm conservation. *Genetic resource and crop evolution* 44: 43-55.

Amsal Tarekegn, Adamu Molla, Amanual Gorfu and Zewdie Yilma (1996). Malting barley agronomy research. Pp. 85-91. In: *Barley research in Ethiopia*: past work and future prospects (Hailu Gebre and Joopvan Leur eds.). Proceedings of the first barley research review workshop, 16-19 October 1993, Addis Ababa. IAR/ICARDA. Addis Ababa, Ethiopia.

Anderson, J.A., Taliaferro, C.M., and Martin, D.L. (1993). Evaluating freeze tolerance of bermuda grass in a controlled environment. *HortScience* **28** (9): 955.

Asmare Yallow, Alemu Hailye and Halima Hassen (1998 a). Farmers' food habit and barley utlization in North West Ethiopia. Pp 107-112. In: *Barley-based farming systems in the highlands of Ethiopia* (Chilot Yirga, Fekadu Alemayehu and Weldeyesus Senebo eds.). Ethiopian Agricultural Organization, Addis Ababa, Ethiopia.

Asmare Yallow, Hailemichael Shewayirga Asefa Alebachew and Rahel Asrat (1998 b). Barley production practices and constraints in Mekct wereda (North Welo). Pp. 91-95. In: *Barley-based farming systems in the highlands of Ethiopia* (Chilot Yirga, Fekadu Alemayehu and Weldeyesus Senebo eds.). Ethiopian Agricultural Organization, Addis Ababa, Ethiopia.

Beck, E., Schulze, E-D., Senser, M. and Scheibe, R. (1984). Equilibrium freezing of leaf water and extracellular ice formation in afro-alpine ''giant rosette'' plants. *Planta* **162**: 276-284.

Bekele Hundie, Worku Jima, Feyisa Tadese, Mulugeta Amsalu, Mengistu Yadessa and Arfasa Kiros (1998). The dinsho farming systems of bale highlands. Pp.101-106. In: *Barley-based farming systems in the highlands of Ethiopia* (Chilot Yirga, Fekadu Alemayehu and Weldeyesus Senebo eds.). Ethiopian Agricultural Organization, Addis Ababa, Ethiopia.

Berhane Lakew, Hailu Gebre and Fekadu Alemayehu (1996). Barley production and research. Pp. 1-8. In: *Barley research in Ethiopia*: past work and future prospects (Hailu Gebre and Joopvan Leur eds.). Proceedings of the first barley research review workshop, 16-19 October 1993, Addis Ababa. IAR/ICARDA. Addis Ababa, Ethiopia.

Berhane Lakew, Yitbarek Semeane and Fekadeu Alemayehu (1995). Evaluations of Ethiopian barley landraces for disease and agronomic characters. In: *Rachis*; barley and wheat news letter ICARDA, Aleppo, Syria **14** (1/2): 21-25.

Bjorkman, O. and Demmig, B. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origin. *Planta* **170**: 489-504.

Boef, W.S. Berg, T. and Haverkort, B. (1996). Crop genetic resources. In: *Biotechnology: building on farmers' knowledge* (Bundres,J. Havrkort, B. and Hiemstra, W. eds.). MACMILLAN EDUCATION LD, London. Pp. 103-128.

Boorse, G.C., Bosma, T.L., Meyer, A.-C., Ewers, F.W. and Davis, S.D. (1998). Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. *Int. J. Plant Sci.* **159** (3): 513-521.

Bothmer, R.Von and Jacobson, N. (1985). Origin, taxonomy and related species. In: *Barley* (Rasmusson ed.). American society of Agronomy Inc., USA. Pp. 19-53.

Burke, M.J., Gusta, L.V., Quamme, H.A., Weiser, C.J., Li, P.H. (1976). Freezing and injury in plants. *Ann. Rev. Plant Physiol.* **27**: 507-528.

Calkins, J.B. and Swanson, B.T. (1990). The distinction between living and dead plant tissue; viability tests in cold hardiness research. *Cryobiology* **27**: 194-211.

Cardona, C.A., Duncan, R.R., and Lindstron, O. (1997). Turfgrass science: low temperature tolerance assessment in paspalum. *Crop Sci.* **37**: 1283-1291.

Chilot Yirga, Agagie Tesfaye and Elias Zerfu (1998). Barley-oat farming system Grar-Jarsso and Dejem weredas (North Shewa). Pp.41-65. In: *Barley-based farming systems in the highlands of Ethiopia* (Chilot Yirga, Fekadu Alemayehu and Weldeyesus Senebo eds.). Ethiopian Agricultural Organization, Addis Ababa, Ethiopia.

Chilot Yirga and Elias Zerfu (1998). The barley-enset based farming systems of Ambo and Tikur Enchini highlands, West shewa. Pp. 11-25. In: *Barley-based farming systems in the highlands of Ethiopia* (Chilot Yirga, Fekadu Alemayehu and Weldeyesus Senebo eds.). Ethiopian Agricultural Organization, Addis Ababa, Ethiopia.

Demmig, B. and Bjorkman, O. (1987). Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. *Planta* **171**: 171-184.

Dexter, S.T., Toffingham, W.E., and Graber, L.F. (1932). Investigations of the hardiness of plants by measurement of electrical conductivity. *Plant Physiol.* 7: 63-78.

Endashaw Bekele (1983). A different rate of regional destribution of barley flavonoid patterns in Ethiopia, a view on the center of origin of barley. *Hereditas* **98**: 269-280.

Fekadu Alemayehu and Hailu Gebre (1987). Barley breeding in Ethiopia. In: Barly and wheat News letter (Srivastava, J.P., Habib Ketata, Varma, S. and Tarek Abdel-Malak eds.). *Rachis* **6**: 1987.

Fracheboud, Y., Haldimann, P. Leipner, J., and Stamp, P. (1999). Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). *J. Exp. Bot.* **50** (338): 1533-1540.

Harlan, J.R. (1976). Barley. Pp. 93-98. In: *Evolution of crop plants* (Simmonds, N.W. ed.). Longman, London and New York.

Hansen, J. and Beck, E. (1987). Evidence for ideal and non-ideal equilibrium freezing of leaf water in frost hardy Ivy (*Hedera helix*) and winter barley (*Hordeum vulgare*). *Bot. Acta* **101**: 76-82.

Hedberg, O. (1995). *Features of afro alpine plant ecology*. EKBLADS, Sweden. Pp. 8-20.

Krause, G.H. and Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basis. *Ann. Rev. Plant Physiol. Mol. Biol.* 42: 313-349.

Larcher, W. (1980). *Ecophysiological plant ecology*. Springer-Verlag, Germany. Pp. 32-60.

Lazar, D. and Naus, J. (1998). Statistical properties of chlorophyll fluorescence induction parameters. *Photosynthetica* **35** (1): 121-127.

Levitt, J. (1980). *Responses of plants to environmental stresses*: V.I: Chilling, freezing, and high temperature stresses. Academic press, New York. Pp. 1-497.

Malhotra, R.S., and Saxena, M.C. (1993). Screening for cold and heat tolerance in cool-season food legumes. Pp 227-244. In: *Breeding for stress tolerance in cool-season food legumes* (Singh, K.B. and Saxena, M.C. Eds.). John Wily and Sons, New York.

Marshall, D.R. and Brown, A.H.D. (1975). Optimum sampling strategies in genetic conservation. Pp. 33-80. In: *Crop genetic conservation for today and tomorrow* (Frannel, Q., and Hawks, J.G. eds.) Cambridge University Press, Cambridge.

Masresha Fetene, Nauke, P. Luttge, U. and Beck, E. (1997). Photosynthesis and photoinhibition in tropical alpine giant rosette plant, *Lobelia rhynchopetalum*. *New Phytol*. **137**: 453-461.

McKersie, B.D. and Leshem, Y.Y. (1994). *Stress and stress coping in cultivated plants*. Kluwer Academic Publishers, London. Pp. 104-128.

Mulugeta Negassa (1985). Patterns of phenotypic diversity in Ethiopian barley collection, and the Arsi-Bale highlands as a center of origin of barley. *Hereditas* **102**: 139-150.

Orlov, A.A. (1928). The barley of Abyssinia and Eritrea. *Bulletin of Applied Botany, Genetics and Plant Breeding*. Leningrad. **20**: 228-243. English summery is used.

Palta, J.P., Levitt, J. and Stadelmeann, J. (1977 a). Freezing injury in onion bulb cells: I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. *Plant Physiol*. **60**: 393-397.

Palta, J.P., Levitt, J. and Stadelmeann, J. (1977 b). Freezing injury in onion bulb cells: II. Post thawing injury or recovery. *Plant Physiol.* **60**: 398-401.

Pearce, R.S. (1980). Relative hardiness to freezing of laminae, roots and tillers of tall Fescue. *New Phytol.* **84**: 449-463.

Pearce, R.S., and Willison, J.H.M. (1985). A freezing-etch study of the effects of extracellular freezing on cellular membranes of wheat. *Planta* **163**: 304-316.

Poehlman, J.M. (1985). Adaptation and distribution. Pp. 1-17. In: *Barley* (Rasmussen, D.C. ed.). American society of Agronomy Inc., USA.

Purseglove, J.W. (1972). *Tropical crops: Monocotyledons.* Longman group limited, London. Pp.120-342.

Qiquan, S. (1981). The evolution of cultivated barley. Pp. 22-25. In: *Barley Genetics* IV. Proceedings of the Fourth International Barley Genetics Symposium (Whitehouse, R.N.H. ed.). Edinburgh University press, Edinburgh.

Sakai, A., and Larcher, W. (1987). *Frost survival of plants: responses and adaptation to freezing stress.* Springer-Verlag, Berlin. Pp 1-321.

Schreiber, U., Bilger, W. and Neubar, C. (1995). Chlorophyll fluorescence as a non-intrusive indicator for a rapid assessment of vivo photosynthesis. Pp.40-70. In: *Ecophysiology of photosynthesis, ecological studies* (Schulze, E.D. and CaldwellM.M., eds.). Vol. 100, springer, Berlin.

Srinivasan, A., Takeda, H. and Senboku, T. (1996). Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques. *Euphytica* **88**: 35-45.

Stanca, A.M., Crosatti, C., Grossi, M., Lacerenza, N.G., Rizza, F. and Cattivelli, L. (1996). Molecular adaptation of barley to cold and drought conditions. *Euphytica* **92**: 215-219.

Vavilov, N.I. (1951). *The origin, variation, immunity and breeding of cultivated plants*. The Ronald Press Company, NewYork. Pp. 1-364.

Wanner, L.A. and Junttila, O. (1999). Cold-induced freezing tolerance in Arabidopsis. *Plant Physiol*. **120**: 391-399.

Weltzien, H.C. and Strivastava, J.P. (1981). Stress factors and barley productivity and their implications in breeding straegies. Pp. 351- 361. In: *Barley Genetics IV*. Proceedings of the Fourth International Barley Genetics Symposium (Whitehouse, R.N.H.). Edinburgh University press, Edinburgh.

Yitbarek Semeane, Bekele Hundie, Getaneh Woleab and Dereje Tadesse (1996). Disease surveys and loss assessment studies on barley. Pp. 105-115. In: *Barley research in Ethiopia*: past work and future prospects (Hailu Gebre and Joopvan Leur eds.). Proceedings of the first barley research review workshop, 16-19 October 1993, Addis Ababa. IAR/ICARDA. Addis Ababa, Ethiopia.

Zemede Asfaw (1988). Variation in the morphology of the spike within Ethiopian barley, *Hordeum vulgare* L (Poaceae). *Acta Agric. Scand.* **38**: 277-288.

Zemede Asfaw (1990). An ethnobotanical study of barley in the central highlands of Ethiopia. *Biol. Zent. bl.* **109**: 51-62.

Zemede Asfaw (2000). The barleys of Ethiopia. In: *Genes in the field*: on farm conservation of crop diversity (Brush, S. B. ed.). Lewis Publishers of CRC press LLC, USA. Pp. 77-108.

Zhang, M.I.N. and Willison, J.H.M. (1987). An improved conductivity method for the measurement of frost hardiness. *Can. J. Bot.* **65**: 710-715.

Zohary, N. (1969). Is *Hordeum agriocrithon* the ancestor of six-rwed cultivated barley? *Evolution* **13**: 279 - 280.

9 APENPENDICES

9.1 Appendix 1 Accessions provided from IBCR for experimental work.

Experimental number	Accession number	Local variety name	Area of collection			Altitude (m)
Acc1	3268	Garboo	Bale	Wabe	Ginir	1630
Acc2	3267	Chenka	Bale	Wabe	Ginir	1650
Acc3	3235	*Belga*-Gebs	Gonder	Gonder	Gonder-zuria	2350
Acc4	1696	Senef-kollo	Arsi	Ticho	Tena	2400
Acc5	3260	Kokufa	Bale	Mendeyo	Agarfa	2430
Acc6	3231	Menso	Gojam	Agewmidir	Banja	2460
Acc7	3232	Wenteka	Gojam	Agewmidir	Banja	2460
Acc13	64091	-	Arsi	Kofele	-	2800
Acc8	1759	Balemi	Shewa	Jibat and Mecha	Dondi	2870
Acc9	4839	-	Welo	Were-Himono	Legambo	3500
Acc10	235079	Gebs	Welo	Were-Himono	Legambo	3500
Acc11	4941	Tikur-Gebs	Welo	Were-Illu	-	3550
Acc12	235096	Gebs	Shewa	Menz and Gishe	-	3600

9.2 Appendix 2 The guide questions used during ethnobotanical data collection

This questionnaire was prepared to gather indigenous knowledge of the farming communities of the North Ethiopia in cultivation and utilization of barley as part of a research conducted for the fulfillment of the master's thesis on the problem entitled FROST RESISTANCE IN INDIGENOUS BARLEY LANDRACES CULTIVATED AT DIFFERENT ALTITUDINAL RANGES. November 1999.

Status of interviewee

1. Sex 1. Male ___ 2. Female ____

2. Age 1. 1-18 ____ 2. 19-30 ____ 3. 31-45 ____ 4. 46 and above ____

3. Marital status 1.Single _____ 2. Married ____ 3. Divorced ___ 4. Widow (er) ____

4. Occupation 1. Farmer ____ 2. Merchant ___ 3. Daily laborer ___ 4. Other (specify)________

5. Religion 1. Christian ___ 2. Moslem _____ 3. Other (specify) __________

6. Ethnicity 1. Amhara ____ 2. Tigai _____ 3. Other (specify) ________

7. Family size 1. 1-2 ___ 2. 3-5. ____ 3. 6 and above ________

8. Is your locality: 1. *Kolla* ____ 2. *Weinadega* __ 2. *Dega* ____ 3. *Wurch* ____

9. Kindly, Rate and list the first six crops based on production.

____

10.What proportion of your field is covered with barley? 1. Less than half _____

2. About half _____ 3. Greater than half ______ 4. Other (specify) ____

11. What purposes do you use barley for? 1. Food________.

2. Means of income ___ 3. Feed for animals_____ 4. Beverages_______

5. For construction _____ 6. Fuel source _____ 7. Others (specify) _________

2. Means of income ___ 3. Feed for animals_____ 4. Beverages_______

5. For construction _____ 6. Fuel source _____ 7. Others (specify) _________

12. Would you please enumerate the foodstuffs (food types) that are prepared from barley.

1.Bread (*ambashaa, kita*, ..)______ 2. Loaf (*enjera*) _____ 3. Porridge ______ 4. *besso* ___

5.Roasted grain (*kolo*)______ 6. Other (specify) ______

13. How is each prepared? Which landraces are most preferred for which food type? Why?

Local name	Meaning	Food types prepared	Method of preparation

14. Types of beverages prepared from barley and their method of preparation?

Beverage type	Method of preparation	Barley landraces used	Meaning

15.Which food types prepared from barley are usually served for the social group?

Social groups — Barley food types usually served

Children

Adolescents

Male-elderly

Female-elderly

Mothers

16. Are barley foods used in ceremonies? 1. Yes __ 2. No ____

17. If your answer in question no. 16 is yes, would you please enumerate the food types commonly served in different ceremonies:

<u>Ceremonies</u> <u>Food types</u>

Religious

Cultural

18. Which crop can substitute barley? 1. No crop can substitute barley______ 2. Wheat ________

3. Maize_______ 4. Teff _______ 5. Others (specify) __________

19. Is there any tale, songs, sayings, believes and incantations concerning barley in your locality?

____

____

20. Which barley landraces are cultivated in your locality?

Vernacular name	Meaning of the vernacular name	Extensively cultivated landraces

21. Is barley production and/ or cultivation declining? 1. Yes______ 2. No ________

23. If the answer (s) for question no. 22 are more than one, would you please enumerate in order of decreasing severity

____

____

24. Are there landraces that are known tolerant to environmental stresses? Enumerate them, please.

Stress	resistant landraces
Frost	
Poor soil fertility	
Water deficit	
Others	

25. Is selection of landraces for frost common practice in your locality?

1.Yes______ 2. No _______

26. If your answer in question no. 25 is yes, how do you identify the frost resistant landraces?

____

27. Who selects seeds for cultivation? 1. Youngsters _______ 2. Female elderly _____

3. Male elderly ____ 4. Other (specify)______

28. Sources of seed to be sown is (are) from: 1. Stored in self-barns ___ 2. Neighborhood ____

3. Market ____ 4. Other (specify) ______

29. In which season do you cultivate barley in your locality? 1. Long rainy season _____

2. Short rainy season _______ 3. Both seasons ______

36. If your answer in question no. 35 is yes, which landscapes are used for short or long rainy seasons? Why?

____

____

37.Have you ever brought seeds for cultivation from areas, which are either extremely higher up or lower than your area? If yes, what differences do you see? If not, why not?

____

____

____

38. Is frost one of the major constraints of barley production? If yes, do you have any mechanism that helps you to minimize decline in production as a result of frost stress?

____

____

39. How can we improve barley production?

____

____