



American Journal of  
**Plant Physiology**

ISSN 1557-4539



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## **Effect of Chilling Stress on the Chlorophyll Fluorescence, Peroxidase Activity and Other Physiological Activities in *Ipomoea batatas* L. Genotypes**

Shahidul Islam, Ehiorobo Izekor and J.O. Garner

Department of Agriculture, Mail Slot 4913, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601, USA

*Corresponding Author: Shahidul Islam, Department of Agriculture, Mail Slot 4913, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601, USA*

### **ABSTRACT**

The growth and adaptation of tropical and subtropical herbaceous plants including sweet potatoes (*Ipomoea batatas* L.) are restricted by deleterious effects of low temperature stress or chilling injury. Chilling injury is the morphological and physiological damage sustained by plant tissue exposed to freezing temperatures. The aim of this investigation study was to evaluate the physiological functions of sweet potato genotypes to possibly explain tolerance to chilling injury. An experiment was conducted using four chilling tolerant sweet potato genotypes such as 105MS1, 108MS2, 180MS3, 183MS4 which were selected from seventy nine lines from three major variety crosses for tolerant to chilling injury. The effects of chilling exposure on chlorophyll fluorescence, peroxidase enzyme activity and other physiological parameters, such as specific leaf dry and fresh weight, percent leaf dry weight, percent leaf water content and stomata density were investigated. Following chilling treatment, qualitative visual ratings of the tolerant genotypes were different but the lowest score of the four on the scale of five was considered chilling tolerant. Chilling exposure decreased transpiration rate and stomata conductance but increased Electrolytic Leakage (EL) and Peroxidase Enzyme Activity (PEA). Genotypes differences were found in EL, PEA, chlorophyll fluorescence yield and other physiological functions following chilling exposure. Difference were also found among the genotypes in percent leaf dry weight and percent leaf water content which indicated the degree of water loss among the genotypes. The result suggest that breeding and selecting for chilling tolerance could enhance chilling tolerance in sweet potatoes.

**Key words:** *Ipomoea batatas*, chilling stress, chlorophyll fluorescence, peroxidase enzyme, leaf physiology

### **INTRODUCTION**

Sweet potato (*Ipomoea batatas* L. Lam.) is an important food crop which is enriched in health beneficial components (Ibaraki and Murakami, 2007; Islam *et al.*, 2009a). Usually sweetpotato is growing well under the warm climate, low temperature caused chilling damaged. Chilling injury is the physiological damage sustained by plant tissues exposed to nonfreezing temperature of approximately 0-15°C. A number of mechanisms have been proposed based on the physiological and biochemical functions associated with chilling injury (Al-Shoaibi, 2008; Saleh, 2007; Khorshidi and Nojavan, 2006; Anjum and Khatoon, 2003; Ali *et al.*, 2000). The severity and length of time

required to cause an irreversible dysfunction are generally determined by the temperature extreme, duration of exposure to cold conditions, plant species and morphological and physiological conditions of the plant material at time of exposure (Islam *et al.*, 2009b). Chilling injury disrupts metabolic and physiological processes of higher plants, so it is unlikely that a single basic cause could explain injury. These includes an increased in the concentration of cytosolic calcium; a marked decreased in protoplasmic streaming, an alteration in the cytoskeleton, a conformational change in some enzymes and temperature induce change in the molecular ordering of membrane lipids (Kim *et al.*, 2007). The plasma membrane is regarded as the sensitive site of injury during low temperature stress in herbaceous plants (Zhang and Tian, 2009). Limited component begins to freeze at chilling temperature, the opposite occurred in chilling-resistance species (Sthapit and Witcombe, 1998).

Chlorophyll fluorescence is a non-destructive assay used to estimate the intrinsic capacity of photosynthesis in green plants. Fluorescence emitted from photosystem II (PSII) have been used as an intrinsic cellular probe to study cellular injuries caused by a wide range of environmental stresses (Panda *et al.*, 2006; Aroca *et al.*, 2001). When a leaf is placed to the dark for 30 min or more and the light is switched on, the yield of chlorophyll fluorescence changes over the first 3 min until steady-state photosynthesis occurs. During the first few seconds of illumination, there is an almost instantaneous increase in Fluorescence term ( $F_0$ ) from the baseline. Further rise in fluorescence is termed variable Fluorescence ( $F_v$ ) which reaches maximum value ( $F_m$ ) and slowly decays to a lower steady state. Various parameters of chlorophyll fluorescence including relative quantum yield from the PSII reaction centers (Panda *et al.*, 2006), chlorophyll fluorescence ratio ( $F_0:F_m$ ), prompt and delayed fluorescence at chilling temperature (Ibaraki and Murakami, 2007) have been used as a diagnostic probe to select genotype response to chilling stress. The yield of chlorophyll fluorescence is greatest when the yield of photochemistry is at a minimum; this happens when the primary electrons acceptors of PSI are opened or fully reduced and is unable to extract energy from the reactive center of PSI. On the other hand, fluorescence yield is at minimum when the electrons acceptors are oxidized and less active to extract energy from reaction centers. Inhibition of photosynthesis electron transfer on the photo reducing side of PSI blocking reoxidation of the primary electron acceptors will increase the fluorescence and subsequently result in quenching of the induced chlorophyll fluorescence (Ibaraki and Murakami, 2007; Panda *et al.*, 2006). Ibaraki and Murakami, 2007) using chlorophyll fluorescence measured the decrease in induced  $F_R$  of three maize populations at °C and found that variable rate of  $F_R$  occurred linearly with time. FI progenies of *Solanum lycopersoides* which had relatively low  $F_0:F_m$  ration were observed to be tolerant to chilling temperature stress as further evidenced by low symptoms of chilling related damages (Sthapit and Witcombe, 1998). Woods *et al.* (1991) working with selecting chilling tolerant sweet potato genotypes using chlorophyll fluorescence assay observed 85% loss of relative chlorophyll fluorescence in chilling susceptible genotype compared with 63% loss in the chilling resistant genotype following a 5°C chilling treatment for 24 h. The results showed that the chlorophyll fluorescence transient of chilling sensitive plants was substantially higher compared with those of chilling resistant plants.

Chemical analyses of peroxidase isoenzymes in acclimated chilled plants suggest possible association with cell wall lignification. Bassal and El-Hamahmy (2011), Cao *et al.* (2011) and Lee and Lee (2000) asserted that location of peroxidase enzymes in cell wall of acclimated seedling is an indicative that antioxidants likely improved and maintained the mechanical strength of chilling sensitive mesocotyl. The lower level of antioxidants in chilling sensitive plants at early stage of

chilling treatment suggests the elevated toxicity of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>. Reduction in catalase was catalase was observed under low light intensity as compared to high light intensity (39% under high light intensity vs 15% for low light intensity). Hodgson and Raison (1991) reported that carbohydrates were less utilized in comparison with photosynthetic rate by chilling sensitive plants compared with tolerant plant. Enzymes activity increased in sensitive plant partly due to productions of more chilling tolerant isoenzymes. Hetherington *et al.* (1989) showed that high light intensity hasten symptoms of chilling injury. Under chilling temperature of 4°C and high light intensity (800 µmol m<sup>-2</sup>sec<sup>-1</sup>), approximately 10-15% of the leaf area of mug bean (*Vigna radiate*) were necrotic, whereas under low light intensity (155 µmol m<sup>-2</sup>sec<sup>-1</sup>) no symptoms of chilling damages were apparent. The objectives of this study were: to evaluate chlorophyll fluorescence, peroxidase enzyme activity and other physiological functions among the selected genotypes of sweetpotatose to possibly explain tolerance to chilling injury.

## MATERIALS AND METHODS

**Plant materials and cultural methods:** Plant selection to determine genotypes tolerant to chilling injury from lines of a genetically diverse population was commenced in the greenhouse. Four genotypes namely 105MS1, 108 MS2, 180MS3, 183MS4 were selected from seventy nine lines from three major variety crosses for tolerant to chilling injury (Islam *et al.*, 2009c). The experiment was conducted during 1998 to 2002 at the Mississippi State University, USA. Stem cuttings from each line, approximately 12 cm, were placed in 3.8 L plastic pots containing peat-perlit vermiculite medium (2:1:1 by volume). The medium was amended with 4.56 kg dolomitic lime, 1.82 kg ON-9P-OK, 1.40 kg calcium nitrate and 0.17 kg fritted trace element per cubic meter. Following planting, the plants were kept in a glasshouse under intermittent mist to initiation roots. After five days, plants were moved to another bench in the same glasshouse where they were manually watered once a day for five days. Ten day old plants of each genotype were moved from the glasshouse to walk-in temperature controlled growth chamber maintained at 5°C and a control room 25°C with 85% relative humidity, 10 h photoperiod for duration of 72 h chilling treatment and a fluorescence light intensity of (12 µE M<sup>-2</sup> ses<sup>-1</sup>) suspended over the canopy.

**Visual rating:** Visual rating of the chilled plants from each genotypes were recorded after three and six days respectively according to the degree of chilling damage sustained and the following guideline was used, 1, 75% leaf necrosis, 2, 50% leaf necrosis, 3, wilting leaf necrosis, 4, wilting and 5, no wilting symptoms. Average score of the four replications on each line represented the capacity of chilling tolerance of that line. A higher score indicated chilling tolerance of that line while lower score showed susceptibility.

**Electrolyte leakage of chilling tolerant sweet potato leaf:** Leaf effusates conductivity test was performed on previously selected chilling tolerant genotypes (105MS1, 108 MS2, 180MS3, 183MS4) with a YSI conductivity Meter model 35 (YSI Scientific Instrument Company, Yellow Springs, OH.). The experimental design was Completely Randomized Design (CRD) with genotype as the main plot and temperature as subplot. Two temperature regimes, 5 and 25°C, respectively in two different rooms and the plants were completely randomized within each temperature controlled rooms. Stem cuttings and root initiation was as previously described. Following 24 h of chilling treatment, conductivity test on the leaf was done as follows. Twelve one-centimeter leaf discs were excised with a metal cork borer from the interveinal area of leaf lamella. Diced leaves

were then placed in 16×100 mm glass Pyrex culture tubes containing 10 mL of deionized water. The culture tubes were placed on a Rito-torque rotator (Cole-Palmer Instrument Company, Chicago, IL, USA) for 30 min. An initial conductivity of the solutions was obtained following the 30 min incubation period. Total electrolyte leakage was obtained by heating the culture tubes to boiling for five minutes and taking the final reading after cooling to room temperature. Final leakage was expressed as percentage leakage. The formula used was the percent leakage was expressed as (Conductivity before boiling/conductivity after boiling)×100. Data was analyzed using the SAS GLM technique and means separated by fisher protected LSD.

**Chlorophyll fluorescence:** Chlorophyll fluorescence induction transients were measured using a portable fluometer (Model SF10, Richard Branker Research LTD, Ottawa, Canada) and the fluorescence induction curves displayed on a strip chart recorder. Stem cutting from each line, approximately 12 cm, were placed in 3.8 L plastic pots contains peat-perlit vermiculite medium (2:1:1 volume). The medium was amended with 4.56 kg dolomite lime, 1.82 kg ON-9P-OK, 1.40 kg calcium nitrate and 0.17 kg fritted trace elements per cubic meter. Following planting, the plants were moved to another bench in the same greenhouse where they were manually 3watered once a day for five days. Ten day old plants of each genotype were moved from the greenhouse to walk-in temperature controlled growth chamber maintained at 5°C and a control room 25°C with 85% Relative Humidity (RH), 10 h photoperiod for duration of 72 h chilling treatment and a fluorescence light intensity of 12  $\mu\text{E m}^{-2}\text{sec}^{-1}$  suspended over the canopy. Plants were removed from the cold room after 24 h of exposure and dark adapted for 60 min at room temperature (25°C). White tags with string were tied to the most expanded proximal leaf blade from the apex to avoid variations due to leaf arrangement (Meir *et al.*, 1997). Red light from the photodiode was applied for 10 sec with irradiance of 13  $\mu\text{mol M}^{-2} \text{sec}^{-1}$ . The experimental design was a split plot with temperature (5 and 25°C) and genotype as the main plot. The data arranged in a Completely Randomized Design (CRD) with three replications. The results were presented as follows:  $F_v = F_m - F_o$ ,  $F_v/F_m$ , where  $F_v$  = Variable fluorescence rise,  $F_o$  = The initial minimal fluorescence level,  $F_m$  = Maximum rise fluorescence. The ratio,  $F_v/F_m$ , according to Ibaraki and Murakami (2007), the ratio  $F_v/F_m$  is the quantum yield of photosystem II primary photochemistry. Efficiency of photosystem II is determined by the rate of non-radioactive energy dissipation and the donor side activity of electrons transport chain (Schapendonk *et al.*, 1990).

**Peroxidase enzyme activity:** The activity of peroxidase as crude soluble enzymes and cell wall bound enzymes was determined from 10 days sweet potato leaves. The experimental design was a split-plot with genotypes as the main plots and temperature as the subplots. Data was analyzed in a Completely Randomized Design (CRD) with three replications. The extracts for enzyme analysis were prepared by grinding 3 g fresh weight of deveined leaf tissue in 9 mL cold citric phosphate buffer (pH 4.5), using a pre-chilled mortar and pestle. The homogenate was transferred to plastic centrifuge tubes and centrifuged in a Sorvall RC-5B automatic super speed refrigerated centrifuge (Sorvall Instrument, Du point Co., Wilmington, DL) at 10,000 g for 10 min. The supernatant was saved as the soluble enzymes fraction. The remaining pellets were washed with 9 mL deionized water and recentrifuged at 10,000 g, for 10 min. The supernatant was discarded and the pellets resuspended in 9 mL of 0.2 M calcium chloride ( $\text{CaCl}_2$ ) solution and centrifuged at 10,000 g, for 10 min. The supernatant was saved in 7 mL borosilicate glass vial and analyzed as crude cell wall bound enzymes. Peroxidase, activity was measured according to Venkatarayappa *et al.* (1984) and Islam *et al.* (2009b).

**Statistical analysis:** A randomized complete block design with five replications was used. Data for the different parameters were analyzed by analysis of variance (ANOVA) using the General Linear Models procedures of SAS version 8.1. Mean separations were done using Fisher's protected Least Significant Difference (LSD) tests.

**RESULTS AND DISCUSSION**

**Electrolyte leakage and visual rating of chilling tolerant sweet potato leaf:** There were no genotype temperature interactions for Transpiration Rate (TR), Diffusive Resistance (DR) and Electrolyte Leakage (EL) when the genotypes were exposed to chilling treatment (Table 1). Exposure to 5°C chilling for 24 h decreased TR and increased DR and EL of all genotypes tested. This indicated that the response of these genotypes to chilling were not different. Therefore, no difference in their chilling tolerance mechanism could be attributed to these factors as a response to temperature. Genotypic differences in EL were found (Fig. 1). Genotypes 108MS2 exhibited a higher percentage of EL compared to genotypes 105MS1 and 183MS4. No other difference was observed among the genotypes for EL. The higher EL for 108MS2 suggested that the cell membrane of these genotypes may be more susceptible to chilling injury, although all lines were considered chilling tolerant. Data in Table 2 gives the tolerance rating of the four genotypes selected for further study of their tolerance characteristics. All four genotypes selected received a rating of at least 4, following 24 h exposure to 5°C chilling at 85% RH. Average score of each genotype represented the capacity of chilling tolerance of that genotype. A high score indicated chilling tolerance of that line while lower score showed susceptibility.

Table 1: Effect of temperature on transpiration rate (TR), diffusive resistance (DR) and leaf electrolytic (EL) activity of chilling tolerant sweetpotato genotypes following chilling exposure

Temperature	Transpiration rate ( $\mu\text{g cm}^{-2} \text{sec}^{-1}$ )	Diffusive resistance ( $\text{cm sec}^{-1}$ )	Electrolyte leakage (%)
5°C	3.30b <sup>z</sup>	5.69a <sup>z</sup>	16.04a <sup>z</sup>
25°C	8.35a	1.28b	10.54b
LSD (<0.05) <sup>y</sup>	4.39	2.65	0.10

z = Means in column not followed by the same letter are different at the (0.05) level. y = LSD (0.05) compare the means between temperature treatment

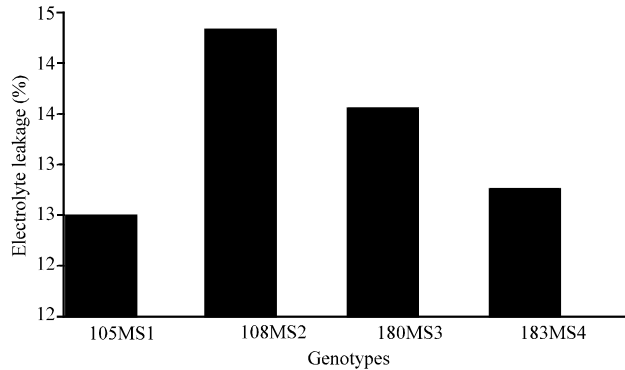


Fig. 1: Effect of genotypes on leaf electrolyte leakage (%) of chilling tolerant sweetpotato genotype following chilled exposure

Table 2: Effect of temperature and genotypes on qualitative plant visual rating of chilling tolerant sweetpotato genotypes following 24 h of 25 and 5°C chilling at 85% RH

Genotypes	Plant survival (scale of 1-5) <sup>z</sup>	
	Chilled	Not-chilled
105MS1	4.5a <sup>y</sup>	5.0 <sup>z</sup>
108MS2	4.0b	5.0
180MS3	4.8a	5.0
183MS4	4.0b	5.0
LSD (0.05)	0.54	ns

z = Qualitative plant visual rating as expressed; 1 = 75% leaf necrosis; 2 = 50% leaf necrosis; 3 = wilting leaf necrosis; 4 = wilting, 5 = no wilting symptoms. Y = Means in column not followed by the same letter are differ at 0.05 level

Table 3: Effect of genotypes and temperature on specific leaf weight and specific dry leaf weight of chilling tolerant genotypes of sweetpotato following 24 h of 5°C chilling exposure at 85% relative humidity

Genotypes	Specific leaf fresh weight (mg cm <sup>2</sup> )		Specific leaf dry weight (mg cm <sup>2</sup> )	
	Chilled	Not chilled	Chilled	Not chilled
105MS1	21.30a <sup>z</sup>	21.30a <sup>z</sup>	4.88b <sup>z</sup>	4.63a <sup>z</sup>
108MS2	11.91b	18.79b	5.58ab	3.51b
180MS3	19.42a	22.05a	4.88b	4.00ab
183MS4	12.53b	20.60ab	5.95a	3.82b
LSD (p<0.05)	2.39		0.70	

<sup>z</sup> = Means in column not followed by the same letter are significantly different at p<0.05

**Characteristics chilling tolerant sweetpotato leaf:** Specific leaf weight revealed differences among the genotypes (Table 3). The not chilled leaves of genotypes 108MS2 had the lowest specific leaf fresh weight (SLFW) (18.8 mg cm<sup>-2</sup>) of the genotypes tested. Genotype 105MS1, 180MS3 and 183MS4 are not statistically different in their SLFW. SLFW of the chilled leaves did follow the same pattern to a considerable degree. Genotype 108MS2 and 183MS4 had the lowest SLFW when chilled and were both different from the remaining two genotypes. Specific Leaf Dry Weight (SLDW) was showed different among the genotypes (Table 3). When the plants were not chilled, genotype 105MS1 had higher SLDW, than genotypes 108MS2 and 183MS4. When chilled, 183MS4 was shown to have a higher SLDW and was different from genotype 180MS3 and 105MS1. Specific leaf weight (SLW; weight and dry) gives an indication of leaf thickness and/or mass. The thicker leaf could be advantageous under stress conditions such as a chilling environment. Barden (1974) hypothesized that SLW might be a useful index for photosynthetic potential. The not chilled leaves of 105MS1 had the highest specific leaf weight on both fresh and dry weight basis except when compared to 183MS4. Genotypes 105MS1 and 183MS4 had the highest SLW when chilled. The drastic reduction in SLFW and SLDW may have indicated that the vulnerability of genotypes 108MS2 and 183MS4 to chilling injury may be due to water loss than other genotypes tested. 108MS2 and 183MS4 to chilling injury may be due to water loss than other genotypes tested in this study.

The effect of chilling on percent leaf dry weight and leaf water content was different among the genotypes (Table 4). When chilled, percent dry leaf weight of genotype 108MS2 was higher (35.2%) than that observed for the tree other genotypes tested (23-27%). Under the not chilled treatment, genotype 108MS2 and 183MS4 had higher leaf dry weight (18.07-18.66%) than

Table 4: Effect of temperature and genotypes on percent specific leaf dry weight and leaf water content of chilling tolerant genotypes of sweetpotato following 24 h of 5°C chilling exposure at 85% relative humidity

Genotypes	Leaf dry weight (%) <sup>w</sup>		Leaf water content (%)	
	Chilled	Not chilled	Chilled	Not chilled
105MS1	23.11b <sup>z</sup>	16.31b	76.88a	83.28 <sup>y</sup>
108MS2	35.91a	18.67a	64.09b	81.33
180MS3	23.66b	16.07b	78.78a	84.82
183MS4	26.25b	18.66a	73.64a	81.29
LSD (p<0.05)	5.45		4.29	

<sup>z</sup> = Means in column not followed by the same letter are significantly different at p<0.05. <sup>y</sup> = No significant difference among the genotypes. <sup>w</sup> = Dry leaf weight (mg) based on 5 cm<sup>-2</sup> of leaf area

Table 5: Effect of genotype on stomata density of chilling tolerant sweetpotatoes

Genotypes	Stomata density (#/mm <sup>-2</sup> )	
	Abaxial leaf surface	Adaxial leaf surface
105MS1	177b <sup>z</sup>	74b
108MS2	167b	72b
180MS3	225a	102a
183MS4	225a	105a
LSD (p<0.05)	33.72	58.21

<sup>z</sup> = Means in column not followed by the same letter are significantly different at p<0.05

genotype 105MS1 and 180MS3 with (16.07-16.31%). Leaf dry weight of genotype 108MS2 was higher at both temperature treatments. There was no statistical difference among the not chilled genotypes with respect to leaf water content. When chilled, leaf water content of genotype 108MS2 was lower (64.09%) than other genotypes (74-79%). This data suggest that the higher leaf dry weight of genotype 108MS2 was due to greater water loss from chilling. Oren *et al.* (1986) concluded that the combined structural index (leaf biomass×relative specific leaf weight) could, when calibrated, predict the total annual carbon uptake by different parts of the crown. If direct measurements of photosynthesis are not available, the combined structural index may still serve as a comparative estimator of annual carbon up-take. Leaf stomata density of the four chilling tolerant sweetpotatoes showed differences among genotypes (Table 5). Genotype 108MS2 and 105MS1 were not different from each other but had lower stomata densities on both adaxial and abaxial leaf surfaces. Yu *et al.* (2008), Jones (1998), Beerling and Chaloner (1993), Hunt *et al.* (1991) and Curtis *et al.* (1990) have showed that environmental stress and genotypes significantly influenced stomata density. These are in support of our results. It would seem that lower stomata number may be advantageous to plants under chilling stress. We have concluded that the lower stomata number for genotype 105MS1 and 108MS2 could be related to their tolerant level.

**Chlorophyll fluorescence:** An interaction for genotypes and chilling treatments was observed for chlorophyll fluorescence ration (Fv/Fm) (Fig. 2). When the genotypes were not chilled, genotype 183MS4 had a lower Fv/Fm ration than the others studied. The chilling treatment reduced Fv/Fm ration of genotypes 105MS1, 108MS2 and 183MS4 compared to their not chilled control. The Fv/Fm ration of 180MS3 was not affected by the chilling treatment and was higher than the Fv/Fm ration obtained for the three other genotypes when chilled. There was also a difference between the



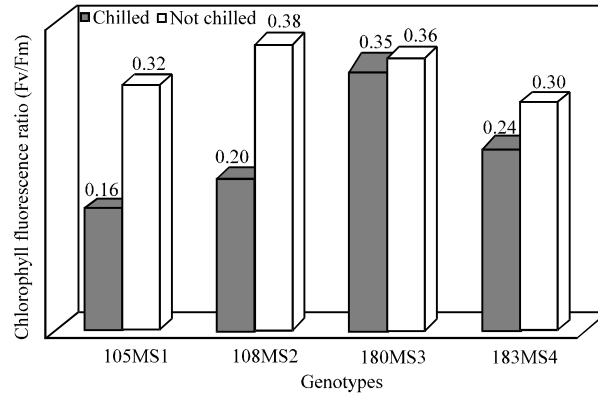


Fig. 2: Effect of temperature and genotypes on chlorophyll fluorescence ratio (Fv/Fm value) of chilling tolerant sweetpotato genotype following 24 h of 5°C chilling and 25°C not chilled exposure

Fv/Fm ratio recorded for 105MS1 and 183MS4 when chilled. Woods *et al.* (1991) showed that genotype MS26-1 had a higher Fv/Fm ratio when chilled and added that this was an indication of chilling tolerance. The data in this experiment indicated that these genotypes responded differently to chilling conditions and that 180MS3 was more tolerant to chilling with respect to chlorophyll fluorescence ratio. There, the photosynthetic process of genotype 180MS3 may be more stable to chilling environment. Several researchers reported that chilling injury depends on the severity and duration of exposure to cold conditions, plant species and morphological and physiological conditions of the plant materials at time of exposure (Panda *et al.*, 2006; Allen and Ort, 2001). These are in agreement with our present study. Plant size, shape and ability to survive during imposed stresses are largely determined by the number, morphology and arrangement of the plant cells (Osborne *et al.*, 2008; Nakayama *et al.*, 2007). The basic need to increase chilling resistant genotypes from a diverse population of gene pool, has led individuals to pursue different systems of selecting and determine genotypes tolerant to chilling injury. Therefore, it was concluded that selecting parents for chilling tolerance would increase the possibility of developing a chilling tolerant cultivar with desired quality criteria(s) of sweetpotatoes.

**Peroxidase enzyme activity:** The genotypes 180MS3 and 183MS4 had higher peroxidase enzyme activity compared to the genotypes 105MS1 and 108MS2 (Fig. 3). The chilling temperature showed significantly higher peroxidase activity as compared to higher temperature (25°C) studied. Similar results obtained by El-hilali *et al.* (2003). They reported that the peroxidase activity increases continuously at 4°C over the period of storage in fortune mandarin fruits. If the peroxidase enzyme activity is a factor in chilling tolerance as suggested by Woods *et al.* (1991), a difference in the tolerance mechanism is indicated. Chilling sensitive and chilling tolerant plants responses to low temperature stress by producing differential level of toxic oxygen compounds and these compounds were suggested to be the products metabolisms (Li *et al.*, 2011; Zahra *et al.*, 2009; Lin *et al.*, 2006; Hodgson and Raison, 1991). Superoxide Dismutase (SOD) Catalyzes (CAT) and various peroxidases and ascorbate peroxidases, glutathione reductases constitute the cellular defense mechanism against oxidative stress (Bowler and Chua, 1994). The results revealed that genotypes 180MS3 and 183MS4 may have a better protective mechanism with respect to

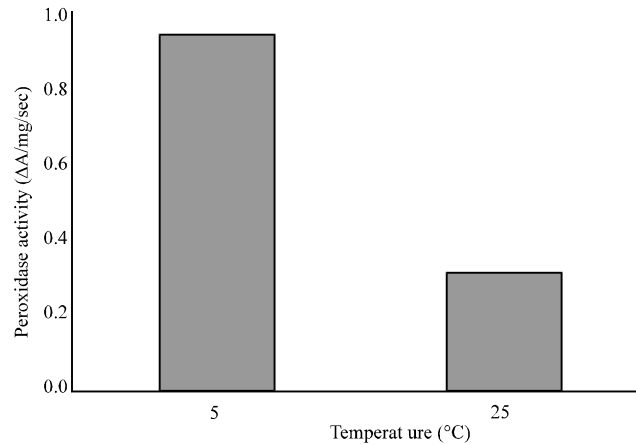


Fig. 3: Effect of temperature on the peroxidase enzyme activity of chilling tolerant genotypes of sweetpotatoes following chilling exposure

peroxidase enzyme activities. Usually peroxidases are ubiquitous enzymes that have diverse biochemical functions in higher plants and are involved in the response of plants to chilling stress (Li *et al.*, 2011; Safizadeh *et al.*, 2007). Thus, the tolerance of 105MS1 and 108MS2 could be from other physiological mechanisms.

## CONCLUSION

From the aforementioned results and discussion it was concluded that breeding and selecting for chilling tolerance could enhance chilling tolerance in sweetpotatoes. The basis or physiological mechanism for chilling tolerance was not the same for the four sweetpotato genotypes tested; therefore combining traits for tolerance could lead to higher tolerance levels.

## REFERENCES

- Al-Shoaibi, A.A., 2008. Photosynthetic response to the low temperature in elephant grass (*Pennisetum purpureum*) and *Zea mays*. *Int. J. Bot.*, 4: 309-314.
- Ali, A., C. Stushnoff and D.L. Johnson, 2000. Negative association of endogenous sorbitol with cold hardiness in lentil. *Pak. J. Biol. Sci.*, 3: 2026-2029.
- Allen, D.J. and D.R. Ort, 2001. Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Sci.*, 6: 36-42.
- Anjum, Z.I. and A. Khatoon, 2003. Chilling effect on germination and seedling vigour of some cultivated species of *Gossypium*. *Asian J. Plant Sci.*, 2: 297-299.
- Aroca, R., J.J. Irigoyen and M.S. Diaz, 2001. Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. *Plant Sci.*, 161: 719-726.
- Barden, J.A., 1974. Net photosynthesis, dark respiration, specific leaf weight and growth of young apple trees as influenced by light regime. *J. Am. Soc. Hort. Sci.*, 99: 547-551.
- Bassal, M. and M. El-Hamahmy, 2011. Hot water dip and preconditioning treatments to reduce chilling injury and maintain postharvest quality of Navel and Valencia oranges during cold quarantine. *Postharvest Biol. Tech.*, 60: 186-191.

- Beerling, D.J. and W.G. Chaloner, 1993. The impact of atmospheric CO<sub>2</sub> and temperature change on stomatal density: Observations from *Quercus robur* lammad leaves. *Ann. Bot.*, 71: 231-235.
- Bowler, C. and N.H. Chua, 1994. Emerging themes of plant signal transduction. *Plant Cell*, 6: 1529-1541.
- Cao, S., Z. Yang, Y. Cai and Y. Zheng, 2011. Fatty acid composition and antioxidant system in relation to susceptibility of loquat fruit to chilling injury. *Food Chem.*, 127: 1777-1783.
- Curtis, P.F., L.M. Balduman, B.G. Drake and D.F. Whigham, 1990. Elevated atmospheric CO<sub>2</sub> effects on below ground processes in C<sub>3</sub> and C<sub>4</sub> estuarine marsh communities. *Ecology*, 71: 2001-2006.
- El-hilali, F., A. Ait-Oubahou, A. Remah and O. Akhayat, 2003. Chilling injury and peroxidase activity changes in Fortune mandarin fruit during low temperature storage. *Bulg. J. Plant Physiol.*, 29: 44-54.
- Hetherington, S.E., J. He and R.M. Smillie, 1989. Photoinhibition at low temperature in chilling-sensitive and -resistant plants. *Plant Physiol.*, 90: 1609-1615.
- Hodgson, R.A.J. and J.K. Raison, 1991. Superoxide production by thylakoids during chilling and its implication in the susceptibility of plants to chilling-induced photoinhibition. *Planta*, 183: 222-228.
- Hunt, R., D.W. Hand, M.A. Hannah and A.M. Neal, 1991. Response to CO<sub>2</sub> enrichment in 27 herbaceous species. *Funct. Ecol.*, 5: 410-421.
- Ibaraki, Y. and J. Murakami, 2007. Distribution of chlorophyll fluorescence parameter Fv/Fm within individual plants under various stress conditions. *Acta Hortc.*, 761: 255-260.
- Islam, I., A.U. Shaikh and I.M. Shahidul, 2009a. Antioxidative and antimutagenic potentials of phytochemicals from *Ipomoea batatas* (L.) Lam. *Int. J. Cancer Res.*, 5: 83-94.
- Islam, S., E. Izekor and J.O. Garner, 2009b. Development of selection procedures of sweetpotato genotypes according to chilling tolerance. *J. Food Agric. Environ.*, 7: 237-245.
- Islam, S., R.C.R. Carmen and J.O. Garner Jr., 2009c. Carbohydrate compositions and peroxidase activity in ungerminated, cotyledon and embryo tissues of *Vigna unguiculata* L. Walp seed grown under stress temperatures. *Am. J. Plant Physiol.*, 4: 9-17.
- Jones, H.G., 1998. Stomatal control of photosynthesis and transpiration. *J. Exp. Bot.*, 49: 387-398.
- Khorshidi, M. and A.M. Nojavan, 2006. The effects of abscisic acid and CaCl<sub>2</sub> on the activities of antioxidant enzymes under cold stress in maize seedlings in the dark. *Pak. J. Biol. Sci.*, 9: 54-59.
- Kim, Y.H., S.M. Han, J.C. Lee, W.K. Song and J.W. Bang *et al.*, 2007. Differential expression of 10 sweetpotato peroxidases in response to sulfur dioxide, ozone and ultraviolet radiation. *Plant Physiol. Biochem.*, 45: 908-914.
- Lee, D.H. and C.B. Lee, 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: In gel enzyme activity assays. *Plant Sci.*, 159: 75-85.
- Li, Q., B. Yu, Y. Gao, A.H. Dai and J.G. Bai, 2011. Cinnamic acid pretreatment mitigates chilling stress of cucumber leaves through altering antioxidant enzyme activity. *J. Plant Physiol.*, 10.1016/j.jplph.2010.11.025
- Lin, K.H., F.H. Pai, S.Y. Hwang and H.F. Lo, 2006. Pre-treating paclobutrazol enhanced chilling tolerance of sweetpotato. *Plant Growth Regul.*, 49: 249-262.
- Meir, S., R. Ronen, S. Lurie and S.P. Hadas 1997. Assessment of chilling injury during storage: Chlorophyll fluorescence characteristics of chilling susceptible and triazole-induced chilling tolerant basil leaves. *Postharvest Biol. Tech.*, 10: 213-220.

- Nakayama, N., H. Saneoka, R.E.A. Moghaieb, G.S. Premachandra and K. Fujita, 2007. Response of growth, photosynthetic gas exchange, translocation of  $^{18}\text{C}$ -labelled photosynthate and N accumulation in two soybean (*Glycine max* L. Merrill) cultivars to drought stress. *Int. J. Agric. Biol.*, 9: 669-674.
- Oren, R., E.D. Schnlze, R. Matyssek and R. Zimmermann, 1986. Estimating photosynthetic rate and annual carbon gain in conifers from specific leaf weight and leaf biomass. *Oecologia*, 70: 187-193.
- Osborne, C., E.J. Wythe, D.G. Ibrahim, M.E. Gilbert and B.S. Ripley, 2008. Low temperature effects on leaf physiology and survivorship in the  $\text{C}_3$  and  $\text{C}_4$  subspecies of *Alloteropsis semialata*. *J. Exp. Bot.*, 59: 1743-1754.
- Panda, D., P.K. Dash, N.K. Dhal and N.C. Rout, 2006. Chlorophyll fluorescence parameters and chlorophyll content in mangrove species grown in different salinity. *Gen. Applied Plant Physiol.*, 32: 175-180.
- Safizadeh, M.R., M. Rahemi and M. Aminlari, 2007. Effect of postharvest calcium and hot-water dip treatments on catalase, peroxidase and superoxide dismutase in chilled lisbon lemon fruit. *Int. J. Agric. Res.*, 2: 440-449.
- Saleh, A.A.H., 2007. Amelioration of chilling injuries in mung bean (*Vigna radiata* L.) seedlings by paclobutrazol, abscisic acid and hydrogen peroxide. *Am. J. Plant Physiol.*, 2: 318-332.
- Schapendonk, A.H.C.M., O. Dolstra and O. Kooten, 1990. The use of chlorophyll fluorescence as a screening method for cold tolerance in maize. *Photosynth. Res.*, 20: 235-247.
- Sthapit, B.R. and J.R. Witcombe, 1998. Inheritance of tolerance to chilling stress in rice during germination and plumule greening. *Crop Sci.*, 38: 660-665.
- Venkatarayappa, T., R.A. Fletcher and J.E. Thompson, 1984. Retardation and reversal of senescence in bean leaves by benzyladenine and decapitation. *Plant Cell Physiol.*, 25: 407-418.
- Woods, F.M., J.O. Garner, J.L. Silva and C. Phromtong, 1991. Estimation of chilling sensitivity in leaves of sweet potato by chlorophyll fluorescence and electrolyte leakage. *Phyton*, 51: 33-37.
- Yu, H., X. Chen, Y.Y. Hong, Y. Wang and P. Xu *et al.*, 2008. Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. *Plant Cell*, 20: 1134-1151.
- Zahra, P., R. Majid and B. Amin, 2009. Seasonal changes of peroxidase, polyphenol oxidase enzyme activity and phenol content during and after rest in pistachio (*Pistacia vera* L.) flower buds. *World Applied Sci. J.*, 6: 1193-1199.
- Zhang, C. and S. Tian, 2009. Crucial contribution of membrane lipids unsaturation to acquisition of chilling-tolerance in peach fruit stored at  $0^\circ\text{C}$ . *Food Chem.*, 115: 411-415.