

MORPHO-PHYSIOLOGICAL TRAITS ASSOCIATED WITH COLD STRESS TOLERANCE IN TROPICAL MAIZE (*ZEA MAYS* L.)

P.H. Zaidi^{1,*}, M. Yadav², P. Maniselvan², R. Khan², T.V. Shadakshari², R.P. Singh², D. Pal³

¹ CIMMYT-Asia Regional Maize Program, ICRISAT, Patancheru-502 324, India;

² Directorate of Maize Research, Pusa Campus, New Delhi-110012, India;

³ Regional Research Station, C.C.S. Haryana Agriculture University (HAU), Uchani, Karnal, India;

Received July 21, 2010

ABSTRACT - Winter season maize (*Zea mays* L.) has emerged a new crop in many parts of South and South-east Asia, where the crop has to face low temperature regimes (<5°C) for few weeks during vegetative growth stage. The objective of this study was to identify the morphological and physiological traits associated with cold stress tolerance during vegetative growth period, when maximum dry matter is accumulated and floral primordia are formed. A total of 80 cultivars, including hybrids and open pollinated varieties (OPVs) from International Maize and Wheat Improvement Center (CIMMYT) and Indian maize program were evaluated in replicated trials at Indian Agricultural Research Institute (IARI), New Delhi and at Regional Research Station, Haryana Agriculture University, Karnal, India during the winter season, where critical period of vegetative growth and floral primordia developmental stage was exposed to <10°C temperature. Data on various growth and developmental traits and key physiological traits were recorded during the low temperature regime. Except ears per plant and physiological maturity, the cold stress significantly affected all the growth and developmental traits and also physiological traits studied. However, significant genotypic variability was observed for most of the traits studied. Genotypes with relatively high leaf appearance and extension rate, less cold injury symptoms and cell membrane damage showed good level of cold tolerance in terms of reproductive behavior and eventually grain yield under cold stress. These secondary traits could be used in selection index along with days to anthesis, anthesis-silking interval (ASI) and grain yield for selection and improvement of tropical maize for low temperature adaptation.

KEYWORDS: Maize; "*Zea mays*"; Cold stress; Low temperature; Secondary traits.

INTRODUCTION

Maize (*Zea mays* L.) is a *thermophilic* plant

species and highly sensitive to low temperature at all stages of development (MIEDEMA, 1982; STAEBLER, 2001). Current trend of growing maize in non-traditional areas during winter seasons has increased the likelihood that a maize plant will spend most part of early development under suboptimal temperature conditions. In Indo-Gangetic plains (IGP) region of South Asia the winter season maize crop invariably face severe low temperature regime during winter months. The average minimum temperature may fall below 5°C or even less, especially in the North-West Plains of IGP. An example of extreme low temperature stress was experienced by maize crop in northern India during winter season of 2002-03 and again northern India and Nepal during 2009-10, which caused severe yield losses in large maize growing areas of IGP. Adaptation of maize to winter season in IGP and similar environment in tropics requires genetic improvement for cold tolerance, which implies vigorous seedling growth without suffering with cold injuries under low temperature conditions.

Being cold sensitive plant maize is prone to physiological damages during non-freezing suboptimal temperatures. Leaves of maize, which develop under low temperature conditions, are characterized by a lower photosynthetic capacity, lower quantum efficiency of CO₂-fixation, and lower quantum efficiency of electron transfer at PSII (/PSII) than leaves which develop under more favorable conditions (NIE *et al.*, 1992; LEIPNER *et al.*, 1999). One reason for the lower photosynthetic performance might be the perturbation of chloroplast development, specifically, the limited ability of maize leaves to develop a functional photosynthetic apparatus at low temperature (NIE and BAKER, 1991). MIEDEMA *et al.* (1987) studied the effect of low temperature on seedling growth from germination to 6th leaf stage, and suggested that resistance to chlorosis and rapid leaf ex-

* For correspondence (e-mail: phzaidi@cgiar.org).

pansion at low temperature are considered major selection criteria for the improvement of low-temperature adaptation. YING *et al.* (2000) observed that maize hybrids differ significantly in leaf CER response to cold night temperature during flowering/grain filling period, which directly affects effectiveness of grain filling. However, genetic variation does exist for the severity of the response to low temperatures for several parameters, such as leaf CER, quantum efficiency of PSII, rate of development, and rate of dry matter accumulation (YING *et al.*, 2002). Several studies have been conducted on cold tolerance in temperate and highland maize growing areas, largely focusing on germination and early growth stages. However, limited information is available on cold tolerance in maize grown during winter season in tropical/sub-tropical environment. Keeping in view the current trend of maize cultivation during winter cycle South Asian tropics and other areas with similar ecology crop improvement for low-temperature tolerance has emerged an important issue. In the present study we attempted to identify those morphological and physiological traits that help in discriminating tolerant and susceptible genotypes, their relationship with yield under cold stress, and those traits could be used as selection criteria for further improving cold tolerance in tropical maize.

MATERIAL AND METHODS

Germplasm

A total of 80 cultivars were used in the present study, which includes 46 genotypes from CIMMYT highland maize program including hybrids, open pollinated varieties (OPVs) and synthetics, and 32 tropical/sub-tropical hybrids from regional research station, Haryana Agriculture University (HAU), Uchani, Karnal, India. Two tropical hybrids (Buland and Sheetal) were released from Punjab Agriculture University (PAU), Ludhiana, India as cold tolerant hybrids for cultivation during winter season in North India were used as check entries. CIMMYT hybrids, OPVs and synthetics were derived from genetically broad-based tropical highland populations and pools, which were selected and improved for cold tolerance, including highland early white semi-dent populations 86, 900, 902 and 903, highland late yellow semi-dent population 88, highland early white semi-dent pool 10A and highland early yellow semi-dent pool 13A. Population 86 was constituted involving 55% highland germplasm, 20% tropical/sub-tropical germplasm and 25% US corm belt temperate germplasm. Population 900 was developed using diverse highland germplasm from colder highland areas. Population 902 was constituted by crossing seventeen S3 to advanced lines largely from population 85 with good combining ability (GCA) to CML-246, a CIMMYT highland maize inbred line from population 800. Population 903 was developed using 22 S3 to advanced lines

principally from CIMMYT highland population 800 with good GCA with CML-242, a highland line derived from and OPV 'Batan-8785'. The constitution of population 88 was same as population 86, except that it was a late maturity population. Pool 10A is primarily based on a Mexican highland race called 'Conico', in which about 20% germplasm traces of diverse origins from North and South America, and Asia were introgressed. Similarly pool 13A was developed by introgressing germplasm traces from North America, Europe, Africa, Guatemala, and Columbia in a Mexican highland race 'Chalqueno'. Further details about the CIMMYT tropical highland pools and populations can be found in CIMMYT's report on maize germplasm (CIMMYT, 1998). The lines involved in developing 30 hybrids from HAU, Uchani, Karnal, India were derived from various sources including Indian OPVs NAD 126, BC 175 and BC 182, few lines from CIMMYT tropical pool 28 and lines from elite commercial hybrids, such as Cargil 633, HM-1, CH-11, Cargil 501, CH-9 and HM-6. The lines CM-146 and CM-147 involved in developing hybrid Buland were derived from Suwan-1 and a temperate pedigree population J54 x Mo17, respectively. Another cold tolerant hybrid Sheetal was developed by crossing LM-9 x LM-10, which were derived from CIMMYT tropical pools MS pool 21 and Pool 28 TSR, respectively. Further details about Indian maize inbred lines involved in developing the hybrids used in the present study can be found in the catalog of Indian maize inbred lines (KAUL *et al.*, 2009).

Experimental site, cultural practices and stress treatment

Experiments were conducted during the *Rabi* (winter season) of 2006-07 at the maize research farm, Indian Agricultural Research Institute (IARD), New Delhi, India (28.4°N, 77.1°E, 228.1 masl) and Regional Research Station, C.C.S. Haryana Agriculture University (HAU), Uchani, Karnal, India (29.43°N, 76.58°E, 245.0 masl). Soil of the IARI experimental station is characterized as sandy loam with a pH of 7.8, while HAU, Karnal is clay loam with a pH 7.4. At both the locations planting was done during second week of November. The time of planting date was chosen on the basis of long-term (10 years) climatic data of the two

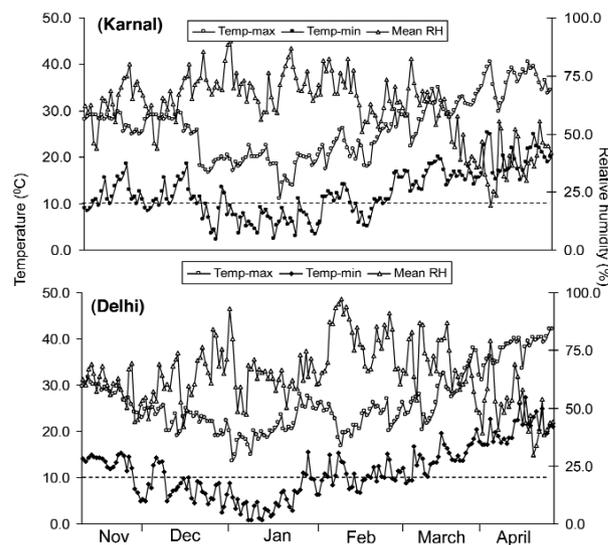


FIGURE 1 - Maximum, minimum temperature (°C) and mean relative humidity (%) during the winter crop season at Regional Research Station, Haryana Agriculture University, Karnal, Haryana and Indian Agricultural Research Institute, New Delhi, India.

locations in order to get vegetative stage (V6-V8 stage, tassel and ear primordia emergence stage) exposed to low temperature regimes (<10°C). Climate data recorded during experimental period showed that at both the locations minimum temperature was less than 10°C for over one month during mid-December to mid-January, and occasionally dropped down below to 2°C (Fig. 1).

All the entries were over-sown and thinned to one plant per hill at V₂ growth stage to give a population density of 66600 plants ha⁻¹. Each entry was planted in one row plot, each 4.0 m long, with 20 cm spacing within and 75 cm between rows. Before planting 60 kg nitrogen (N) ha⁻¹ in the form of urea, 60 kg phosphorous ha⁻¹ as single super phosphate, 40 kg potassium ha⁻¹ as muriate of potash and 10 kg zinc as zinc sulfate were applied as a basal dressing. Second and third doses of N (each 30 kg N ha⁻¹) were side-dressed at knee-high and tassel emergence stages. Pre-emergence application of pendimethalin and atrazine (both at 0.75 kg ha⁻¹ a.i., tank mixed) were used for weed management in the experimental plots. Experiments were kept free from insect, weeds and diseases using recommended post-emergence chemical measures, and managed under optimal agronomic practices.

Observations

In vivo chlorophyll content in the upper-most fully expanded leaf was recorded on five plants in each plot using a Minolta SPAD-502 chlorophyll meter in each plot twice, first during 4th week of December and second during 2nd week of January, and averaged. Plant height, number of fully appeared leaves (the leaves with fully visible collar) and leaf area was recorded at weekly interval during the low temperature regime (<10°C), on five plants from each plot and averaged. During this period, starting from 4th week of December until 3rd week of January, the low temperature remained below 10°C, except very few exceptions at Karnal location (Fig. 1). Leaf length and maximum width of the each fully expanded leaf was recorded on five plants in each plot; leaf area per leaf was calculated using the formula: length x maximum width x 0.75 (Montgomery, 1911). Leaf area of a single leaf was multiplied by total number of leaves to estimate the total area for each plant, and averaged for five plants to calculate the leaf area per plant. Increase in these traits during the one month period (15 December to 15 January) was divided by total number of days and expressed in terms of percent growth rate per day in plant height, leaf appearance and leaf area. Visual cold tolerance score (1-3 scale) was recorded twice, first during 4th week of December and second during 2nd week of January, and averaged. The cold tolerance was scored on the basis of visible cold stress symptoms, i.e. hybrids with least stress symptoms (chlorosis, necrosis/leaf firing, leaf etiolation and stunted growth) were scored as 3 for cold tolerance and vice-versa.

Days from planting to anthesis and silking, indicated when 50% of plants had extruded anther or produced silk, were recorded by daily visual observations during the flowering period. Anthesis-silking interval (ASI) was calculated as the difference between the number of days to 50% silking and 50% anthesis. Days to physiological maturity was noted when the black layer (abscission layer) was first visible at the base of kernels present in middle of the ear. Black layer formation was observed on five plants in each plot by daily visual observations during late grain filling stage. At maturity, ears were harvested, excluding two plants close to alley from both end of the rows; ear number per plot was determined, and ears per plant (EPP) was calculated.

Ears were dried and grain yield was recorded on shelled grain basis and adjusted to 15% moisture level. Per day yield was computed by dividing grain yield with total number of days taken to reach to physiological maturity.

Electrolyte leakage, anthocyanin concentration and total soluble sugar was measured in selected best (9) and worst (17) hybrids in terms of cold tolerance score, along with nine other randomly selected hybrids. All the three traits were measured twice, first during 4th week of December and second during 2nd week of January, and averaged. Cold-induced cell membrane injuries in leaf tissues were quantified by estimating the electrolyte leakage (%) in top most fully expanded leaf. The electrolyte leakage was measured using the standard protocol (<http://www.plantstress.com/methods/index.asp>). Anthocyanin pigments were extracted from the 50mm leaf disc of top-most fully expanded leaves using acidified ethanol (95% ethanol in 1.5 N HCl). Total anthocyanin concentration was measured using spectrophotometer at 546 nm wave-length (BEGGS and WELLMANN, 1985). Preserved leaf samples in ethanol (95%) were used to extract soluble sugar content through repeated boiling and extraction in 80% ethanol (MC CREADY *et al.*, 1950). An aliquot was used for determining the reducing sugars through Nelson's arsenomolybdate method (NELSON, 1944) using improved copper reagent (SOMOGYI, 1952). Clarified extract was hydrolyzed in 0.5N HCl and the solution was used for determination of total sugar similarly as reducing sugar.

Statistical design

Trials were grown with two common check entries using an alpha (0, 1) lattice design (Patterson *et al.*, 1978) with two replications, wherein each pair of entry appeared together in a block only once or not at all. Analysis of variance (ANOVA) was calculated using ALPHA-program (CIMMYT, 1999), considering entries as fixed and replicates, plots and incomplete blocks within replicates as random factors, and lattice-adjusted means were computed for each trial. Data for two locations (IARI, New Delhi and HAU, Karnal) was pooled after testing homogeneity of error variance using Hartley's F_{max} test (OTT, 1988). 'Best' and 'Worst' entries were selected on the basis of their phenotypic score using a selection index (CIMMYT, 1999). Test of significance of differences between selected best and worst hybrids was computed using Student's t-test. Pearson phenotypic correlation coefficients and simple linear regression between grain yield and different morpho-physiological traits under cold stress were computed using MSTATc (MSTATC, 1990). Linear regressions between different traits and grain yield under cold stress were compared by conducting analysis of variance of slope and intercept values (BÄNZIGER *et al.*, 1999).

RESULTS AND DISCUSSION

All the traits, except ears per plant and physiological maturity, were significantly affected by cold stress (Table 1). Cold stress resulted in severe leaf chlorosis, which was evident in terms of highly significant (P<0.01) genotypic variability for leaf chlorophyll content. Reduced chlorophyll content in maize leaves under cold stress has also been reported by others (LEIPNER *et al.*; 1999; LEE *et al.*, 2002). Inhibition of chlorophyll accumulation at low tem-

TABLE 1 - Mean, standard error and variation range of various morpho-physiological traits observed on of maize hybrids under cold stress.

Traits	Mean	SEm	Best	Worst
Chlorophyll (SPAD unit)	23.2	1.754	43.8	19.62
Cold tolerance score (0-3 scale)	1.13	0.206	2.04	0.74
Leaf emergence rate (% day ⁻¹)	1.63	0.255	2.48	0.59
Leaf area growth rate (% day ⁻¹)	1.22	0.313	3.96	1.12
Growth in plant height (% day ⁻¹)	1.61	0.137	3.16	0.67
50% anthesis (days)	119.4	3.016	103.7	123.9
Anthesis-silking interval (days)	4.99	0.707	2.9	7.63
Ears per plant	0.68	0.051	0.87	0.63
Physiological maturity (days)	158.9	6.036	153.5	166.2
Grain yield (t ha ⁻¹)	3.97	0.359	7.16	2.13
Per day yield (kg ha ⁻¹)	24.5	4.033	44.13	13.14
Anthocynin (OD cm ⁻²)	1.11	0.064	2.83	0.46
Total soluble sugar (mg g ⁻¹ fresh weight) ^b	14.05	1.469	26.04	11.08
Electrolyte leakage (%)	19.57	2.017	10.87	28.92

TABLE 2 - Mean squares (MS), percent coefficient of variation (CV) and standard error (SE) of the hybrid trial for chlorophyll content, cold tolerance score, leaf emergence rate, leaf area growth rate, plant height growth rate, per day yield and grain yield of hybrids grown under cold stress.

Source	DF	Chlorophyll	Cold tolerance score	Leaf emergence rate	Leaf area growth rate	Plant height growth rate	Per day yield	Grain yield
Genotype	79	163.29**	2.14**	2.034**	3.104**	3.98*	143.58**	5.59**
Error	79	4.208	0.09	0.02	0.14	0.16	5.39	0.12
CV (%)		18.69	26.35	31.16	21.36	16.98	14.53	19.63
SE		1.45	0.07	0.10	0.12	0.09	1.64	0.24

* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

perature was attributed to the photo-oxidation of the chlorophyll at a faster than it was being synthesized. However, it did not significantly correlate with stress sensitivity (MIEDEMA *et al.*, 1982); probably because chlorotic plants recover at the rise of temperatures but growth remain inhibited in comparison with non-chlorotic plants. Anthocynin pigmentation in leaves showed significant variation among genotypes under cold stress, which was more pronounced when best and worst type of entries were compared ($P < 0.01$). Maize lines capable of accumulating high amounts of anthocynin in the illuminated leaf surface may present an adaptive response to harmful conditions of low temperature associated with high light and it is not limiting to photosynthesis (PIETRINI *et al.*, 2002). Highland maize, which usually exposed to cold stress, accu-

mulates anthocynin pigments in stems and leaves (CHONG and BRAWN 1969). According to Gould *et al.* (2000), the role of anthocynin pigments is not clear and may depend on whether their location is in the vacuoles of the abaxial or adaxial leaf epidermis, in the cytosol of mesophyll cells, in roots, or in stems. Apart from visual score on cold injuries, genotypic variability for the developmental traits, such as leaf appearance and leaf area growth rate, was highly significant ($P < 0.01$). This is in agreement with the observations made by LEE *et al.* (2002) on maize inbred lines, where they found that leaf appearance rate was about three times slow in cold stressed maize seedling (15/3°C; 16-h photoperiod) than grown under normal temperature (25/15°C; 16-h photoperiod). Leaf area growth was also reported to be a function of prevailing temperature regime. Tol-

LENAAR *et al.* (1979) reported that the optimum temperature for leaf extension in maize was 30°C, and the extrapolated minimum 7°C. MIEDEMA *et al.* (1982) also found genetic variation in leaf extension rate at day/night temperatures of 15/10, 20/15, and 25/20°C.

Cold stress significantly affected days to 50% male flowering, which resulted in significant variation in anthesis-silking interval (ASI). MIEDEMA *et al.* (1987) suggested that resistance to chlorosis and rapid leaf expansion at low temperature are considered major selection criteria for the improvement of low-temperature adaptation. BECHOUX *et al.* (2000) reported that chilling significantly affected the tassel morphogenesis, and significantly reduced number of tassel branches and spikelet pairs in maize. Cold waves during winter cycle of 2002-03 in North India severely affected male flowering aspects of maize crop, including tassel development, limited growth of anther lobes and filaments and pollen shedding, which resulted in severe yield losses (SAMRA *et al.*, 2003). The effect of cold stress on leaf appearance resulted in significant ($P < 0.05$) genotypic variability in vertical growth, which was expressed in terms of significant effects on growth in plant height. Cold stress significantly reduced cell division and cell elongation, which eventually resulted in stunted growth (MIEDEMA *et al.*, 1982).

Apart from morphological traits, the physiological traits including total soluble sugars in leaf and electrolyte leakage (indicator of cell membrane damage due to cold stress) also showed significant ($P < 0.05$) genotypic variability under cold stress. Soluble sugars have been found to play an important role during cold acclimation process (YUANYUAN *et al.*, 2009). Soluble sugars exert their positive effects to protect plant cells from damage caused by cold stress through several ways, including serving as osmo-protectants, nutrient as well as interacting with the lipid bi-layer. LUKATKIN (2003) observed a gradual increase in leakage of ions from the cells upon prolongation of chilling exposure, with the maximum attained by the end of 24-h chilling treatment. FAROOQ *et al.* (2008) found that reduced membrane permeability contributed towards chilling tolerance in maize hybrids. The overall effect of cold stress on various morpho-physiological traits eventually resulted in highly significant ($P < 0.01$) genotypic variability for grain yield under stress. Though, the effect of cold stress was statistically non-significant on physiological maturity, but it has significant effects on per day yield ($P < 0.01$). The traits such as - ears

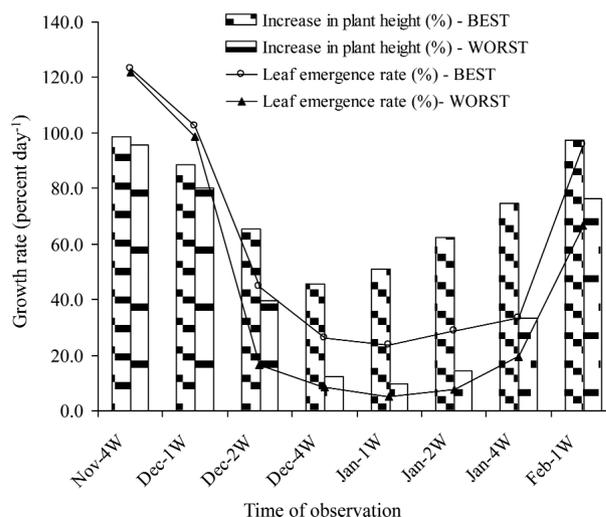


FIGURE 2 - Leaf emergence rate and increase in plant height in best and worst maize hybrids during low temperature regime.

per plant and physiological maturity in leaves showed non-significant genotypic variation across all genotypes, however the variation was statistically significant when the best and worst genotypes were compared (Table 1). Fast early growth and early flowering with long grain-fill duration, and extended stay-green have provided the basis for hybrids with high yield potential under low temperature stress (FREI, 2000). Analysis of variance (Table 2) of various traits showed highly significant contribution of genotype in genotypic variability under cold stress. Though coefficient of variation was on higher side for some of the traits, for example - leaf appearance, cold tolerance score and area growth rate, but it was largely because of low mean of the trials, not because of high error of the trial.

Data indicated that there was a sharp decrease in leaf appearance rate during cold temperature regime (Fig. 2). In genotypes with poor cold tolerance it decreased significantly during 2nd week of December (86.4%) in comparison to their leaf appearance rate during last week of November. The decrease was maximum during 1st week of January (95.9%), and the low rate of leaf appearance was continued until end of January. The leaf appearance rate slowed down in case of even best cold tolerant genotypes; however, the effect was comparatively less severe, except during first week of January (80.7%), when low temperature dropped down below 2°C (Fig. 1). Similar effect of cold temperature regime was observed on rate of increase in plant height as well (Fig. 2). Vertical growth was worst af-

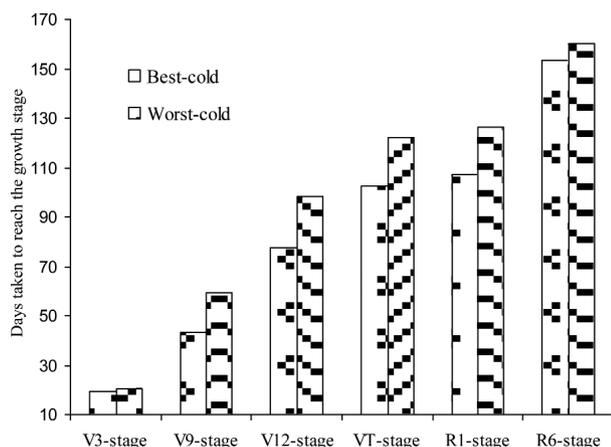


FIGURE 3 - Days taken to reach at various growth stages by the best and worst maize hybrids during winter crop season exposed to cold stress during late vegetative stage.

ected during last week of December and first week of January. With increase in temperature during end of January the recovery was comparatively faster in vertical growth than leaf appearance rate. Variation in susceptibility to low temperature on leaf appearance rate directly affected overall plant growth rates of best and worst group of genotypes (Fig. 3). The rate at which successive new leaves emerge at the stem apex in gramineae (phyllochron) is largely controlled by temperature (WILHELM and McMASTER, 1995; VAN ESBROECK *et al.*, 2008), which was found curvilinear over a range of temperature regime (WARRINGTON and KANEMASU, 1983). After V3 stage, when temperature dropped down below 10°C, the worst group of genotypes took significantly more number of days to achieve the various growth stages in comparison to best entries for cold tolerance; for example – worst group of genotypes reached to tassel appearance (VT) growth stage about 20 days later than best group of entries. However, the differences between best and worst group of genotypes gradually reduced at later stages (after pollination), which resulted in comparatively less grain filling period for worst group of genotypes, and eventually affected final grain yield.

Phenotypic correlation and regression analysis showed significant relationship between various secondary traits with grain yield under cold stress (Table 3). However, intercept and slope of the regression curve for various traits on grain yield varied significantly. Among all the traits, leaf appearances rate showed strongest positive relationship with grain yield under cold stress ($r = 0.69^{**}$; $R^2 = 0.73^{**}$). Cold tolerance score and per day yield also showed

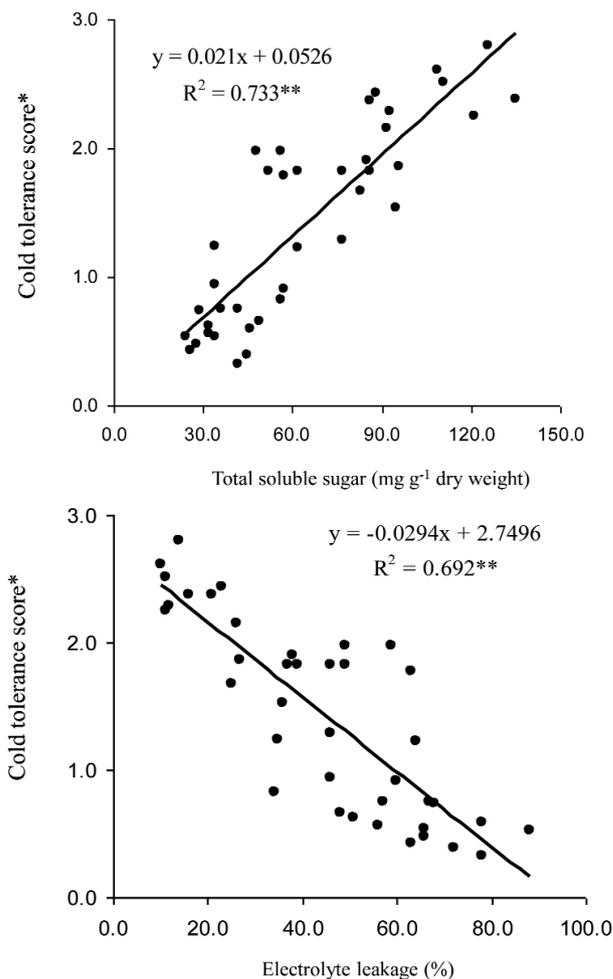


FIGURE 4 - Cold tolerance as function of total soluble sugars and electrolyte leakage in maize hybrids during winter crop season exposed to cold stress (1-2°C) during late vegetative stage. (*Score >2.0 = Tolerant, 2-3 = moderately tolerant, <1 = susceptible); Asterisks (* and **) statistical significance at $P < 0.05$ and 0.01 , respectively.

significant relationship with grain yield under cold stress. Visible scores on cold tolerance was significantly related with total soluble sugar concentration in leaves ($R^2 = 0.560^{**}$) and electrolyte leakage from the leaf tissues ($R^2 = 0.643^{**}$) (Fig. 4), which indicated that availability of total soluble sugar and reduced ion leakage helped in reducing the cold injuries. The low availability of total soluble sugars under cold stress might be related to reduced rate of photosynthesis that resulted in low assimilates starvation, and eventually visible stress symptom on plants. Inhibition of photosynthesis is known to be an early response to low temperature (YING *et al.*, 2002). Regression analysis between per day yield and ratio of days of 50% anthesis and physiological

TABLE 3 - Phenotypic correlation (r) between grain yield and morpho-physiological traits and linear regressions of different morpho-physiological traits on grain yield in maize hybrids under cold stress.

Traits	r	Regression equations	R^2	Significant differences Intercept	Slope
Chlorophyll	0.36	$y = 0.1147x + 1.0029$	0.41	ns	ns
Cold tolerance score	0.62*	$y = 0.8477x + 2.4676$	0.68**	**	**
Leaf emergence rate	0.69**	$y = 0.0801x + 0.923$	0.73**	**	***
Per day yield	0.59**	$y = 4.3601x + 10.131$	0.63**	**	**
Total sugar	0.48*	$y = 1.043x + 3.0112$	0.58**	*	**
Electrolyte leakage	0.54*	$y = 0.7504x + 1.025$	0.63**	*	**

*, ** and *** indicate statistical significance at 0.05, 0.01 and 0.001 probability levels, respectively; ns indicates non-significant differences.

TABLE 4 - Genotypic variability for cold tolerance at vegetative stage ($V_{7.9}$) in tropical maize hybrids under various low temperature regimes.

Cold tolerance score (0-3 scale)*	Temperature range (°C)				
	8.0 to 10.0	5.0 to 7.0	3.0 to 4.0	1.0 to 2.0	0 to -2.0
<1.0	0	6	33	51	80
1.0-2.0	14	19	29	17	0
>2.0	66	55	18	12	0

(* >2.0 = Tolerant, 2-3 = moderately tolerant, <1 = highly susceptible).

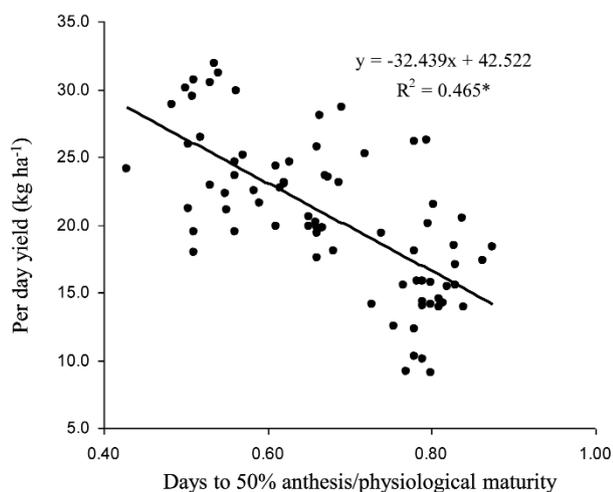


Figure 5 - Per day yield (kg ha^{-1}) as function of days to 50% anthesis/ physiological maturity in maize hybrids during winter crop season exposed to cold stress during late vegetative stage. Asterisks (*) indicates statistical significance at $P < 0.05$.

maturity (Fig. 5) showed significant negative relationship ($R^2 = 0.465^*$). This indicates that the genotypes which required more days to complete their vegetative growth had fewer days for reproductive growth, which resulted in low per day yield, and eventually low final grain yield under cold stress.

Our findings suggest that, in general, tropical maize is highly sensitive to cold stress during vegetative growth stage. However, there is considerable genotypic variability available for various traits, such as – photosynthetic pigments, growth and development traits. Presence of genetic variation for these secondary traits indicates that alleles are available in breeding materials that can improve cold tolerance. CIMMYT gene pools developed for high altitudes contain valuable material for these traits (BECK *et al.*, unpublished data). We conclude that selection of genotypes using secondary traits, such as- leaf appearance and extension rate, visual score for cold injuries, reduced electrolyte leakage along with grain yield under cold stress may be used as a selection index for identification and further improvement of cold tolerance in tropical maize.

ACKNOWLEDGEMENTS - This study was conducted under the Competitive Grant Project of Indian Council of Agricultural Research on “Development of technologies for improved stable yields of winter maize under cold stress in north India” and as part of ICAR-CIMMYT Collaborative Program (2005-08). Thanks to Dr. S.B. Singh and Prof. N.P. Gupta for their fruitful comments and suggestions on earlier drafts of this paper. Thanks to Shri Nanak Chand, Rajesh Pal and other field staff at the Maize Research Farm, IARI for plot management and assistance in data collection.

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