Screening Corn Hybrids for Cold Tolerance using Morphological Traits for Early-Season Seeding

Chathurika Wijewardana, Matthew Hock, Brien Henry, and K. Raja Reddy*

ABSTRACT

Early planting of corn (Zea mays L.) is a strategy to avoid excessive heat and drought that often negatively influence grain production during its reproductive phase. An experiment was conducted by imposing very low (day/night, 21/13°C), low (25/17°C), and optimum (29/21°C) temperatures during seed germination and seedling growth stages under optimum moisture and nutrient conditions. Above- and belowground growth parameters were assessed at 18 d after seeding. Several root morphological traits were assessed using the WinRHIZO root image analysis system. Corn hybrids varied significantly for many traits measured, particularly plant component weights and root morphological parameters. Principal component analysis (PCA) and total low-temperature response index (TLTRI) methods were used to categorize corn hybrid tolerance to low temperature and to group corn hybrids as cold tolerant, moderately cold tolerant, moderately cold sensitive, and cold sensitive. Total leaf and root weights and cumulative root length and length per unit volume were the most important morphological traits in describing hybrid tolerance to cold temperature. Based on the TLTRI method, relative scores were provided for each hybrid, which ranged from 22.45 to 29.52 among the hybrids. The hybrids CR8410VT3P, D57VP51, and R22BHR43 were classified as cold sensitive and AR1262, DKC6697, DKC6804, and M2V707 as cold tolerant based on PCA and TLTRI techniques. Based on the relative scores assigned in this study, corn producers could select hybrids to maximize corn production in an early planting production system.

Dep. of Plant and Soil Sciences, 117 Dorman Hall, Box 9555, Mississippi State Univ., Mississippi State, MS 39762. Received 10 July 2014. Accepted 7 Nov. 2014. *Corresponding author (krreddy@pss.msstate.edu).

Abbreviations: CLTRI, combined low-temperature response index; CVLTRI, combined very low-temperature response index; IVSRI, individual vigor stress response index; LA, leaf area; LW, leaf dry weight; NUE, nutrient uptake efficiency; PCA, principal component analysis; PH, plant height; RAD, average root diameter; RCL, cumulative root length; RL, longest root length; RLPV, root length per volume; RN, number of roots; RNC, number of crossings; RNF, number of forks; RNL, number of roots having laterals; RNT, number of tips; RS, root/shoot ratio; RSA, root surface area; RV, root volume; RW, root dry weight; SD, standard deviation; SPAR, soil-plant-atmosphere-research; SW, stem dry weight; TD, total dry weight; TL, total number of leaves; TRI, temperature response index; TLTRI, total low-temperature response index.


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hybrids that are adapted to U.S. mid-South production system. Therefore, it is important to identify, select, and develop hybrids that are best suited for an early planting production system in the southern United States. Selecting hybrids best suited to planting early should help corn growers optimize early planting and minimize heat and drought injury during flowering and grain-filling stages.

Corn is a chilling-sensitive species (Irigoyen et al., 1996), thus, adaptation of corn to early season planting requires a high-percentage emergence and vigorous seedling growth under cool temperatures. Selection and development of early maturing, cold-tolerant corn hybrids have been considered, and they should be more economic, efficient, and, in some regions, escape a number of important diseases including common rust (Puccinia sorghi) and maize dwarf mosaic virus (Revilla et al., 2003). In the central and delta regions of Mississippi, corn is usually planted between mid-March and late April with plants tasseling in June and July, which are historically the hottest and driest periods of the growing season in Mississippi (Reddy et al., 1996). The major constraint that limits yield in the U.S. mid-South is inadequate and erratic rainfall, particularly during a 2 to 3 wk critical precipitation window around tasseling. Thus, by moving the planting window to early March, under normal growing conditions, the crop may initiate tasseling in May, a month with cooler temperatures, greater solar radiation, lower evaporative demand, and consistent, plentiful precipitation. Early season, cold-tolerant plantings may also promote a higher growth rate; hence, there is a need for a rapid development of a plant canopy, enabling corn plants to be more competitive with weeds. Rapid growth at cool temperatures and quick plant canopy development may reduce unfavorable environmental side effects of cultivation such as nitrate leaching, soil erosion, and the need for the intensive use of herbicides (Verheul et al., 1997). In addition, planting corn early should result in timely completion of the crop and may allow corn producers to plant another crop during the year in the U.S. mid-South. Recently, Revilla et al. (2014) evaluated several maize inbred lines adapted to Europe for cold tolerance under controlled environmental conditions but under low light levels with a photosynthetic photon flux of 228 µmol m⁻² s⁻¹. The results and outcomes may not be portable to the field where we expect much higher solar radiation levels.

Root systems are difficult to study because of their highly structured underground distribution, complexity of vigorous interactions with the environment, and their diversity of functions. A corn seedling consists of three root systems. They include the primary and lateral roots, which belong to the embryonic root system and play a major role in early plant development (Richner et al., 1997). There also are crown roots that are postembryonic shoot-borne roots representing a major part of mature corn root system. Furthermore, lateral roots have a significant role in water and nutrient uptake (McCully and Canny, 1988) and are responsible for the root system architecture (Lynch, 1995). Depending on the genotype, plant species vary in response to environmental conditions such as unfavorable temperatures (Stamp et al., 1997) and drought conditions. At low temperature, nutrient acquisition of corn may be limited by the inhibition of root growth (Hund et al., 2008), and the growth of roots is delayed in cold-sensitive hybrids to a greater extent than shoot development (Stamp, 1984). Therefore, it is important to understand the root system architecture and different root traits to select the best performing corn hybrids that compete better at cold temperatures for introduction into an early season planting system.

Different methodologies have been developed to study root growth under both field and controlled environmental conditions. Coring methods, uprooting plants (Trachsel et al., 2011), and minirhizotrons (Johnson et al., 2001) are the prominent methods that have been used frequently for field studies. Under controlled environments, moistened papers, hydroponics, or Petri dishes have been used for screening of root mass and architecture (Reynolds et al., 2012). Root scanning based on the WinRHIZO optical scanner (Regent Instruments, Inc.) is one of the efficient methods that allow image analysis and examining the root morphological traits. This technique provides data that can easily be analyzed by established software protocols in a way of simple and rapid accurate screening of root characteristics. Therefore, this method was suited for screening of root traits of corn hybrids grown under controlled environmental conditions.

Temperature tolerance is a multigenic trait and, therefore, simple, consistent, and applicable methods are required to assess genetic variability and cold tolerance in crops. Also, experimental facilities are needed to impose such stresses that mimic field environments including solar radiation (Reddy et al., 2001). Several plant morphophysiological traits have been used to identify temperature tolerance among crop genotypes (Singh et al., 2007). To assess response of genotypes to temperature stress, selection indices based on relative rankings using single-value indices, cumulative indices, percentiles, or groupings based on statistical separation of means under single or multiple stresses have been developed. They area known as total temperature response indices (TRI) and they represent the multigenic nature of stress in crops (Koti et al., 2004; Salem et al., 2007). Quantitative relationships determined by principal component analysis (PCA) (Singh et al., 2008) have also been proposed. Moghaddam and Hadi-Zadeh (2002) developed a stress-tolerant index that is a useful tool to select for favorable corn hybrids under stressful and stress-free conditions. Principal component analysis, which is a multivariate technique, allows reducing a large number of observed traits into a smaller
set of traits that have the maximum contribution in separating the genotypes or hybrids. The TRI method uses all traits of interest that may potentially contribute to a given stress condition, tolerance, or sensitivity, and each trait will have an equal contribution. Therefore, combining both techniques to identify stress tolerance among corn hybrids will be an effective procedure.

We hypothesize that corn hybrids with prolific root systems would be able to withstand low temperature to produce greater leaf area (LA) for optimum photosynthesis during early seedling growth and developmental stages. Also, we hypothesize that morphological variability can be exploited among corn hybrids adapted to the U.S. mid-South for cold tolerance. The objectives of this study were to (i) determine if there is a variation in cold tolerance among commercially available corn hybrids, (ii) determine which traits among commercial hybrids are best suited for screening cold tolerance, and (iii) classify and rank corn hybrids based on a combined stress response for cold tolerance. We expect that this information will be useful to identify cold-tolerant traits that could be used in the development of improved hybrids or for use by farmers and crop consultants as a decision aid to select hybrids best suited for an early planting production system.

MATERIALS AND METHODS

Seed Material and Experimental Conditions

Thirty-three corn hybrids grown commercially in the southern United States were used for this study (Table 1). The experiment was conducted during the early growing season in sunlit soil-plant-atmosphere-research (SPAR) chambers located at the Rodeny Foil Plant Science Research facility of Mississippi State University, Mississippi State, MS. Each SPAR chamber consists of a steel soil bin (1-m deep × 2-m long × 0.5-m wide) and a 1.27-cm thick Plexiglas chamber (2.5-m tall × 2.0-m long × 1.5-m wide) to accommodate root and aerial plant parts, respectively. The Plexiglas allows 97% of the visible solar radiation to pass without spectral variability in absorption (wavelength 400–700 nm; Zhao et al., 2003). During the experiment, the incoming daily solar radiation measured with a pyranometer (Model 4–8; The Eppley Laboratory Inc.) outside the SPAR units ranged from 5.5 to 29.2 MJ m−2 d−1 with an average value of 20.14 ± 1.65 MJ m−2 d−1. More details of the SPAR chamber operations and control have been described by Reddy et al. (2001). Briefly, air ducts located on the northern side of each SPAR unit were connected to the heating and cooling devices. Conditioned air was passed through the plant canopy with sufficient velocity to cause leaf flutter (4.7 km h−1) and was returned to the air-handling unit just above the soil level. Chilled ethylene glycol was supplied to the cooling system via several parallel solenoid valves that opened or closed depending on the cooling requirement. To fine-tune the air temperature, two electrical resistance heaters provided short pulses of heat as needed. Chamber air temperature, CO₂ concentration, and soil watering in each SPAR unit, as well as continuous monitoring of environmental and plant gas exchange variables, were controlled by a dedicated computer system (Reddy et al., 2001) (Supplementary Table S1). The relative humidity of each chamber was monitored with a humidity and temperature sensor (HMV 70Y, Vaisala, Inc.) installed in the returning path of airline ducts. The vapor pressure deficits in the units were estimated from these measurements as per Murray (1967) (Supplementary Table S1).

Fungicide-treated seeds were sown in 297 polyvinyl-chloride pots (15.2-cm diameter and 30.5-cm height) filled with the soil medium consisting of 3:1 sand/top soil classified as sandy loam (87% sand, 2% clay, and 11% silt) with a 500 g of gravel at the bottom of each pot. Initially, four seeds were sown in each pot, and 7 d after emergence the plants were thinned to one per pot. Pots were arranged as a randomized complete block in 11 rows with three pots per row in each SPAR chamber. Three temperature treatments were randomly arranged in nine SPAR units. Except for the three temperature treatments, the other growth conditions were the same during the experiment for all the units. For each treatment, three replications were maintained by using one SPAR unit as one replication.

<table>
<thead>
<tr>
<th>Company</th>
<th>Hybrid</th>
<th>Abbreviated number</th>
<th>Days to maturity</th>
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<tr>
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<tr>
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Table 1. Thirty-three corn hybrids used in the study with their company name, hybrid name, abbreviated number, and days to maturity obtained from the respective companies.
Plants were irrigated three times a day through an automated, computer-controlled drip system with full-strength Hoagland’s nutrient solution (Hewitt, 1952), delivered at 0700, 1200, and 1700 h, based on treatment-based evapotranspiration values. Evapotranspiration rates expressed on a ground area basis (L d⁻¹) throughout the treatment period were measured in each SPAR unit as the rate at which the condensate was removed by the cooling coils at 900-s intervals (McKinion and Hodges, 1985; Reddy et al., 2001; Timlin et al., 2007). They were obtained by measuring the mass of water in collecting devices connected to a calibrated pressure transducer. Season-long average evapotranspiration values for each treatment are provided in Supplementary Table S1.

Temperature Treatments
Each hybrid appeared randomly within each of the three replicates for the three temperature treatments, 21/13 (day/night), 25/17, and 29/21°C. The daytime temperatures were initiated at sunrise and returned to the nighttime temperature 1 h after sunset. The environmental data for mean temperature are presented in Supplementary Table S1. Throughout the experiment, three temperature treatments, 21/13, 25/17, and 29/21°C, were considered as very low, low, and optimum temperatures, respectively, for corn growth and development.

Measurements
Phenology and Growth
Total number of leaves (TL) was counted and plant height (PH) was measured on all plants at 18 d after planting. Leaf area was measured using the LI-3100 leaf-area meter (LI-COR, Inc.). Plant component total dry weights (TDW) were measured from all plants after oven drying at 75°C until constant weight was reached.

Major Elemental Analysis
To determine concentrations of major mineral nutrients, plant components from all the hybrids were combined for each temperature treatment and they were ground using a Wiley Mill with a 20-mesh sieve. Leaf potassium (K), phosphorus (P), and nitrogen (N) were quantified at the Soil Testing Laboratory, Mississippi State University, Mississippi State, MS, using an inductively coupled plasma spectrophotometer (PerkinElmer Instruments). Methods followed those described by Donohue and Aho (1992), Cataldo et al. (1974), Fiske and Subbarow (1925), and Nelson and Sommers (1972). The individual hybrid plant-component mineral analysis was performed by multiplying dry weight with concentration. Nutrient uptake efficiency (NUE) was estimated for whole plant and shoot and root components.

Root Image Acquisition and Analysis
Roots were cut and separated from the stems and washed thoroughly avoiding any disturbance to the root system. Longest root length (RL) was determined using a ruler. The cleaned individual root systems were floated in 5 mm of water in a 0.3-by 0.2-m Plexiglas tray. Roots were untangled and separated with a plastic paintbrush to minimize root overlap. The tray was placed on top of a specialized dual-scan optical scanner (Regent Instruments, Inc.), linked to a computer. Gray-scale root images were acquired by setting the parameters to high accuracy (resolution 800 × 800 dpi). Acquired images were analyzed for the cumulative root length (RCL), root surface area (RSA), average root diameter (RAD), root length per volume (RLPV), root volume (RV), number of roots (RN), number of roots having laterals (RNL), number of tips (RNT), number of forks (RNF), and number of crossings (RNC) using WinRHIZO Pro software (Regent Instruments, 2009).

Data Analysis, Definitions, and Calculations of Stress-Tolerance Indices
Data were subjected to analysis of variance (SAS Institute, 2011) with a completely randomized design considering hybrids and temperature as sources of variance. Replicated values for TL, PH, LA, stem weight (SW), leaf weight (LW), root weight (RW), RL, RCL, RSA, RAD, RLPV, RV, RN, RNL, RNT, RNF, RNC, root/shoot ratio (RS), and TD were analyzed using one-way ANOVA of general linear model, PROC GLM, in SAS (SAS Institute, 2011) to determine the effect of temperature on the morphological and developmental parameters. Fisher protected least significant difference tests at $P = 0.05$ were employed to test the differences among treatments for measured parameters, and the standard errors of the mean were calculated and presented in the figures as error bars.

Classification of Hybrids Based on Total Low-Temperature Response Index
Corn hybrids were classified into cold-tolerant groups based on the summation of the individual temperature response index (TLTRI) values (Koti et al., 2004) with modifications. Initially, individual vigor stress response index (IVSRI) for very low temperature (21/13°C) of each parameter was calculated as the value of a parameter ($P_v$) at very low temperature of a given hybrid divided by the value for same parameter ($P_o$) at optimum temperature (29/21°C) (Eq. [1]). The IVSRI for low temperature (25/17°C) was determined by dividing the value of a parameter ($P_v$) at low temperature of a given hybrid by the value for same parameter ($P_o$) at optimum temperature (Eq. [2]). Then, combined very low-temperature response index (CVLTRI) and combined low-temperature response index (CLTRI) for each hybrid (Eq. [3] and [4]) were calculated as the sum of 19 IVSRIs derived from TL, PH, LA, SW, LW, RW, RL, RCL, RSA, RAD, RLPV, RV, RN, RNL, RNT, RNF, RNC, RS, and TD. Finally, TLTRI was calculated (Eq. [5]) as the sum of CVLTRI and CLTRI (Eq. [3] and [4]), respectively. Hybrids were classified into four categories of cold tolerance based on TLTRI values of 19 parameters and standard deviation (SD) as cold tolerant [greater than (minimum TLTRI + 3SD)], moderately cold tolerant [(between (minimum TLTRI + 2SD) and (minimum TLTRI + 3SD)], moderately cold sensitive [(between (minimum TLTRI + SD) and (minimum TLTRI + 2SD)], and sensitive [between (minimum TLTRI and minimum TLTRI + SD)].

\[
\text{IVSRI (very low)} = \frac{P_v}{P_o} \quad [1] \\
\text{IVSRI (low)} = \frac{P_v}{P_o} \quad [2]
\]
Principal Component Analysis and Classification of Corn Hybrids

Principal component analysis, which is a multidimensional preference analysis technique that allows the identification of parameters that best describe the tolerance to response variables, was used to separate hybrids into tolerant groups. It was performed by producing loadings for response variables, also known as eigenvectors for hybrids, termed as eigenvalues (PC scores). These loadings were used to identify the correlation of response variable vectors and hybrids across the ordination space. The data matrix for the analysis included hybrid means as rows and variable vectors and hybrids across the ordination space. The presence of large absolute values for some eigenvectors compared with other eigenvectors indicates having a strong relationship with a particular hybrid. The values of eigenvectors and PC scores were used to classify corn hybrids into cold-tolerant groups.

RESULTS AND DISCUSSION

This is the first study providing data for shoot and root morphological parameters to assess genetic variability and cold tolerance of corn hybrids adapted to the U.S. mid-South production system (Table 1). Knowledge of hybrid performance in low-temperature conditions will be valuable for manipulating breeding strategies for yield improvement and for developing or selecting hybrids best suited for early season planting in many corn-growing areas.

Performance of Hybrids and Their Relationship with Temperature

The analysis of variance revealed significant (P ≤ 0.001) differences among the hybrids, temperature, and hybrid × temperature combination for all the measured traits with the exception of the TL (P > 0.05) (Table 2). The relationships between temperature and both shoot and root parameters were significant (Table 2). The significant variations among hybrids and hybrid × temperature under three different temperature conditions indicate that adequate genetic variation existed among the tested corn hybrids. Total leaf number, PH, LA, SW, LW, RW, RL, RCL, RSA, RAD, RLPV, RV, RN, RNL, RNT, RNF, RNC, RS, and TD were positively correlated with temperature (Supplementary Table S2). In contrast, RAD and RS showed a negative correlation with temperature and all other parameters. Increase in temperature plays a key role in stem elongation, LA, and TL, which, in turn, affects RS. The phenotypic relationships between RAD × RL and RAD × RSA were not significant (P > 0.05). The relationship between the RAD and the length of the roots

Table 2. Analysis of variance across the hybrids (Hyb) and temperature (T) treatments and their interaction (Hyb × T) with corn morphological parameters measured 18 d after treatment; total leaf (TL), plant height (PH), leaf area (LA), stem weight (SW), leaf weight (LW), root weight (RW), root length (RL), cumulative root length (RCL), surface area (RSA), average root diameter (RAD), length per volume (RLPV), root volume (RV), number of roots (RN), number of roots having laterals (RNL), number of tips (RNT), number of forks (RNF), number of crossings (RNC), root/shoot ratio (RS), and total dry matter (TD).

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<th>Source of variance</th>
<th>TL</th>
<th>PH</th>
<th>LA</th>
<th>SW</th>
<th>LW</th>
<th>RW</th>
<th>RL</th>
<th>RCL</th>
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<th>RAD</th>
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<th>RNC</th>
<th>RS</th>
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<tr>
<td>21/13°C</td>
<td>NS</td>
<td>***</td>
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<td>Hyb [25/17°C]</td>
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** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
† NS, significance level P > 0.05.
was negative, possibly due to the effect of the low soil temperature, at which corn roots grew thicker with fewer branches, which supports data from Cutforth et al. (1986).

**Plant Height, Leaf Area, and Leaf Number**

Corn hybrids grown at optimum temperature had taller stems and differed significantly from those grown at the two low-temperature treatments. At very low temperature, hybrid plant PH ranged from 2 to 6 cm with an overall average of 4 cm (Fig. 1a). At low temperature, it ranged from 6 (M2V714) to 12 cm (CR6640VT3P) with the average of 9 cm, whereas at optimum temperature, it varied from 11 (M2V714) to 20 cm (CR6640VT3P) with the average of 16 cm. The very low temperature caused a significant decrease

![Figure 1. Temperature effects on (a) plant height and (b) leaf area of corn hybrids. Measurements were taken at 18 d after sowing. Horizontal dotted lines indicate the average value of a given parameter at each temperature treatment. Bars indicate standard error of the mean ± 3 replications.](image-url)
from optimum in PH with maximum decrease observed for AG6533VT2RJB (3 cm), M2V707 (3 cm), and M2V714 (3 cm) hybrids (Fig. 1a). This is in agreement with earlier observations (Miedema, 1982) that cold stress significantly reduced cell division and cell elongation, which resulted in stunted plant growth. Pioneer P1319HR (6 cm), DKC6804 (6 cm), and CR6926VT3P (6 cm) showed the maximum PH at very low temperature. Among the 33 hybrids, 16 hybrids (48%) showed higher PH compared with the overall average value (4 cm) at very low temperature.

Both total LA and LN followed a similar trend to that of PH across treatments and hybrids. Leaf growth, LA, and, more importantly, the vegetative and reproductive shoot development strongly depend on temperature. Leaf growth in field conditions might also be limited by insufficient root development, which is strongly related to soil temperature (Richner et al., 1996). Subsequently, the direct effect of temperature on the shoot meristem, or its effect on reduced nutrient uptake through the roots, will affect shoot development at suboptimal soil temperatures (Engels and Marschner, 1990). Generally, cold temperature slows the rate of leaf initiation, which may reduce the LN and directly lowers the rates of both leaf cell division and elongation (Ben-Haj-Salah and Tardieu, 1995), resulting in lower LA and leaf dry weight. In the present study, hybrid AR1262 exhibited the highest values of LA (58 cm²) closely followed by P1319HR (57 cm²), and CR6926VT3P (57 cm²) at very low temperature (Fig. 1b) with the smallest value recorded for M2P886 (31 cm²) and overall average of 43 cm². At low temperature, LA ranged from 62 to 143 cm² with the average of 112 cm², and at optimum temperature it varied from 201 to 347 cm² with the average of 283 cm². Hybrid M2P886 showed the smallest LA at both low and optimum temperatures, while DKC6757 and D55VP77 showed the largest LA at low an optimum temperatures (Fig. 1b). Hybrids CR6926VT3P and D57VP75 showed maximum cold tolerance at very low temperature for leaf number (Supplementary Fig. S1a). Leaf number among the hybrids ranged from 3.0 to 3.3 with an overall average of 3.1 at very low temperature. Among the 33 hybrids, 11 hybrids (33%) showed a higher leaf number than the overall average value. At low temperature, SYN68B3111 (3) and GHT7240VT2P (5) showed minimum and maximum leaf numbers, ranging from 5 to 6 at optimum temperature with the average of 5. Leaf appearance rate is almost exclusively determined by soil temperature during seedling growth stages (Giauffret et al., 1995). Tollenaar et al. (1979) reported that the minimum and optimum temperatures for leaf extension in corn were 7 and 30°C. Miedema (1982) also recorded genetic variation in leaf extension rate in corn populations at day/night temperatures of 15/10, 20/15, and 25/20°C and they suggested the LA expansion at low temperature was a major selection criterion for the improvement of low-temperature adaptation. Because, plant growth and development play essential roles in crop production, any factor governing the production of new leaves, the duration of LA expansion, and stem extension will affect canopy development and radiation interception and thus crop yield (Reddy et al., 1997).

**Root Developmental Parameters**

Total root number and RNLs varied with temperature. They were increased at optimum temperature and significantly reduced at low temperature (Supplementary Fig. S1b, S1c). This trend was also evident for each individual hybrid at very low temperature. Total root number varied greatly with values ranging from 6 and 9 for DKC6188 and AR1262, respectively (Supplementary Fig. S1b). The overall mean for total root number was 8 at very low temperature. At low and optimum temperatures total root numbers ranged from 7 (P1498YHR) to 11 (DKC6757) and 9 (DKC6697 and AG6489VT2RJB) to 14 (R22BHR43) with the averages of 9 and 12, respectively. Hybrid AR1262 exhibited the highest value (4) for RNL, and smallest was observed for AR1550 (2) at very low temperature (Supplementary Fig. S1c). Among the 33 hybrids, 24 hybrids (72%) showed a higher RNL than the overall average value (4). Hybrids CR8621, AR1550, DKC6929, and SYN68B3111 showed lowest RNL, while M2V707 exhibited the highest at low temperature. At optimum temperature, RNL ranged from 7 (AG6489VT2RJB, AR1550, DKC6804, and DKC6697) to 11 (ST11504VT3) with the average of 8 (Supplementary Fig. S1c). Generally, if root length exceeds a certain size, the branching process starts by initiation, emergence, and growth of lateral roots from the root pericycle and epidermis (Morita, 1993). Lateral roots are responsible for the larger quantities of water and nutrient absorption (Yoshida and Hasegawa, 1982) because they account for approximately 77% of the surface area of the root system in any crop (Jills et al., 2000). In addition, lateral roots generally influence plant architecture (Yamauchi et al., 1987) and have a large effect on water uptake (Varney, et al., 1993), particularly under drought conditions (Banoc et al., 2000).

Plant roots optimize their root architecture to acquire water and essential nutrients. Number of root tips, forks, and crossings play an important role on root architecture because they have potential to enhance penetration through soil layers, resulting in a positive effect on plant nutrient uptake. In the present study, root tips, forks, and crossings densities differed significantly among hybrids at suboptimal temperature treatments compared with optimum growing conditions (Supplementary Fig. S2a, b, c). Among the hybrids, RNT ranged from 1826 (M2P886) to 8462 (DKC6188) with an overall average of 3686 at very low temperature (Supplementary Fig. S2a). At low and optimum temperatures, RNT ranged from 7598.
fewer numbers of those traits. Imposing nutrient and water uptake than corn hybrids with tolerance extreme environmental conditions better by maximizing nutrient and water from the soil profile. Thereby, a particular hybrid means that hybrid can compete better to the plant. Higher RNCs, RNTs, and RNFs in a particular temperature it ranged from 13,819 (M2P886) to 36,453 (AR1550) with the average of 26,765 (Supplementary Fig. S2c). Nine hybrids (27%) had a significantly higher RNT than hybrids mean value, and 15 (45%) and 14 (42%) hybrids had higher RNFs and RNCs at very low temperature (21/13°C). Root forks ranged from 9124 (DKC6929) to 20,040 (CR6640VT3P) with the average of 13,750 at low temperature whereas at optimum temperature it ranged from 13,819 (M2P886) to 36,453 (AR1550) with the average of 26,765 (Supplementary Fig. S2c). Hybrids AG6489VT2RIB (866) and CR6640 (2526) showed minimum and maximum RNCs at the low-temperature treatment, while M2P886 (1647) and D55VP77 (4788) exhibited the highest and the lowest values at optimum temperature (Supplementary Fig. S2c). Number of crossing, RNT, and RNF densities suggests the development of a more branched root system and this is directly related to water and nutrient uptake potential of the plant. Higher RNCs, RNTs, and RNFs in a particular hybrid means that hybrid can compete better to forage nutrients and water from the soil profile. Therefore, hybrids with more tips, forks, and crossings should tolerate extreme environmental conditions better by maximizing nutrient and water uptake than corn hybrids with fewer numbers of those traits.

## Root Growth Parameters

Total root length, RSA, and RAD have been used to characterize root systems and to evaluate their functional size (Costa et al., 2002). These characteristics are useful to predict nutrient uptake ability and performance under stress conditions. Several studies have characterized the rooting system patterns of corn hybrids (Hammer et al., 2009; Rosolem et al., 1994), but these studies were all conducted on plants that were similar in aboveground architecture, and their root systems at early developmental stages have not been properly characterized. In the present study, a set of root structural parameters were investigated that includes root development with respect to RL, RCL, RSA, RV, RAD, root distribution pattern in the soil column, RS, and root branching, which are very important to establish differences among hybrids in different temperature treatments. Evaluation of these root traits could provide important insights to distinguish root growth and development at suboptimum temperatures.

In the current study, significant genetic variation and temperature effects were detected among different corn hybrids for root morphological traits. Root length (Fig. 2a), RV (Supplementary Fig. S3a), RSA (Supplementary Fig. S3b), and RL (data not presented) increased for all hybrids at optimum temperature and reduced significantly at very low and low–temperature conditions. Maximum root length is affected by environmental conditions (Yoshida and Hasegawa, 1982), but also influenced by genetics. If the roots do not encounter a physical limit to growth, roots will reach a maximum depth in a particular soil profile. In this study, RCL values ranged from 736 cm (AURB25) to 1197 cm (DKC6757) with an overall mean value of 970 cm for the 33 hybrids tested (Fig. 2a). Within the very low–temperature treatment, 15 hybrids (45%) exhibited the higher RCL than overall mean root length. Hybrids M2P886 (1373 cm) and DKC6757 (2553 cm) showed smallest and largest RCL at low temperature, whereas at optimum temperature, M2P886 (2230 cm) and AR1262 (5111 cm) exhibited minimum and maximum values with the average of 3480 cm.

Most of the hybrids demonstrated the greatest RV at low temperature. That means the higher temperature treatment affected the majority of hybrids by decreasing their RV. Hybrids AG6489VT2RIB (3 cm²) and R28HR20 (4 cm²) had higher RV at very low temperature compared with the other hybrids at same temperature (Supplementary Fig. S3a). Five hybrids had a higher RV value than the mean volume value (2 cm³). At low temperature, RV ranged from 2 (M2V714) to 5 cm³ (CR8621VT3P) with the average of 4 cm³, whereas at optimum temperature it varied from 2 (M2P886) to 6 cm³ (AG6533VT2RIB) with the average of 4 cm³. The highest RSA (cm²) was observed for DKC6757 (267 cm²), H68B (259 cm²), and AG6489VT2RIB (232 cm²) at very low temperature (Supplementary Fig. S3b). The overall mean for RSA was 139 cm² at very low temperature and 10 hybrids exhibited a higher value than the mean value. Hybrids GHT7240VT2P (219 cm²) and CR8621VT3P (408 cm²) showed minimum and maximum RSA at low temperature, while M2P886 (312 cm²) and AR1550 (535 cm²) exhibited minimum and maximum at optimum temperature. The average values for RSA ranged from 320 to 419 cm² at low and optimum temperatures. These data support the possibility that these hybrids may possess the potential to explore greater soil volume and uptake nutrients more successfully at suboptimal temperatures than other hybrids evaluated in this study.

Corn hybrids responded differently with respect to RAD. A significant increase in RAD was detected for hybrids AG6489VT2RIB, H68B, R28HR20, SYN68B3111, and DKC6929 at very low temperature compared with other temperatures and other hybrids (Supplementary Fig. S3c). Additionally, most of the hybrids showed a greater RAD at low temperature than at optimum temperature. Among hybrids, RAD values ranged from 0.4 (P2088YHR) to 0.8 mm (AG6489VT2RIB) with an overall average value of 0.6 mm at very low temperature, whereas at low and optimum temperatures those
values were from 0.4 (P2088YHR) to 0.6 mm (AR1550), respectively, with average of 0.5 mm, and 0.3 (AR1262) to 0.5 mm (AUGB25) with mean of 0.4 mm. These data suggest the presence of larger RADs at suboptimal temperatures than the optimum temperature condition, probably because of temperature effects on cell elongation. In a study by Yambao et al. (1992), RAD was recorded as an effective selection criterion for xylem size in plant root systems. The assumption is that larger RADs provide drought resistance because of enhanced penetration ability (Clark et al., 2008; Materechera et al., 1992), branching (Fitter, 1991), a greater xylem vessel radius, and lower axial resistance to water flux (Yambao et al, 1992).

The quantity of root length in layers within the soil profile is usually expressed in terms of RLPV (cm m$^{-3}$) of soil. Root length per soil volume among the 33 hybrids...
at very low temperature varied significantly with values ranging from 610 cm m\(^{-3}\) for P1636YHR to 1246 cm m\(^{-3}\) for DKC6188 with an average of 971 cm m\(^{-3}\) (Fig. 2b). At low and optimum temperatures, RLPV ranged from 1373 to 2551 cm m\(^{-3}\) and 2239 to 4444 cm m\(^{-3}\) with the average of 2115 and 3483 cm m\(^{-3}\), respectively. Hybrid M2P886 showed the lowest RLPV at both low and optimum temperatures, while at low temperature, DKC6208, and at optimum temperature, AR1262, showed the largest RLPV. Generally, RLPV reflects the development of lateral roots and it is directly related to water uptake ability of the plants because water is mostly absorbed passively. Water uptake usually increases as RLPV increases, but only up to a given length, which is termed critical root length density (Gowda et al., 2011).

**Total Biomass and Partitioning**

Leaf dry weight, SW, and RW were increased with increasing temperature treatments. Hybrids CR6926VT3P and D57VP75 showed the maximum tolerance at low temperature for LW and SW. Leaf dry weight among the 33 hybrids varied significantly with values ranging from 0.06 g in M2V707 to 0.14 g in D57VP75 with an average of 0.09 g at very low temperature (Supplementary Fig. S4a). Hybrids M2P886 (0.18 g) and R22BHR43 (0.4 g) had the minimum and maximum LWs at low temperature, respectively; whereas GHT7240VT2P (0.6 g) and DKC6757 (1 g) exhibited the lowest and the highest LWs at optimum temperature. Sixteen hybrids had a higher SW value than the mean value (0.03 g) and SW varied from 0.01 to 0.06 g (Supplementary Fig. S4b). At low and optimum temperatures, SW values ranged from 0.08 (M2V714) to 0.19 g (R22BHR43) and 0.24 (M2P886) to 0.56 g (DKC6929) with the average of 0.13 and 0.4 g, respectively. In the present study, RW varied significantly among the 33 corn hybrids. Hybrid D57VP75 exhibited highest value of RW (0.15 g) closely followed by hybrids DKC6929 (0.13 g), CR6926VT3P (0.13 g), and AR1262 (0.13 g) with the smallest value recorded for M2P886 (0.05 g) and an overall average of 0.10 g at very low temperature (Supplementary Fig. S4c). Average values of RW ranged from 0.19 to 0.3 g at low and optimum temperatures. Mycogen 2P886 showed the smallest RW at both low and optimum temperatures. At low temperature DKC6208 (0.26 g) had the highest RW, whereas R22BHR43 (0.47 g) had the highest at optimum temperature.

Biomass partitioning between shoot and root systems varied among the hybrids and treatments. The RS across 33 corn hybrids averaged 0.8 with a high and low value of 1.1 and 0.5 for DKC6697 and M2P886, respectively (Supplementary Fig. S5). At low and optimum temperatures, RS varied from 0.3 (M2P886) to 0.7 (M2V707) and 0.2 (DKC6929) to 0.3 (DKC6697) with the average of 0.5 and 0.3. Root/shoot ratio is a measure of the distribution of resources between different plant constituents. In the present study, RS increased significantly as the temperature decreased. Root/shoot ratio was closely related to both above- and belowground traits. The allocation of resources such as dry matter toward the shoot and root was high at optimum temperature but decreased markedly at very low temperatures. As a result of this, the RS decreased as the temperature increased.

The effect of cold stress on TD accumulation varied among corn hybrids. Dry matter content was increased significantly for all hybrids at optimum temperature and reduced as temperature treatment decreased in both very low and low temperatures. Among the 33 hybrids, significant variation was observed for TD content, with a high and low value of 0.4 and 0.2 g for D57VP75 and M2P886, respectively, with an overall mean of 0.2 g at very low temperature (Fig. 3). Hybrids 2P886 and R22BHR43 had smallest and highest TD content under both low and optimum temperatures. Among the 33 hybrids, D57VP75 exhibited the maximum tolerance at low temperature whereas GHT7240VT2P (0.6 g) and DKC6757 (1 g) showed the maximum cold tolerance for N and K, respectively (Fig. S6b). In this study, R22BHR43 (1.95%) and M2V714 (0.74%) showed maximum and minimum total N uptake respectively (Fig. 4). Among the 33 hybrids tested,
Figure 3. Temperature effects on total dry matter content of corn hybrids. Measurements were taken at the 18 d after sowing. Horizontal dotted lines indicate the average value of a given parameter at each temperature treatment. Bars indicate standard error of the mean ± 3 replications.

Figure 4. Temperature effects on N, P, and K uptake efficiency by corn plants. Measurements were taken at the 18 d after sowing. Horizontal dotted lines indicate the average value of a given parameter at each temperature treatment. Bars indicate standard error of the mean ± 3 replications.
15 hybrids showed higher values for N uptake efficiency than the average values (1.23%). At very low temperature, P uptake efficiency ranged from 0.05 (M2V714) to 0.15% (R.22BHR43) with an average of 0.09%, and K uptake efficiency varied from 0.60 (M2V714) to 1.74 (R.22BHR43) with an average of 1.10 (Fig. 4).

Root length, RV, RAD, RSA, and RNL affect the plant's ability to extract nutrients from the rooted soil profile (Barber, 1995). Consequently, differences in NUE in corn plants influence the alterations in absorption, translocation, and root and shoot dry matter production, and thereby, tolerance to extreme environmental conditions. Therefore, identification of hybrids having different nutrient uptake efficiencies will allow breeders to develop hybrids with high NUE to optimize crop production systems, particularly under stressful environments.

**Performance of the Hybrids Based on Root Morphology**

The assessment of root morphological parameters and shoot traits can produce significantly more information about the performance of a hybrid. To identify capable corn hybrids, a prompt and accurate assessment of root morphological characteristics is needed. In the present study, scanner-based image analysis was used to investigate root morphology in an accurate manner for corn seedlings at various temperature conditions. Observations of the root systems of hybrids CR.8410VT3P and DKC6804 revealed differences that were visually distinct based on the temperature tolerance (Fig. 5). The hybrid DKC6804 contributed a strong, well-structured root system a higher RN as well as abundant root hairs, while hybrid CR.8410VT3P exhibited a less-structured root system with reduced RN and RNL at the suboptimum temperature treatments. Relatively, all corn hybrids designated as cold tolerant in this study had larger, more robust and branched root systems with well-organized root morphology with higher values for root traits, whereas cold sensitive hybrids showed less organized root structures with low values for root traits. Therefore, the hybrids with highly structured root systems are associated with vigorous plant development that may potentially tolerate cold stress successfully under field conditions during seedling development.
Classification of Corn Hybrids

The TLTRI values for each corn hybrid were derived by summing individual temperature response indices for all root and shoot parameters among the corn hybrids evaluated in this study. The TLTRI-based technique was used to identify hybrid variability for very low and low temperature (very low–CTRI and low–CTRI) tolerance in corn hybrids. Cold-tolerant TLTRI varied from 29.52 for hybrid DKC6804 to 22.45 for hybrid D57VP51. Based on cold tolerance, four hybrids were classified as cold tolerant, 12 were moderately cold tolerant, 15 were moderately cold sensitive, and three were cold sensitive hybrids (Table 3).

The correlation between shoot and root traits using TLTRI for cold tolerance is positively correlated \( r^2 = 0.68, P = 0.0001 \) and implies that either component (shoot or root traits) could be used in identifying corn hybrids for cold tolerance (Fig. 6). However, screening based on both shoot and root traits should be robust to provide a better means of classification.

Assessment of Cold Tolerance Using Principal Component Analysis

Principal component analysis reduces the dimensionality of a given data set while retaining most of the variations by producing numbers in absolute values for the response variables. These response variables and the reduction are accomplished by identifying directions, called principal components. This statistical tool assigns eigenvalues (PC scores) for each hybrid and eigenvectors for each principal component (response variables). By using this method, the parameters that best describe the cold tolerance can be represented by selecting relatively few numbers of traits instead of many variables. Data samples can then be plotted, making it conceivable to visually assess similarities and differences and accordingly categorize them into groups (Singh et al., 2008). In the present study, PCA was performed to identify the principal components of shoot and root parameters of corn hybrids that best described the response to temperature, and, hence, to identify cold-tolerant and cold-sensitive hybrids. For this analysis, only the very low temperature treatment (21/13°C) was considered because our goal was to categorize the hybrids according to their cold-tolerance level. The first three PCs accounted for 34.21, 23.05, and 12.98% of the total variation among the hybrids. The total variability of 70.24% was explained by three principal components and it was efficiently summarized by 57.26% variability from first two principal components and 47.19% variability from the first and third principal components.

The first principal component (PC1) can be interpreted as representing higher values for TD, RW, LW, and RCL, and to a lesser extent, LC, RAD, RN, RLN, and
and PC2. In contrast, hybrids D57VP51, R22BHR43, and cold tolerant because of its relatively higher scores for PC1. Therefore, the hybrid Mycogen 2V707 is considered as with strongest differences for these traits are placed in the PC2 (Fig. 7) should separate the hybrids that have higher scores for PC2 should have high RAD, RSA, RV, RN, and RS. Hence, hybrids with strongest differences for these traits are placed in the upper-right and lower-left corner of the graph (Fig. 7). Therefore, the hybrid Mycogen 2V707 is considered as cold tolerant because of its relatively higher scores for PC1 and PC2. In contrast, hybrids D57VP51, R22BHR43, and CR8410VT3P can be classified as cold sensitive as they have relatively small values for TD, RW, LW, and RCL as well as moderately small scores for LC, RAD, RN, RLN, and RS. As a result, the biplot of PC1 vs. PC2 grouped the hybrids CR8410VT3P, D57VP51, and R22BHR43 as cold sensitive and AR1262, DKC6697, DKC6804, and M2V707 as cold tolerant. The TLTRI analysis (Table 3) also identified CR8410VT3P, D57VP51, and R22BHR43 as cold sensitive and AR1262, DKC6697, DKC6804, and M2V707 as cold-tolerant hybrids based on temperature response indices. Therefore, the findings from PCA were in reasonable agreement with the TLTRI method where all traits were used in the analysis and in the classification corn hybrids for low-temperature tolerance.

The third PC contrasted with PC1 by providing higher scores for RTN, RFN, and RCN with comparatively low negative values for PH, LA, SW, LC, LW, RW, TD, LD, and RN (Table 4). However, the biplot of PC1 vs. PC3 showed considerable similarity in the categorization of hybrids for their cold tolerance as it identified the hybrids CR8410VT3P, D57VP51, and R22BHR43 as cold sensitive to moderate cold sensitive and AR1262, DKC6697, DKC6804, and M2V707 as cold tolerant to moderate cold tolerant in each quadrants (Fig. 8).

The scores of PC1, PC2, and PC3 collectively contributed greater importance in the hybrid separation for cold tolerance. We used PCA to group the hybrids into cold tolerant and cold sensitive. Finally, 33 corn hybrids were classified into four groups as cold tolerant, moderately cold tolerant, moderately cold sensitive, and cold sensitive based on their responses to low temperature. Further, by analyzing the first two principal components (because they expressed 57.26% of variability), we determined that TD content, LW, RW, CRL, and RLPV were the parameters that best described cold tolerance.

**CONCLUSIONS**

The 33 corn hybrids examined in this study exhibited substantial variability in their responses for all the traits measured. The TLTRI derived from root parameters showed a significant positive correlation with shoot parameters. This suggests that screening based on root parameters may provide a robust analysis and classification for low-temperature tolerance. By PCA, we identified TD, RW, LW, CRL, and RLV as the variables to best describe cold tolerance of corn hybrids. The TLTRI and PCA methods used together in the present study identified cold-tolerant hybrids and demonstrated that variability existed among the commercially available hybrids that we tested. Based on these methods, corn hybrids CR8410VT3P, D57VP51, and R22BHR43 were recorded as cold sensitive.

<table>
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Figure 7. Principal component analysis (PCA) biplot for the first two principal component (PC) scores, PC1 vs. PC2, related to the classification of 33 corn hybrids (solid star symbols) for cold tolerance. The eigenvectors (PC1 and PC2) for the variables (solid circle symbols) are superimposed with the PC biplot scores at the similar scale that reflects the contribution of the parameters (variables) in determination of cold tolerance. The directional eigenvectors represent the traits: total number of leaves (TL), plant height (PH), leaf area (LA), longest root length (RL), leaf dry wt. (LW), stem dry wt. (SW), root dry wt. (RW), total dry wt. (TD), cumulative root length (RCL), root surface area (RSA), root diameter (RAD), root length per volume (RLPV), root volume (RV), root/shoot ratio (RS), number of roots (RN), number of roots having laterals (RNL), number of tips (RNT), number of forks (RNF), and number of crossings (RNC). The eigenvector values were multiplied by five to obtain a clear and superimposed figure. The arrows along the right y axis and the bottom x axis indicate the interpretation of the PCs.

Figure 8. Principal component analysis (PCA) biplot for the first and third principal component (PC) scores, PC1 vs. PC3, related to the classification of 33 hybrids (solid star symbols) for cold tolerance. The eigenvectors (PC1 and PC3) for the variables (solid circle symbols) are superimposed with the PC biplot scores at the similar scale that reflects the contribution of the parameters (variables) in determination of cold tolerance. The directional eigenvectors represent the traits: Total number of leaves (TL), plant height (PH), leaf area (LA), longest root length (RL), leaf dry wt. (LW), stem dry wt. (SW), root dry wt. (RW), total dry wt. (TD), cumulative root length (RCL), root surface area (RSA), root diameter (RAD), root length per volume (RLPV), root volume (RV), root/shoot ratio (RS), number of roots (RN), number of roots having laterals (RNL), number of tips (RNT), number of forks (RNF), and number of crossings (RNC). The eigenvectors were multiplied by 5 to obtain clear and superimposed figure. The arrows along the right y axis and the bottom x axis indicate the interpretation of the PCs.
and AR1262, DKC6697, DKC6804, and M2V707 were identified as cold-tolerant hybrids. Selected cold-tolerant corn hybrids may be useful for breeders to develop new corn hybrids that can withstand low-temperature conditions. The identified cold-tolerant hybrids may perform better than other hybrids planted early in cool growing conditions. However, these results should be validated under field conditions to evaluate their performance before recommending them to the producers and breeders to accomplish the maximum benefit of early season seeding.

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