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ScienceDirect
Europ. J. Agronomy 27 (2007) 52–61
European Journal of Agronomy
www.elsevier.com/locate/eja

Root morphology and photosynthetic performance of maize inbred lines at low temperature

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Received 17 August 2006; received in revised form 18 December 2006; accepted 8 January 2007

Abstract

At low temperature, as occurs in the spring, a high photosynthetic performance of maize (*Zea mays* L.) in combination with a large leaf area is an important measure for early vigor. However, little is known about adaptation of root morphology to low-temperature conditions. The objectives were (i) to characterize a set of 21 modern inbred lines for photosynthesis-related traits and root morphology at 15/13 °C (day/night) and (ii) elucidate relationships between shoot and root traits. Plants were grown in sand substrate until the two-leaf (V2) stage; the operating efficiency of photosystem II (Φ_{PSII}), chlorophyll content (SPAD), and leaf area were used to estimate the rate of CO_2 assimilation per plant ($\hat{A}_p$). The genotypes were separated as follows: those that maximize leaf area and those that maximize Φ_{PSII}. The morphological organization of the root systems of the genotypes varied to a great extent. Using a principal component analysis (PCA) of root traits (*i.e.* length of the primary, seminal, and crown roots), genotypes with homogeneous (similar primary and seminal roots) and heterogeneous (lateral roots of the primary root generally longer than the lateral roots of the seminal roots) root systems were identified. The length of the primary lateral roots was most closely associated with all $\hat{A}_p$-related traits and with high plant dry weight. Therefore, most of the genotypes with an heterogeneous root system outperformed those with an homogeneous root system with regard to dry matter accumulation and photosynthetic performance. In conclusion, differences in the organization of the embryonic root system are associated with early vigor.

Keywords: Root morphology; Chilling stress; Chlorophyll fluorescence; *Zea mays*

1. Introduction

Improving early vigor is a major goal for the adaptation of maize to cool and humid conditions. Early vigor of maize is the ability to quickly produce assimilates for autotrophic growth after the endosperm reserves are exhausted. Typically, early vigor is measured as the integration of canopy size, leaf greenness, and the superior overall appearance of young plants about 3 weeks after emergence (Revilla et al., 1999). Low temperature is one of the most significant abiotic stress factors affecting early maize growth. The critical low-temperature threshold of maize growth under controlled conditions ranges from 10 to 17 °C depending on the examined trait and the cultivar (Blacklow, 1972; Bowen, 1991; Haldimann et al., 1996). The low-temperature threshold for photosynthesis can be reduced by selecting for high operating efficiency of photosystem II (Φ_{PSII}) at low temperature (Fracheboud et al., 1999), since the quantum yield of the photosynthesis and Φ_{PSII} are highly correlated under controlled conditions (Genty et al., 1989; Edwards and Baker, 1993) and in the field (Leipner et al., 1999). Further selectable traits for improving early vigor are the carbon exchange rate and the rate of development from the seven- to eight-leaf tip stage (Lee et al., 2002). The first objective of this study was to characterize genotypes according to their aboveground appearance based on leaf area and photosynthetic performance.

However, evaluating only aboveground traits does not take the genetic variability in the development of the root system into consideration. According to the literature on root morphology

Abbreviations: $\hat{A}_p$, estimated rate of CO_2 assimilation per plant; PCA, principal component analysis; DW, dry weight; CV_G, genetic coefficient of variation; CrAx, crown axile root; PPFD, photosynthetic photon flux density; PrAx, primary axile root; PrLat, primary lateral roots; SeAx, seminal axile roots; SeLat, seminal lateral roots; SPAD, chlorophyll content (soil plant analysis development); Φ_{PSII}, operating efficiency of photosystem II

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doi:10.1016/j.eja.2007.01.003

there is a correlation between a large seedling root system and ear weight, plant height, earliness (Andrew and Solanki, 1966; Richner et al., 1997), and silage yield under field conditions (Richner et al., 1997).

Furthermore, according to Engels (1994), inhibition of root growth is the most limiting factor for the early acquisition of nutrients by maize at low temperature. This is supported by the findings of Stamp (1984) who also reported that, under chilling conditions, the growth of the roots of chilling-susceptible genotypes was hindered to a much greater extent than the development of the shoots. When examining the morphological components of the roots, two points must be considered: the different root types (primary, seminal, and crown) can be examined separately and the axile and lateral roots can be treated as different root populations (Cahn et al., 1989). QTL studies (Tuberosa et al., 2002; Hund et al., 2004) and mutation experiments (Feix et al., 2000) proved that these traits are under different genetic control.

The primary and seminal roots are part of the embryonic root system, which can be classified as homogeneous or heterogeneous. For an homogeneous root system both root types, the primary and seminal roots, have similar ratios of the lateral-to-axile roots. In contrast, for heterogeneous embryonic root systems the ratio of lateral-to-axile roots of the primary root is much higher and consequently the primary root represents a large portion of the embryonic root system. Our second objective was to characterize the genotypes according to their root morphology by comparing the lengths of the different root types and their first-order lateral roots.

From a breeding perspective root morphological traits, such as an heterogeneous or homogeneous root system, can provide important information about early vigor under long-term mild chilling stress. Information about selectable root traits is scarce and usually limited to the root–shoot dry weight ratio. The extent, to which the root morphology is reflected by the expression of shoot parameters is unknown; thus, we present information about the root morphology and how it is associated with early vigor.

2. Materials and methods

2.1. Genotypes

A set of 21 inbred lines was chosen on the basis of (i) scores for vigor in field tests and (ii) the operating efficiency of photosystem II (Φ_{PSII}) in growth chambers (Table 1; vigor/Φ_{PSII}). The ETH lines are experimental lines derived from a divergent selection for Φ_{PSII} (Fracheboud et al., 1999); S335 showed greater vigor under conditions in Poland (Sowinsky et al., 1998). For the cross of the lines (Ac7643 × Ac7729/TZSRW, Lo964 × Lo1016, and ETH-DH7 × ETH-DL3) linkage maps of their segregating offspring are available (Fracheboud et al., 2002, 2004; Tuberosa et al., 2002).

Table 1
Genetic material used

Line	Label and kernel type[a]	Vigor/Φ_{PSII}[b]	Origin[c]
Ac7643	a	Φ_{PSII}+	CIMMYT
Ac7729/TZSRW	b	Φ_{PSII}−	CIMMYT
CM105	c	L	AgCanada
DSP-1387C	d	M	DSP
DSP-1387F	e	H	DSP
DSP-1771F	f	M	DSP
ETH-DH1	g	Φ_{PSII}+	ETH
ETH-DH7	h	Φ_{PSII}+	ETH
ETH-DL3	i	Φ_{PSII}−	ETH
Lo1016	k	na[d]	CRA
Lo964	l	na	CRA
S335	m	H	IHAR
D167	n	L	HOH
D171	o	H	HOH
DSP-1639H	p	H	DSP
DSP-1911D5	q	H	DSP
ETH-EH3	r	Φ_{PSII}+	ETH
ETH-FH1	s	Φ_{PSII}+	ETH
ETH-FL1	t	Φ_{PSII}−	ETH
ETH-FL8	u	Φ_{PSII}−	ETH
Z7	v	Φ_{PSII}+	Zelder

[a] Letter labels to identify individual lines in Figs. 1–3; a–m = dent, n–v = flint kernel types.

[b] Plants were chosen on the basis of high (Φ_{PSII}+) and low (Φ_{PSII}−) Φ_{PSII} in previous growth chamber experiments or on high (H), medium (M), and low (L) vigor, based on scores of DSP.

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[d] na = no information about Φ_{PSII} or vigor rating.

2.2. Experimental design and growing conditions

The experiments were carried out in growth chambers (PGW36, Conviron, Winnipeg, Canada) with a 12 h photo- and thermoperiod and at 60/70% (day/night) relative air humidity and 500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) in the centre of the growth chamber. The temperatures were set to obtain 15/13 °C (day/night) soil temperature and a corresponding air temperature at the canopy level of 16/13 °C (day/night). The growth substrate was a mixture of quartz sand (particle size 0.08–0.2 mm) and 5% (w/w) vermiculite powder (Vermex Pulver E, Vermica AG, Bözen, Switzerland) with a volumetric nutrient solution content of 15%. The nutrient solution was a modified Hoagland solution containing 7.0 mM $Ca(NO_3)_2$, 2.0 mM $MgSO_4$, 1.0 mM H_3PO_4, 1.5 mM K_2SO_4, 0.16 mM FeNa–EDTA, 0.05 mM KCl, 18.0 μM $MnSO_4$, 12.0 μM H_3BO_3, 1,5 μM $ZnSO_4$, 0.6 μM $CuSO_4$, and 4.2 μM MoO_3. A pH of 7 was obtained by adding H_2SO_4 to the nutrient solution. Moist substrate was packed into PVC growth columns (5.6 cm diameter and 50 cm height) to a bulk density of 1.25 Mg m^{-3}; after planting, the surface was covered with an isolation layer (1 cm) of Perlit (PePe® Pflanzen Perlit, Otto Hauenstein Samen AG, Switzerland).

Twenty-five seeds per genotype with a line-specific weight (average seed weight of the line ±10%) were surface-sterilized for 12 min with 2.5% NaOCl and pre-germinated in plastic boxes

on filter paper at 25 °C. Germination was recorded and germinated seeds with a 0.3–2 cm-long primary root were placed in the substrate at a depth of 2 cm. The growth columns were covered with transparent plastic foil to prevent evaporation. Slits were cut into the foil above the emerging coleoptile. Once the coleoptile had reached a length of ~1 cm, the columns were covered with aluminum foil to prevent warming of the upper zone of the substrate due to the light radiation.

2.3. Measurements

Germination was recorded twice a day for the first 4 days and then daily until the seventh day. From these data, a germination index (GI) was calculated using the formula given by Smith and Millet (1964):

$$\frac{\sum[\text{number of seeds germinated on a given day} \times \text{number of days after imbibition}]}{\text{total number of seeds germinated}} \quad (1)$$

The percentage of germinated plants was recorded 7 days after imbibition.

Photosynthesis-related measurements were performed on the second leaf between the collar and the leaf tip at a leaf temperature of 16 °C. The chlorophyll *a* fluorescence of the second leaves was measured with a pulse-amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). The operating efficiency of photosystem II (Φ_{PSII}) was measured at PPFD by always moving the plants to the same position in the growth chamber. The PPFD during the measurements was recorded with the quantum sensor of the leaf-clip holder (2030-B, Walz). The chlorophyll content of the second and third leaf was measured with a SPAD-502 Chlorophyll Meter (Minolta Corporations, Ramsey, NJ, USA). The estimated leaf absorption (α_L) was calculated from SPAD values using the relation (Earl and Tollenaar, 1997):

$$\alpha_L = \min + (\max - \min) \times (1 - e^{-k\,\mathrm{SPAD}}) \quad (2)$$

where min, max, and k values were 0.548, 0.981, and 0.0517, respectively. The constants were derived from the relationship between the SPAD values and α_L ($R^2 = 0.78$), measured with an integrating sphere on 232 plants in the second replication. The estimated photosynthesis per unit leaf area ($\hat{A}_a$) was calculated as

$$\hat{A}_a = \mathrm{PPFD} \times \alpha_L \times \Phi_{PSII} \times 12^{-1} \ [\mu\mathrm{mol\,CO_2\,m^{-2}\,s^{-1}}] \quad (3)$$

where PPFD is the flow of the photosynthetic active photons recorded by the leaf-clip holder of the PAM-2000, α_L the portion of applied photons that were absorbed by the leaf, and Φ_{PSII} the portion of absorbed photons that were used for electron transport at PSII. It is assumed that 12 mol of transported electrons are required to fix 1 mol CO_2 (Edwards and Baker, 1993). The estimated rate of CO_2 assimilation per plant was calculated as

$$\hat{A}_p = \hat{A}_a \times \text{leaf area} \ [\mu\mathrm{mol\,CO_2\,plant^{-1}\,s^{-1}}] \quad (4)$$

The leaf area of the first, second, and partially developed successive leaves were measured with a leaf-area meter (LI-COR 3100, Lincoln, NE, USA). The plants were harvested 29 days after imbibition when more than half the plants had reached the two-leaf (V2) stage. The stage was defined by the number of leaves with visible collars (Ritchie and Hanway, 1984).

The roots were taken from the growth columns by removing the soil under pressurized tap water and were separated into primary, seminal, and crown roots. The axile roots, *i.e.* the main axes of the primary (PrAx), seminal (SeAx) and crown (CrAx) roots, were counted and their length measured. The length of the longest primary lateral root (PrLat) was measured on a centimeter scale. The individual seminal lateral roots (SeLat) were not measured. A figure illustrating the root terminology can be found in Hund et al. (2004). The roots were stored at −18 °C until further processing. For the digital measurement of the root morphology, the roots were cut and distributed evenly on a glass tray in a thin layer of water. The tray was placed on a flatbed scanner (Scanjet 4c, Hewlett Packard, CA, USA) to obtain 8 bit images (resolution 600 dpi × 600 dpi) and covered with a black box to obtain a uniform background. The root images were analyzed with the digital image analysis program RD (Root Detector, ETH Zurich, Switzerland; Walter and Bürgi, 1996). The dry matter of the leaf blades, shoots, and roots was determined separately after drying at 65 °C for 42 h.

2.4. Experimental design

A randomized complete-block design, combined over two growth chamber runs (replication) with four sub samples per run, was used. Each sub sample within a replication consisted of a complete, randomized set of 21 inbred lines. The experimental unit was one growth column with one plant. The data were processed according to the general linear models procedure (PROC GLM) of SAS 8.02 (1999). The factor "replication" was tested against the "sub samples within replication" mean squares. The factor "genotype" and the "genotype × replication" interaction were tested against the pooled error mean squares (McIntosh, 1983). The repeatability (ρ) was calculated according to (Falconer and Mackay, 1996)

$$\rho = \frac{V_G + V_{Eg}}{V_P} \quad (5)$$

where the numerator includes both the genetic variance (V_G) plus the general environmental variance (V_{Eg}) associated with the permanent differences between individuals (in this case inbred lines) and V_P represents the phenotypic variance. Thus, the repeatability across the $t = 2$ replications with $b = 4$ sub samples was calculated as

$$\rho = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_G + \hat{\sigma}^2_{GT}/t + \hat{\sigma}^2_e/bt} \quad (6)$$

where σ^2_G, σ^2_{GT}, and σ^2_e are the ANOVA estimates of the variance for genotype, genotype × replication, and error, respectively, and $\hat{\sigma}^2_G$ includes both V_G and V_{Eg}. The genetic coefficient of

Table 2
Summary statistics with minimum (Min.), maximum (Max.), and mean values, genetic coefficient of variation (CV_G), and repeatability (ρ) of seed, shoot, and root traits of 21 inbred lines grown at 15/13 °C (day/night) until the two-leaf stage

Trait	Max.	Min.	Mean	CV_G[a]	ρ[b]
Seed					
Grain weight (mg)[c]	348	164	250***	18	–
Germination index (d)	4.39	1.78	2.86***	22	–
Germination (%)	100	68.0	90.2***	12	–
Plant					
Overall dry weight (mg)	452	107	306***	27	87
Leaf area/root length (cm^2/cm)	0.16	0.04	0.09***	33	90
Shoot/root dry weight	1.62	0.81	1.21**	9	75
Shoot					
Shoot dry weight (mg)	231	66	155***	26	93
Leaf area (cm^2)	47.1	21.2	31.3***	21	89
Photosynthesis					
SPAD values	37.3	12.6	27.5***	26	96
Φ_{PSII}	0.531	0.014	0.365***	36	96
Est. photosynthesis ($\hat{A}_p$)[d]	0.073	0.001	0.043***	41	96
Roots					
Root dry weight (mg)	244	41	152***	7	85
Overall root length (cm)	810	152	397***	21	95
Axile root length (cm)	206	60	127***	16	88
Lateral root length (cm)	604	30	270***	30	95
Primary root					
Axile (PrAx) length (cm)	46.1	15.0	29.9***	28	88
Lateral (PrLat) length (cm)	516.7	21.2	218.0***	55	93
Max. length of ind. lateral (cm)	17.3	2.2	8.3***	38	95
Seminal roots					
Axile (SeAx) number	5.38	1.13	3.66**	25	76
Axile (SeAx) length (cm)	126.8	20.0	67.4***	37	79
Lateral (SeLat) length (cm)	181.0	3.3	52.1***	44	94
Crown roots					
Axile (CrAx) number	6.88	3.00	4.33**	19	70
Axile (CrAx) length (cm)	62.3	6.5	30.0***	21	85

** and ***: comparison of mean values of lines significant at $P \leq 0.01$ and 0.001, respectively.
[a] Genetic coefficient of variation (%), calculated from the ANOVA estimate of variance according to Eq. (7).
[b] Repeatability (%), calculated from the ANOVA estimates of variance according to Eq. (6).
[c] Average grain weight ±10%.
[d] μmol CO_2 $plant^{-1}$ s^{-1} (Eqs. (1)–(3)).

variance was calculated as

$$CV_G = \frac{\hat{\sigma}_G}{\bar{x}} \times 100 \quad (7)$$

where $\bar{x}$ is the mean value of the traits. Principal component analyses (PCA) of morpho-physiological traits were calculated from the between-traits (means across the two replications) correlation matrix using the PRINCOMP procedure of SAS.

3. Results

3.1. Genetic variation and repeatability

The analysis of variance revealed highly significant ($P \leq 0.001$) differences among genotypes in all the measured traits, with the exception of the shoot/root dry weight and the number of seminal axile roots and crown axile roots ($P \leq 0.01$) (data not shown).

The genetic coefficient of variation of the factor "genotype" (CV_G) was used to detect traits with high variability among lines (Table 2). The highest CV_G values (>40%) were found for the length of the PrLat and the SeLat as well as for the estimated photosynthesis ($\hat{A}_p$). The repeatability was taken as an estimate of the proportion of variability of a trait that is due to differences among genotypes rather than to uncontrolled variability among individuals. High repeatability (≥95%) was found for all the photosynthesis-related traits, the overall root length, the length of the lateral roots, and the length of the longest PrLat. The repeatability of the shoot/root dry weight ratio and the CrAx number was particularly low (≤75%).

3.2. Trait averages

A low germination index of ∼2.5 days (*i.e.* fast germination) and a high germination percentage of >90% was observed

Table 3
Pearson correlation coefficients between mean values of seed, root, and shoot traits and indicators of early vigor of 21 maize inbred lines grown at 15/13 °C day/night until the two-leaf stage

Trait	SPAD values	Φ_{PSII}	Leaf area	Overall root length	Plant DW
Seed					
Grain weight	0.19 ns	0.00 ns	0.28 ns	0.31 ns	0.32 ns
Germination index	−0.01 ns	0.11 ns	−0.05 ns	−0.24 ns	−0.19 ns
Germination	−0.07 ns	0.01 ns	0.17 ns	0.13 ns	0.12 ns
Plant					
Overall dry weight	0.72***	0.75***	0.63**	0.80***	1.00
Leaf area/root length	−0.68***	−0.54*	−0.11 ns	−0.79***	−0.61**
Shoot/root dry weight	−0.34 ns	−0.43 ns	0.04 ns	−0.53 *	−0.49 *
Shoot					
Shoot dry weight (mg)	0.69***	0.73***	0.76***	0.68***	0.93***
Leaf area	0.21 ns	0.33 ns	1.00	0.57**	0.63**
Photosynthesis					
SPAD values	1.00	0.87***	0.21 ns	0.59**	0.72***
Φ_{PSII}	0.87***	1.00	0.33 ns	0.56**	0.75***
Est. photosynthesis	0.78***	0.90***	0.68***	0.44*	0.89***
Roots					
Root dry weight	0.66**	0.69***	0.45*	0.81***	0.94***
Overall root length	0.59**	0.56**	0.57**	1.00	0.80***
Axile root length (cm)	0.26 ns	0.37 ns	0.41 ns	0.57**	0.38 ns
Lateral root length	0.59**	0.52*	0.52*	0.97***	0.79***
Primary roots					
Axile (PrAx) length	0.27 ns	0.37 ns	0.52*	0.67***	0.52*
Lateral (PrLat) length	0.58**	0.46*	0.46*	0.89***	0.79***
Max. length of laterals	0.47*	0.47*	0.45*	0.73***	0.75***
Seminal roots					
Axile (PrAx) number	0.01 ns	0.10 ns	0.29 ns	0.13 ns	0.22 ns
Axile (PrAx) length	0.19 ns	0.35 ns	0.27 ns	0.45*	0.25 ns
Lateral (PrLat) length	0.30 ns	0.42 ns	0.42 ns	0.67***	0.38 ns
Crown roots					
Axile (PrAx) number	0.47*	0.39 ns	0.02 ns	0.06 ns	0.32 ns
Axile (CrAx) length	0.26 ns	0.18 ns	0.41 ns	0.45*	0.38 ns

*, **, ***: correlation coefficients are significant at $P \leq 0.05$, 0.01, and 0.001, respectively. ns: not significant.

for two-thirds of the lines. The poorest seed performance was observed for the lines DSP-1639 and Ac7643 with a germination index of ~4 days (*i.e.* slow germination) and a germination percentage of <80% (data not shown).

The mean dry weight of the roots and shoots was each about 150 mg per plant; the plant dry weight at harvest thus exceeded the seed dry weight. The overall length of the axile roots was roughly half that of the overall length of the lateral roots (Table 2). The maximal length of the individual laterals roots depended on the genotype and root type. PrLat showed the most extreme variation among genotypes ($CV_G = 55\%$) and high repeatability (93%). The maximal individual PrLat ranged from 17 cm for Z7 to as low as 2.2 cm for Lo1016. The primary roots of the latter line were hardly distinguishable from its seminal roots. The PrLat length contributed, on average, to 81% of the fine root structure and to 55% of the overall root length.

3.3. Single-trait correlations with measures of early vigor

To reduce the correlation matrix only five traits, that were considered to provide important information about early vigor, were correlated with all the other traits: the SPAD values, Φ_{PSII}, and the leaf area as components of photosynthetic carbohydrate supply; the overall dry weight (DW) of the plant for the ability to use carbohydrate for growth; the overall root length as a measure of the potential for nutrient and water uptake (Table 3).

Despite the relatively early harvest stage, dry matter accumulation was not significantly associated with seed characteristics (Table 3). In contrast, a close relationship between Φ_{PSII} and plant DW indicates that the autotrophic carbohydrate supply already played an important role.

The leaf area/root length ratio was significantly negatively correlated with the SPAD values, Φ_{PSII}, plant DW, and the overall root length, indicating that the root length increased for plants with a high overall performance. The shoot/root dry weight ratio followed the same pattern, but the correlation was not always significant. The overall root length was strongly correlated with plant DW and modestly with the photosynthesis-related traits. The length of the lateral roots was significantly correlated with the SPAD values, Φ_{PSII}, and the leaf area, whereas the axile root length was not.

Table 4
Principal components of photosynthesis-related traits

Statistic	Prin 1	Prin 2	Prin 3
Eigenvalues and variability			
Eigenvalue	2.03	0.85	0.12
Proportion of variability (%)	68	28	4
Variable	Attribute loading for		
	Prin 1	Prin 2	Prin 3
Eigenvectors			
Leaf area	0.37	0.92	0.12
Φ_{PSII}	0.67	−0.18	−0.72
SPAD values	0.64	−0.34	0.68

Eigenvalues, proportion of variability, and eigenvectors of the first 3 principal components are given. For further information see Fig. 1.

3.4. Principal component analysis

We used three principal component analyses (PCAs) to address the three objectives of the study: to characterize genotypes according to: (i) photosynthesis-related traits; (ii) root structural traits; (iii) relate root and shoot traits and their relation to early vigor, as indicated by early accumulation of plant dry matter.

3.5. PCA of photosynthesis-related traits

The first PCA model was calculated based on the three traits of $\hat{A}_p$ (leaf area, Φ_{PSII}, and SPAD values). The total variability (96%) of the three-dimensional space is efficiently summarized by the first 2 principal components, which account for 68 and 28% of the variability (Table 4). The first component (Prin 1) can be interpreted as representing the overall photosynthesis, which is based primarily on Φ_{PSII} and SPAD and, to a lesser extent, on the total leaf area per plant. The second principal component (Prin 2) has high positive loadings for the leaf area and moderate negative loadings for Φ_{PSII} and SPAD. It differentiates the two strategies for achieving a large supply of photosynthetic carbohydrates: a large leaf area and a high efficiency of the photosynthetic apparatus.

Plotting the individual inbred lines by means of their component scores (Fig. 1) reinforces this interpretation. The first component distinguishes lines with the highest $\hat{A}_p \leq 0.058\ \mu mol\ CO_2\ plant^{-1}\ s^{-1}$ (Fig. 1A>) and the lowest $\hat{A}_p \leq 0.024\ \mu mol\ CO_2\ plant^{-1}\ s^{-1}$ (Fig. 1A<). The second component distinguishes lines with the largest leaf area > 40 cm^2 per plant (Fig. 1B>) from those with the smallest leaf area of ~20 cm^2 per plant (Fig. 1B<).

3.6. PCA of root structural traits

A second PCA was performed on the length of the different root types (PrAx, SeAx, CrAx, PrLat, and SeLat). The first 3 principal components account for 56, 22, and 17% of the variability (Table 5). Prin 1 is interpreted as a measure of the overall root length, because it shows approximately equal loadings for all variables. Prin 2 has high positive loadings for CrAx and PrLat and high negative loadings for SeAx and SeLat (Table 5) as well as a weak positive loading for PrAx. Therefore, high positive values indicate an heterogeneous embryonic root system with a dominant primary root, while high negative values indicate an homogeneous (homogenous) embryonic root system with similar sized primary and seminal roots. Prin 3 measures the contribution of axile and lateral roots to the total root length. It has high positive loadings for PrLat and SeLat and a high negative loading for CrAx as well as moderate to weak negative loadings for PrAx and SeAx.

Fig. 2 supports this interpretation. The first component separates lines with the slowest root growth (CM105 = 152, Lo1016 = 177, Ac7729/TZSRW = 209, and ETH-FL8 = 251 cm overall root length; Fig. 2C<) from those with the fastest growing roots (ETH-FH1 = 810, ETH-DH1 = 689, ETH-DL3 = 566, and Z7 = 564 cm overall root length; Fig. 2C>). The second component distinguishes lines with a heterogeneous root system with

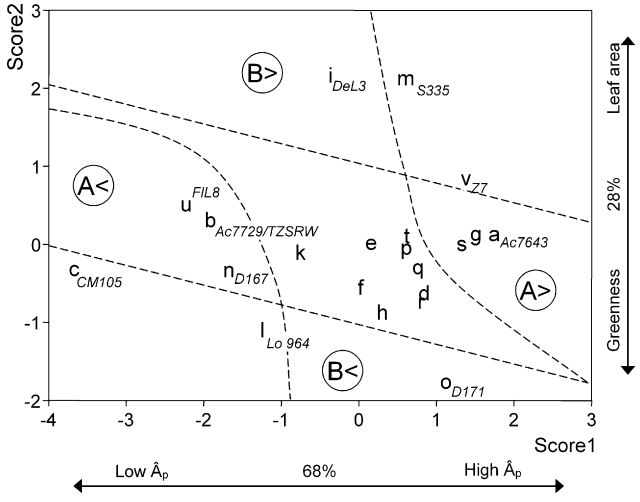

Fig. 1. Scores of the principal components 1 and 2 of the photosynthesis-related traits (leaf area, operating efficiency of photosystem II (Φ_{PSII}), and chlorophyll content (SPAD values)). Components were calculated from the correlations between traits. Letters indicate individual genotypes and are given in the materials and methods. a–m = dent, n–v = flint kernel type. Outstanding *inbred lines* are given the corresponding letter. Dashed lines combine genotypes with highest (A>) and lowest (A<) estimated photosynthesis per plant ($\hat{A}_p$) and biggest (B>) and smallest (B<) leaf area. Arrows indicate the interpretation of the principal component and the proportion of explained variability is given (see Table 4).

Table 5
Principal component analysis of root length data

Statistic	Prin 1	Prin 2	Prin 3
Eigenvalues and variability			
Eigenvalue	2.81	1.08	0.86
Proportion of variability (%)	56	22	17
Variable	Attribute loading for		
	Prin 1	Prin 2	Prin 3
Eigenvectors			
Length of primary axile roots (PrAx)	0.56	0.09	−0.20
Length of seminal axile roots (SeAx)	0.50	−0.47	−0.15
Length of crown axile roots (CrAx)	0.40	0.49	−0.52
Length of primary lateral roots (PrLat)	0.28	0.60	0.67
Length of seminal lateral roots (SeLat)	0.45	−0.41	0.47

Eigenvalues, proportion of variability, and eigenvectors for the first 3 principal components are given. For further information see Fig. 2.

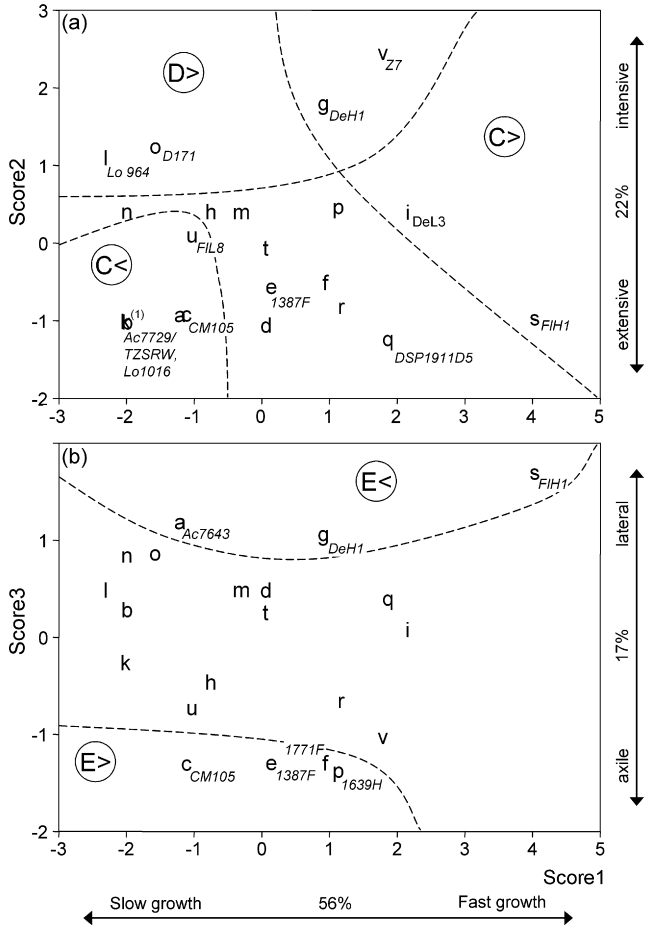

Fig. 2. Principal components analysis of root length (primary, seminal, and crown axile roots as well as primary and seminal lateral roots). Plot of scores for components 1 and 2 (a) and 1 and 3 (b). Dashed lines combine *inbred lines* with highest (C>) and lowest (C<) overall root length; highest (D>) portion of the primary root of the embryonic root system and highest (E>) and smallest (E<) share of crown roots of the overall root system. Arrows indicate the interpretation of the principal component and the proportion of explained variability is given (see Table 5). For more information see Fig. 1. (1) b and k are overlapping.

the highest portion (>80%) of the primary root on the embryonic root system (Fig. 2D>) from those with a more homogenous root system with the lowest portion (≤51%) of the primary root on the embryonic root system (Lo1016, CM105, DSP-1911D5, and DSP-1387F). The third component discriminates between a relatively fine root structure and a strong root framework, the latter of which is dominated by axile roots. Thus, the four lines with the highest portions (≥13%) of the CrAx length of the overall root system have the highest negative scores (Fig. 2E>). In contrast, three of seven lines with the lowest portion (≤6%) of the CrAx length (Fig. 2E<) have highest positive scores.

3.7. PCA of root and shoot traits

A final PCA model was used to elucidate relationships among photosynthesis-related traits, root structural traits, and plant DW as well as to discriminate between high-vigor and low-vigor genotypes. The first 3 principal components explain 51, 18, and 12% of the total variability. Prin 1 (Table 6) is an overall measure of vigor since it shows modest loadings for all attributes. Prin 2 shows modest negative loadings for SPAD values, Φ_{PSII}, plant DW, and PrLat length as well as modest to high positive loadings for SeAx, PrAx, and SeLat length. Therefore, it distinguishes lines with a relatively heterogeneous root system, high photosynthesis capacity, and high plant DW from relatively poorly performing lines with an homogeneous root system. Prin 3 also distinguishes homogeneous and heterogeneous rooting. In contrast to Prin 2, the measures of an homogeneous root system (high SeAx and SeLat length) of Prin 3 are associated with SPAD values and Φ_{PSII}. PrLat length is the only trait associated with plant DW in all three principal components.

Based on the first 2 components the lines can be split into three groups according to their overall performance (Fig. 3; F<, F, and F>). Lines with poor performance (Fig. 3F<) a plant DW < 250 mg are clearly separated from high-performance lines (Fig. 3F>) with a plant DW of ∼450 mg. Within the three groups, the lines can be separated into genotypes with homogeneous (CM105, ETH-DL3, and ETH-FH1) and heterogeneous (Lo964,

Table 6
Principal components of morpho-physiological traits of shoots and roots

Statistic	Prin 1	Prin 2	Prin 3
Eigenvalues and variability			
Eigenvalue	4.62	1.62	1.11
Proportion of variability (%)	51	18	12
Variable	Attribute loading for		
	Prin 1	Prin 2	Prin 3
Eigenvectors			
Plant dry weight	0.41	−0.28	0.10
Leaf area	0.32	0.07	0.33
Φ_{PSII}	0.36	−0.29	−0.35
SPAD values	0.33	−0.41	−0.23
Length of primary axile roots (PrAx)	0.37	0.37	0.16
Length of seminal axile roots (SeAx)	0.29	0.51	−0.35
Length of crown axile roots (CrAx)	0.27	0.24	0.56
Length of primary lateral roots (PrLat)	0.32	−0.33	0.24
Length of seminal lateral roots (SeLat)	0.30	0.30	−0.44

Eigenvalues, proportion of variability, and eigenvectors for the first three principal components are given. For further information see Fig. 3.

D171, and ETH-DH1) embryonic root systems. In summary, the genotypes with the best performance (e.g. ETH-DH1) plot in the lower right corner of Fig. 3 while those with the poorest performance (e.g. CM105) plot in the upper left corner.

4. Discussion

4.1. Autotrophic versus heterotrophic carbohydrate supply

In general, the evaluation of early vigor is conducted at the autotrophic growth stage, which starts approximately when

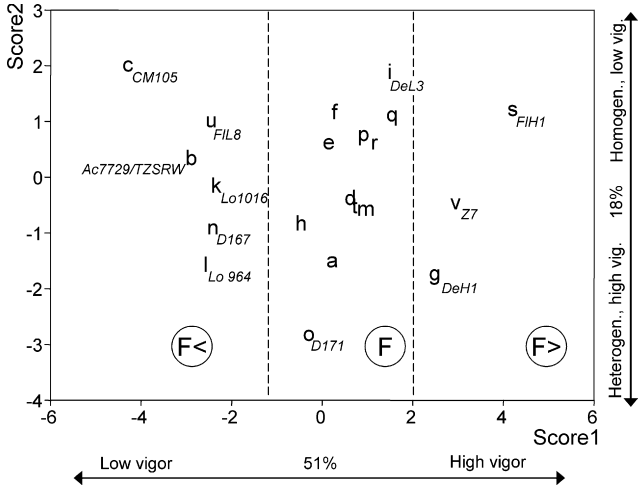

Fig. 3. Scores of principal components 1 and 2 of morpho-physiological traits of shoots (leaf area, operating efficiency of photosystem II, and chlorophyll content), root length (primary, seminal, and crown axile roots as well as primary and seminal lateral roots), and plant dry weight. Dashed lines combine genotypes with highest (F>), intermediate (F), and lowest (F<) overall vigor. Arrows indicate the interpretation of the principal component and the proportion of explained variability is given (see Table 6). For further information see Fig. 1.

the third leaf is fully expanded and endosperm reserves are exhausted (Derieux et al., 1989). This evaluation was performed at the relatively early V2 stage to reduce the time required for the root morphology analyses. Seeds were germinated at 25 °C to minimize the influence of differences in cold tolerance during germination, which was not the focus of the study. Using seed traits (Table 2) as covariates in a multiple regression model, with plant DW as the dependent variable, did not increase the precision of the model (data not shown). We therefore conclude that differences in seed size and germination did not influence early plant performance in this study. However, since seeds were produced in different environments, seed size and germination effects on plant DW could be masked by other seed quality effects. The close relationship of Φ_{PSII} with plant DW indicates that, under long-term mild chilling stress, strong photosynthetic performance plays an important role in plant development even prior to the V3 stage. This is supported by the results of two QTL studies based on material reported here: (i) for a QTL mapping population derived from ETH-DL3 × ETH-DH7 plant DW and Φ_{PSII} were co-located at the major locus on chromosome 6, explaining about 10% of the phenotypic variability (Fracheboud et al., 2004) and (ii) for a QTL mapping population derived from Lo964 × Lo1016 two loci, each explaining about 10% of the phenotypic variability of the above traits, were detected at the end of chromosome 10 (Hund et al., 2004).

4.2. Shoot morphology and photosynthesis-related traits

The inbred lines in this study were separated by means of PCA into those maximizing the leaf area at the expense of the efficiency of the photosynthetic apparatus and those maximizing the photosynthetic efficiency at the expense of the leaf area. Furthermore, Lee et al. (2002) reported different responses of traits that underlie $\hat{A}_p$. In their study, the maintenance of the carbon exchange rate, which is related to Φ_{PSII} and SPAD, and

the rate of development from the seven- to the eight-leaf stage, which is related to the leaf area, were identified as promising discriminators for cold tolerance. As Lee et al. (2002) pointed out, it is a challenge to determine whether the maintenance of photosynthesis per unit leaf area or rapid leaf development is the more desirable trait. The selection of the strategy will probably depend on the typical duration of the cold stress in the target environment.

4.3. Root morphology

The genotypes were clearly separated according to root morphology. Apart from the overall root length, ranging from 138 cm (CM105) to 810 cm (ETH-FH1), the organization of the embryonic root system provided the most information. Lo964 and D171 had the most heterogeneous root system with the greatest portion of the primary root; those with the most homogeneous root system were Lo1016 and CM105 with the lowest portion of the primary root. The differences were due to (i) a much greater ratio of the PrLat/PrAx length of Lo964 and D171 (15.9 and 13.1, respectively) compared to the PrLat/PrAx length ratio of Lo1016 and CM105 (3.1 and 0.8, respectively) and (ii) a lower number of SeAx of Lo964 and D171 (1–2) compared to the number of SeAx of Lo1016 and CM105 (4–5). The organization of the embryonic root system may be due to independent genetic factors, each controlling certain root traits. This is supported by the presence of root mutants, showing distinct root types such as rtcs (Hetz et al., 1996), lacking all shoot-borne crown and brace roots as well as embryonic seminal roots. However, a QTL study of a population derived from the Lo964 × Lo1016 cross indicates that pleiotropic effects may be also involved in organization of the embryonic root system (Hund et al., 2004), since two QTLs, controlling the primary root diameter, were associated with QTLs controlling seminal root length. Our observation that PrLat of some genotypes (e.g. Z7) was considerably longer than the SeLat is in accordance with the observation of Cahn et al. (1989), who reported the same finding for the maize cultivar Cornell 175. The maximum length of SeLat was not determined here, but the findings of McCully (1999) support our visual impression that most of the SeLat at the early stage are short (mode ≤ 3 cm). This is further supported by results of Richner et al. (1997), who found that the mean length of the individual PrLat of hybrids at the V3 stage was about 2.7 cm longer than the mean length of individual SeLat. In contrast to earlier findings (Wiggans, 1916; Siemens, 1929), the kernel type (flint versus dent) was not associated with a certain root morphology.

4.4. Relationship between root and shoot traits

Information about the relationship between root and shoot traits of maize under chilling stress is scarce. If the root morphology were closely related to aboveground traits, then the assessment of root morphological parameters would not produce significantly more information about the performance of a genotype. However, in accordance with Stamp (1984) the root growth of chilling sensitive genotypes in our study was hindered to a much greater extent than shoot growth. This was indicated by a significant, negative correlation of the leaf area/root length ratio with SPAD values, Φ_{PSII}, plant DW, and root length. This correlation was particularly driven by the large differences in the PrLat length of the genotypes, ranging from a minimum length of 21 cm for CM105 to a maximum length of 517 cm for ETH-DH1 (Table 2). Furthermore, the significant correlation between the PrLat length on one hand and SPAD values and Φ_{PSII} on the other suggests that there is a genetic relationship between these traits (Table 3). The second component of the final PCA model confirmed the association of a very long PrLat with the above traits and with DW, in contrast to all the other root traits (Table 6). Therefore, based on the first 2 principal components, the genotypes were separated into high-vigor genotypes with heterogeneous root systems (e.g., line ETH-DH1) and low-vigor genotypes with homogeneous root systems (e.g., line CM105). It is unclear as to, why a more heterogeneous rooting is associated with a better photosynthetic performance. The hypothesis that the population stratification (flint versus dent) severely biased the association between root morphology photosynthetic performances was not supported. Root morphology was not associated with a certain kernel type. Therefore, pleiotropic effects are likely. However, an heterogeneous root system was not always associated with superior photosynthetic performance or a higher DW. For example, the two previously mentioned lines with a heterogeneous root system, where among the lines with moderate (D171) to poor (Lo964) dry matter accumulation. Furthermore, a QTL mapping of the root morphology for the population derived from heterogeneous rooting Lo964 and homogenous rooting Lo1016 did not yield a co-location of QTLs of PrLat length and photosynthesis-related traits (Hund et al., 2004).

The three genotypes with the highest scores in Prin 1 (Z7, ETH-FH1, and ETH-DH1) were superior with respect to overall root length, plant DW, and photosynthetic performance. Z7 is a European flint line, developed in the 1980s, and widely used for cold tolerance studies of maize. Interestingly, of the modern inbred lines, only ETH-FH1 and ETH-DH1, derived by the selection for high Φ_{PSII} (Fracheboud et al., 1999), were equal or superior to Z7.

5. Conclusions

The estimated CO_2 assimilation rate per plant ($\hat{A}_p$) accounted for as much as 79% (Table 3; $r = 0.89$) of the variation in plant DW, more than any other trait, with the exception of shoot DW. Genotypes with a heterogeneous embryonic root system could be clearly separated from genotypes with a homogenous embryonic root system. Mostly the genotypes with a more heterogeneous root system and with longer lateral roots, also had a higher plant dry weight and improved photosynthetic performance, whereas the axile root length was not associated with better performance. It is still unclear whether this relationship is due to pleiotropic effects (e.g. better photosynthetic performance resulting from better nutrient acquisition by lateral roots), or due to undetected population structure influencing the results. It is important to elucidate how differences in root morphology,

such as the increase of lateral roots as opposed to axile roots, are expressed in hybrids and how they affect field performance.

Acknowledgements

We would like to thank the breeders listed in Table 1 for the supply for the genetic material and the anonymous reviewers for their valuable comments. This study was supported by the Swiss Federal Office for Education and Science, International Research Programs—COST.

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