

## Original Paper

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## Early growth of field-grown swiss flint maize landraces

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### Abstract

Mild cold stress (chilling) limits early growth of maize (*Zea mays* L.) in central and northern Europe. Introgression of chilling tolerance from landraces has been proposed, because the genetic basis for chilling tolerance of European Flint x Dent hybrids is small. Therefore, the aim of this study was a detailed characterization of the chilling tolerance of Swiss maize landraces, hypothesizing a relatively good performance in marginal thermal environments. The environments were set up by different sowing dates in two years. A functional growth analysis of the shoot from the one-leaf to the six-leaf stage was conducted with eight Swiss landraces and a check hybrid (Magister). The mean air temperature calculated across the six environments was above 15°C. Under these conditions, none of the landraces grew consistently better than Magister. Some landrace-specific relative growth reactions were observed compared to Magister, apparently due to strong changes in the temperature course. However, based on this study direct use of Swiss maize landraces in breeding for the improvement of chilling tolerance is not recommended. More detailed investigations of promising landraces are proposed.

**Keywords:** maize, flint, landrace, chilling tolerance, early growth

### Introduction

Sowing of maize (*Zea mays* L.) in central and northern Europe is constrained by the temperature. Sowing too early may mean that plants are exposed to low temperature, i.e. a mild cold stress (chilling) (Leipner and Stamp, 2009); sowing too late may result in plants not reaching maturity. Therefore, better chilling tolerance of maize in spring is essential. Chilling gradually represses growth without a loss of cellular functions (Fraoocq et al, 2009) and usually occurs between 10 and 15°C (Stamp et al, 1997). Although maize yields in Europe have increased dramatically in the last decades due to the hybridization of Northern European Flint with high-yielding American Corn Belt Dent (Reif et al, 2005), the chilling tolerance of these hybrids is relatively low, because Corn Belt Dent is usually chilling-sensitive (Hardacre and Eagles, 1986; Schnell, 1992; Stehli et al, 1999; Leipner and Stamp, 2009). However, the chilling-adapted traditional European Flint maize (*Zea mays* var. *indurata* L.) is a valuable source of early vigor (e.g. Frei, 2000; Mieg et al, 2001; Ordas et al, 2006; Revilla et al, 2006; Peter et al, 2009a and b). Since the actual genetic basis of the breeding pool of Northern European Flint is relatively narrow with regard to alleles for chilling tolerance (Stehli et al, 1999; Frei, 2000), introgression of such traits from landraces has been proposed.

Some of the original maize germplasm, introduced into Europe from North America, was pre-adapted to relatively high latitudes. It is assumed that this facilitated its proliferation in the cool, temperate climate of northern Europe (Rebourg et al,

2003; Dubreuil et al, 2006). However, further adaptation in Europe is possible, because the chilling tolerance of some European maize races is outstanding (Leng et al, 1962; Miedema et al, 1987; Peter et al, 2009a and b). Considerable natural selection for chilling tolerance might have played a role because of the changeable climate in northern Europe, alternating between warm and cool periods (cf. Ackermann, 1995). As a result, specific European maize populations may have evolved (Brandolini, 1971; Gouesnard et al, 2005). It was found that Swiss Flint maize landraces vary considerably with regard to chilling tolerance and early vigor, sometimes combining good early establishment with good early production of biomass, thereby outperforming even a vigorous modern hybrid (Peter et al, 2009a and b).

The present study focused on the analysis of the heterotrophic and early autotrophic growth of eight Swiss maize landraces (open-pollinated populations), chosen from the two main clusters of landraces identified by Peter et al (2009a and b) and Eschholz et al (2008 and 2010), and a modern hybrid. In 2008 and 2009 six field experiments with different sowing dates between mid-April (early) and end of May (late), were established with the aim of exposing maize to contrasting air temperature environments. The objectives of the growth analysis were to (1) obtain precise information about specific growth reactions to the temperature regimes and (2) determine whether some landraces respond better to these regimes. Our hypotheses were: (1) The relatively better adaptation of Swiss maize landraces to chilling stress becomes more

**Table 1** - Information about the maize landraces and the check hybrid.

Variety name	Accession identity (BDN, 2010)	Canton of origin	Place of origin	Altitude (m a.s.l.)	Latitude (°N)	Longitude (°E)	TKW <sup>1</sup> (g)
003PR	13001003	Grisons	Rodels	625	46.7	9.4	307
025VS	13001025	Valais	Drône	894	46.3	7.4	369
074TM	13001074	Ticino	Preonzo	250	46.3	9.0	206
098RV	13001098	St Gallen	Eichberg	465	47.3	9.5	314
103RV	13001103	St Gallen	Au	403	47.2	8.6	365
125PV	13001125	Grisons	Brusio	780	46.3	10.1	236
179RV	13001179	St Gallen	Buchs	447	47.2	9.4	398
198TM	13001198	Ticino	Ludiano	477	46.4	9.0	115
Magister	Hybrid	-	-	-	-	-	336

<sup>1</sup> TKW: thousand kernel weight

evident as the thermal environment becomes more marginal for maize growth. (2) In marginal temperature environments, some Swiss maize landraces may outperform modern hybrids.

## Materials and Methods

### Plant material

Eight Swiss maize landrace accessions (Table 1) were selected from the core collection proposed by Eschholz et al (2008). The main criteria for the choice of accession were: maximal degree of genetic diversity among the chosen landraces and minimal inbreeding coefficient within the landraces. Furthermore, the accessions were chosen to represent the principal regions of origin. Three of the landraces stem from the northern part of Switzerland [lower Rhine valley (RV)], three from the southern part [Ticino (TM) and the Poschiavo valley (PV)] and two from the central alpine regions [the Posterior Rhine valley (PR) and Valais (VS)]. Figure 1 shows the climate diagrams of central locations in these three regions (Bad Ragaz in the Rhine valley, Sion in Valais and Locarno in Ticino). The hybrid Magister (Syngenta Seeds AG, Basel, Switzerland) was included as a check due to its outstanding early growth under cold conditions (Menzi et al, 2007; Peter et al, 2009a and b). Hereafter, both the landraces and the hybrid are referred to as “accessions”.

### Growth conditions

The experiments were conducted in 2008 and 2009 on the Swiss plateau (Lindau, 540m a.s.l.), near the experimental station of the ETH Zurich in Eschikon (47°26'N / 8°40'E). The soil is a Cambisol (IUSS Working Group WRB, 2006). There were two (2008) and four sowing dates (2009). Table 2 gives the temperature data of the six environments. Cumulative growing degree days (GDD) were calculated according to Jones and Kiniry (1986), starting on the sowing date. The base temperature was set at 8°C. Air temperature data were obtained from a nearby (ca. 2 km) weather station (Agrometeo, Lindau, Switzerland).

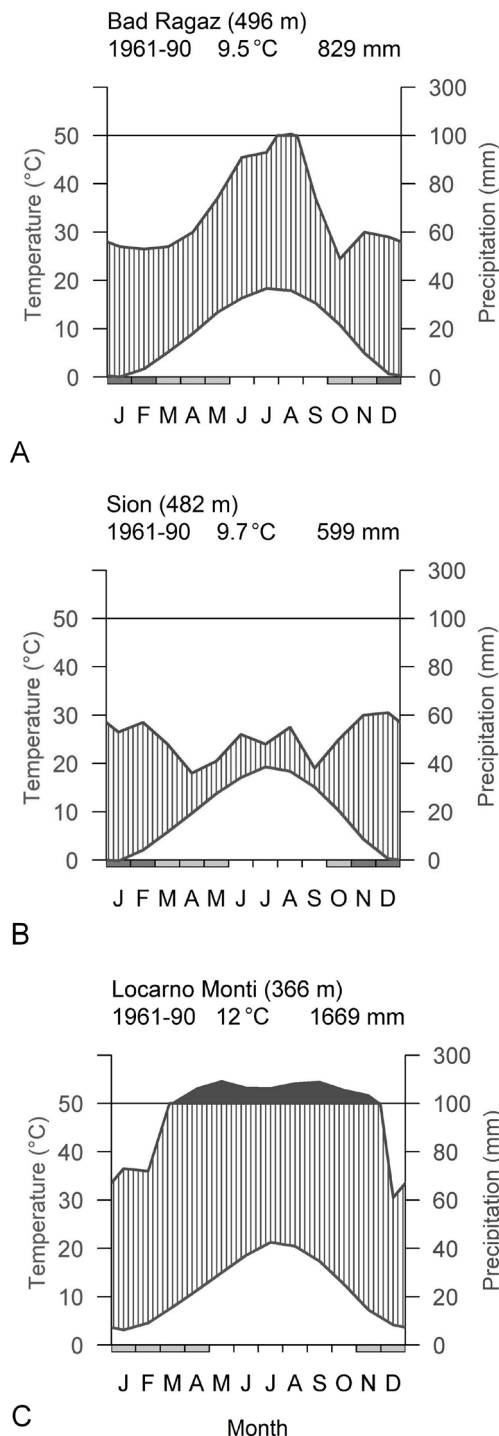
The maize plots consisted of one row, 5 m long, in 2008 and three rows, 3 m long, in 2009, separated by 0.75 m. The seeds of the landraces were multiplied at the research station in the field season preceding the experiments (in 2007 and 2008). Sowing was done with an automatic air-seeder (Wintersteiger AG, Ried, Austria), starting in April as soon as the soil water content was suitable for tractor-powered cultivation. To prevent the seeds from rotting and pests, they were dressed with Fludioxonil/Metalaxyl-M (Maxim® XL, Syngenta Agro, Dielsdorf, Switzerland) and Methiocarb (Mesuroi® liquide, Bayer, Leverkusen, Germany). Thiamethoxam (Cruiser®, Syngenta Agro, Dielsdorf, Switzerland) was applied only in 2009 to prevent a higher risk of damage by wireworms due to the preceding cover crop of grass. Seeds were sown at a depth of 0.06 m and intra-row spacing of 0.15 m, resulting in a density of 8.9 plants m<sup>-2</sup>. All the plots were treated with the broad-band herbicide Atrazine (Gesaprim® Quick, Syngenta Agro, Dielsdorf, Switzerland). Nitrogen was applied as ammonium nitrate fertilizer with 27.5% N. In 2008, 25 kg N ha<sup>-1</sup> were applied at the three-leaf stage (3-LS) of Magister to all the plots, whereas 75 kg N ha<sup>-1</sup> were applied right after planting in 2009.

### Measurements

Emergence of the maize accessions was defined as the moment when 50% of the plants had emerged (e50). Plants were counted every other day up to 30 days after planting. To measure the dry weight, maize shoots were sampled four (2008) or five times (2009) from 0.75 m<sup>2</sup> (1-m section of a row) from the one-leaf stage (1-LS) until all the accessions had at least six fully developed leaves (6-LS). Depending on the experiment, samples were taken on between eight and 20 days, from one or several blocks a day. The maximum interval between consecutive sampling dates was seven days. Shoot samples were dried at 80°C to constant weight.

### Statistical analysis

In 2008 and 2009, the experiments consisted of



**Figure 1** - Climate diagrams from locations in A) the Rhine valley (north of the Alps), B) Valais (central alpine valley), C) Ticino (south of the Alps). The lower and upper curves represent temperature and precipitation, respectively. Frost days are indicated below the zero line (white: no frost days, light grey: one to ten frost days, dark grey: more than ten frost days per month). Data: [MeteoSwiss \(2011\)](#). R package [climatol \(Gujjarro, 2011\)](#).

four and six completely randomized blocks, respectively. The statistical analysis of the data was performed with R ([R Development Core Team, 2010](#)). Emergence counts were analyzed with a generalized linear model (`glm()`, R package MASS; [Venables and Ripley, 2002](#)) assuming a Poisson distribution of the counts. Plant development (untransformed) and the dry weight of the maize shoot (SDW) (log-transformed) were analyzed separately for each experiment according to a general linear mixed effects modeling procedure ([Pinheiro and Bates, 2000](#)) using the function `lme()` from the R package nlme ([Pinheiro et al, 2009](#)). The model always included the main effects and the interactions of genotype (G) and a polynomial function of thermal time (T), i.e. cumulative growing degree days, as well as blocks (BL) and plots (PL) nested within blocks. G and T and their interactions were treated as fixed, BL and PL as random effects. The model for the dry weight of the maize shoot included two additional parameters, D and P entering the model as fixed effects, and representing correction factors for the effect of varying plant development and plant density, respectively ([Causton and Venus, 1981](#)). For a given experiment, parameter D represented the residuals of the corresponding fit of plant development. The effect of D accounted, at least in parts, for random variation (i.e. variation not accounted for by the experimental factors) in seed germination and plant emergence. P for a given plot was calculated as the deviation of plant density from the median plant density over all the plots in a given experiment. The suitable polynomial degree for thermal time was selected following residual analysis. This resulted in the following model equations for the fixed effects:

$$\log(\text{SDW}) \sim T * G + T^2 * G + T^3 * G + D + P$$

(Experiments 1 to 4)

$$\log(\text{SDW}) \sim T * G + T^2 * G + D + P$$

(Experiments 5 and 6).

Heteroscedasticity was accounted for as required. Comparisons of genotypes are based on confidence intervals, typically at the 95% probability level, which were extracted from the fitted model by using the function `contrast()` in the R package `contrast` ([Kuhn et al, 2010](#)). Function `dotplot()` from R package `Hmisc` ([Harrell Jr, 2010](#)) was used for drawing the dot plot.

**Definitions**

“Emergence phase” refers to the period from planting to 50% emergence (e50, see above). “Heterotrophic growth phase” refers to the period from e50 to the day, on which the accumulated daily mean air temperatures exceeded 200°C days, i.e. until around the 3-LS. By this time the kernel reserves are assumed to have been dissimilated by the seedling (cf. [Verheul et al, 1995](#)). “Early autotrophic phase” refers to the period between 200 and 360°C days, i.e. until around the 6-LS.

**Table 2** - Thermal environment during the experiments (EX).

Ex	Sowing date	Duration (d)			Average temperature (°C)			Growing degree days (°C days)		
		sowing to e50 <sup>1</sup>	sowing to 6-LS <sup>2</sup>	e50 to 6-LS	sowing to e50	sowing to 6-LS	e50 to 6-LS	sowing to e50	sowing to 6-LS	e50 to 6-LS
1	28.04.2008	17	49	35	14.0	15.2	15.7	103.3	211.4	354
2	22.05.2008	13	37	30	17.4	17.8	17.7	134.4	200.8	372
3	15.04.2009	23	53	34	11.3	14.4	16.0	83.0	201.0	348
4	01.05.2009	16	45	32	14.1	16.1	16.8	90.9	208.4	356
5	13.05.2009	13	40	31	16.7	16.7	16.8	131.4	204.3	357
6	28.05.2009	12	37	29	15.2	17.1	17.6	85.7	202.6	344

<sup>1</sup> e50: time when 50% of the plants had emerged; <sup>2</sup> 6-LS: six-leaf stage (six fully developed leaves)

## Results

### Environments

The average mean air temperature of the six experiments from planting to 6-LS was 16.2°C, ranging from 14.4 to 17.8°C in the single experiments (Table 2). In both years the average daily mean air temperature increased from the early to the later sown experiments due to seasonal increases in temperature. The mean air temperature in the first experiments in 2008 and 2009 (Experiments 1 and 3) was about 2.6 °C lower than in the experiments with the highest average air temperature. Experiment 1 had the most marginal thermal growth environment. In Experiment 3 the coolest days were concentrated mainly around the phase of emergence. However, a few chilling spells occurred in all the experiments.

### Plant emergence

There was a clear effect of the experiment ( $p < 0.0001$ ), on the time of emergence (e50), but the genotype and genotype x environment effects were not significant (data not shown).

### Duration of the experiments

Experiment 3 lasted 53 days from planting to the accumulation of 360°C days at approximately the 6-LS (Table 2). In contrast, Experiments 2 and 6 lasted only 37 days. The time to e50 was longest in Experiment 3 (19 d) and shortest in Experiment 2 (7 d). The duration from e50 to 360°C days was more similar among the six experiments than the duration between sowing and e50; it was longest in Experiment 1 (35 d) and shortest in Experiment 6 (29 d). With growing degree days as a time scale, most of the remaining differences among the experiments were eliminated, which enabled a comparison of the growth patterns throughout the experimental periods.

### General patterns of shoot growth

In all but Experiment 6, there was a statistically significant genotype x time interaction. In Experiment 6, the main effects of genotype and time were highly significant ( $p < 0.0001$ ) (data not shown). Growth was nearly exponential in all the experiments, with dry

matter ranging from 46.0 to 124.6 g m<sup>-2</sup> by 360°C days for the hybrid Magister (Figure 2). Within experiments the growth of all the accessions was similar. Differences in dry matter accumulation were most pronounced between 2008 and 2009. However, particularly in 2009, there were differences within the year, as well. Contrary to the duration of the experiments, the dry matter accumulation was not clearly related to the average daily mean air temperature in the six experiments: in Experiment 6, dry matter accumulation at 360°C days was almost as low as in Experiment 3, although the average daily mean air temperature of Experiment 6 was the highest of all the experiments.

### Growth performance and grouping of landraces

The yields of shoot dry matter of the landraces by 360°C days ranged from 21.6 to 122.8 g m<sup>-2</sup> (Figure 2). The landraces grouped according to their geographic origin: the northern group, consisting of 098RV, 103RV and 179RV, with growth performance close to that of Magister, and the southern group (074TM, 198TM and 125PV) with poor growth. Two other landraces (003PR and 025VS), both from inner alpine valleys, showed an intermediate performance. Only in Experiment 2, with the warmest conditions, the north-south ranking was more or less reversed, because the two landraces from Ticino performed better than those from the north (Figures 3 and 4).

The northern Swiss landrace 179RV performed (except in Experiment 5) as well as or even better than Magister in the heterotrophic phase and only occasionally worse in the early autotrophic phase (Figure 3). Especially in Experiment 3, 179RV was significantly superior to Magister at the beginning of the sampling period, indicating good early heterotrophic growth under these chilling conditions. The growth dynamics of other landraces from the north were similar but they were sometimes slightly less productive. The landrace 198TM, with southern origin, did not usually perform as well as Magister up to the 6-LS, except for the beginning of Experiments 1 and 3, which again indicates good early heterotrophic

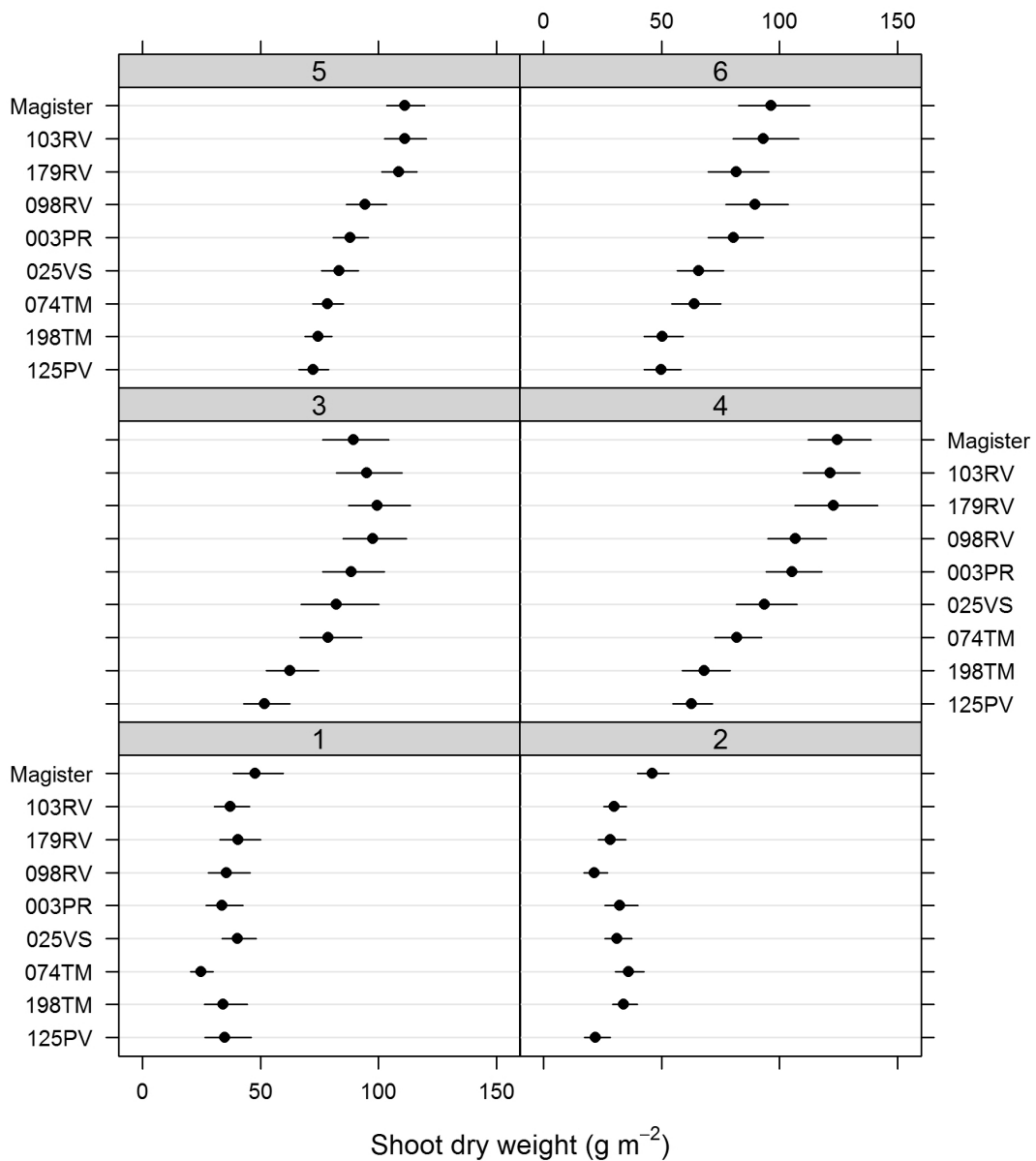


Figure 2 - Shoot dry weight (g per m<sup>2</sup>) in the six experiments (1 to 6) by 360°C days with 95%-confidence intervals.

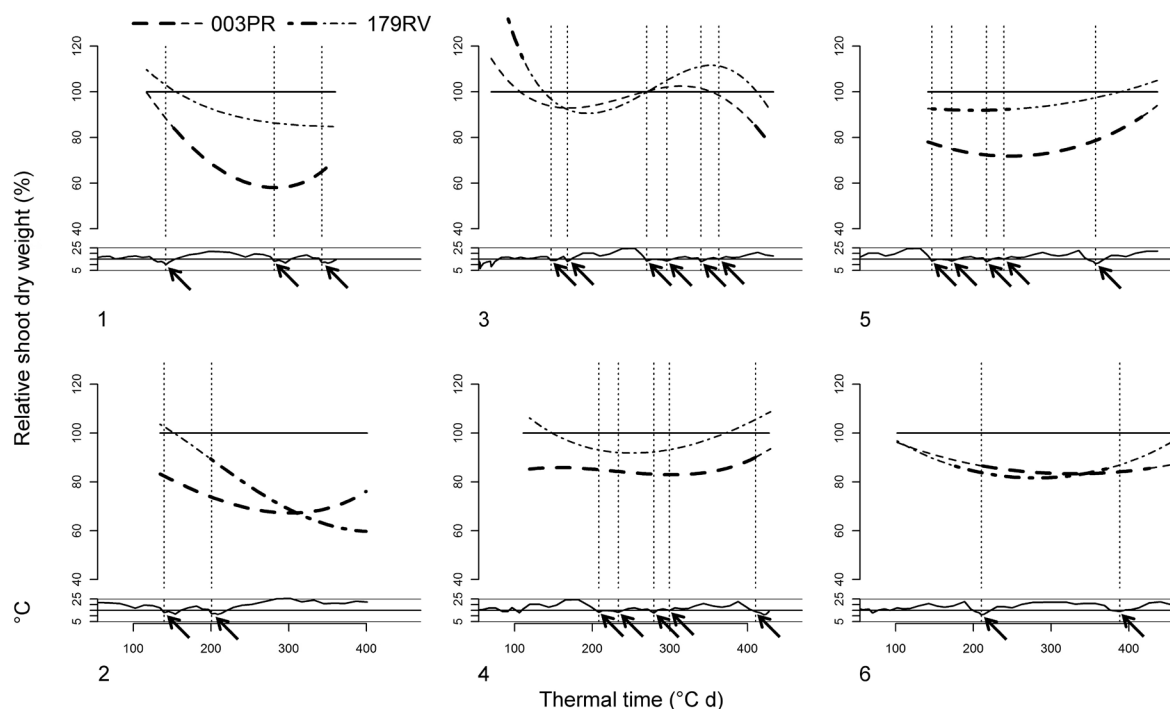
growth under marginal temperature conditions (Figure 4). The two other landraces of the southern group performed similar to 198TM.

In general, the southern landraces responded better to higher temperatures, particularly during early autotrophic growth. The two intermediate landraces, 003PR and 025VS, were sometimes more similar to Magister and sometimes to the southern landraces.

**Relative growth patterns of the landraces compared to the hybrid Magister**

The sowing date had an effect on the relative performance of the landraces compared to that of the

hybrid by 360°C days (cf. Figure 2). Magister always performed at least as well as the landraces. However, the relative differences between the hybrid and the landraces varied considerably. For example, in Experiment 3 the average dry weight of the landraces was 92% of that recorded for Magister. In the other experiments the relative performance of the landraces was between 64 and 80%. During the heterotrophic phase the growth of the landraces was usually slower than that of Magister (see the negative slopes in Figures 3 and 4) but accelerated compared to Magister during the first part of the early autotrophic



**Figure 3** - Relative dry weight of the shoot of the landraces 003PR and 179RV in percent with regard to the check hybrid Magister (= 100%). Numbers 1 to 6 correspond to the six experiments. Bold lines indicate where the landraces performed significantly different from the check ( $P=0.05$ ). The course of the ambient temperature in degrees Celsius is indicated below the growth curves. The black flashes designate chilling spells of less than 15°C and the vertical, dotted lines guide to the corresponding part of the relative growth curve.

phase. Marked changes in the performance of the landraces relative to Magister often coincided with changes in the temperature course, particularly when the air temperature was below 15°C (vertical dotted lines) or increased rapidly (Figures 3 and 4).

In Experiment 3, after a long cold period of germination and heterotrophic growth, a sudden increase in temperature occurred at the 3-LS. Except for 198TM, the landraces reacted to it with a stronger increase in growth than Magister, as shown by the positive slopes in the profiles in Figures 3 and 4. However, this effect was brief and lasted only until the 6-LS, where Magister caught up with the landraces.

## Discussion

### *Environmental and genetic variability*

In a maritime climate, varying sowing dates within and across seasons imply that the temperature course is different in each experiment and, thus, so is plant growth. This variability makes it difficult to interpret the field trials. Furthermore, the heterogeneous character of open-pollinated maize populations was another important source of variability. Nonetheless, from the present study it appears that this approach allows identifying maize populations of potential interest for further studies. In the search of useful traits of landraces future investigations might involve doubled

haploid lines from pre-screened landraces. Doubled haploids showed less severe decreases in viability than inbreds in former studies (Wilde et al, 2009).

### *Marginality of the environments in terms of air temperature*

Chilling stress has been reported to occur at temperatures below about 15°C (Leipner and Stamp, 2009). In the two earliest experiments (1 and 3), the average daily mean air temperature (15.2 and 14.4°C, Table 2) was in the upper range of chilling stress, whereas the temperature in the other experiments was slightly higher. Accordingly, there were only relatively small differences between the experiments in terms of mean air temperature. Moreover, the extreme values of the daily mean air temperature differed only slightly among the experiments (data not shown). On the other hand, the six environments had specific cool and warm periods and, thus, varied with regard to the temperature course. In Experiment 3, for example, chilling stress occurred shortly after planting, whereas in Experiment 1 there were chilling days over the entire growth period. This is important, because temperature has different effects on the three main growth phases (emergence, heterotrophic growth and early autotrophic growth). For example, maize can germinate at air temperatures below 10°C (Leipner and Stamp, 2009), whereas heterotrophic

growth is very limited below 10°C (Brandolini et al, 2000). Dolstra and Miedema (1986) reported a temperature minimum of 13°C for autotrophic growth. Furthermore, acclimation appears to play a significant role in accession-specific reactions to different temperature histories (Hardacre and Greer, 1989; Verheul et al, 1995; Leipner et al, 1997). Verheul et al (1995) suggested that chilling-tolerant genotypes do better in the long run, especially at fluctuating temperatures, because they recover more quickly once they have adapted to the environmental conditions. Overall, despite the rather small differences with regard to the marginality of the mean air temperature, temperature differences might still have contributed considerably to the variation of the experiment- and accession-specific growth patterns.

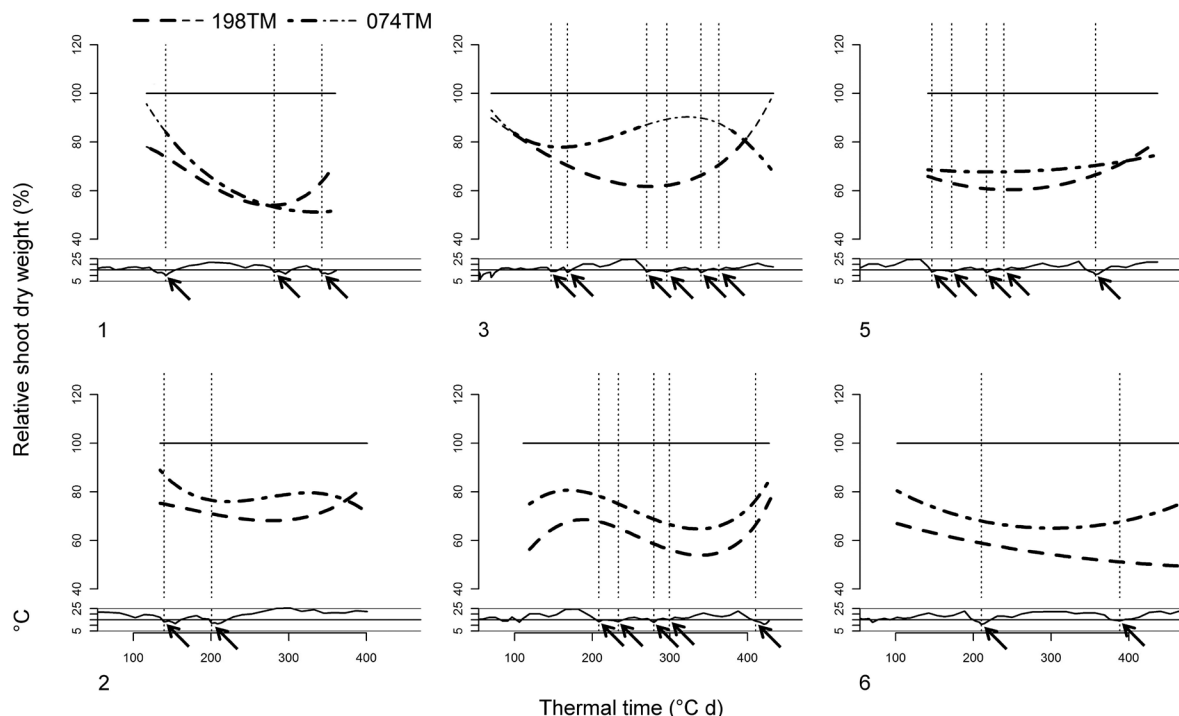
At the beginning of Experiments 1, 2, and 3 the landraces performed relatively well, possibly due to a faster transfer of kernel reserves to the seedling (cf. Soldati et al, 1999) and the relatively long phases of emergence. Such advantages may be beneficial to growth. Nonetheless, our experiments showed clearly that initial advantages can be lost very rapidly, because the further heterotrophic growth of the landraces was often slower than that of the hybrid.

The simultaneous changes in the temperature course and in the relative growth patterns of landraces with regard to Magister may indicate different re-

sponses to changes in temperature and may support the hypothesis that landraces often perform relatively better than Magister as the temperature decreases. Nevertheless, there were no clear indications of chilling tolerance in the present study. None of the landraces significantly outperformed the vigorous hybrid Magister, probably because the stress in this study was milder than in the study of Peter et al (2009b).

Experiment 2 was the only one, in which emergence and growth prior to the 1-LS occurred at air temperatures around 20°C (data not shown), resulting in the fast and regular emergence of all the accessions. However, at later growth stages, landrace 098RV and, to a lesser extent, 179RV showed very weak growth, even though Peter et al (2009b) reported that 179RV is chilling tolerant. One explanation might be that both landraces did not tolerate the six days of chilling at the end of the heterotrophic phase, because they had not yet become acclimated to chilling temperature.

In the future, maize must adapt to unusually low and moderate temperature in spring as the climate changes. Both are expected to occur more frequently (Schär et al, 2004). Testing the landraces under more severe conditions (high and low temperature) than in the present study will clarify the potential of Swiss landraces to be a source of genes for breeding programs, which aim at improving the tolerance of maize



**Figure 4** - Relative dry weight of the shoot of the landraces 074TM and 198TM in percent with regard to the check hybrid Magister (= 100%). Numbers 1 to 6 correspond to the six experiments. Bold lines indicate where the landraces performed significantly different from the check ( $P=0.05$ ). The course of the ambient temperature in degrees Celsius is indicated below the growth curves. The black flashes designate chilling spells of less than 15°C and the vertical, dotted lines guide to the corresponding part of the relative growth curve.

to different temperature environments.

### Conclusions

Our hypotheses were not confirmed; the tested Swiss landraces did not grow better than the modern check hybrid at low mean air temperature. Nevertheless, there are indications that some landraces are relatively tolerant to marked decreases in temperature during early growth. This suggests that there are still some quantitative trait loci to uncover in these genetic resources. However, even if Swiss landraces contain genes for better chilling tolerance, it will probably not be straightforward to use them, because the negative reaction to fluctuating temperature, as observed in some of our experiments, may hinder the achievement of yield consistency.

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