Identification of QTLs involved in the response to cold stress in maize (*Zea mays* L.)

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ABSTRACT

The effect of low temperature on the physiology of maize has been well studied, but the genetics behind cold tolerance is poorly understood. To better understand the genetics of cold tolerance we conducted a QTL analysis on a segregating population from the cross of a cold-tolerant (EP42) and a cold susceptible (A661) inbred line. The experiments were carried under cold (15 ºC) and control (25 ºC) conditions in phytotron. Cold temperature reduced the shoot dry weight, number of survival plants and quantum yield of electron transport at PSII (ΦPSII) and increases the anthocyanin content in maize seedlings. Low correlations were found between characters under low and optimum temperature. Ten QTLs were identified, six of them identified at control temperatures and four under cold temperatures. Through a meta-QTL analysis we identified three genomic regions placed in chromosomes 2, 4 and 8 that regulate the development of maize seedlings under cold conditions and are the most promising regions to be the target of future MAS breeding programs or to perform fine mapping to identify genes involved in cold-tolerance in maize.

Keywords: Cold tolerance; maize; photosynthesis; meta-QTL.

INTRODUCTION

Most of the world's important food crops are nowadays cultivated outside their original climate zones where yields are constrained by the so-called thermal thresholds for optimal growth. Maize is considered a cold-sensitive species with a relative high temperature threshold for germination and vegetative growth. Chilling temperatures affect maize development and physiology throughout its ontogeny, albeit cold-thresholds vary during development. In general terms, stages at early development are more vulnerable to chilling temperatures than mature ones. Roughly, two stages could be considered during early maize development. The first stage (the heterotrophic phase) covers from germination to approximately the three-leaf stage and growth relies mainly on seed reserves, whereas the second stage (the autotrophic phase) initiates with the development of a functional photosynthetic apparatus (Cooper and MacDonald 1970). Apparently, both stages have different temperature requirements (Revilla et al. 2000). A thermal threshold for germination and emergence has been established at 5 ºC, though temperatures below 10 ºC slow these processes down, leading to poor crop establishment and reduced yields (Bochicchio 1985; Orr et al. 1983; Greaves 1996). The development of the photosynthetic apparatus seems to require higher temperatures and different studies have reported a significant reduction of the amounts of chlorophyll in plants developed at temperatures below 15 ºC (Rodríguez et al. 2013; Greaves 1996). These low temperatures also reduce the electron transport in the photosystems and the activity of the enzymes involved in carbon fixation (Greaves 1996). A consequence of the inhibition of the photosystem functionality is the production of ROS species potentially harmful for the plant. Different protective mechanisms have been developed by plants to avoid the accumulation of this oxygen-derived species and one of these

mechanisms is the accumulation of anthocyanins in the leaves. Pietrini et al.(2002) found that the anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. Similar results have been reported in other plant species (Hughes et al. 2005; Steyn et al. 2009).

The major difficulty that maize breeders face is the complexity of the response to low temperatures. Epistatic, additive, and dominant gene effects were reported to be significant for most traits evaluated under cold conditions, which along with maternal effects and low heritability has limited the success of breeding programs (Revilla et al. 2005; Revilla et al. 2000). In the last decades, efforts have been focused on the development of marker-assisted selection (MAS) programs that could provide a plausible alternative to classical breeding for improving cold tolerance in maize. In spite of several studies identifying QTLs related to cold-tolerance in maize, so far none of them were reliable enough to be used in a MAS program (Jompuk et al. 2005; Rodriguez et al. 2008). The integration of QTL information, through meta-QTL analysis, from studies performed under different environmental conditions (i.e. field or growth chambers, diverse temperature regimes or evaluation of diverse organs), would allow the identification of consistent genomic regions with a higher potential to be target regions for MAS selection.

In a recent study, we identified a maize inbred line (A661) which shows a conditional albino-phenotype when exposed to suboptimal temperatures (Rodríguez et al. 2013). The levels of chlorophyll are dramatically reduced in this inbred line when grown at temperatures below 15 ºC, which compromise seedling survival beyond the heterotrophic phase. Therefore, this genotype is of special interest to identify QTLs involved in cold tolerance, due to its remarkable susceptibility to low temperatures.

Therefore the objective of this work was to identify QTLs for cold tolerance-related traits during early development on a segregating population from the cross of a cold-tolerant (EP42) and a cold susceptible (A661) inbred line and combine our results with previous reports through a meta-QTLs analysis.

MATERIAL AND METHODS

Plant material

The inbred lines EP42 (flint) and A661 (dent) were used to identify those chromosome regions regulating cold tolerance on maize during early development. The inbred line EP42 was released from a local open-pollinated variety (Tuy) from the Miño valley (Galicia, Northewestern Spain) whereas A661 comes from the early maize population AS-A from Minnesota (USA) (Geadelmann and Peterson 1976). Based on preliminary evaluations the inbred line A661 was identify as susceptible to low temperatures suffering a drastic reduction on chlorophyll content when grown at cold stressful conditions, whereas the inbred line EP42 showed an intermediate cold-tolerance. A total number of 210 $F_{2:3}$ families were used for phenotypic and QTL analysis. The $F_{2:3}$ was obtained by hand pollination in Northwestern Spain in 2004.

Phenotypic analysis

Genotypes were evaluated under cold and control conditions in a phytotron (20 m^3) equipped with very high-output fluorescent lamps with a photosynthetic photon flux of

228 µmol m^2 s^{-1}. The 210 $F_{2:3}$ families, along with the parental inbred lines, were evaluated following a randomized complete block design with two replications. Maize seeds were planted in seedbeds filled with sterilized peat with one kernel per hill. Each plot consisted on 8 hills. Experiments were watered after planting and afterwards trials were watered as needed. Temperature conditions were set up at 14 ºC/14 h light and 8 ºC/10 h dark for cold experiments and 25 ºC/14h light and 20 ºC/10h dark for control experiment. Data were recorded at V3 stage to assure that plants were on the same developmental stage. Four cold-tolerance related traits were recorded: 1.Number of survival plants: for each genotype determined as the number of plants still alive at the end of the experiment. 2. Dry weight (g/plant): shoots were harvested from each genotype and dried in an oven (80 °C) until constant weight. 3. Quantum yield of PSII (ΦPSII): recorded using an OS-30p Chlorophyll Fluorometer (Opti-Sciences, Inc., USA) in all plants. 4. Total anthocyanin content: samples of 0.28 cm^2 were collected from the second leaf of each plant within each family in the growth chamber and stored at -20 °C for later determinations. Anthocyanins were extracted with 1 ml of acidified methanol (1% HCl, v/v) overnight at 4 °C with continuous shaking. After that, the samples were centrifuged at 4000 g at 4 °C for 10 min and anthocyanin content quantified from the supernatant with a Spectra MR^{TM} Microplate Spectrophotometer (USA) as described in Sims and Gamon (2002).

Comparisons among LSMEANS of the parental inbred lines and the $F_{2:3}$ population and simple correlation coefficients between traits were computed with SAS, version 9.2 (SAS Institute Inc. 2008). An ANOVA of the $F_{2:3}$ families, was performed for each trait by using the procedure PROC MIXED of SAS, version 9.2. The $F_{2:3}$ families were considered random effects, and best linear unbiased prediction (BLUPs)

was employed in order to obtain the estimate of each $F_{2:3}$ family score for each condition. Broad-sense heritability was calculated as described by Holland et al (2010).

QTLs analysis

A final set of 98 SSR markers that were polymorphic between parental inbred lines and give clear bands patterns were used for linkage mapping and QTL analysis. The linkage map was built using MAPMAKER 3.0b (Lander et al. 1987). Composite interval mapping was performed separately for data recorded at low- and optimal-temperature conditions with the PLABQTL software (Utz and Melchinger 1996). A likelihood odds (LOD) threshold of 2.3 was chosen for declaring the putative QTL significant. The LOD score was obtained by the permutation test method (Churchill and Doerge 1994). The analysis and cofactor election were carried out following PLABQTL's recommendations, using an ''F-to-enter'' and an ''F-to-delete'' value of 3.5. The proportion of phenotypic variance explained by all QTLs was determined by the adjusted coefficient of determination of regression (R^2adj), fitting a model including all detected QTLs. Fivefold cross validation (CV/G) was performed following the procedures described by Utz et al. (2000). Four of these subsets were combined to estimation set (ES), and the remaining subset formed the test set form the estimation (TS), in which predictions derived from the ES were tested for their validity by correlating predicted and observed data. We used 1000 replicated CV/G runs. Estimates of medians and frequency of QTL detection in the ES and TS were calculated over all replicated CV/G.

Meta-QTL analysis

In order to compare our results with previous studies we performed a meta-QTL analysis using the Biomercator v3 software (Sosnowski et al. 2012). Reliable data on QTLs identified for cold-tolerance in maize were collected from six studies (Fracheboud et al. 2004; Fracheboud et al. 2002; Jompuk 2005; Leipner et al. 2008; Presterl et al. 2007; Hund et al. 2004; Guerra-Peraza et al. 2011), along with data herein presented. Data collected cover information about the genetic map, traits recorded and QTLs identified. A consensus map was developed for those chromosomes where QTLs were identified in the present work, using the maize IBM2 2008 Neighbors as a reference map. The final consensus map included four chromosomes, 3585 markers (with an average distance of 0.98 cM between markers) and covered a total length of 3278 cM. QTLs were projected onto the consensus map in a way that the QTL confidence interval (CI) is resized on according to a scaling factor which takes into account the marker distance variations between the original and the consensus map (Veyrieras et al. 2005). Whether the CI was not available from the original literature, it was calculated using the formula proposed by Darvasi and Soller (1997):

$$CI = \frac{530}{N \times R^2}$$

where N is the population size and R^2 is the proportion of phenotypic variance explained by the QTL.

The meta-QTL algorithm developed by Goffinet and Gerber (2000) was used to determine the number of meta-QTLs (mQTLs) on each chromosome. This algorithm determines whether N-QTLs identified in the same chromosome region are consistent with a 1-, 2-, 3-, 4- or N-QTL models. The Akaike-type statistical criterion was used to select the best model among the five ones proposed.

182 RESULTS AND DISCUSSION

183

184 Based on preliminary observations we selected the inbred lines EP42 and A661 as
185 parental inbred lines due to their differential cold-tolerance. As expected, no differences
186 were observed among inbred lines and the mean of the $F_{2:3}$ segregating population for
187 any trait evaluated under control conditions (Table 1). Under cold temperature
188 conditions, the parental inbred lines and the $F_{2:3}$ differ for the quantum yield of PSII
189 (ΦPSII). The inbred line A661 showed the lowest value for ΦPSII, which was below the
190 detection limits, suggesting that photosynthesis activity in this inbred line is completely
191 inhibited by cold temperatures. Besides, the mean of ΦPSII of the $F_{2:3}$ population was
192 significantly higher than that observed in the parental inbred lines, indicating that
193 dominance effects could be important for this trait. When comparing both temperature
194 conditions, plants developed under cold conditions showed significantly less dry weight
195 and ΦPSII than plants growth under control conditions. These results agree with those
196 previously reported in cold chamber (Rodriguez et al. 2008; Haldimann 1999) and in
197 the field (Leipner et al. 1999). Concerning the other two characters, no significant
198 differences were observed, tough a tendency to decrease the number of survival plants
199 and accumulate a higher amount of anthocyanins was shown by plants grown under
200 cold conditions compared to those grown under control temperatures for the two inbred
201 lines and the $F_{2:3}$ populations.

202 Interestingly, in cold conditions the inbred A661 accumulated twice as much
203 anthocyanins than the inbred EP42, which could be explained by the induction of
204 anthocyanin production caused by the inhibition of ΦPSII in A661. It is well known that
205 anthocyanins play a protective role under photoinhibitory conditions (Pietrini et al.
206 2002). The heritability observed for the number of survival plants and dry weight were

remarkably high compared with those observed in previous studies under similar conditions (Fracheboud et al. 2004). Low heritabilities were observed for the anthocyanin content and ΦPSII indicating that these traits are highly affected by the environment. Nevertheless, contrary to expectations, heritabilities under cold conditions for these traits were higher than those observed under control conditions (Table 1). Variability among the $F_{2:3}$ families was observed for the four traits recorded (Table 2). Analysis of variance performed individually for each temperature showed that genotypes differ for all traits under cold conditions, whereas just the number of survival plants and the dry weight showed significant differences under control conditions (data not shown). These results support the suitability of this population to identify QTLs related to cold tolerance.

In general, low correlation coefficients for the different traits evaluated were observed at both cold and control conditions (Table 3) what is typical of studies performed under stress conditions (Hund et al. 2004; Rodriguez et al. 2008). The highest correlation was found between the dry weight and ΦPSII under cold conditions (r=0.54) which indicates that part of the reduction on dry weight under cold conditions is due to the negative impact of low temperature on the photosynthesis rate.

Ten QTLs identified in the present analysis were displayed along four chromosomes (2, 4, 7 and 8) (Table 4). Six of these QTLs (QTL-1, -2, -3, -4, -5 and -6) were found under control temperature conditions and four (QTL-7, -8, -9 and -10) under cold conditions. A region of 79 cM in the short arm of the chromosome 4 encompasses five QTLs (QTL-1, -2, -3, -9, -10), three of them regulate the dry weight, ΦPSII and the number of survival plants under control conditions and two regulate the ΦPSII and the number of survival plants under cold conditions (Table 4).

231 Two QTLs were exclusively identified under cold conditions. One is located in the long
232 arm of the chromosome 8 for dry weight (QTL-8) and explains a 6.9 % of the
233 phenotypic variance which increases to a 13.1 % when estimated based on 200 fivefold
234 CV/G runs (Table 4). This QTL was identified in the 73.5 % of the cross-validation runs
235 supporting its reliability. To our knowledge, none QTLs related with the maize response
236 to cold temperatures have been previously reported in this chromosome region.
237 The other QTL identified only under cold conditions is located in the chromosome 2
238 (QTL-8) and is involved in the maintenance of the ΦPSII under cold conditions. This
239 QTL explains the highest percent of phenotypic variance (19.0 %) of all detected QTLs
240 and was detected in the 56.7 % of the cross-validation runs which is quite high
241 compared with other QTLs detected under similar experimental conditions (Rodriguez
242 et al. 2008). A QTL located in the same region was previously identified regulating
243 chlorophyll content under cold conditions (Rodríguez et al. 2013).
244 In spite of its role protecting the photosystem under stressful conditions and the
245 significant differences observed in the anthocyanin accumulation between the parental
246 inbred lines, no QTLs were identified regulating this character under cold conditions
247 and just one QTL was identified regulating the anthocyanin content under control
248 conditions. The high coefficients of variation observed under cold conditions and the
249 low heritability could explain the lack of significant QTLs for this trait.
250 The relative importance of additive and dominance effects depended on the
251 temperature regime, i.e. under cold conditions dominance effects were higher than
252 additive effects while under control conditions half of the QTLs have higher additive
253 effects and half higher dominance effects. Dominance effects were of higher magnitude
254 than additive effects for ΦPSII under cold conditions, in agreement with the higher
255 value observed for the $F_{2:3}$ population compared to the parents (Table 2). Contrarily,

256 previous reports have shown that the genetics of cold tolerance was mainly due to
257 additive genetic effects (Revilla et al. 2005; Revilla et al. 2000). Interestingly enough,
258 QTLs for ΦPSII under cold conditions (QTL-8 and 9) had opposite signs for the
259 additive effects, indicating that favorable alleles were contributed by different parents
260 for each QTL.

261 In order to compare our results with previous studies we performed a meta-QTL
262 analysis. A total number of 85 QTLs located in any of the four chromosomes where
263 QTLs were identified in the present study, were projected onto a consensus map using
264 the IBM2 2008 Neighbors as a reference map. Sixty four of these QTLs where
265 identified in experiments performed under cold conditions, either in growth chamber or
266 in the field, whereas 21 were identified in the corresponding control experiments.
267 Through the meta-QTL analysis we identified 20 mQTLs regions (Figure 1). With the
268 exception of an mQTL located at the bottom of the chromosome 4, which was
269 integrated from a single QTL, the other 19 mQTLs integrate more than one QTL from
270 different publications.

271 Nine out of the 10 QTLs identified in our mapping population were integrated in
272 six mQTLs (only QTL-4 was not integrated in any detected mQTL). In our QTL
273 analysis, we localize three genomic regions that regulate maize physiology exclusively
274 under cold conditions (QTL-7, -8 and -9). A mQTL in the chromosome 8 includes the
275 QTL-7, which regulates the shoot dry weight under cold conditions, along with two
276 QTLs regulating the maximum fluorescence yield of PSII (Fm) (Guerra-Peraza et al.
277 2011) and the activity of the malate dehydrogenase (MDH) under cold conditions
278 (Leipner and Mayer 2008). The malate dehydrogenase is a key enzyme in the
279 photosynthetic process. Either a reduction of the electron transport rate in the PSII or a
280 decrease in the activity of the enzymes involved in the C4 cycle, would reduced the

281 photosynthesis rate which could lead to reduced plant growth and therefore a reduced
282 dry weight. In a similar way, QTL-9 was included in an mQTL in chromosome 4
283 together with two QTLs regulating the maximum fluorescence yield of the PSII
284 (Guerra-Peraza et al. 2011) and the shoot dry weight (Presterl et al. 2007). These results
285 indicate that two major regions regulating seedling growth under cold conditions
286 localize in chromosome 4 and 8.
287 The QTL-8, which regulates the quantum yield of PSII (ΦPSII), was integrated
288 in a mQTL along with a QTL regulating the photochemical quenching factor of PSII
289 (qP) (Fracheboud et al. 2004), and a QTL regulating the activity of a soluble vacuolar
290 invertase (Guerra-Peraza et al. 2011). Soluble invertases play a central role in the
291 adjustment of carbohydrate metabolism under stress conditions (Roitsch and González
292 2004). Although the activity of invertases has been associated to the maintenance of the
293 activity of the PSII in plants growth under stress conditions (Leipner and Mayer 2008),
294 the fact that the QTL regulating the invertase activity was identified under both control
295 and cold conditions, whereas the QTLs regulating the qP and ΦPSII were identified
296 only under cold conditions, suggest that these traits are regulated by different loci.
297 In conclusion, based on meta-QTL analysis we identified three genomic regions, placed
298 on chromosomes 2, 4 and 8, which regulate early development of maize seedlings under
299 cold conditions that could be potentially used in further breeding programs due to its
300 reliability.
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307 REFERENCES

308

309 Bochicchio A (1985) *Zea mays* L.and chilling conditions at sowing time: a review.
310 Maydica 30:241-256.
311 Cooper C, MacDonald P (1970) Energetics of early seedling growth in corn (*Zea mays*
312 L.). Crop Sci 10:136-138.
313 Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait
314 mapping. Genetics 138 (3):963-971.
315 Darvasi A, Soller M (1997) A simple method to calculate resolving power and
316 confidence intercal of QTL map location. Behavioural Genetics 27:125-132.
317 Fracheboud Y, Jompuk C, Ribaut J-M, Stamp P, Leipner J (2004) Genetic analysis of
318 cold-tolerance of photosynthesis in maize. Plant Mol Biol 56:241-253.
319 Fracheboud Y, Ribaut J-M, Messmer R, Stamp P (2002) Identification of quantitative
320 trait loci for cold-tolerance of photosynthesis in maize (*Zea mays* L.). J Exp Bot
321 53:1967-1977.
322 Geadelmann JL, Peterson RH (1976) Registration of five maize parental lines (Reg. No.
323 PL 43 to 47). Crop Sci 16:747.
324 Goffinet B, Gerber S (2000) Quantitative Trait Loci: A Meta-analysis. Genetics 155
325 (1):463-473.
326 Greaves JA (1996) Improving suboptimal temperature tolerance in maize- the search for
327 variation. J Exp Bot 47 (3):307-323.
328 Guerra-Peraza O, Leipner J, Reimer R, Thuy Nguyen H, Stamp P, Fracheboud Y (2011)
329 Temperature at night affects the genetic control of acclimation to cold in maize
330 seedlings. Maydica 56:366-377.

331 Haldimann P (1999) How do changes in temperature during growth affect leaf pigment
332 composition and photosynthesis in Zea mays genotypes differing in sensitivity
333 to low temperature?. J Exp Bot 50 (333):543-550.
334 Holland JB, Nyquist WE, Cervantes-Martínez CT (2010) Estimating and interpreting
335 heritability for plant breeding: an update. In: Plant Breeding Reviews. John
336 Wiley & Sons, Inc., pp 9-112. doi:10.1002/9780470650202.ch2
337 Hughes NM, Neufeld HS, Burkey KO (2005) Functional role of anthocyanins in high-
338 light winter leaves of the evergreen herb *Galax urceolata*. New Phytol 168
339 (3):575-587.
340 Hund A, Fracheboud Y, Soldati A, Frascaroli E, Salvi S, Stamp P (2004) QTL
341 controlling root and shoot traits of maize seedlings under cold stress. Theor Appl
342 Genet 109 (3):618-629.
343 Jompuk C (2005) Mapping of quantitative trait loci associated with chilling tolerance in
344 maize (Zea mays L.) seedlings grown under field conditions. J Exp Bot 56
345 (414):1153-1163.
346 Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA
347 (1987) MAPMAKER: an interactive computer package for constructing primary
348 genetic linkage maps of experimental and natural populations. Genomics 1
349 (2):174-181.
350 Leipner J, Fracheboud Y, Stamp P (1999) Effect of growing season on the
351 photosynthetic apparatus and leaf antioxidative defenses in two maize genotypes
352 of different chilling tolerance. Environ Exp Bot 42 (2):129-139.
353 Leipner J, Jompuk C, Camp K-H, Stamp P, Fracheboud Y (2008) QTL studies reveal
354 little relevance of chilling-related seedling traits for yield in maize. Theor Appl
355 Genet 116 (4):555-562.

356 Leipner J, Mayer E (2008) QTL mapping in maize seedlings reveals little relevance of
357 C4 cycle enzymes and antioxidants for genotypic differences in chilling
358 tolerance of photosynthesis, vol 53. vol 3-4. Maydica, Bergamo, ITALIE
359 Orr W, Roche AIDL, Singh J, Voldeng H (1983) Imbibitional chilling injury in
360 cultivars of soybeans differing in temperature sensitivity to pod formation and
361 maturation periods. Canadian Journal of Botany 61 (12):2996-2998.
362 Pietrini F, Iannlli MA, Massacci A (2002) Anthocyanin accumulation in the illuminated
363 surface of maize leaves enhances protection from photo-inhibitory risks at low
364 temperature, without further limitation to photosynthesis. Plant Cell Environ
365 25:1251-1259.
366 Presterl T, Ouzunova M, Schmidt W, Möller E, Röber F, Knaak C, Ernst K, Westhoff P,
367 Geiger H (2007) Quantitative trait loci for early plant vigour of maize grown in
368 chilly environments. Theor Appl Genet 114 (6):1059-1070.
369 Revilla P, Butron A, Cartea ME, Malvar RA, Ordas A (2005) Breeding for cold
370 tolerance. In: Ashraf M, Harris PJC (eds) Abiotic stresses: plant resistance
371 trough breeding and molecular approaches. Haworth, New York, pp 301-398
372 Revilla P, Malvar RA, Butron A, Ordas A (2000) Inheritance of cold tolerance at
373 emergence and during early season growth in maize. Crop Sci 40:1579-1585.
374 Rodríguez V, Velasco P, Garrido J, Revilla P, Ordás A, Butrón A (2013) Genetic
375 regulation of cold-induced albinism in the maize inbred line A661. J Exp Bot
376 doi: 10.1093/jxb/ert189.
377 Rodriguez VM, Butron A, Malvar RA, Ordas A, Revilla P (2008) Quantitative trait loci
378 for cold tolerance in the maize IBM population. Int J Plant Sci 169 (4):551-556.
379 Roitsch T, González M-C (2004) Function and regulation of plant invertases: sweet
380 sensations. Trends Plant Sci 9 (12):606-613.

SAS Institute Inc. (2008) SAS® 9.2 Enhanced Logging Facilities. SAS Inst., Cary, North Carolina.

Sims DA, Gamon JA (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens Environ 81 (2-3):337-354.

Sosnowski O, Charcosset A, Joets J (2012) BioMercator V3: an upgrade of genetic map compilation and QTL meta-analysis algorithms. Bioinformatics.

Steyn WJ, Wand SJE, Jacobs G, Rosecrance RC, Roberts SC (2009) Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel. Physiol Plant 136 (4):461-472.

Truntzler M, Barriere Y, Sawkins MC, Lespinasse D, Betran J, Charcosset A, Moreau L (2010) Meta-analysis of QTL involved in silage quality of maize and comparison with the position of candidate genes. Theor Appl Genet 121 (8):1465-1482.

Utz HF, Melchinger AE (1996) PLABQTL: A program for composite interval mapping of QTL. Journal of Agricultural Genomics 2:1-5.

Utz HF, Melchinger AE, Schon CC (2000) Bias and Sampling Error of the Estimated Proportion of Genotypic Variance Explained by Quantitative Trait Loci Determined From Experimental Data in Maize Using Cross Validation and Validation With Independent Samples. Genetics 154 (3):1839-1849.

Veyrieras J-B, Goffinet B, Charcosset A (2005) MetaQTL, Version 1.0. INRA, France

Table 1. Comparison of means among the parental inbred lines and the $F_{2:3}$ population for four cold-tolerance related traits.

Trait	Control				Cold			
	A661	EP42	$F_{2:3}$	H^2 [d]	A661	EP42	$F_{2:3}$	H^2 [d]
Survival plants [a]	8.0 a	7.5 a	7.0 a	0.72 ± 0.05	4.5 a	5.0 a	3.9 a	0.60 ± 0.07
Dry weight [b]	0.10 a	0.09 a	0.09 a	0.86 ± 0.02	0.03 b	0.02 b	0.03 b	0.48 ± 0.10
ΦPSII	0.71 a	0.72 a	0.71 a	0.21 ± 0.15	0.00 d	0.19 c	0.27 b	0.54 ± 0.09
Anthocyanin [c]	0.36 b	0.56 b	0.57 b	0.04 ± 0.20	5.37 a	1.76 b	2.02 b	0.14 ± 0.21

[a] Number of survival plants at the end of the experiment. [b] Dry weight (g) per plant. [c] Anthocyanin content (μmol/ml cm^2 FW). [d] Broad-sense heritability ± SE. Means followed by the same letter within the same row are not significantly different at $P<0.05$.

Table 2. Mean squares of the ANOVA of 210 $F_{2:3}$ families evaluated in growth camber under optimum and cold conditions.

Source of variation	df	Number of survival plants	Dry weight plant	ΦPSII	Anthocyanin content
Genotype	209	7.12**	0.0010**	24315.4**	4.0**
Temperature	1	2024.3**	0.7019**	35926117.6**	414.8**
Rep (Temperature)	2	222.9**	0.0029**	71767.4**	8.4
Temperature*Genotype	209	2.05	0.0003**	21900.9**	3.4

df: degrees of freedom

*, **: significant differences at P≤ 0.05 or P≤ 0.01, respectively

Table 3. Simple correlation coefficients among traits recorded under control (above diagonal) and cold (below diagonal) conditions.

	Survival plants	Anthocyanins	ΦPSII	Dry weight
Survival plants	-	-0.27**	-0.12*	0.31**
Anthocyanins	-0.35**	-	0.04	-0.11*
ΦPSII	0.09	-0.10	-	0.03
Dry weight	0.18**	-0.06	0.54**	-

*, ** significant at P≤ 0.05, P≤ 0.01, respectively

Table 4. Detected QTLs for ΦPSII, number of survival plants (N.S. plants), anthocyanin content and dry weight traits in the maize (A661× EP42) $F_{2:3}$ population.

QTL	Trait	Chromosome	Confidence interval (cM)	LOD score	Flanking markers	R^2	CV (k=5)[a]			
							R^2_{adj}	Frequency (%)	Median	
									Additive	Dominant
Control										
QTL-1	Dry Weight	4	50-87	3.2	bnlg1318, umc1963	7.0		58.0	-0.01	0.00
		Final fit					6.2			
QTL-2	ΦPSII	4	50- 86	2.5	bnlg1318, umc1963	5.5		19.9	0.05	-1.49
		Final fit					10.8			
QTL-3	N.S.Plants	4	59- 85	4.0	bnlg1318,umc1963	8.6		82.6	-0.31	0.22
QTL-4	N.S.Plants	8	204- 218	4.5	bnlg240,umc1055	9.7		40.1	-0.68	0.06
QTL-5	N.S.Plants	8	234- 244	3.8	umc1384,phi015	8.1		66.8	0.28	0.23
		Final fit					15.7			
QTL-6	Anthocyanin	7	0-39	2.3	umc1545, phi112	5.3		44.0	0.01	-0.03

		Final fit					7.2			
Cold										
QTL-7	Dry weight	8	77-110	3.2	umc1872, phi115	6.9		73.5	0.00	0.01
		Final fit					13.1			
QTL-8	ΦPSII	2	69-104	2.3	umc1823, umc1185	19.0		56.7	-30.10	48.08
QTL-9	ΦPSII	4	8-41	2.6	umc2150, bnlg1318	5.8		34.2	9.91	32.65
		Final fit					12.0			
QTL-10	N.S.Plants	4	45-87	2.8	bnlg1318, umc1963	6.0		45.5	-0.36	0.32
		Final fit					11.3			

LOD: likelihood odds; $\mathbf{R^2}$: percentage of phenotypic variance explained by a putative QTL; $\mathbf{R^2_{adj}}$: percentage of phenotypic variance explained by detected QTL based on 200 fivefold CV/G runs; [a] Frequency and median of QTL detection calculated based on 200 fivefold CV/G runs. *, ** significantly different from zero at $P < 0.05$ and $P < 0.01$, respectively.

FIGURE LEGENDS

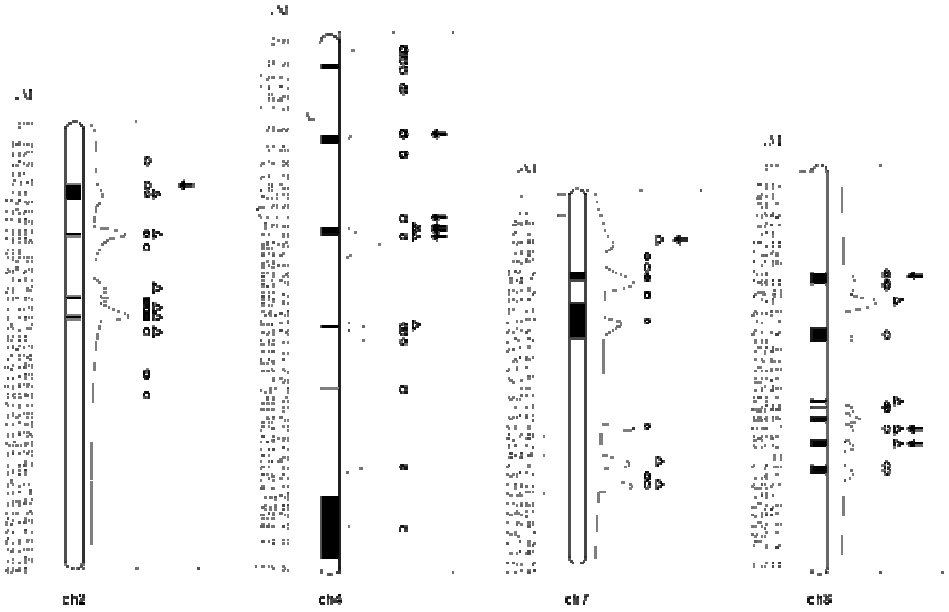

Figure 1

Figure 1. Meta-analysis overview of QTLs involved in cold-tolerance in maize. The black regions in chromosomes represent mQTLs. Original QTLs projected onto the consensus map are represented as circles (cold conditions) or triangles (control conditions). Arrows indicate the position of QTLs identified in the present study. Curves at the right of each chromosome represent the average overview $U_{0.5}$ value, calculated as described in Truntzler et al (2010).