**Response to selection for the timing of vegetative phase transition in a maize
population**

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ABSTRACT

Variability for the timing of transition from juvenile to adult vegetative phases in maize (*Zea mays* L.) is genetically regulated and has been associated with disease and pest tolerance. Epicuticular wax is present in juvenile leaves and absent in adult leaves. The objective of this work was to assess the potential modification through selection of the timing of vegetative phase transition. Three cycles of divergent phenotypic selection for early and late vegetative phase transition performed on a synthetic population were evaluated by using a randomized complete block design with two replications, in two locations of northwestern Spain across two years. Selection for early transition was made by recombining plants with fewer leaves with epicuticular wax, while, for late transition, plants with more leaves with epicuticular wax were recombined. Selection response was significant and more efficient for late phase transition than for early phase transition. Other changes observed cannot be considered correlated responses to selection because they changed in the same way for both directions of selection. We conclude that selection for the timing of vegetative phase transition was efficient and there was no detrimental correlated responses on any agronomic trait.

1 Maize has two vegetative phases, juvenile and adult. Juvenile and adult leaves, internodes, and
2 axillary buds differ in anatomy and physiology (Lawson and Poethig, 1995; Poethig, 1988,
3 1990). The traits used to distinguish vegetative phases include internode length, brace root
4 production, morphology of axillary shoots, epicuticular wax production, and trichome
5 production (Bongard-Pierce et al., 1996). The most apparent phase transition related trait is the
6 presence of epicuticular wax in juvenile tissue; while adult leaves lack epicuticular wax. Abedon
7 et al. (1996) found that the number of leaves with epicuticular wax was the best indicator of
8 the timing of vegetative phase change. Vegetative phase transition occurs at a predictable time
9 in shoot growth, generally between leaves six and eight (Bongard-Pierce et al., 1996).

10 The existence of heterochronic mutants in maize, that alter the timing of vegetative
11 development, suggests that the timing of vegetative phase transition has a genetic basis
12 (Poethig, 1988; Schnable et al., 1994). Mutations that prolong the juvenile phase do not affect
13 the adult vegetative phase or reproductive development (Bongard-Pierce et al., 1996; Lawson
14 and Poethig, 1995). The regulation of phase change in leaves is independent of the
15 development of the shoot apical meristem (Orkiszewski and Poethig, 2000).

16 Earlier vegetative phase transition has been associated with resistance to common rust,
17 European corn borer, fall armyworm, and southwestern corn borer in maize (Williams et al.,
18 1998, 2000; Abedon et al., 1999; Abedon and Tracy, 1996; Lawson and Poethig, 1995) and
19 other crops (Wang et al., 1999; Brink, 1962). These authors have shown that adult tissue is
20 more resistant to pathogens than juvenile tissue.

21 The genetics of phase transition has been indirectly studied by Abedon et al., (1996),
22 who found that general combining ability was more important than specific combining ability
23 for phase transition related traits, in a diallel among six sweet corn populations. These authors
24 concluded that there was variability in the timing of vegetative phase change in sweet corn and

1 that it was not associated with yield or yield components. Abedon and Tracy (1998), based on
2 lack of inbreeding depression, concluded that the timing of vegetative phase transition is
3 mainly governed by additive effects.

4 The objective of our study was to assess the potential modification through selection of
5 the timing of vegetative phase transition.

6

MATERIALS AND METHODS

A maize synthetic population, EPS5, where the letters E, P, and S stand for España (Spain), Pontevedra, and Synthetic, respectively, was made from 16 maize inbreds: EP1, EP19, EA2087, PB57, PB60, PB130, A251, A554, A556, A624, A637, A652, A654, A662, MS1334, and W182B. These 16 inbreds were released from European flint and several American Corn Belt varieties including a wide range of genetic backgrounds unrelated to the inbred B14. This synthetic is currently used in several breeding programs. The 16 inbreds were crossed in pairs to produce eight single crosses, which were then crossed to produce four double crosses, and so on until the 16-inbred cross was made in 1981. The synthetic was recombined two times and multiplied two more times before it was used for this study. For each recombination or multiplication, 150 plants were randomly crossed plant-to-plant, using each plant once, either as a female or a male. Consequently, the effective population size was greater than 100 individuals.

A divergent selection program for early and late phase transition was started in 1995. Selection for early transition was conducted by recombining plants with fewer leaves with epicuticular wax, while selection for late transition was conducted by recombining plants with more leaves having epicuticular wax. Although genotype $\times$ environment interaction is not significant for phase transition related traits (Abedon et al., 1996), there is an environmental effect on the production of epicuticular wax, which could be reduced or extended for a given genotype grown in different years. For that reason, the numbers of leaves with epicuticular wax in early and late phase transition changed in different years. In 1995, 300 plants of EPS5 were used as the base selection population. Fifty-four plants without epicuticular wax on the sixth leaf were recombined plant-to-plant to obtain the first cycle of early phase transition (EPS5(EPT)C1) and 22 plants with epicuticular wax on the ninth leaf were recombined for

obtaining the first cycle of late phase transition (EPS5(LPT)C1), where EPT stands for Early Phase Transition and LPT for Late Phase Transition. In 1996, 150 EPS5(EPT)C1 plants were grown and 40 plants without epicuticular wax on the seventh leaf were recombined to obtain the EPS5(EPT)C2. Forty six plants with epicuticular wax on the tenth leaf, out of 150 plants of EPS5(LPT)C1, were recombined to make EPS5(LPT)C2. Finally, in 1997, EPS5(EPT)C3 was produced by recombining 26 plants without epicuticular wax on the sixth leaf from 150 plants from EPS5(EPT)C2; and EPS5(LPT)C3 was formed by recombining 40 plants with epicuticular wax on the ninth leaf from 150 plants from EPS5(LPT)C2.

Response to selection was measured by comparing the three cycles for early phase transition (EPS5(EPT)C1, EPS5(EPT)C2, and EPS5(EPT)C3), the three cycles for late phase transition (EPS5(LPT)C1, EPS5(LPT)C2, and EPS5(LPT)C3), the original synthetic EPS5, and a commonly grown commercial hybrid D.M.B.15-70. Trials were grown in 1998 and 1999 in two locations (Cotobade and Pontevedra) in the northwest of Spain. Pontevedra is approximately at 20 m and Cotobade 500 m above sea level with average annual rainfall of about 1600 mm. Entries were arranged in a randomized complete block design with two replications.

Each plot consisted of two rows with 15 plants per row. Plants were spaced 0.21 m apart and rows were spaced 0.80 m, corresponding to a density of approximately 60 000 plants ha⁻¹. Hills were overplanted and thinned after emergence. In each plot, the following agronomic data were taken: early vigor (using the scale from 1 = weak to 9 = vigorous), pollen and silking dates, plant height (cm), number of ears per plant, ear length (cm), 100-kernel weight (g), seed-set (proportion of ear length filled with kernels), grain moisture at harvest (g kg⁻¹), and grain yield (kg ha⁻¹ adjusted to a moisture content of 140 g kg⁻¹). Also number of leaves with epicuticular wax, number of leaves below the main ear, and number of adult leaves

below the main ear were recorded as traits associated with the timing of vegetative phase transition.

Each location-year combination was considered as one environment in the analyses of variance. The sources of variation were environments, replications within environments, varieties (cycles of selection plus check), and the varieties $\times$ environments interaction. All sources of variation, except cycles of selection, were considered random. Genetic progress from selection was estimated by using the model suggested by Eberhart (1964). The sums of squares of population were partitioned into sums of squares due to linear and quadratic regression and deviations from regression. Further, regression sums of squares were partitioned into average linear regression, between linear regressions, quadratic regressions, and between quadratic regressions. This analysis is appropriate when two or more populations are developed from the same base population by different methods of selection, as in our study, where early and late phase transition are compared. Linear and quadratic regression coefficients were estimated from each selection method. Estimates of average linear and quadratic coefficients from both selection directions were also calculated following Eberhart (1964). All analyses were performed using the SAS program (SAS, 2000).

RESULTS AND DISCUSSION

Selection for phase transition was efficient because both linear and quadratic regressions were significant for leaves with epicuticular wax in the analysis of variance. Besides, differences between linear and quadratic regression coefficients were significant, indicating that the selection response for early and late phase transition were different. Deviation from the regression model was also significant (data not shown). However, linear and quadratic coefficients of selection for early phase transition were not significant. The third cycle of selection for late phase transition reached 10.4 leaves with epicuticular wax, while the third cycle of selection for early phase transition reached 6.2 leaves with epicuticular wax (Table 1). Since the original synthetic had 7.3 leaves with epicuticular wax, progress has been greater for increasing than for decreasing the number of leaves with epicuticular wax. In fact, the first cycle of selection for early phase transition did not significantly change from the original population (Table 1). The environment $\times$ cycle interaction was not significant for number of leaves with epicuticular wax (data not shown), which agrees with the results of Abedon et al. (1996).

Changes observed after divergent selection for phase transition were significant for half of the vegetative traits, particularly, the average coefficient of linear regression was significant for stalk lodging, and the average quadratic regression for plant height and leaves below ear (Table 1). However, for early phase transition, correlated responses were not significant for any vegetative trait, while for late phase transition, plant height and adult leaves below ear had significant response. Linear regression coefficients were significantly different between selection for early and late phase transition for leaves below ear and adult leaves below ear, although all these coefficients had the same sign. Besides, for leaves below ear, linear regression coefficients were not significant when quadratic effects were considered in the

1 model. Differences between quadratic regression coefficients were not significant for any trait.
2 Therefore, the observed changes cannot be considered correlated response to divergent
3 selection for phase transition. Vegetative traits increased with selection for phase transition,
4 either for improving performance, as early vigor, or for worsening as lodging.

5 Among the vegetative traits, changes observed after divergent selection were significant
6 for flowering dates, kernel weight, seed set, and grain moisture (Table 2). Selection for early
7 phase transition significantly affected almost the same traits than for late phase transition,
8 except that the first one affected grain yield and the last one affected seed set. Some yield
9 components, such as ears per plant and ear length did not change significantly after selection
10 for phase transition. Neither linear nor quadratic regression coefficients were significantly
11 different between directions of selection for any reproductive trait, suggesting that changes
12 were not due to correlated response to selection.

13 Generally, performance improved with selection for phase transition, though
14 regressions were not significant for some traits. When significant for a trait in one of the
15 directions of selection, coefficients of linear and quadratic regression had opposite sign. If
16 selection for early or late phase transition are considered separately, these results could allow
17 the conclusion that there are correlated responses to selection for phase transition. However,
18 when both directions of the divergent selection are considered, these changes cannot be
19 considered correlated responses because improvements happened in both directions of the
20 divergent selection, and coefficients of regression were not generally different between both
21 directions of selection. The most likely explanation for the observed changes is that selection
22 methodology requires rejection of plants showing poor performance and early plants have
23 larger probability of being chosen than late plants. We conclude that selection for the timing of
24 vegetative phase transition was efficient and there was no detrimental correlated responses on

1 any agronomic trait.

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Table 1. Means and significant regression coefficients for vegetative traits following three cycles of divergent selection for phase transition.

Trials were conducted at Cotobade and Pontevedra, Spain, during 1998 and 1999.

Cycles	Leaves			Leaves		Adult	
of	Early	with	Plant	below	leaves	Stalk	Root
selection	vigor	epic. wax	height	ear	below ear	lodging	lodging
	(1-9) [†]	No.	cm	No.	No.	%	%
EPS5	5.5	7.3	211	10.8	4.3	14.4	9.8
Early phase transition							
EPS5(EPT)C1	5.4	7.4	203	10.2	3.7	12.8	3.3
EPS5(EPT)C2	5.5	6.5	206	10.3	4.2	19.3	8.2
EPS5(EPT)C3	5.6	6.2	203	10.0	4.3	19.9	6.5
Linear regression	0.1±1.0	-0.2±0.3	-7±5	-0.5±0.3	-0.4±0.7	0.1±4.6	-3.8±5.0
Quadratic regression	0.0±0.3	-0.1±0.1	1±2	0.1±0.1	0.2±0.2	0.4±1.5	1.1±1.6
Late phase transition							
EPS5(LPT)C1	4.1	9.7	205	10.5	2.8	17.6	3.4
EPS5(LPT)C2	6.0	10.1	203	10.6	2.8	17.9	4.5

EPS5(LPT)C3	6.4	10.4	213	11.0	2.9	25.2	5.4
Linear regression	-0.9±0.9	2.5±0.3**	-11±5*	-0.4±0.3	-1.5±0.7*	1.0±4.6	-5.8±5.0
Quadratic regression	0.4±0.3	-0.5±0.1**	4±2*	0.2±0.1	0.4±0.2	0.8±1.5	1.6±1.6
Average regression							
Linear	0.4±0.2	0.2±0.1**	0±1	0±0.1	-0.1±0.2	3±1.0*	-0.3±1.2
Quadratic	0.1±0.2	-0.2±0.1**	3±1*	0.13±0.06*	0.2±0.1	1.0±1.0	1.4±1.1
LSD (P=0.05)	1.7	0.5	9	0.5	1.3	8.8	9.6
DMB1570	6.1	8.7	234	12.1	4.1	19.2	11.8

† Early vigor recorded with the scale 1 = weak to 9 = vigorous.

*, ** Regression coefficient significant at P=0.05 and 0.01, respectively.

Table 2. Means and significant regression coefficients for reproductive traits following three cycles of divergent selection for phase transition. Trials were conducted at Cotobade and Pontevedra, Spain, during 1998 and 1999.

Cycles of selection	Pollen date	Silking date	Ears per plant	100-kernel weight	Ear length	Seed set	Grain yield	Grain moisture
	d	d	No.	%g	cm	%	kg ha ⁻¹	g kg ⁻¹
EPS5	77	80	0.91	31.2	15.5	88.1	3456	267
Early phase transition								
EPS5(EPT)C1	73	74	0.99	24.9	15.3	91.6	4560	221
EPS5(EPT)C2	74	75	1.00	26.5	15.5	91.2	4906	219
EPS5(EPT)C3	74	75	1.03	25.2	15.9	92.2	4454	213
Linear regression	-4±1**	-5±1**	0.07±0.09	-4.9±1.3**	-0.4±0.6	3.1±15	1570±712*	-4±1*
Quadratic regression	1±0*	1±0**	-0.01±0.03	1.1±0.4*	-0.2±0.2	-0.6±0.5	-406±229	1±0*
Late phase transition								
EPS5(LPT)C1	74	75	0.94	25.4	15.7	90.7	3739	226
EPS5(LPT)C2	74	75	0.94	26.9	15.5	91.9	4587	228
EPS5(LPT)C3	75	76	0.96	26.5	15.6	90.6	4666	224

Linear regression	-4±1**	-5±1**	0.01±0.09	-4.7±1.0*	-0.2±0.5	35±1.5*	599±712	-4±1*
Quadratic regression	1±0*	1±0*	0.00±0.03	1.2±0.4**	-0.1±0.5	-0.9±0.5	-51±229	1±0*
Average regression								
Linear	-1±0	-1±0*	0.02±0.02	-1.0±0.3**	0.1±0.2	0.8±0.4*	348±169	-1±0*
Quadratic	1±0**	1±0**	-0.01±0.02	1.1±4.1**	0.1±0.1	-0.7±0.3	-223±157	1±0*
LSD (P=0.05)	2	3	0.18	2.5	1.2	2.9	1367	15
DMB1570	80	82	1.00	29.5	18.2	94.1	6072	232

* Regression coefficient significant at P=0.05.