

1 **Breeding maize for traditional and organic agriculture**

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11

1 **Abstract**

2 Breeding maize (*Zea mays* L.) for traditional agriculture can increase quality and added value of
3 agricultural products and allow the recovery of traditional foods. The objectives of this work were to
4 evaluate improved open-pollinated populations under organic and conventional agriculture in order to
5 determine the effects of selection for yield and flour yield and the relationship between agronomic and
6 quality traits under both cropping systems. We have selected open-pollinated maize populations for flour
7 yield and bakery quality under organic conditions, improved them under conventional conditions and
8 evaluated the breeding programs under organic and conventional conditions. Breeding was efficient for
9 grain and flour yield under organic agriculture for Meiro (an open-pollinated population with black
10 grains) but not for the other populations neither in organic nor in conventional conditions. Yield ranks of
11 varieties were moderately correlated under both conditions, and genotype \times environment interaction (GE)
12 was significant for most traits when the analyses of variance were made over all environments but also
13 when organic and conventional environments were separated. GE was higher under organic agriculture.
14 Correlations between traits were higher under conventional agriculture and there were important
15 discrepancies between correlations in organic and conventional agriculture. We concluded that selection
16 under conventional agriculture was efficient for one population under organic agriculture. Selection under
17 the target environment could increase the possibilities of success.

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19 **Key words:** organic agriculture; yield; quality; germplasm; breeding

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1 **Introduction**

2

3 Traditional agriculture was similar to organic agriculture until the advent of inorganic fertilizers and
4 phytosanitary synthetic products in the second half of the Twentieth Century. According to Kovacevic
5 and Lazic (2012), organic agriculture is based on strong ecological principles and the absence of
6 application of agrochemicals, GMO, etc. Organic agriculture is a holistic way of farming besides
7 production of goods of high quality; conservation of the natural resources and richness of biodiversity.

8 When breeding crops for traditional or organic agriculture, some breeders consider that selection
9 need not be accomplished under both organic and conventional agriculture because varieties developed
10 for conventional agriculture are also suitable for organic agriculture (Ardelean et al. 2012; Burger et al.
11 2008; Lorenzana and Bernardo 2008). Contrarily, other authors emphasize the need to develop effective
12 strategies for improving crop performance in organic systems through plant breeding and that varieties
13 should be improved for specific adaptation (Löschenberger et al. 2008; Murphy et al. 2005; Wolfe et al.
14 2008; Kovacevic and Lazic 2012; Van Bueren et al. 2011). These last authors have suggested that
15 breeding for organic conditions has specific requirements for some crops and that cultivars that perform
16 very well under conventional agriculture are not interesting for organic agriculture. Nevertheless, no
17 specific maize breeding program has been reported so far although Rodrigues de Oliveira et al. (2011)
18 point out that election of base breeding material must be made in the specific environment for the
19 expression of favorable alleles that confer advantages for adaptation to this system. Some authors also
20 tested under organic conditions hybrids released under conventional conditions and found an acceptable
21 agreement (Lorenzana and Bernardo 2008; Burger et al. 2008); however, yield was reduced under organic
22 conditions (Burger et al. 2008). Several breeders have released improved varieties under conventional
23 agriculture, but there are few reports of plant breeding under organic agriculture.

24 Maize open-pollinated populations have been cultivated for centuries in traditional agriculture
25 and selected for adaptation and quality but have lower yield than modern hybrids under conventional
26 agricultural conditions. Since the introduction of maize in Europe, diverse maize varieties have been
27 selected for adaptation to a wide range of environments and consumer preferences. Such wide genotypic
28 and environmental diversity can have caused also significant genotype × environment interactions with
29 specific adaptation to environmental conditions and farmer's preferences (Duarte et al. 2005; LeFord and
30 Russell 1985; Malvar et al. 2008; Revilla et al. 2008).

1 Maize was traditionally used for bread, which is a peculiarity of the north of Spain and Portugal
2 but also of other countries. Maize bread is made traditionally with whole flint maize grains. Consumers
3 prefer flint grains because their flour has better cooking characteristics and flavor than dent grains (Landa
4 et al. 2006). Quality requirements are important for human consumption (Watson 1988) but bread quality
5 is not defined for maize as for wheat and baking quality is lower for maize than for wheat (He and
6 Hoseney 1991). Although there are no defined criteria for bakery quality for maize bread, some criteria
7 are generally accepted, such as large grain size, uniformity, high density, and lack of physical damage,
8 pests, and diseases (Alonso-Ferro et al. 2008; Serna-Saldivar et al. 2001; Watson 1988). Yellow maize is
9 used normally for feed because it is a source of carotenoids for animals (Troyer 1999), while white maize
10 is preferred for human consumption because pigments cause strong aroma and flavor when cooked
11 (Poneleit 2001). Moreover, yellow or black grains are preferred in some areas (Serna-Saldivar et al. 2001;
12 Landa et al. 2006). Actually, in previous studies we concluded that differences in pigment content are
13 directly related to antioxidant activity in maize grains and that traditional methods for maize production
14 and processing maintained quality, pigment content and antioxidant activity (Revilla et al. 2012;
15 Rodríguez et al., 2013).

16 Revilla et al. (2008) identified some local varieties with white, yellow, and black grains,
17 appropriate for bakery that performed well under organic agriculture. In that report we concluded that
18 there was no clear relationship between yield and quality and the varieties improved under conventional
19 agriculture are adequate for organic agriculture as well. Therefore, we began some breeding programs for
20 improving open-pollinated varieties for agronomic performance under conventional agriculture focusing
21 on traditional and organic agriculture.

22 With the introduction of hybrids under intensive cropping systems, the local populations and the
23 traditional uses of maize have been abandoned. The reintroduction of improved traditional varieties
24 suitable for organic agriculture and for manufacturing products for human consumption and organic
25 agriculture would fit the social demands for higher quality and safe foods. The objectives of this work
26 were to evaluate under organic and conventional agriculture several maize varieties improved for grain
27 yield or quality along with other open-pollinated populations of maize grown in the past by farmers under
28 traditional agriculture to find out if selection for grain yield and flour yield under conventional agriculture
29 was useful also for organic agriculture, and to investigate the relationship between agronomic and quality
30 traits under both cropping systems.

1 **Materials and Methods**

2

3 *Plant material and breeding programs*

4 We evaluated for agronomic performance and grain quality under organic and conventional
5 agriculture ten open-pollinated populations, potentially valuable for maize bread or bakery use (Table 1).
6 Five of these open-pollinated populations (Donostia, Meiro, Rebordanes, Sarreaus, and Tuy) have been
7 improved for grain yield or flour yield during one or three cycles. The breeding program for Donostia was
8 carried out in the experimental field of NEIKER (Álava) and consisted on evaluating 100 S₁ families and
9 recombining the 20 S₁ families with the higher ability for making “talo”, a kind of maize bread. One cycle
10 of selection was carried out. The breeding program for Meiro, Rebordanes and Sarreaus was carried out
11 in the experimental field of Misión Biológica de Galicia (Pontevedra) and consisted recombining the 20
12 S₁ families with highest flour yield and quality for maize bread from 100 S₁ families. Flour yield was
13 obtained by multiplying yield by proportion of flour produced after grinding 50 g of whole grain in a
14 coffee mill for 1 minute, and sieving for 1 minute in a sieve with 1 mm orifices (Table 2). Quality for
15 maize bread was assessed by a panel that evaluated maize breads made from the S₁ families with flour
16 yield above average. Bread was made following a traditional recipe (Revilla et al. 2008). Two cycles of
17 selection were carried out for those three populations. Finally, the breeding program for Tuy was carried
18 out in a similar way, but in this case the selection criterion was grain yield. Three cycles of selection were
19 carried out for this population.

20

21 *Experimental design*

22 The resulting 20 open-pollinated maize populations and improved cycles from northern Spain
23 and two commercial checks were evaluated in two farmers’ fields in Galicia and in the Basque Country
24 under organic and conventional agriculture. Five organic environments and five conventional
25 environments were used between 2010, 2011 and 2012 (Table 2). The 22 genotypes were evaluated in
26 trials that followed an experimental design of randomized complete blocks with three replications. The
27 experimental plots of 10 m² had a density of 60,000 plants ha⁻¹, with rows separated 0.8 m and plants
28 within rows 0.21 m. Agricultural practices followed the recommendations of organic agriculture, i.e.
29 nutrients were supplied by adding manure, weeds were removed mechanically, and no chemical treatment
30 was used. Under conventional agriculture, current practices were the usual in the area with inorganic

fertilizers, use of herbicide and no irrigation. On each plot we measured early vigor (scale from 1 = weak to 9 = vigorous on 5-week old plants), plant appearance (the same scale from 1 to 9 but after flowering), days to silking, ears per plant, grain yield (Mg ha^{-1} at $140 \text{ g H}_2\text{O kg}^{-1}$), flour yield (Mg ha^{-1} calculated as described above for the selection programs), grain moisture (g kg^{-1}), 100 grain weight, milling test (% of grinds), and grain density (g ml^{-1}). Milling test was an estimation of the resistance of grains to produce flour in a laboratory mill; it was defined as the percentage of flour produced in a limited time. The milling test consisted on grinding 50 g of whole grain in a coffee mill for 1 minute, sieving for 1 minute in a sieve with 1 mm orifices, and weighting the remaining fraction. Milling test was calculated as $100 \times (\text{grain weight} - \text{remaining fraction}) / \text{grain weight}$. The method for estimating grain density was to pour 50 g of whole grain in a test-tube containing 50 ml of 95% ethanol, and to record the final volume of the mixture. Grain density was estimated as $\text{grain weight} / (\text{final volume} - \text{initial volume})$.

Statistical analyses

Analyses of variance were carried out using the procedure GLM of SAS (SAS Institute Inc. 2010) with 10 environments. Each environment is the combination of one year and one location. Five environments were under conventional conditions and 5 under organic conditions. First, we made combined analyses of variance over the ten environments in order to check the genotype \times environment interaction (GE) and considering random all effects except genotypes. We carried out analyses of variance by type of agriculture (organic and conventional) and considering random all effects except type of agriculture. Then we carried out analyses of variance by environment in order to check if interactions were of rank or of magnitude, considering also random all effects except genotypes.

Comparisons of means were made by using the Fishers' protected LSD at $P=0.05$. Pearson (simple) and Spearman (rank) correlations were calculated between traits with the procedure CORR of SAS. To analyze the efficiency of the selection programs, values of 0, 1, 2, and 3 were assigned to the corresponding cycles of selection, and simple linear regression analyses incorporating random effects in the model [environment and replication (environment)] were performed for yield, flour yield and milling test (dependent variable) and cycles of selection (independent variables).

1 **Results**

2

3 The combined analyses of variance showed that differences between types of agriculture (organic or
4 conventional) were not significant for the main agronomic traits (grain moisture or yield) but differences
5 were significant for most quality traits, including 100 kernel weight, milling test and grain density (Table
6 3). Differences between varieties were significant for few traits and the triple interaction environment \times
7 type \times variety was significant for most traits.

8 When we checked the effects of selection for yield, flour yield, and milling test, we found out
9 that most regression coefficients were not significant, with most of the significant coefficients being
10 negative (Table 4). Only Meiro significantly increased flour yield under organic conditions while it
11 decreased for Sarreaus, and the response was not significant under conventional conditions. The response
12 was similar for yield, except for Donostia with a significant decrease of yield in both organic and
13 conventional conditions. For milling test, Rebordanes increased grain hardness under both conditions and
14 Sarreaus decreased hardness under conventional conditions.

15 In the combined analyses of variance over the ten environments, genotypes were significantly
16 different for all traits except early vigor, and environments for all traits except early vigor and plant
17 appearance. Genotype \times environment interaction (GE) was significant for all traits except ears per plant
18 and grain density and interactions were mainly of rank. We carried out analyses of variance by type of
19 agriculture (organic vs. conventional) and found that the genotypes were significantly different for all
20 traits except early vigor, plant appearance and silking under conventional agriculture, and for all traits
21 except early vigor, silking, ears per plant and grain density under organic conditions. Environments were
22 also significantly different for most traits, and GE was significant for most traits including grain yield,
23 flour yield and grain moisture. Interactions were mostly of rank (Tables 5 and 6).

24 Combined means for flour yield were highest for the commercial checks and Meiro(P)C2 (Table
25 4). Ranks under organic and conventional conditions were similar. Both commercial checks had the
26 highest yield in both conditions. Many varieties were not significantly different for milling test from those
27 with hardest grains across conditions, namely Carballeira, Rebordanes, and Getaria. The softest grains
28 under organic conditions were those of Meiro(P)C2, and of NKThermo under conventional conditions,
29 while across conditions the softest grains were produced by Oroso, the commercial check PR36B08, and

1 Sarreaus(P)C2. Rank correlations between organic and conventional conditions were moderate for milling
2 test ($r^2=0.65$, $P=0.001$), flour yield ($r^2=0.70$, $P>0.001$), and yield ($r^2=0.69$, $P>0.001$) (Figure 1).

3 The highest and the lowest yielder for each location were different both between and among
4 organic and conventional conditions (Table 5). However, the most stable across organic conditions was
5 Meiro(P)C2 followed by Tuy(S)C2 and NKThermo. Under conventional conditions, the most stable was
6 the commercial check PR36B08.

7 GE was significant for milling test under conventional conditions but not under organic
8 conditions. Differences among genotypes were not significantly different for three organic and one
9 conventional environments. The hardest grains across locations were those of Rebordanes(P)C2 both
10 under organic and conventional conditions followed by Tuy (Table 6). The varieties with hardest grains
11 differed for each location except for Rebordanes(P)C2 and Carballeira that had the hardest grains in two
12 and three locations, respectively. Differences among genotypes for early vigor were not significant in
13 either type of agriculture, for plant appearance only in organic conditions, and for silking, ears per plant,
14 and grain density only in conventional conditions (Table 7). The varieties with fewer days to silking were
15 Sarreaus and its improved cycles, Donostia and its improved cycle, and Martikoenea, while those with
16 high yield, such as Meiro and its improved cycles and the commercial hybrid PR36B08, were among
17 those with the longest growth cycle. Grain moisture is another measure of earliness that has low
18 correlation with silking (Table 8) but varieties with lowest grain moisture had also few days to silking and
19 vice versa (Table 7). Varieties also performed differently for grain moisture under organic and
20 conventional conditions. Ears per plant were below one under organic conditions while most varieties had
21 around one ear per plant in conventional conditions and the lowest values were for Martikoenea followed
22 by DonostiaC1 and Txalin.

23 Correlations between traits under organic and conventional conditions were different for grain
24 yield and grain moisture under conventional ($r^2=0.79$ $P>0.01$) and organic ($r^2=-0.68$ $P>0.01$) conditions,
25 for flour yield with grain moisture under conventional ($r^2=0.78$ $P>0.01$) and organic ($r^2=-0.67$ $P>0.01$)
26 conditions (Table 8). Silking and early vigor, and silking and plant appearance had significant correlation
27 under conventional conditions, but not under organic conditions. Agronomic traits had weak correlations
28 with quality traits, except for milling test that had a negative correlation with yield, flour yield, and grain
29 moisture; these correlations were even weaker under organic conditions

30

1 **Discussion**

2

3 Breeding was efficient for Meiro that increased yield and flour yield only under organic conditions.

4 However, selection was not efficient for the other open-pollinated varieties. Other authors have shown
5 that intrapopulation recurrent selection with selfed families was efficient under conventional agriculture
6 (Romay et al, 2011, Vales et al. 2001, Weyhrich 1998). The explanations of our results could be that the

7 evaluation was carried out in different fields (with more stressful conditions) than those used for
8 selection, that the variability available for yield was not enough or was exhausted early, or that the
9 selection reached a ceiling for example in the second cycle of Tuy. Whatsoever the reason could be, it is

10 also true that the selection programs were similarly inefficient for yield and for flour yield both under
11 organic and conventional conditions. Accordingly, Burger et al. (2008) stated that no specific adaptation
12 to conventional or organic agriculture was observed in maize hybrids and concluded that including

13 organic experimental sites among the evaluation fields increased the chances of success when selecting
14 for organic conditions. Similarly, Lorenzana and Bernardo (2008) concluded that high-yielding cultivars
15 for organic systems can be developed by screening conventional inbreds and hybrids for their

16 performance under organic systems. Boller et al. (2008) concluded that varieties of grasses should be
17 chosen based on performance under organic conditions but yields in organic and conventional conditions
18 were high enough to expect that selection under either condition would be similarly efficient. However,

19 most of the breeding programs we carried out under conventional agricultural conditions in our station
20 were not efficient when evaluated under organic conditions, suggesting that breeding programs carried
21 out in the target environment could be more efficient, as other authors have concluded, e.g. Rodrigues de

22 Oliveira et al. (2011) believe that maize breeding for organic conditions should be carried out in the target
23 environment; Löschenberger et al. (2008) recommended a winter wheat breeding program specifically
24 designed for organic agriculture since the election of the base germplasm until the final evaluation; and

25 Murphy et al. (2005) recommend a specific method for breeding for organic conditions that they call an
26 evolutionary participatory breeding method for improving inbred small grain crop species on a large
27 number of low-input and organic farms.

28 In the current trials, GE was very important probably due to the variety of environments and
29 cultural practices involved; nevertheless, evaluations of wide collections of maize varieties for human
30 consumption under conventional or organic agriculture show large diversity also for the importance of

1 GE (Duarte et al. 2005; LeFord and Russell 1985; Malvar et al. 2008; Revilla et al. 2008). Other reports
2 combining organic and conventional agriculture have shown inconsistent results concerning GE (Lazcano
3 et al. 2012; Ardelean et al. 2012).

4 Meiro(P)C2 and some of the commercial checks had the highest flour yield and, although the
5 rank for flour yield was not the same under organic and conventional conditions, there was a reasonable
6 agreement for the best and the worst genotypes. Grain yield was highest for both commercial checks,
7 followed by Meiro(P)C2 and Martikoenea. Milling test was not as discriminating as yield and many
8 varieties were not significantly different from those with hardest grains across conditions. Comparisons of
9 means were more discriminating under organic than under conventional agriculture because the organic
10 fields used here were less heterogeneous than the conventional fields and, thus, GE was higher under
11 conventional agriculture. Rank correlations between organic and conventional conditions were moderate
12 for flour yield, yield, and milling test. Other authors have reported low correlations between organic and
13 conventional conditions (Löschenberger et al. 2008). Burger et al. (2008) reported moderate phenotypic
14 correlations between organic and conventional agriculture for grain yield and strong correlation for grain
15 dry matter content but not consistent genotypic correlations for maize hybrids. Boller et al. (2008) found
16 good correlations between yield of grasses under organic and conventional conditions and Lorenzana and
17 Bernardo (2008) reported that genetic correlations for performance in the two production systems were
18 0.84 for grain yield; greater than 0.90 for grain moisture, plant height, and ear height; and about 0.50 for
19 root lodging and stay green for maize hybrids.

20 As the organic and the conventional trials were carried out in different locations we cannot make
21 direct comparisons between organic and conventional conditions. Nevertheless, genotypes performed
22 under organic conditions better than under conventional conditions for early vigor and plant appearance,
23 and had fewer days to silking, while conventional conditions were superior for ears per plant, yield and
24 flour yield, grain moisture (drier grains), 100 grain weight, and grain density; also grains were harder
25 under organic conditions. Burger et al. (2008) found that maize hybrids yielded 16% less under organic
26 than under conventional conditions. As a general trend, our open-pollinated varieties performed better in
27 the organic environments for vegetative traits and in the conventional environments for yield components.
28 Indeed, these results depend both on the genotypes and locations involved in these experiments.

29 The earliest varieties, based on flowering and grain moisture, were Sarreaus and its improved
30 cycles, Donostia and its improved cycle, and Martikoenea. The varieties with longest growth cycle had

1 also the highest yield, such as Meiro and its improved cycles and some commercial checks. The
2 correlation between flowering and grain moisture was low, but several varieties performed differently for
3 grain moisture under organic and conventional conditions but there were also some agreements between
4 organic and conventional conditions.

5 Quality traits varied between organic and conventional conditions, as under organic conditions
6 grains were harder and had lower weight and density. Although quality is important for these populations
7 initially intended for human consumption, it is not clear which are the parameters that define quality. In
8 previous works we have considered that grain weight, milling test, and grain density were important
9 quality factors (Revilla et al. 2008) following a general opinion that considers purity of the white color,
10 large uniform size of grains, high specific density, hard endosperm, and white cob (Watson 1988). Here
11 we include black and yellow varieties because in these regions they are used also for human consumption.
12 The lack of response to selection for quality traits can be partially due to the genetic complexity involved
13 in its regulation (Alonso Ferro et al. 2008; Malvar et al. 2008).

14 Generally, correlations between traits were higher under organic than under conventional
15 conditions and correlations among traits under organic and conventional conditions were different for a
16 number of pairs of traits, such as grain yield and grain moisture under conventional and organic
17 conditions, which were both significant but with opposite signs, as were for flour yield with grain
18 moisture under conventional and organic conditions. There were also pairs of traits with significant
19 correlation under conventional conditions, but not under organic conditions; the reason for these
20 discrepancies could be that the conventional environments where the trials were carried out were more
21 similar to the environments used for selection than the organic environments. Besides, the relationships
22 between agronomic performance and quality were not strong, except for milling test that had a negative
23 correlation with yield, flour yield, and grain moisture; these correlations were even weaker under organic
24 conditions. In a previous work we also found a weak relationship between yield and quality under organic
25 conditions (Revilla et al. 2008).

26 As conclusion, breeding has been efficient for Meiro in organic conditions while for the other
27 populations was equally inefficient under organic and conventional conditions. The GE and the
28 complexity of these traits can be partially responsible for these negative results because their
29 improvement is not straightforward.

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Table 1. Maize varieties, origin, type of germplasm and growth cycles evaluated in Northern Spain under organic and conventional agriculture		
Genotype	Origin	Cycle, grain color
Carballeira	Galicia	Medium, black
Donostia	Basque Country	Medium, yellow
DonostiaC1	Basque Country	Medium, yellow
Martikoenea	Basque Country	Medium, yellow
Meiro	Galicia	Medium late, black
Meiro(P)C1	Galicia	Medium late, black
Meiro(P)C2	Galicia	Medium late, black
NKThermo	Commercial check	
Oroso	Galicia	Medium, yellow
PR36B08	Commercial check	
Rebordanes	Galicia	Medium, white
Rebordanes(P)C1	Galicia	Medium late, black
Rebordanes(P)C2	Galicia	Medium late, black
Sarraus	Galicia	Early, Yellow
Sarraus(P)C1	Galicia	Early, Yellow
Sarraus(P)C2	Galicia	Early, Yellow
Tuy	Galicia	Medium, yellow
Tuy(S)C1	Galicia	Medium, yellow
Tuy(S)C2	Galicia	Medium, yellow
Tuy(S)C3	Galicia	Medium, yellow
Txalín	Basque Country	Medium, yellow

Table 2. Environments used for selection and evaluation of 23 maize varieties, improved cycles and checks in Northern Spain under organic and conventional agriculture						
Environment	Location	Year	Type	Latitude	Longitude	Altitude
1	Lobeira (Ourense, Galicia)	2010	Organic	41° 60' N	8° 02' W	600 m
2		2011				
3		2012				
4	Heredia (Álava, Basque Country)	2010		42° 53' N	2° 26' W	567 m
5	2012					
6	Pontevedra ^a	2010	Conventional	42° 24' N	8° 38' W	20 m
7	(Galicia)	2011				
8		2012				
9	Arkaute ^a (Álava, Basque Country)	2010		42° 51' N	2° 38' W	512 m
10	2011					

^a Selection sites

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Table 3. Degrees of freedom (df) and mean squares of the main agronomic and quality traits from the analyses of variance of 22 maize varieties, improved cycles and checks (see table 1) evaluated under organic and conventional conditions in five environments (see table 2) in Northern Spain							
Sources of variation	df	Grain moisture (g / kg)	Yield (kg / ha)	Flour yield (kg / ha)	100 kernel weight (g)	Milling test (%)	Grain density (g/cm ⁻³)
Environment	3	776.84**	768115455**	611681861**	1784.13**	177.02**	0.0516*
Type	1	367.72	46217972	41889517	401.28**	17.09*	0.0619**
Repetitions (env.)	8	18.81**	4924875**	3874024**	11.75	23.08**	0.009*
Variety	21	64.68**	9129773	6709382	154.65**	9.7**	0.0054
Env × Var	57 ^a	11.42	8713440	7021260	36.23	3.79	0.0047
Type × Var	21	38.36	1857704	1482925	19.26	2.68	0.0044
Env × Type × Var	53 ^b	134.27**	15912305**	11640576**	37.16**	4.25**	0.0053*
Error	287 ^c	3.067	987340	817366	9.076	2.004	0.00357
*, ** Significant at P = 0.05 and 0.01, respectively							
^a DF (Env × Var) = 54 for Moisture, Yield and Flour yield							
^b DF (Env × Type × var) = 35 for Moisture and Yield and 34 for Flour yield							
^c DF (Error) = 263 for moisture, 257 for yield, 247 for flour yield, and 279 for density							

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Table 4. Mean ^a yield, flour yield (kg ha ⁻¹ at 140 g kg ⁻¹ grain moisture) and milling test (% of grinds) for 22 maize varieties, improved cycles and checks (see table 1) evaluated in five environments (see table 2) in Northern Spain under organic (Org) in five environments under conventional (Con) agriculture and significant coefficients of regression for cycles of selection						
	Flour yield		Yield		Milling test	
	Org	Con	Org	Con	Org	Con
Carballeira	4118def	5199cd	4672def	5456def	11.87a	11.34ab
Donostia	4148def	5194cd	4570def	5715c-f	9.25de	10.03b-f

DonostiaC1	3338ef	5512bcd	3347fg	4987ef	9.71de	10.42a-e
Donostia b ^b			-1222.6*	-1170.7*		
Getaria	1679g	3917d	1884g	4406f	11.00abc	11.20abc
Martikoenea	5830abc	7831ab	6503bc	8727ab	10.27bcd	10.11a-f
Meiro	5096bcd	6614abc	5641bcd	7274b-e	9.57de	9.51d-g
Meiro(P)C1	5110bcd	6026a-d	5644bcd	6920b-f	9.61de	9.80b-g
Meiro(P)C2	6205ab	6830abc	6833b	7584a-d	9.01e	9.47d-g
Meiro b ^b	554.6*		596.0*			
NKThermo	6860a	8129a	9720a	8140abc	10.24bcd	8.30g
Oroso	3129f	6600abc	3429efg	6389b-f	9.53de	8.80gf
PR36B08	5747abc	8107a	10592a	10052a	9.47de	8.89efg
Rebordanes	4451c-f	5982a-d	4951c-f	6118c-f	9.80cde	10.27b-h
Rebordanes(P)C1	4785cd	6184 a-d	5318bcd	6878b-f	10.24bcd	10.72a-f
Rebordanes(P)C2	4035ef	4890cd	4528def	5711c-f	11.24ab	11.57a
Rebordanes b ^b					0.72**	0.51*
Sarreus	4923bcd	5575bcd	5485bcd	6191b-f	10.09b-e	10.63a-d
Sarreus(P)C1	4560cde	5041cd	5056b-f	5583c-f	9.77de	10.47a-d
Sarreus(P)C2	4187def	5513bcd	4613def	6054c-f	9.63de	9.37d-g
Sarreus b ^b	-368.4*		-435.8*			-0.68**
Tuy	4690cde	6162 a-d	5219cde	6872b-f	10.15b-e	10.84a-d
Tuy(S)C1	4793cd	6239 a-d	5308bcd	6482 b-f	9.68de	10.06a-f
Tuy(S)C2	4965bcd	6562abc	5513bcd	7255 b-e	9.80cde	10.37b-e
Tuy(S)C3	4715cde	6541abc	5147b-e	7293b-d	10.12b-e	10.56a-d
Tuy b ^b						
Txalin	5314bcd	5428cd	6786b	5931c-f	10.30bcd	9.70c-g
LSD (5%)	1407	2375	1797	2582	1.20	1.55
^a Means followed by the same letter within the same column are not significantly different (P=0.05)						
^b Coefficient of regression of each trait on cycles of selection. +, *, ** significant at P = 0.1, 0.05, and 0.01,						

respectively

Table 5. Mean^a yield (Mg ha⁻¹ at 140 g kg⁻¹ grain moisture) for 22 maize varieties, improved cycles and checks (see table 1) evaluated in ten environments (see table 2) in Northern Spain under organic and conventional agriculture

	Organic conditions					Conventional conditions				
Genotype	1	2	3	4	5	6	7	8	9	10
Carballeira	2966.7c	2532.0cde	2787.0a-d	6999.7b-f	5146.3h	881.7a-d		5283.3de	8167.0cde	7491.3hg
Donostia	2717.0c	1825.3efg	1544.7e	7936.3a-e	8076.7g	1200.0a-d		2883.0fg	9791.0abc	8985.7fge
DonostiaC1	3321.7bc	1200.7g	388.0f	7135.3b-f	8824.7fg	99.5d		1580.7g	8980.0b-e	7660.0hg
Getaria			1884.0de					4405.7ef		
Martikoenea	2862.3c	2110.7d-g		9144.7a	4689.3h	304.0dc			9110.0b-e	11151.7c
Meiro	4286.3abc	3228.3abc	2983.3a-d	7610.0a-f	11893.7bc	1261.0a-d		7227.3c	9427.0a-d	11179.7c
Meiro(P)C1	2739.7c	3918.0a	2219.7b-e	7927.3a-e	10098.3def	1194.3a-d		7770.3c	9768.0abc	8945.7fge
Meiro(P)C2	5653.3a	3640.7ab	2884.3a-d	8520.0ab	11413.7cde	1042.7a-d		9758.3b	8804.0b-e	10728.7c
NKThermo				7633.3a-e	13468.3ab	1834.3ab		12488.7a	7167.0e	11071.7c
Oroso	2774.3c	1514.0fg		6000.0fg	11807.3bcd	532.7bdc			10645.0ab	7991.0fgh
PR36B08				6356.0efg	14828.3a	2185.0a		13248.0a	8523.0b-e	16251.3a
Rebordanes	3300.3bc	2958.7bcd	3118.3abc	5353.3g	10026.0ef	833.7a-d		6796.0cd	7174.0e	9668.3dce
Rebordanes(P)C1	4169.0abc	2403.0c-f	2622.0a-e	8354.3ab	9042.3fg	1091.0a-d		6394.3cd	11578.0a	8449.3e-h
Rebordanes(P)C2	2604.7c	2234.5def	2587.7a-e	6580.0d-g	7869.3g	527.0bcd		5988.7cde	8662.0b-e	7667.0hg
Sarreaus	3561.0bc	1981.0efg	2035.7cde	7748.7a-e	12099.3bc	521.0bcd		5934.0cde	9249.0b-e	9058.7d-g
Sarreaus(P)C1	3161.0bc	2534.7cde	2352.3a-e	6007.3fg	11225.7cde	1186.5a-d		5203.7de	7507.0de	6969.7h
Sarreaus(P)C2	3296.3bc	1196.0g	1541.0e	7679.7a-e	9354.0fg	749.0bcd		4361.7ef	9176.0b-e	8161.7e-h
Tuy	3066.7c	1907.0efg	3338.3a	8800.3a	8980.7fg	1075.3a-d		6359.3cd	10458.0ab	9595.7c-f
Tuy(S)C1	3835.0abc	2366.3c-f	2400.7a-e	7994.0a-d	9943.3ef	1502.3a-d		6477.3cd	9161.0b-e	8788.7efg
Tuy(S)C2	5085.3ab	2670.3cde	3153.0ab	8237.0abc	8417.3fg	603.3bcd		6046.0cde	9433.0a-d	12938.0b
Tuy(S)C3	3522.0bc	3176.3abc	3011.3abc	6667.0c-g	9358.3fg	1098.0a-d		7192.0c	8167.0cde	10648.7dc
Txalin	3223.7bc			8488.7ab	8645.3fg	1549.3abc			9209.0b-e	7034.0h
LSD (0.05)	1946.9	927.3	1100.3	1610.4	1739.4	1413.5		1872.8	2163.5	1639.5

^a Means followed by the same letter within the same column are not significantly different (P=0.05)

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Table 6. Mean ^a milling test (% of grinds) for 22 maize varieties, improved cycles and checks (see table 1) evaluated in ten environments (see table 2) in Northern Spain under organic and conventional agriculture											
		Organic conditions					Conventional conditions				
Genotype		1	2	3	4	5	6	7	8	9	10
Carballeira	11.74b-e	11.00a	13.34	9.94	13.34	10.8ab	13.20a	13.94a	9.94	9.26b-f	
Donostia	9.06f	8.66b-f	10.34	7.86	10.34	10.74ab	11.00bc	10.80de	7.86	9.74b-e	
DonostiaC1	10.06def	8.86b-e	12.20	8.54	12.20		13.00a	11.40bcd	8.54	9.60b-f	
Getaria			11.00					11.20cde			
Martikoenea	11.94b-e	8.66b-f		9.46	11.00	9.60ab	10.26b-e		9.46	10.94ab	
Meiro	11.46b-e	6.60f	10.86	8.06	10.86	8.34bc	10.46b-e	12.94a-d	8.06	7.74f	
Meiro(P)C1	11.2fcde	7.14def	11.06	7.60	11.06	9.14ab	10.34b-e	12.80a-d	7.60	8.80c-f	
Meiro(P)C2	9.66ef	6.94ef	10.06	8.34	10.06	8.60bc	8.80ef	11.60bcd	8.34	10.00b-e	
NKThermo	10.90c-f			9.80		5.20c	8.94def	8.00f	9.80	8.54def	
Oroso	12.20bcd	8.66b-f		7.74		8.50bc	10.90bc		7.74	8.66c-f	
PR36B08				9.46		8.46bc	8.20f	8.94ef	9.46	10.40a-d	
Rebordanes	11.00c-f	7.46c-f	11.66	7.20	11.66	10.10ab	10.54bcd	13.06a-d	7.20	10.40a-d	
Rebordanes(P)C1	11.80b-e	9.26abc	10.66	8.80	10.66	11.60ab	10.60bcd	13.60ab	8.80	9.00c-f	
Rebordanes(P)C2	13.60ab	9.74ab	12.00	8.86	12.00	12.66a	11.66ab	12.60a-d	8.86	12.30a	
Sarreaus	10.46c-f	9.14a-b	11.14	8.60	11.14	12.60a	10.00b-e	12.34a-d	8.60	9.60b-f	
Sarreaus(P)C1	11.20c-f	9.14a-b	10.26	8.00	10.26	11.80ab	11.00bc	12.66a-d	8.00	8.86c-f	
Sarreaus(P)C2	10.26def	9.66ab	10.26	7.66	10.26	9.00b	9.30c-f	11.54bcd	7.66	9.20b-f	
Tuy	10.26def	9.40abc	11.14	8.80	11.14	10.46ab	11.66ab	12.74a-d	8.80	10.54abc	
Tuy(S)C1	10.54c-f	8.66b-f	10.40	8.40	10.40	9.00b	10.14b-e	13.26abc	8.40	9.14b-f	
Tuy(S)C2	9.94def	8.20b-f	11.14	8.60	11.14	10.94ab	11.14b	12.40a-d	8.60	8.80c-f	

Tuy(S)C3	14.60a	9.14a-d	10.20	9.46	10.20	11.30ab	10.66bc	11.40bcd	9.46	10.20b-e
Txalin	12.74abc			7.86		11.74ab	13.00a		7.86	8.40ef
LSD (0.05)	2.32	2.10				3.58	1.72	2.30		1.94
^a Means followed by the same letter within the same column are not significantly different (P=0.05)										

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Table 7. Means ^a for 22 maize varieties, improved cycles and checks (see table 1) evaluated in ten environments (see table 2) in Northern Spain under organic (Org) and conventional (Con) agriculture													
	Plan appearance (1-9)			Silking (days)		Ears per plant		Grain moisture (%)		100 grain weight (g)		Grain density (g cm ⁻³)	
Genotype	Con	Org	Con	Org	Con	Org	Con	Org	Con	Org	Con	Org	Con
BastoxBlanco		3.7cde		78.7				22.6cd					
Carballeira	3.3	3.7cde	4.0	69.8	82.8ab	0.78	0.88a	21.8de					
Donostia	2.7	3.3e	3.2	72.0	73.5gh	0.80	0.91a	f	20.7e-i	32.7c-f	35.1f-i	1.23	1.26ab
DonostiaC1	3.0	2.3f	2.5	67.8	71.9gh	0.55	0.54c	21.0e-h					
Getaria					81.7abc	0.95	1.03a	h	21.4d-h	36.3ab	39.8b	1.14	1.25abc
Martikoenea	2.8	4.3abc	3.5	75.0	72.3gh	0.54	0.25d	20.8fg					
Meiro	3.8	4.5ab	4.3	68.0	80.0b-e	0.74	0.92a	h	19.7ij	36.1ab	39.7b	1.20	1.25a-d
Meiro(P)C1	3.5	4.5ab	4.5	75.5	86.6a	0.81	0.96a	36.4a	23.2abc	29.5gh	40.2b	1.12	1.20gh
Meiro(P)C2	3.3	4.5ab	4.7	77.8	80.8a-e	0.75	0.95a	20.2g-j	20.4g-j	37.45a	42.9a	1.20	1.20b-f
NKThermo	3.5	4.7a	4.3	78.3	79.5b-f		1.03a	24.0c	21.4d-h	31.8d-g	35.2f-i	1.18	1.24a-e
Oroso	3.7	4.3abc	4.7	78.0	81.4a-d	0.74	0.85ab	26.0b	24.1a	28.2hi	33.6hij	1.20	1.30a
PR36B08	3.5	3.8b-e	3.7	79.7	81.3a-d		1.04a	26.2b	23.4ab	30.9d-h	34.9ghi	1.17	1.20c-f
Rebordanes	3.2	4.27a-d	3.7	75.5	76.8c-g	0.70	0.86ab	18.0k	21.1d-h	30.5e-h	32.0j	1.17	1.16h
Rebordanes(P)C1	3.8	4.27a-d	4.2	75.5	79.9b-e	0.75	0.95a	23.9c	24.0a	33.6bcd	33.4ji	1.23	1.21-cf
Rebordanes(P)C2	3.3	2.0f	3.2	76.3	81.0a-e	0.76	0.93a	19.0jk	21.6d-g	30.6e-h	35.5fg	1.15	1.20f-h
Sarreaus	3.3	4.5ab	4.0	70.7	71.9gh	0.72	0.83ab	21.8d-g					
Sarreaus(P)C1	3.2	3.5de	3.3	74.2	70.2h	0.77	0.79abc	g	21.4d-h	35.4abc	39.5cb	1.20	1.23a-f
Sarreaus(P)C2	3.3	3.3e	2.8	70.7	75.8d-h	0.70	0.92a	22.9cd	21.5d-g	32.6c-f	35.4fgh	1.19	1.23d-f
Tuy	3.0	4.3abc	4.5	68.7	76.0c-g	0.80	0.94a	23.8c	21.4d-h	31.8d-g	35.0f-i	1.19	1.24a-e
								20.7f-i	19.2j	33.4b-e	35.4fgh	1.18	1.23b-f
								20.7f-i	20.5f-j	30.2fgh	34.0ghi	1.19	1.21c-f
								19.9hij	20.1hij	25.5i	29.8k	1.17	1.22c-f
								22.0de	21.8def	33.7bcd	36.9def	1.17	1.24a-e

								f					
Tuy(S)C1	3.7	4.3abc	4.2	72.8	76.2c-g	0.72	1.00a	22.6cd	22.1bcd	35.4abc	39.3bc	1.19	1.21def
								22.6cd					
Tuy(S)C2	3.0	3.8b-e	3.8	70.3	76.1c-g	0.77	0.92a	e	22.0cde	36.0ab	38.4bcd	1.19	1.21edf
Tuy(S)C3	3.8	3.8b-e	3.8	73.0	75.4e-h	0.91	1.06a	22.9cd	20.9d-i	34.9abc	37.7cde	1.20	1.2b-f
Txalin	3.3	4.3abc	3.8	70.8	73.8fgh		0.59bc	19.2ijk	20.3g-j	36.2ab	35.8efg	1.21	1.21efg
LSD (5%)		0.76			5.79		0.270	1.61	1.34	2.93	1.93		0.04
^a Means followed by the same letter within the same column are not significantly different (P=0.05)													

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Table 8. Simple correlations for ten traits recorded in 22 maize varieties, improved cycles and checks (see table 1) evaluated in ten environments (see table 2) in Northern Spain under organic (below the diagonal) and conventional (above the diagonal) agriculture										
	Early vigor	Plant appearance	Silking	Ears per plant	Grain moisture	Grain yield	Flour yield	100 grain weight	Grain density	Milling test
Early vigor		0.49*	0.43*		0.24	0.10	0.10	-0.51*	-0.14	-0.23
Plant appearance	0.55**		0.47*		0.56**	0.30	0.39	-0.24	-0.06	-0.19
Silking	0.14	0.15		0.39	0.40**	0.18	0.17	-0.44**	0.05	-0.08
Ears per plant			0.00		0.65**	0.74**	0.70**	-0.16	0.06	-0.19
Grain moisture	-0.07	-0.06	0.42	0.66**		0.79**	0.78**	0.00	0.09	-0.59**
Grain yield	0.35	0.53*	0.11	0.19	-0.68**		0.99**	0.07	0.00	-0.73**
Flour yield	0.33	0.48*	-0.10	0.06	-0.67**	0.97**		0.09	0.02	-0.76**
100 grain weight	-0.24	-0.07	-0.41	-0.35	-0.29	0.10	0.15		0.24	-0.02
Grain density	-0.23	-0.06	-0.35	-0.35	-0.25	0.07	0.15	0.28		0.01
Milling test	-0.30	-0.21	-0.17	0.18	0.28	-0.41**	-0.43**	-0.06	0.13	
*, ** Significant at P=0.05 and 0.01, respectively										