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Response to farmer mass selection in early generation progeny of bread wheat landrace crosses

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Abstract

A participatory plant breeding (PPB) program involving the French farmers' association 'Réseau Semences Paysannes' and the French National Agricultural Research Institute (INRA) at Le Moulon was initiated in 2005. In the process of designing the breeding scheme, we evaluated the impact of farmer selection at an early stage (F_2) on bread wheat cross progeny populations. The objectives were to characterize the effect of farmer selection, to evaluate the impact of farmer selection on intra-varietal diversity, to provide farmers with relevant information that they can use to improve their selection practices. Early selection was found efficient for some traits and for some of the 35 F_2-derived F_3 families. For traits of interest such as thousand kernel weight or grain weight per spike, when the response was significant, it was always positive. For most of the traits studied, the among-family genetic variance increased after selection while the average within-family genetic variance decreased. This study provides the first quantitative results for this PPB program and information that will help optimize it in the future.

Key words: bread wheat, participatory plant breeding, population varieties, biodiversity management, organic breeding

Introduction

Organic farming often leads to specific environmental conditions, which are more stressful for the plants since the use of chemical inputs is not permitted[1]. While modern varieties fit the needs of conventional farming in industrialized countries (homogeneity, adaptation to mechanization and high input management), they often are not adapted to agronomic practices that decrease the use of inputs and fossil energy. In addition, dimensions of integrated systems such as the need for biomass for animal feed, competitive ability with weeds, efficient uptake and utilization of nitrogen and end-use quality for traditional foods are often not taken into account in conventional breeding programs[2–5]. This often leads farmers in North Africa, Latin America or Asia to cultivate landraces or historic varieties instead of modern cultivars[6,7], since landraces may be adapted to heterogeneous environments and specific objectives[8].

To succeed in developing new varieties adapted to these kinds of heterogeneous environments, participatory plant breeding (PPB) has been implemented in several cases[9,10]. PPB aims at developing varieties adapted to farmers' needs in contrasted low input environments (such as organic management in Europe), while maintaining genetic diversity. It is based on (i) accounting for genotype × environment × management interactions through decentralized selection; (ii) collaboration between researchers, breeders, farmers and other stakeholders; and (iii) the development and use of appropriate genetic diversity for breeding.

Efficient breeding in stressful environmental conditions will require that environmental complexity is taken into account[11]. Decentralized selection in many sites is needed in order to conduct direct selection in the target environment[9], which has proved more efficient than indirect selection from favorable to stressful environments[2]. In addition, participation of farmers is required to

benefit from their experience and expertise in varietal evaluation in their particular environment[12], and to implement selection in a way that will respond to their specific needs. This participation also empowers farmers and leads to more autonomy with respect to varietal choices and the promotion of farmers' rights[3,13].This approach has already proved to be efficient in developing countries[7,12,14–18] but has started only recently in Europe, with new programs showing promising results[10,19–23].

In France, the demand of organic farmers for adapted varieties first resulted in the cultivation of landraces and historic varieties[24]. In 2003, a group of organic farmers who wanted to conserve agricultural biodiversity and enhance their seed autonomy founded the association Réseau Semences Paysannes (RSP, the Farmers' seed network). The RSP is a network of farmers' associations that conserve, multiply and exchange landraces, old varieties and other farmers' varieties[25]. On-farm management has been shown to be an effective method to conserve agricultural biodiversity, complementary to *in situ* conservation on research stations and *ex situ* conservation in gene banks[26,27]. On-farm management is a key activity for the conservation of genetic resources, as underlined by the 2004 International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) under the control of the FAO. Nevertheless, an *in situ* conservation system solely based on landraces and old varieties may not fit all farmers' needs. Farmers from the RSP have become interested in the development of new varieties that both conserve crop biodiversity and are adapted to the current organic farming practices[25]. Because in the context of industrialized agriculture and an institutionalized seed supply system, the classical intergenerational transmission of knowledge has disappeared, PPB development presents unique challenges both at the technical (genetic, agronomic and analytical) and at the organizational level[24,28].

Several strategies can be applied to start a new breeding scheme, such as crossing, mixing or selecting in the available landraces or locally adapted populations[29]. Here, we chose to create new populations through manual crosses, thus generating a broad range of new allelic combinations. The farmer–baker who initiated the project, Jean-François Berthellot (J.F.B.), chose the parents according to their baking and milling quality, their history and geographical area of cultivation and their agronomic behavior, based on the knowledge he has acquired by growing them on his farm for the past 5 years, and through collaborations with other farmers from the RSP. The goal of making crosses was to combine the bread-making quality and agronomic resilience of landraces with historic varieties (first half of the 20th century) and a few more recent varieties, which are more resistant to lodging. Before the populations derived from these crosses were distributed to a large network of farms, mainly in France, an experiment was performed to assess the impact of farmer's mass selection in an early generation.

From conversations with farmers, it is clear that while they are looking for certain characteristics in their varieties, they are also looking to maintain more phenotypic diversity within varieties than is normally present in modern varieties. They often mention that one of the benefits of more heterogeneous population-varieties is their increased stability over years, due to the within-variety heterogeneity that buffers environmental fluctuations. Within-field varietal diversity has been shown to increase the functionality, resilience and stability of agricultural ecosystems[30,31]. Genetically diverse varieties may combine quantitative and qualitative resistance, thus providing more durable disease resistance[32–34]. Phenotypic variability within varieties has also been found to be associated with an increase in associated biodiversity[35,36]. Within-field varietal diversity may not only contribute to yield stability but also to the stability of quality, as shown in wheat where varietal mixtures increase uptake efficiency of nitrogen[37]. Finally, the on-farm management of such diverse varieties contributes to the *in situ* conservation of genetic resources for plant breeding[8,10,26,38–40].

While for an autogamous plant like wheat, most of the selection is often done among varieties that are nearly pure lines, at a more advanced generation, and not within segregating populations[6,9,41,42], this study assessed the diversity created in the program and the response to farmers' mass selection in early generations after crossing (F_2) in terms of trait means and genetic variance within and among families. Collaboration with farmers (participation) at all stages of the research study was critical to reach these objectives.

Materials and Methods

Context and experimental design

On the initiative of J.F.B., a farmer active in the RSP, a PPB project was started in 2005 with researchers from INRA Le Moulon. The project was extensively discussed with all farmers, RSP coordinators and researchers. Ninety crosses were made on J.F.B.'s farm between different historic wheat varieties, landraces and modern varieties created for organic agriculture. These landraces, historic varieties and modern varieties had been cultivated on his farm for at least 5 years. Most of these populations came from the national seed banks at INRA Clermont Ferrand, France; and from Switzerland (varieties created by Peter Kuntz) and Germany (varieties created by Bertold Heyden).

The first (F_1) and second (F_2) generations of progeny of the 90 different families (one family is derived from each cross, numbered 1–90) have been grown on his farm in 2006–2007 and 2007–2008. Selections were made of individual spikes in a sub-set of the F_2 families. Seed of these spikes was bulked for each family and 35 of these families were evaluated in the F_3 generation with their

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corresponding unselected bulk at INRA Le Moulon (Gif sur Yvette, France) in 2008–2009. Three families had two different selected versions, so the total number of selected populations was 38. When two selections were made within a family, this was indicated by a letter following the number (14a and 14b, 34a and 34b, 42a and 42b). There were three complete block replicates of the 35 families, with paired rows of each version (selected or bulk) for each family. Rows were 1.20 m long with 20 seeds sown per plot. Paired versions within families were randomized in each block but the selected and the bulk families of each pair were always grown side by side. This maximized the power to detect differences between the two versions. In addition, Renan, a pure line cultivar frequently used in organic agriculture in France was used as a check variety and as a point of comparison for the farmers. Renan was included twice in each replicate of the experiment.

Measurements

Qualitative observations and quantitative measures were taken on the main tiller of each of five plants for each version of each family in each replicate (i.e., a total of 15 plants for each version of each family). The traits measured and their abbreviations are listed in Table 1. These traits were chosen by farmers and also based on phenotypic descriptors used for variety registration. After field measurements were made on each plant, the spike was cut and individually bagged. At the technical facilities at INRA Le Moulon, measurements were taken on all spikes collected. Grain from each replication of each version within families was analyzed for technology traits at INRA Clermont Ferrand using near infrared spectroscopy (Foss NIRSystem 6500), using whole grain. Although this prediction is less precise than on whole grains flour, it was chosen because it is not destructive and this allowed us to replant the seeds. The correlation between estimated and true value using this method in the calibration sample was (Table 1): protein (0.86), hardness (0.77), test weight (TW; 0.80), mixing time integral (MTI; 0.7) and dough strength (W; 0.75). This was assessed on modern cultivars (G. Branlard, personal communication).

Statistical analysis

First, we tested for an overall version effect using an ANOVA model with all effects fixed:

$$Y_{ijkl} = \mu + \text{family}_i + \text{rep}_j + \text{version}_k + \varepsilon_{ijkl} \qquad (1)$$

Where Y_{ijkl}, is the phenotypic value measured for plant l of version k of family i in replication j, μ is the general mean, family_i is the effect of family i, rep_j is the effect of repetition j, version_k is the effect of the version (k = selected or non-selected) and ε_{ijkl} is the random error term.

Model (2) was used to test for specific selection effect dependent on the family. This was similar to Model 1 but the version effect (selected or non-selected) was nested within family ($version(family)_{ik}$).

The statistical analysis was implemented in SAS v 9.2 proc GLM[43]. Two-sided tests between the least-square (LS) means for selected and non-selected versions within each family were made with Tukey's multiple comparison procedure in SAS. This was done using the SLICE function on the LS Means for version (family), which tests for an effect of version within each family. To visualize the response to selection for multiple traits and families, the log(p-value) of the two-sided test described above were recorded in a matrix of traits × families (18 × 38). We used a log transformation in order to weigh the significant changes. When the means of the selected versions were higher than the bulk, then log(p-value) were multiplied by −1 (so that the values were positive), else by +1 (so that the values remained negative). Ward's clustering procedure was used on the data in this matrix to group each version of the different families by the similarities in their responses to selection. This clustering and the resulting dendrogram and heatmap visualization were done with the heatmap function in R[44].

Repeatability is a measure of the proportion of phenotypic variation due to genetic causes. It was estimated using the genetic and residual variance estimated for each version (selected or bulk) from the ANOVA mixed model:

$$Y_{ijk} = \mu + \text{family}_i + \text{rep}_j + R_{ijk} \qquad (3)$$

Where Y_{ijkl} is the phenotypic value measured for plant k of family i in repetition j, μ is the general mean, family_i is the family i (random effect), rep_j is the repetition j (fixed effect) and R_{ijk} is the error. Mixed models were used to estimate variance components using the function VarCorr in the R package lme4[44]. Thus the repeatability was estimated as:

$$r = \frac{\text{var}(G)}{\text{var}(G) + \frac{\text{var}(R)}{3}}$$

Where var(G) was the estimated among-family genetic variance, and var(R) the estimated residual variance. As the families are progenies of F_2 spikes, the error R_{ijk} of the model includes both the within-family genetic variance plus the environmental variance.

We estimated the average within-family genetic variance as the difference between the error variance (var(R) from model (3)) and the residual variance of the check variety Renan. As the check is a pure line, i.e., genetically homogeneous, the variation observed was only due to the environment. More information on the calculation of within-family variance is provided in supplemental material. For the data where there was no individual data but only plot mean values, only the replication effect was used in the model.

Correlations among traits before and after selection were also calculated, methods and results are given in the supplementary information.

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Table 1. Traits measured. The traits analyzed are in bold.

Traits	Measures methods
Field measurements:	
Earliness	number of degree-days when the main tiller spike is 50% emerged, recorded for each plant by daily observations in the field from 24/05/2009 to 19/06/2009, and converted to degree-days using meteorological data collected on the research station
Awns	on a scale from 0 (awnless) to 2 (fully awned)
Color	on a scale from 0 (white) to 2 (dark red)
PH	plant height in cm, without the awns, at the top of the highest spikelet, even if sterile
SB	height in cm of the bottom of the lowest spikelet, even if it was sterile
LI	height in cm of the insertion of the stem into the flag-leaf in mm
LLSD	last-leaf-to-spike distance = SB − LI
Spike measurements:	
SL	length of the spike in cm
SpTot	total number of spikelets, including those that may be sterile or missing
SpMi	missing spikelets that had fallen off in the field or the bag, used for correction of other measurements
SpSt	sterile spikelets at the base or summit of the spike that had zero kernels
SpW	weight of the spike before threshing measured to the nearest 0.01 g, after the stem was cut to the base of spike
KN	number of kernels per spike counted after threshing for each spike individually
KN_Spikelet	kernels per spikelet = KN/(SpTot − SpSt − SpMi)
KN_Spike	KN + KN_Spikelet*SpMi
GW	grain weight measured for each spike to the nearest 0.01 g
GW_Spike	KN_Spike*KN/GW
TKW	thousand kernel weight = GW_Spike/KN_Spike*1000
Density	SpTot/SL (spikelets/cm)
Sterility	SpSt/KN_Spike
Grain composition measurements:	
Protein	Protein content (%)
W	Dough strength in 10^{-4} J. It is the force needed to break the dough.
Hardness	Hardness
MTI	Mixing time integral, associated with W
TW	Test weight, which is similar to mass density

If the end of the spike was broken, the existing spike length was used for calculations of spike density, and the SL itself was treated as missing data for the analysis. If only the last spikelet was missing, 5 mm was added to the spike length measurement.

Results

Trait variation

Plant height (PH) was rather high in both the selected and bulk versions with a mean of 134.5 and 132.7 cm, respectively. This was also the case for last-leaf-to-spike distance (LLSD) with means of 28.6 and 27.7 cm, respectively, and for spike length (SL) with means of 12.8 and 12.7 cm, respectively (Table 2). Such high values are typical of landraces and historic varieties. In general, the range of variation of the trait values was quite large, indicating the high level of diversity generated in these populations.

In the models (1) and (2), the family effect was significant for all traits (Tables 3a and 3b), while the effect of replication was significant only for protein, W and MTI, which might be due to the fact that the grain composition traits are highly dependent on the environment[45,46].

Response to selection for trait means

Seven traits changed significantly after selection with a common trend over all families among which thousand kernal weight (TKW) and protein showed highly significant changes (respectively positive and negative). Six other traits changed significantly but the direction of the response depended on the family (Fig. 1). For 24 families out of 38, significant differences between bulk and selected versions were found for at least one trait.

The overall version effect was significant at $P < 0.01$ for TKW and protein (Table 3a), and significant at $P < 0.05$ for PH, earliness, GW_Spike, TW, W and MTI. This indicated a unidirectional response to selection for these traits: although the response was not always significant for the different families, we found a trend towards an increase for TKW, GW_Spike and PH and towards a decrease for protein (Fig. 1). The effect of the version within family was significant for seven traits out of 16: PH, LLSD, SL, total number of spikelets (SpTot), TKW,

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Table 2a. Summary for each trait for the data for selected version.

	PH (cm)	LLSD (cm)	SL (cm)	SpTot	KN_Spikelet	KN_Spike	GW_Spike (g)	TKW (g)	Density	Sterility	Earliness	Protein (%)	Hardness	TW	W
Min.	55.0	3.00	7.7	14	1.05	17.0	0.37	21.53	0.00	0.00	1155	7.6	25.0	76.6	127
1st Qu.	122.7	23.0	11.6	21	2.22	46.0	2.22	45.43	1.64	0.00	1266	9.0	51.3	79.5	184
Median	136.5	29.2	12.8	22	2.46	52.8	2.61	49.57	1.77	0.05	1302	9.5	57.0	80.7	216
Mean	134.5	28.6	12.8	23	2.47	52.9	2.62	49.51	1.80	0.06	1298	9.5	57.4	80.7	212
3rd Qu.	148.2	34.7	14.0	24	2.72	60.0	3.00	53.36	1.94	0.09	1329	10.0	65.0	81.7	241
Max.	172.4	51.6	18.8	29	3.72	85.6	5.10	91.02	2.74	0.97	1548	11.5	91.0	85.1	302

Table 2b. Summary for each trait for the data for bulk version.

	PH (cm)	LLSD (cm)	SL (cm)	SpTot	KN_Spikelet	KN_Spike	GW_Spike (g)	TKW (g)	Density	Sterility	Earliness	Protein (%)	Hardness	TW	W	PH (cm)
Min.	76.0	15.0	8.2	17	1.17	21.0	0.26	10.88	1.32	0.00	1155	7.9	31.0	76.3	118	309
1st Qu.	122.4	22.3	11.5	21	2.18	44.7	2.12	44.15	1.65	0.00	1246	9.3	51.8	78.7	190	337
Median	134.8	28.5	12.5	22	2.45	52.0	2.49	48.13	1.78	0.05	1285	9.7	59.0	80.3	223	358
Mean	132.7	27.7	12.7	23	2.45	52.7	2.53	47.98	1.81	0.05	1291	9.8	59.5	80.2	221	359
3rd Qu.	146.2	34.0	14.0	24	2.71	59.9	2.94	52.59	1.94	0.09	1329	10.3	66.0	81.5	253	378
Max.	173.3	49.8	18.3	30	4.23	97.4	4.23	64.21	2.81	0.35	1481	12.9	93.0	83.8	328	428

Table 3a. Results of the ANOVA (model 1) with DF(family) = 37, DF(version) = 1, DF(rep) = 2.

	PH (cm)	LLSD (cm)	SL (cm)	SpTot	KN_Spikelet	KN_Spike	GW_Spike (g)	TKW (g)	Density	Sterility	Earliness	Protein (%)	Hardness	TW	W	MTI
Family	32.37***	21.51***	7.39***	20.64***	4.68***	5.76***	3.44***	7.86***	5.3***	3.41***	15.12***	2.36***	3.95***	4.56***	3.88***	3.15***
Rep	0.71	2.81	1.29	1.43	1.44	2.13	2.03	1.13	0.02	0.78	3.9*	10.03***	1.74	0.79	6.52**	12.41***
Version	4.15*	2.1	0.66	0.24	0.19	0.19	5.1*	14.39***	1.48	0.19	3.2	11.38***	2.55	5.98*	4.44*	6.47*

F values are presented. Stars indicate the significance of the *F* test; *: 0.05 > *P*-value > 0.01; **: 0.01 > *P*-value > 0.001; ***: *P*-value < 0.001.

Table 3b. Results of the ANOVA (model 2) with DF(family) = 37, DF(version in family) = 38, DF(rep) = 2.

	PH (cm)	LLSD (cm)	SL (cm)	SpTot	KN_Spikelet	KN_Spike	GW_Spike (g)	TKW (g)	Density	Sterility	Earliness	Protein (%)	Hardness	TW	W	MTI
Family	34.54***	22.45***	4.10***	21.14***	4.66***	5.83***	3.48***	7.95***	5.65***	3.48***	15.20***	2.36***	3.78***	4.77***	3.78***	3.05***
Rep	0.76	2.93	2.39	2.96	1.28	1.29	2.15	2.05	1.15	3.9*	0.28	10.04***	1.66	0.83	6.35**	12.02***
Version (family)	2.83***	2.15***	1.47*	1.74*	0.79	1.3	1.38	1.66**	1.4	1.2	1.47*	1.28	0.83	1.37	0.96	0.99

F values are presented. Stars indicate the significance of the *F* test; *: 0.05 > *P*-value > 0.01; **: 0.01 > *P*-value > 0.001; ***: *P*-value < 0.001.

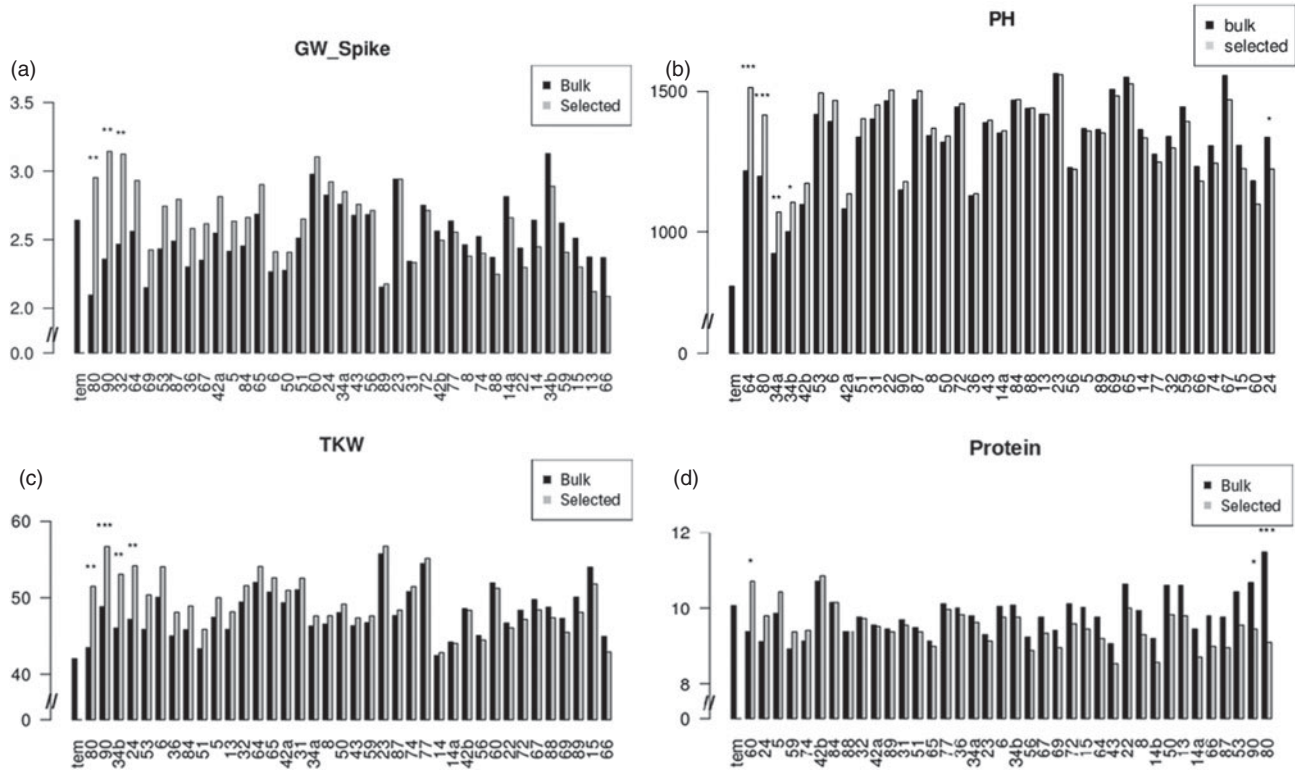

Figure 1. Evolution between selected (grey) and bulk (black) versions for four traits: (a) grain weight per spike (GW_Spike) in grams, (b) plant height (PH) in cm, (c) thousand kernel weight (TKW) in grams and (d) protein concentration (Protein) in %. Stars represent significant differences between the means: *, $0.05 < P\text{-value} < 0.01$; **, $0.01 < P\text{-value} < 0.001$; ***, $P\text{-value} < 0.001$. tem = control (Renan).

sterility and earliness (Table 3b) indicating that for LLSD, SL, SpTot and sterility the response was dependent on the family.

While there was a significant global version effect for PH (Table 3a), the magnitude of the selection response was specific to each family (Table 3b). PH significantly increased from 120 to 150 cm for family 64; from 118 to 140 cm for family 80; from 70 to 110 cm for family 34a, and from 100 to 115 cm for family 34b, whereas PH decreased significantly from 130 to 125 cm for family 24 (Fig. 1b).

As can be seen in Figs 1a, 1c and 2, selection for GW_Spike, KN_Spikelet, TKW, earliness and TW always increased trait values when response was significant. Moreover, TKW increased for 26 families over the 38, of which four cases were significant (Fig. 1c). In contrast, hardness, W and MTI selection always decreased trait values when the response was significant. Finally, PH, LLSD, sterility, awns, SpTot, color, density, protein, and SL changed in both directions. It is interesting to point out that protein tended to decrease after selection for 30 families over the 38 selected, but only two families decreased significantly. For one family (family 60), protein increased significantly after selection. There were 14 families that did not respond to selection for any of the traits measured, 11 families that responded to selection for only one trait, six families that responded to selection for two traits, six families for three to eight traits and one family for 12 traits.

For family 34, the two selections ('a' and 'b'), led to different responses: eight significant changes for 'b' and two significant changes for 'a' that were in the same direction as for 'b' (for PH and LLSD). For family 42, the two selections ('a' and 'b'), led to similar response patterns. For family 14, neither of the selections led to many changes from the bulk version. The dendrogram shows that variables linked to technological properties (Protein, MTI, W) had similar response patterns (Fig. 2). LLSD and PH also responded similarly to each other, as did TKW and TW.

Response to selection at the variance level

Repeatability ranged from 0.13 for GW_Spike within the bulk versions to 0.81 for PH within the bulk versions, with higher values for morphological traits such as PH, LLSD, SL, earliness and SpTot and for grain composition traits (Table 4). This indicated good control of environmental variation and the ability of the experimental design to discriminate among families both before and after selection. Note that the lowest value

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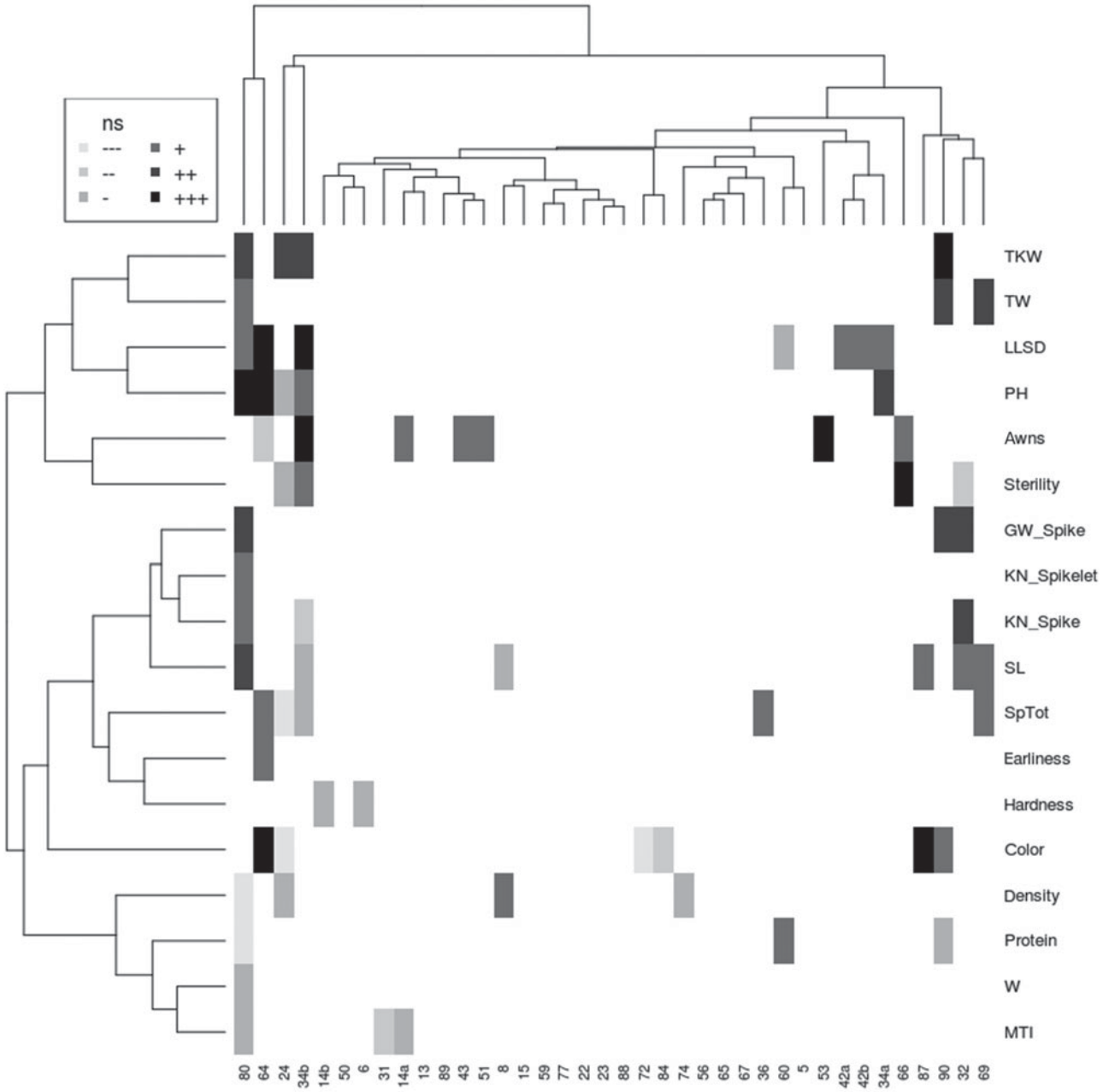

Figure 2. Change in the phenotypic mean between bulk and selected versions of F_3 families for several traits. If the mean decreases: -, $0.05 > P\text{-value} > 0.01$; --, $0.01 > P\text{-value} > 0.001$; ---, $P\text{-value} < 0.001$. If the mean increases: +, $0.05 > P\text{-value} > 0.01$; ++, $0.01 > P\text{-value} > 0.001$; +++, $P\text{-value} < 0.001$. Earliness = days to flowering, so an increase means that a family flowers later. Color that increases means darker spike. Awns that increases means more awns.

was for the trait that is the closest to grain yield (GW_Spike).

GW_spike, TKW, protein and MTI had greater repeatability in the selected version compared to the bulk (increases from +32.2 to +139.4%). Repeatability for SL, TW, W, PH, KN_Spike, SpTot, earliness and density did not change much in the selected version (changes from −4.8 to +10.5%). Repeatability for hardness, sterility, KN_Spikelet and LLSD was lower in the selected version (decreases from −9.2 to −28.3%) (Table 4).

Selection increased the among-family genetic variance for GW_Spike, TKW and MTI (increases from +25.4 to +66.0%) while it was reduced in a more limited proportion for LLSD, KN_Spikelet, earliness and hardness (decreases from −11.4 to −22.7%). Little change was observed for the other traits (Table 4). Selection increased the average within-family genetic variance for sterility (+32.7%) while a marked decrease was observed for GW_Spike, TKW, protein, TW and MTI (between −44.7 and −13.3%) (Table 4).

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Table 4. Differences in repeatability, genetic variance among F_3 and genetic variance within F_3 for each version: bulk and selected for each trait; $\sigma = \sqrt{(variance)}$.

	PH (cm)	LLSD (cm)	SL (cm)	SpTot	KN_Spikelet	KN_Spike	GW_Spike (g)	TKW (g)
Repeatability bulk	0.81	0.77	0.43	0.68	0.29	0.37	0.13	0.36
Repeatability S	0.79	0.67	0.45	0.70	0.26	0.35	0.32	0.51
% between repeatability S and bulk	−2.32%	−13.18%	+4.61%	+3.62%	−10.63%	−4.84%	+139.39%	+42.8%
σ among-family bulk	14.75	6.55	0.81	1.46	0.15	4.88	0.14	2.61
σ among-family S	13.62	5.24	0.83	1.43	0.13	4.47	0.23	3.28
% between σ among-family S and bulk	−7.47%	−19.96%	+2.14%	−1.48%	−11.6%	−8.38%	+65.96%	+25.36%
σ within-family bulk	11.06	5.50	1.35	0.77	0.00	0.00	0.37	4.90
σ within-family S	10.82	5.70	1.31	0.43	0.00	0.00	0.32	4.25
% between σ within-family bulk and S	−2.18%	+3.6%	−2.75%	−44.16%	0%	0%	−14.22%	−13.32%
	Density	**Sterility**	**Earliness**	**Protein (%)**	**Hardness**	**SW**	**W**	**MTI**
Repeatability bulk	0.36	0.31	0.59	0.36	0.62	0.66	0.54	0.34
Repeatability S	0.34	0.22	0.54	0.48	0.50	0.68	0.60	0.60
% between r S and r bulk	−5.21%	−28.35%	−8.74%	+32.2%	−19.26%	+3.93%	+10.53%	+74.28%
σ among-family bulk	0.09	0.02	33.10	0.34	7.12	1.14	23.54	10.43
σ among-family S	0.09	0.02	30.27	0.36	5.57	1.11	21.74	15.56
% between σ among-family S and bulk	−1.12%	−0.52%	−8.55%	+6.64%	−21.77%	−1.99%	−7.64%	+49.3%
σ within-family bulk	0.15	0.05	24.59	0.51	7.80	0.84	18.32	22.20
σ within-family S	0.16	0.06	26.08	0.28	7.75	0.64	0.00	18.86
% between σ within-family bulk and S	+6.09%	+32.69%	+6.03%	−44.73%	−0.57%	−23.64%	0%	−15.03%

Discussion

In this study, we analyzed F_2 derived F_3 families from 35 crosses among a wide range of landraces, historic and more recent varieties and selected by an organic farmer. First we comment on the diversity created by the crosses. Then, we discuss the response to farmer's selection within early generation families (mean and variance). We provide specific examples of directional selection, correlations among selected traits, and the influence of parental varieties on the response to selection in the supplementary information. Finally, we discuss how these results can be integrated in the ongoing PPB program.

Creation of diversity for selection

In conventional selection programs, breeders usually seek to decrease the within-family genetic variance in order to obtain uniform lines. However, in this project, one of the goals was to maintain within-family variance and thus maintain the genetic potential for continuing on-farm selection. Overall, after selection, this variation remained high. This is positive as it will allow the farmers to continue selecting within populations.

Both before and after selection, differences among families were highly significant for all traits (Tables 3a and 3b). This was consistent with the objective of the crosses to generate a large range of diversity in order to increase the chance of developing populations that might adapt to contrasting environmental conditions. Traits where uniformity is of importance for standardized production may also not be as critical in situations where production and value-added processing occurs on-farm or for a small artisanal market. For the specific example of baking force W, see supplementary information.

Because this experiment was done in a common garden with all populations grown in a single environment, the observed phenotypic diversity is likely due to the genetic diversity found in our panel of populations. The populations are in the F_3 generation derived from crosses among very diverse parents. Assessing the relative contribution of among- and within-family variability is of importance to develop appropriate selection procedures in PPB.

In general, genetic among-family variation was high for most traits (Table 4), leading to high repeatability, except for some characteristics of the spike such as KN_spike and GW_spike. This is consistent with classical findings in quantitative genetics for such complex traits. When selecting, selection among families can be the first step[9,17,21,42]. With diverse and distinguishable families, farmers can find populations that better suit their specific environments and practices.

The within-family genetic variance was also quite high for many traits (Table 4). This is expected as we are dealing with segregating populations. After selection among families, selection within families can be the

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second step in PPB. Within-family diversity can be used in an evolutionary plant breeding approach[47] where populations are mainly submitted to natural selection. To increase selection efficiency, farmers may apply mass selection to guide the evolution of the population towards phenotypes of interest for them[7,23,41,48,49]. Researchers and farmers conducting selection have to be careful to monitor competition among individuals within the population (for light and uptake of nutrients in the soil) when using either evolutionary breeding or mass selection, as competition ability might be negatively correlated with some traits desired by farmers (quality and yield)[2,29].

Assessing the impact of farmers' mass selection

A significant positive response was found for morphological and phenological traits such as PH, LLSD, earliness or SL. Within the RSP, farmers are often looking for plants that are tall but resistant to lodging because they have noticed a positive relationship between PH and the maintenance of grain filling under stress which impacts yield and grain quality. From farmers' observations, taller plants also are more competitive with weeds, and farmers can use the extra straw for livestock or for soil fertility management. The measure of LLSD was proposed by the farmers, because they found that a greater distance between the spikes and the foliage may prevent leaf diseases from jumping to spikes. They also observed an improvement in grain filling and grain maturation with longer LLSD if leaves die because of disease or abiotic stress, as the stem resources can be used to continue grain filling. This is corroborated in the scientific literature[50].

Traits such as TKW or GW_Spike, which are more difficult to assess visually in the field, also increased after selection in families where there were significant differences. Family 80 was very heterogeneous and responded to 12 traits out of 18 measured. This can be related to the parents used (see supplementary information). Ceccarelli et al.[42] also showed the ability of farmers to select superior populations when working with early generations.

In discussions among farmers, they said that they may have particular selection criteria but always adapt to the specific populations they observe and take a more holistic approach to selection. As already noticed by Ghaouti et al.[21], farmer selection is integrative, it does not favor individual traits but instead overall plant vigor, field productivity and quality. In our study, traits such as PH, SL, SpTot, density and color are indicators of such vigor for the farmer, leading to homogenization and uniform selection. However, if there are plants that look interesting but do not fit the 'type', the farmers will select them anyway, leading to more heterogeneous samples.

Selection in the F_2 may be early for an efficient response to selection as segregation is not complete (i.e., $(1/2)^2$ heterozygotes expected), and the covariance between F_2 plants and F_3 plants from the same family is expected to be moderate. Moreover, as there is a high level of heterozygosity, differences are difficult to assess. Most of the selection in PPB programs described in the literature are carried out within more advanced generations, for example F_3, F_4, F_5 in barley and bean[6,15,42]; F_5 for rice[41]; and F_3 and F_4 in sorghum[14]. In this study, the farmer's objective was to save time and subject the populations to the conditions of the target environment as soon as possible. An original aspect of this experiment is that all crosses were performed on-farm and the early generations were also cultivated on the same farm by the farmer who initiated the project. In most reported cases of PPB, crosses were made in the research station and the early generations were also grown at the research station[9,14,15,42]. Here, the farmer applied mass selection without being influenced by the researchers. This approach differs from other programs where researchers train farmers[48] or apply selection before the farmers[14,49]. The objective here was to assess specifically the effect of the farmer's selection, which is based on his unique knowledge of his farming system. The efficiency of farmers' selection has been demonstrated in other cases on rice, barley or quinoa but it was most often a screening among families at a later generation[6,17,41].

The farmer whose selections were studied is not representative of all farmers in the RSP. He has extensive experience growing and observing population-varieties and may be described as an 'expert farmer'. The objective of the study was to characterize the response to mass selection by an expert farmer in order to assess the potential of, and limits to, this approach for motivated farmers involved in PPB programs. These results show that mass selection within families can be effective for some traits even at early generations, and that genetic diversity is maintained within families for future selection. These results will contribute to improve farmers' understanding of the impact of their selection and thus may affect their future selection.

Conclusion

The objective of the farmer in this study was to select improved populations, based on farmers' criteria, while maintaining the potential for future selection within populations. Based on our results this goal seems to have been achieved. This study has helped lay out the basis for the implementation of a PPB program by creating new populations with broad diversity which can then be distributed for selection according to farmers' criteria. The understanding and the analysis of the results were possible because of the interaction between farmers and the research team. This collaboration is the basis of the program and has led to a better knowledge of farmer variety management and its impact on genetic diversity.

Selection on-farm is new for farmers in France[28]. More time and exchanges of knowledge are needed for farmers

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to regain the knowledge and skills of selection[51]. These newly created wheat families were sent to farmers all over France and are now managed by around 25 farmers under different environments and practices. These farmers are collaborating in the PPB program which is the basis of the methodology. Several farmers have started mass selection within the populations. The next step will be to study the evolution of these newly created families under farmers' selection and management practices as well as evolutionary pressure in diverse environments in terms of molecular and phenotypic diversity and on-farm agronomic and quality traits.

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Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/S1742170513000343.

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