**Multi-trait genomic prediction model increased the predictive ability for agronomic and malting quality traits in barley (*Hordeum vulgare* L.)**

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Table S1. Basic summary statistics with BLUEs, standard error (SE), minimum, maximum, coefficient of variation (CV; %), broad sense heritability (*H2*), and proportion of the variance component of the genotype by environment interaction (GEI) over the genotypic (G) effect for agronomic and malting quality traits evaluated across multiple locations and years in Uruguay.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Experiment a | Mean | SE | Min | Max | *H2* | GEI/G |
| Grain yield (kg ha-1) | | | | | | | |
| EELE | 7337 | 61.0 | 5271 | 8936 | 0.52 | 0.8 |
| EEMAC | 5134 | 60.2 | 3170 | 7192 | 0.41 | 3.4 |
| MOSA | 7658 | 59.1 | 5988 | 8924 | 0.20 | 5.3 |
| MUSA | 3877 | 62.5 | 2194 | 5357 | 0.29 | 0.6 |
| ALL | 5898 | 42.2 | 3976 | 6924 | 0.66 | 2.1 |
| Number of grains per square m (number) | | | | | | | |
| EELE | 15103 | 146.8 | 9534 | 20452 | 0.61 | 0.5 |
| EEMAC | 12171 | 152.6 | 7553 | 16695 | 0.46 | 2.9 |
| MOSA | 16929 | 150.9 | 12749 | 20288 | 0.47 | 5.8 |
| ALL | 13729 | 122.2 | 7642 | 17479 | 0.71 | 2.0 |
| Thousand grain weight (g) | | | | | | | |
| EELE | 48 | 000.2 | 43 | 54 | 0.80 | 0.3 |
| EEMAC | 43 | 000.3 | 35 | 51 | 0.54 | 2.5 |
| MOSA | 46 | 000.2 | 38 | 51 | 0.42 | 2.2 |
| ALL | 45 | 000.2 | 36 | 49 | 0.64 | 2.6 |
| Plumpness (%) | | | | | | | |
| EELE | 96 | 000.1 | 92 | 98 | 0.42 | 1.6 |
| EEMAC | 84 | 000.5 | 71 | 99 | 0.37 | 4.8 |
| MOSA | 89 | 000.4 | 69 | 98 | 0.25 | 4.6 |
| MUSA | 91 | 000.3 | 82 | 95 | 0.25 | 1.7 |
| ALL | 89 | 000.3 | 78 | 96 | 0.53 | 4.7 |
| Malt extract (%) | | | | | | | |
| ALL15 | 81 | 000.1 | 79 | 82 | 0.67 | 0.4 |
| Soluble nitrogen (mg/100g) | | | | | | | |
| ALL15 | 627 | 003.8 | 530 | 742 | 0.74 | 0.1 |
| Beta-glucan content (ppm) | | | | | | | |
| ALL15 | 268 | 003.1 | 97 | 532 | 0.66 | 0.2 |
| Protein content (%) | | | | | | | |
| ALL15 | 11 | 000.04 | 10 | 12 | 0.68 | 0.04 |

a ALL, 9 experiments (location-year) combined; ALL15, three experiments (EELE, EEMAC, and MOSA) conducted in 2015 were combined. EELE, MOSA, and MOSA were evaluated in 2015 and 2016 whereas EMMAC was evaluated in 2015, 2016, and 2017 growing seasons.

Table S2. Analysis of variance with mean squares for grain yield, plumpness, thousand grain weight (TGW), grain m-2, extract, soluble nitrogen, beta-glucan content, and grain protein content from experiments conducted in different locations and years in Uruguay.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Trait | | | | |
| Combined over all locations and years | | |  |  |
| Source | Yield | Plumpness | TGW | Grain/m2 |
| Genotype (G) | 2545176\*\*\* | 85\*\*\* | 36\*\*\* | 17814893\*\*\* |
| Environment (E) | 524727525\*\*\* | 16338\*\*\* | 3560\*\*\* | 1545255176\*\*\* |
| G x E | 817114\*\*\* | 41\*\*\* | 15\*\*\* | 5734376\*\*\* |
|  |  |  |  |  |
| Year 2015 | | | | |
| G | 1976331\*\*\* | 61\*\*\* | 27\*\*\* | 13968356\*\*\* |
| Location (L) | 772332022\*\*\* | 6563\*\*\* | 3096\*\*\* | 2517077155\*\*\* |
| G x L | 618041\*\*\* | 32\*\*\* | 16\*\*\* | 5238828\*\*\* |
|  |  |  |  |  |
| Year 2016 | | | | |
| G | 1211779\*\*\* | 5\*\*\* | 13\*\*\* | 6017481\*\*\* |
| L | 529495554\*\*\* | 264\*\*\* | 685\*\*\* | 1413867589\*\*\* |
| G x L | 599705 | 3\*\*\* | 7\*\*\* | 2720273 |
|  |  |  |  |  |
| EMMAC | | | | |
| G | 1666739\*\*\* | 106\* | 39\*\*\* | 15406251\*\*\* |
| Year (Yr) | 11522793 | 40824\*\*\* | 4237 | 447874178\* |
| G x Yr | 1032710\*\*\* | 83\*\*\* | 24\*\*\* | 9006373\*\*\* |
|  |  |  |  |  |
| EELE | | | | |
| G | 1082262\*\*\* | 2.594976\*\*\* | 10\*\*\* | 6564439\*\*\* |
| Yr | 658055218\*\*\* | 420.105473\*\*\* | 1240\*\*\* | 1788637099\*\*\* |
| G x Yr | 509851\* | 1.2\*\*\* | 2\*\*\* | 2475388 |
|  |  |  |  |  |
| MOSA | | | | |
| G | 1036515\* | 54 | 14 | 6453761 |
| Yr | 80078645 | 17036\*\* | 6445\*\*\* | 103736379 |
| G x Yr | 780846\*\*\* | 45\*\*\* | 14\*\*\* | 5250458\*\*\* |
|  |  |  |  |  |
| MUSA | | | | |
| G | 1171305\*\* | - | - | 22\* |
| Yr | 318757 | - | - | 4278\* |
| G x Yr | 790031 | - | - | 16 |
|  | | | | |
| Combined over three locations for quality traits (EEMAC, EELE, and MUSA) | | | | |
|  | Extract | Soluble nitrogen | Beta-glucan content | Protein content |
| G | 2.0\*\*\* | 8416\*\*\* | 49018\*\*\* | 1.1\*\*\* |
| L | 47.8\* | 248026\*\* | 1711249\*\*\* | 3.7 |
| G x L | 0.5\*\* | 1328\* | 7341 | 0.3 |

Table S3. Genomic predictive ability for un-phenotyped environments using MT-CV1 and MT-CV2 models.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Testing location | | | | | | | |
|  |  |  |  | EELE | | MOSA | | MUSA | | EEMAC | |
| Set | Training set | Testing year | Trait | MT-CV1 | MT-CV2 | MT-CV1 | MT-CV2 | MT-CV1 | MT-CV2 | MT-CV1 | MT-CV2 |
| By year | All location in the testing year but one | 2015 |  |  |  |  |  |  |  |  |  |
|  |  |  | Yield | 0.11 | 0.15 | 0.26 | 0.41 | 0.18 | 0.41 | -0.04 | 0.25 |
|  |  |  | Plumpness | 0.33 | 0.52 | 0.2 | 0.43 | 0.27 | 0.35 | 0.18 | 0.37 |
|  |  |  | TGW | 0.1 | 0.53 | 0.22 | 0.32 | 0.11 | 0.23 | 0.26 | 0.49 |
|  |  |  | GRM2 | -0.03 | 0.14 | 0.31 | 0.46 | 0.14 | 0.29 | 0.02 | 0.34 |
|  |  | 2016 |  |  |  |  |  |  |  |  |  |
|  |  |  | Yield | 0.1 | 0.09 | 0.21 | 0.23 | 0.07 | 0.13 | 0.03 | 0.22 |
|  |  |  | Plumpness | 0.14 | 0.14 | 0.18 | 0.43 | 0.13 | 0.22 | 0.24 | 0.37 |
|  |  |  | TGW | 0.15 | 0.29 | 0.08 | 0.32 | - | - | 0.06 | 0.19 |
|  |  |  | GRM2 | 0.13 | 0.21 | 0.17 | 0.46 | - | - | 0.14 | 0.2 |
| By location | All years for a location but one | 2015 |  |  |  |  |  |  |  |  |  |
|  |  |  | Yield | 0.08 | 0.29 | 0.14 | 0.12 | 0.15 | 0.18 | 0.02 | 0.26 |
|  |  |  | Plumpness | 0.23 | 0.20 | 0.19 | 0.3 | 0.05 | 0.09 | 0.29 | 0.34 |
|  |  |  | TGW | 0.32 | 0.52 | 0.02 | 0.03 | - | - | 0.21 | 0.46 |
|  |  |  | GRM2 | 0.1 | 0.35 | -0.01 | 0.05 | - | - | -0.03 | 0.29 |
|  |  | 2016 |  |  |  |  |  |  |  |  |  |
|  |  |  | Yield | 0.15 | 0.27 | 0.01 | 0.1 | 0.01 | 0.1 | 0.02 | 0.09 |
|  |  |  | Plumpness | 0.21 | 0.14 | 0.28 | 0.34 | 0.19 | 0.21 | 0.17 | 0.28 |
|  |  |  | TGW | 0.22 | 0.38 | 0.01 | 0.06 | - | - | 0.19 | 0.25 |
|  |  |  | GRM2 | 0.2 | 0.33 | 0.03 | 0.1 | - | - | 0.09 | 0.13 |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 2017 | Yield | - | - | - | - | - | - | 0.27 | 0.32 |
|  |  |  | Plumpness | - | - | - | - | - | - | 0.24 | 0.31 |
|  |  |  | TGW | - | - | - | - | - | - | 0.29 | 0.39 |
|  |  |  | GRM2 | - | - | - | - | - | - | 0.25 | 0.34 |

When sets of environments are grouped by year, the training set includes all genotypes in all locations but one for a given year, while the testing set includes the remaining location; one location at a time is left out of the training set and is being predicted including two multi-trait cross validation schemes. When sets of environments are grouped by location, the training set includes all genotypes in all years but one for a given location, while the testing set includes the remaining year; 1 yr at a time is left out of the training set and is being predicted. CV1, predicting new lines with genotypic information only and CV2, predicting partially phenotyped lines by using genotypic and phenotypic information from all traits from individuals in the training set, and genotypic and phenotypic information from remaining traits in the testing set.

Table S4. The predictive ability for grain yield (YLD), plumpness (PLM), thousand grain weight (TGW), and number of grains per square meter (GM2) using single trait (ST) and multi-trait (MT) models from individual experiments (EELE15, EELE16, EEMAC15, EEMAC16, EEMAC17, MOSA15, MOSA16, MUSA15, and MUSA16), experiments across locations (EELE, EEMAC, MOSA, and MUSA), years (2015, 2016, and 2017), and all experiments combined (ALL).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Model | Trait | ALL | 2015 | 2016 | EELE | EELE15 | EELE16 | MOSA | MOSA15 | MOSA16 | MUSA | MUSA15 | MUSA16 | EEMAC | EEMAC15 | EEMAC16 | EEMAC17 |
| ST-CV1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | GM2 | 0.20 | 0.20 | 0.20 | 0.15 | 0.10 | 0.05 | 0.07 | 0.10 | 0.04 | NA | -0.07 | NA | 0.07 | 0.20 | 0.18 | 0.37 |
|  | PLM | 0.29 | 0.23 | 0.19 | 0.17 | 0.14 | 0.05 | 0.20 | 0.24 | 0.10 | 0.03 | 0.04 | 0.16 | 0.13 | 0.21 | 0.25 | 0.29 |
|  | TGW | 0.11 | 0.12 | 0.11 | 0.25 | 0.26 | 0.38 | 0.03 | 0.10 | -0.08 | NA | 0.02 | NA | 0.15 | 0.15 | 0.22 | 0.24 |
|  | YLD | 0.26 | 0.18 | 0.31 | 0.14 | 0.05 | 0.05 | 0.16 | 0.11 | 0.12 | 0.00 | 0.05 | 0.11 | 0.08 | 0.20 | 0.19 | 0.34 |
| MT-CV1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | GM2 | 0.19 | 0.21 | 0.18 | 0.17 | 0.12 | 0.05 | 0.08 | 0.07 | 0.43 | NA | -0.04 | NA | 0.00 | 0.20 | 0.47 | 0.35 |
|  | PLM | 0.29 | 0.21 | 0.22 | 0.13 | 0.14 | 0.04 | 0.18 | 0.22 | 0.14 | 0.05 | 0.02 | 0.16 | 0.11 | 0.19 | 0.24 | 0.25 |
|  | TGW | 0.15 | 0.12 | 0.16 | 0.23 | 0.25 | 0.39 | 0.02 | 0.11 | 0.07 | NA | 0.02 | NA | 0.13 | 0.16 | 0.22 | 0.22 |
|  | YLD | 0.24 | 0.16 | 0.30 | 0.46 | 0.08 | 0.03 | 0.15 | 0.09 | 0.10 | 0.00 | 0.07 | 0.09 | -0.01 | 0.10 | 0.41 | 0.30 |
| MT-CV2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | GM2 | 0.60 | 0.59 | 0.38 | 0.56 | 0.57 | 0.50 | 0.56 | 0.52 | 0.09 | NA | 0.18 | NA | 0.63 | 0.56 | 0.58 | 0.63 |
|  | PLM | 0.58 | 0.56 | 0.24 | 0.20 | 0.22 | 0.08 | 0.37 | 0.61 | 0.11 | 0.05 | 0.33 | 0.19 | 0.29 | 0.54 | 0.48 | 0.59 |
|  | TGW | 0.56 | 0.56 | 0.33 | 0.48 | 0.44 | 0.50 | 0.35 | 0.59 | 0.23 | NA | 0.33 | NA | 0.43 | 0.54 | 0.51 | 0.58 |
|  | YLD | 0.60 | 0.57 | 0.49 | 0.49 | 0.51 | 0.42 | 0.54 | 0.54 | 0.42 | 0.01 | 0.34 | 0.11 | 0.63 | 0.54 | 0.50 | 0.70 |

CV1, predicting new lines with genotypic information only and CV2, predicting partially phenotyped lines by using genotypic and phenotypic information from all traits from individuals in the training set, and genotypic and phenotypic information from remaining traits in the testing set.

Table S5. The predictive ability for grain yield (YLD), plumpness (PLM), thousand grain weight (TGW), and number of grains square meter (GM2) using single trait (ST) and multi-trait (MT) models from 2015 experiments conducted in three locations (EELE, EEMAC, and MOSA).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Model | Trait | ALL15 | EELE15 | EEMAC15 | MOSA15 |
| MT-CV2\_AGRO |  |  |  |  |  |
|  | GRM2 | 0.59 | 0.57 | 0.56 | 0.52 |
|  | PLUMP | 0.56 | 0.22 | 0.54 | 0.61 |
|  | TGW | 0.56 | 0.44 | 0.54 | 0.59 |
|  | YLD | 0.57 | 0.51 | 0.54 | 0.54 |
| MT-CV2\_A+M |  |  |  |  |  |
|  | GRM2 | 0.77 | 0.78 | 0.76 | 0.73 |
|  | PLUMP | 0.60 | 0.53 | 0.61 | 0.60 |
|  | TGW | 0.61 | 0.58 | 0.60 | 0.62 |
|  | YLD | 0.77 | 0.72 | 0.74 | 0.73 |

ALL15, three combined experiments of the year 2015; CV2, predicting partially phenotyped lines by using genotypic and phenotypic information from all traits from individuals in the training set, and genotypic and phenotypic information from other correlated traits in the testing set.; The traits were: AGRO, agronomic traits (YLD, PLM, TGW, and GM2); or A+M, agronomic and malting quality traits (beta-glucan content, malt extract, protein content, and soluble nitrogen).

Table S6. The predictive ability for malting quality traits using single trait (ST) and multi-trait (MT) prediction models for the experiments conducted in 2015 in three locations (EELE, EEMAC, and MOSA) and for the combined experiments in 2015 (ALL15) and all (ALL).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Trait | Model | Trait Combinations | ALL | ALL15 | EELE15 | EEMAC15 | MOSA15 |
| Beta-glucan content |  |  |  |  |  |  |  |
|  | ST-CV1 |  |  |  |  |  |  |
|  |  | ST | 0.09 | 0.13 | 0.20 | 0.12 | 0.13 |
|  | MT-CV1 |  |  |  |  |  |  |
|  |  | A | 0.17 | 0.17 | 0.25 | 0.08 | 0.16 |
|  |  | A+M | 0.18 | 0.16 | 0.23 | 0.09 | 0.13 |
|  |  | COR1 | 0.18 | 0.20 | 0.20 | 0.09 | 0.15 |
|  |  | COR3 | 0.17 | 0.17 | 0.21 | 0.11 | 0.14 |
|  | MT-CV2 |  |  |  |  |  |  |
|  |  | A | 0.18 | 0.18 | 0.22 | 0.06 | 0.10 |
|  |  | A+M | 0.35 | 0.34 | 0.30 | 0.26 | 0.22 |
|  |  | COR1 | 0.32 | 0.31 | 0.26 | 0.25 | 0.20 |
|  |  | COR3 | 0.28 | 0.28 | 0.29 | 0.26 | 0.17 |
| Extract | ST-CV1 |  |  |  |  |  |  |
|  |  | ST | 0.21 | 0.17 | 0.27 | 0.12 | 0.06 |
|  | MT-CV1 |  |  |  |  |  |  |
|  |  | A | 0.23 | 0.21 | 0.25 | 0.09 | 0.07 |
|  |  | A+M | 0.21 | 0.21 | 0.27 | 0.08 | 0.05 |
|  |  | COR3 | 0.22 | 0.22 | 0.27 | 0.13 | 0.06 |
|  | MT-CV2 |  |  |  |  |  |  |
|  |  | A | 0.50 | 0.26 | 0.24 | 0.07 | 0.52 |
|  |  | A+M | 0.32 | 0.31 | 0.35 | 0.21 | 0.53 |
|  |  | COR3 | 0.27 | 0.27 | 0.33 | 0.18 | 0.32 |
| Grain protein content | ST-CV1 |  |  |  |  |  |  |
|  |  | ST | 0.11 | 0.10 | 0.18 | 0.01 | 0.13 |
|  | MT-CV1 |  |  |  |  |  |  |
|  |  | A | 0.18 | 0.11 | 0.17 | 0.00 | 0.09 |
|  |  | A+M | 0.11 | 0.11 | 0.17 | -0.01 | 0.09 |
|  |  | COR1 | 0.11 | 0.09 | 0.20 | 0.00 | 0.11 |
|  |  | COR2 | 0.10 | 0.08 | 0.18 | 0.00 | 0.09 |
|  | MT-CV2 |  |  |  |  |  |  |
|  |  | A | 0.40 | 0.37 | 0.20 | 0.15 | 0.13 |
|  |  | A+M | 0.46 | 0.44 | 0.34 | 0.25 | 0.31 |
|  |  | COR1 | 0.22 | 0.19 | 0.25 | 0.07 | 0.20 |
|  |  | COR2 | 0.38 | 0.32 | 0.20 | 0.16 | 0.12 |
| Soluble nitrogen | ST-CV1 |  |  |  |  |  |  |
|  |  | ST | 0.11 | 0.14 | 0.17 | 0.12 | 0.14 |
|  | MT-CV1 |  |  |  |  |  |  |
|  |  | A | 0.10 | 0.12 | 0.18 | 0.12 | 0.13 |
|  |  | A+M | 0.12 | 0.10 | 0.16 | 0.12 | 0.13 |
|  |  | COR1 | 0.14 | 0.13 | 0.15 | 0.14 | 0.15 |
|  |  | COR3 | 0.14 | 0.13 | 0.17 | 0.12 | 0.12 |
|  | MT-CV2 |  |  |  |  |  |  |
|  |  | A | 0.12 | 0.15 | 0.14 | 0.18 | 0.22 |
|  |  | A+M | 0.32 | 0.32 | 0.28 | 0.35 | 0.30 |
|  |  | COR1 | 0.29 | 0.29 | 0.27 | 0.26 | 0.23 |
|  |  | COR3 | 0.23 | 0.29 | 0.17 | 0.28 | 0.17 |

ALL, agronomic traits from all nine experiments combined and malting traits from the ALL15; CV1, predicting new lines with genotypic information only; and CV2, predicting partially phenotyped lines by using genotypic and phenotypic information from all traits from individuals in the training set, and genotypic and phenotypic information from other correlated traits in the testing set. Predicted traits were: beta-glucan content; malt extract; protein content; and soluble nitrogen; ST represents single trait, AGRO, agronomic traits (grain yield, plumpness, thousand grain weight, and number of grains per square meter) and trait of interest for multi-trait prediction model; A+M, agronomic and malting quality traits; COR1, denotes correlated traits which are soluble nitrogen, thousand grain weight, beta-glucan content, plumpness, and grain protein content; COR2, denotes traits within principal component 1 (PC1), which are grain yield, thousand grain weight, number of grains per square meter, and grain protein content; COR3, traits within PC2 which are beta-glucan content, plumpness, soluble nitrogen, and extract.

A close up of a map

Description automatically generated

Figure S1. Principal component analysis of 980 INNO lines including 145 lines, that were used in the building and testing the genomic prediction models, showing four subgroupings based on their bi-parental crosses. Five parents (Kalena, Conchita, CLE267, CLE268, and Livia) were involved in the production of 980 lines. The crosses were INNO1, CLE268 x Kalena; INNO2, Kalena x CLE267; INNO3, Kalena x Conchita; and INNO4, Livia x CLE268.

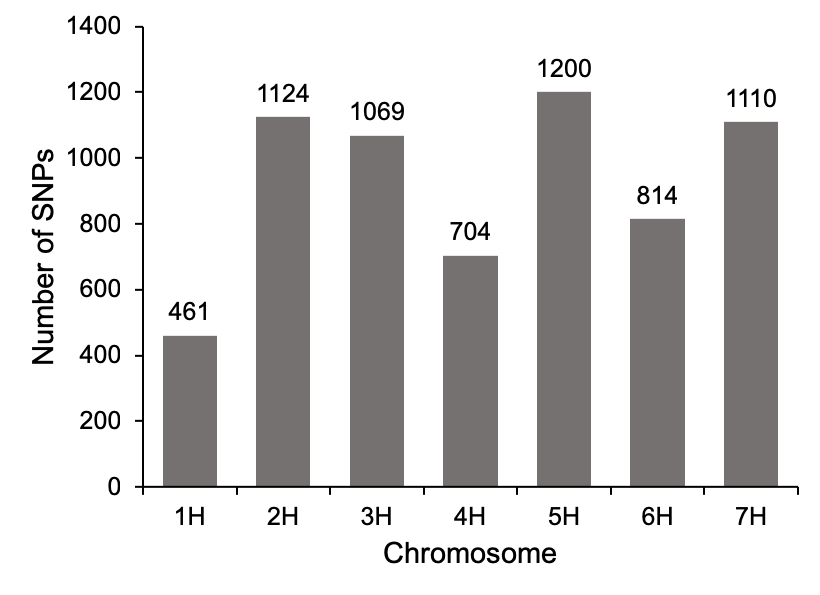


Figure S2. Distribution of the 6,482 single nucleotide polymorphisms (SNPs) across 7 chromosomes in 145 barley genotypes.



Figure S3. Pearson’s correlation for grain yield among environments (locations and years). Locations are EELE15, EELE16, EEMAC15, EEMAC16, EEMAC17, MOSA15, MOSA16, MUSA15, and MUSA16. The numbers 15, 16, and 17 at the end of each location are years 2015, 2016, and 2017, respectively. Environments were sorted to create groups with higher correlation.



Figure S4. Pearson’s correlation for number of grain per square meter among environments (locations and years). Locations are EELE15, EELE16, EEMAC15, EEMAC16, EEMAC17, MOSA15, MOSA16, and MUSA15. The numbers 15, 16, and 17 at the end of each location are years 2015, 2016, and 2017, respectively. Environments were sorted to create groups with higher correlation.

Figure S5. Pearson’s correlation for grain plumpness among environments (locations and years). Locations are EELE15, EELE16, EEMAC15, EEMAC16, EEMAC17, MOSA15, MOSA16, MUSA15, and MUSA16. The numbers 15, 16, and 17 at the end of each location are years 2015, 2016, and 2017, respectively. Environments were sorted to create groups with higher correlation.

Figure S6. Pearson’s correlation for thousand grain weight among environments (locations and years). Locations are EELE15, EELE16, EEMAC15, EEMAC16, EEMAC17, MOSA15, MOSA16, and MUSA15. The numbers 15, 16, and 17 at the end of each location are years 2015, 2016, and 2017, respectively. Environments were sorted to create groups with higher correlation.