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Supplemental File S1 – Estimation of genotype means

For estimation of genotype means, the following linear mixed model was fitted to measurements \( y_{ijk} \), with some modifications depending on the panel and/or trait:

\[
y_{ijk} = g_i + b_j + t_k + (g \times b)_{ij} + (g \times t)_{ik} + (b \times t)_{jk} + e_{ijk}
\]

where \( g_i \) was the mean of genotype \( i \) (fixed), \( b_j \) and \( t_k \) were the effects of replicate \( j \) (random) and year \( k \) (random); \( \times \) indicated interactions (random); \( e_{ijk} \) was the residual. For each random term, the corresponding effects were modelled as independent and identically normally distributed. In the NAP, the effects of sets (blocks within replicates) were accounted for through main effects of sets (random) and interactions between sets and years (random).

For quality traits (C, N and Ash), trait measurements were adjusted for phenology by adding heading date measurements as a covariate in the model, so as to account for the variation in biomass composition over the growing cycle (Vogel and Jung 2001). Heading date was measured in day of year as the time when at least 50% of panicles had emerged (in the NAP) or when at least three panicles had fully emerged (in the SAP).

The linear mixed models used to estimate \( g_i \)'s were fitted using ASREML-R (Gilmour et al. 2009).
Supplemental File S2 – Linkage disequilibrium corrected for population structure and relatedness

Linkage disequilibrium (LD) was characterized by relationships between expected allelic dosages adjusted for population structure, as captured by $Q = [1 \ P]$ ($I$ is a vector of ones), and relatedness, as captured by $XX'$. Following Mangin et al. (2012), for a given set of individuals and pair of markers $(j, k)$, LD correlations were computed as $r_{jk} = \frac{\tilde{x}_j' \tilde{x}_k'}{\sqrt{\tilde{x}_j' \tilde{x}_j \tilde{x}_k' \tilde{x}_k}}$, where $\tilde{x}_j = (XX')^{-1/2}(I - H_Q)x_j$ and $\tilde{x}_k$ are allelic dosages adjusted for population structure and relatedness, with $H_Q$ the matrix of projection onto the column space of $Q$, i.e., $H_Q = Q(Q'(XX')^{-1}Q)^{-1}Q'(XX')^{-1}$. Squared correlations ($r_{jk}^2$) were then compared to physical distances, determined from positions from version 4.1 of the *P. virgatum* reference genome.
null genomic model

For a given panel (the NAP or the SAP) and trait (PH, C, N or Ash), the following model was fitted to estimate the effect of population structure and compute genomic heritabilities:

\[ g = Q\alpha + e \]  \hspace{1cm} (1)

where \( g \) consisted of adjusted genotype means for each of the four traits considered, \( Q \) and associated coefficients \( \alpha \) depicted the effects of population structure, \( e \) consists of marginal residuals depicting random variation by relatedness, as captured by \( XX' \), as well as uncorrelated errors, such that \( e \sim N(0, \Sigma(\lambda)\sigma_e^2) \); \( \Sigma(\lambda) = \lambda^{-1}XX' + I \), \( I \) is the identity matrix, \( \lambda \) is the scaling factor such that the genomic additive variance corresponded to \( \sigma_a^2 = \frac{\sigma_e^2}{\lambda} \sum_j 2p_j(1-p_j) \), with \( p_j \) the alternate-allele frequency of marker \( j \) (VanRaden 2008); \( p_j \) was estimated by half the sample average of allelic dosages at each marker \( x_j \); \( \lambda \) was estimated by restricted maximum likelihood (REML) by the R package rrBLUP (Endelman 2011).

To estimate genomic and phenotypic correlations among traits in a given panel, and genomic correlation between panels for a given trait, model (1) was extended to multiple outcomes (traits or panels) with different estimates of \( \alpha \), \( \sigma_e^2 \) and \( \lambda \) by outcome, correlation among genotypes across outcomes and, only in multiple-trait models, correlation of errors across traits. The multiple-trait models were fitted using the GEMMA software (Zhou and Stephens 2014), and the multiple-panel models were fitted using ASREML-R (Gilmour et al. 2009).
Here, quantile-transformation consisted in applying an inverse normal transformation \( \Phi^{-1} \) to quantiles of marginal residuals \( \hat{\mathbf{e}} \) from model (1): the transformed mean of a genotype of rank \( (i) = 1, \ldots, N \) was \( \tilde{g}(i) = \mathbf{q}'(i) \hat{\mathbf{a}} + \Phi^{-1}\left( \frac{i}{N+1} \right) \) where \( \hat{\mathbf{a}} \) was the estimate of \( \mathbf{a} \) from model (1).

**Marginal marker effects**

The marginal effect of a given marker \( \mathbf{x}_j \) was assessed through the following model:

\[
\mathbf{g} = \mathbf{Q}\mathbf{a} + \mathbf{x}_j \beta_j + \mathbf{e} \tag{2}
\]

where \( \mathbf{g}, \mathbf{Q}, \mathbf{a} \) and \( \mathbf{x}_j \) were as described above; \( \beta_j \) depicted the linear relationship between \( \mathbf{x}_j \) and \( \mathbf{g} \), \( \mathbf{e} \sim N(0, \mathbf{\Sigma} \lambda_j \sigma^2_e) \); \( \mathbf{\Sigma}(\lambda_j) = \lambda_j^{-1} \mathbf{X}\mathbf{X}' + \mathbf{I}, \lambda_j \) was determined by REML specifically for each marker \( j \). The null hypothesis \( \beta_j = 0 \) was tested by a Wald \( \chi^2 \)-test, using the R package lrgpr (v0.1.9) (Hoffman et al. 2014).

**Ecotype-dependent marker effects**

The ecotype-dependent effect of a given marker \( j \) was assessed by extending model (2) for marker × ecotype interactions:

\[
\mathbf{g} = \mathbf{Q}\mathbf{a} + \mathbf{x}_j \beta_j + (\mathbf{x}_j \odot \mathbf{u}) \gamma_j + \mathbf{e} \tag{3}
\]

where \( \mathbf{g}, \mathbf{Q}, \mathbf{a}, \mathbf{x}_j, \beta_j \) and \( \mathbf{e} \) were as described for model (2); \( \mathbf{u} \) indicated whether genotypes belonged to the upland ecotype (0 and 1 referring to lowland and upland genotypes, respectively), \( \odot \) is the element-wise (Hadamard) product, \( \gamma_j \) was the difference in the effect of marker \( j \) from lowland to upland genotypes. The null hypothesis \( \gamma_j = 0 \) was tested by a Wald \( \chi^2 \)-test, using the R package lrgpr (v0.1.9) (Hoffman et al. 2014).
Additive-by-additive marker effects

For a given pair of markers \((j, k)\) among the preselected marker pairs, the \(A \times A\) epistatic effect was assessed by extending model (3) for effects involving each of the two markers individually as well as their \(A \times A\) interaction:

\[
g = Q\alpha + X_j \delta_j + X_k \delta_k + (x_j \circ x_k)\tau_{jk} + e \quad (4)
\]

where \(X_j = [x_j \quad x_j \circ u]\); \(\delta_j = [\beta_j \quad \gamma_j]'\); \(g, Q, a, x_j, u, \beta_j\) and \(\gamma_j\) were as described above, with ecotype-dependent marker effects included in the model so as to avoid detecting spurious epistatic effects through marker \(\times\) ecotype interactions; \(\tau_{jk}\) was the difference in the effect of marker \(j\) as the dosage at marker \(k\) increased (and vice-versa); \(e \sim N(0, \Sigma(\lambda)\sigma_e^2)\); \(\lambda\) was determined by REML based on model (1), using the rrBLUP package (Endelman 2011), regardless of the markers assessed, as in the EMMAX approach of Kang et al. (2010). Whenever there were linear dependencies among predictors of model (4), due to markers \(j\) and \(k\) being in near-perfect LD (as was the case for 2229 to 2249 marker pairs across traits), the association model included single-marker effects for only one of the two SNPs so, e.g., \(g = Q\alpha + X_j \delta_j + (x_j \circ x_k)\tau_{jk} + e\) was fitted instead of model (4). The null hypothesis \(\tau_{jk} = 0\) was tested by a Wald \(\chi^2\)-test.
Supplemental File S4 – Screening for epistatic marker effects

Screens based on single-marker analyses

In screens based on single-marker analyses (SMA screens), markers were tested for an effect in either of the two ecotypes: the lowland ecotype (L4X-NE and L4X-S: 142 individuals) and the upland ecotype (U4X-N, U8X-W, U8X-E and U8X-S: 370 individuals). As was previously suggested, additive marker effects may indicate underlying epistatic effects (Kooperberg and Leblanc 2008). Also, the dependence of marker effects on genetic backgrounds is generally due to interactive effects, which might include epistasis. Under the rationale that A × A effects contribute to marker × ecotype interactions, markers were tested for an additive effect across ecotypes, but also for marker × ecotype interaction. Formally, for any SNP \( j \) within the set of \( m \) considered markers, the null hypothesis \( \beta_j = \gamma_j = 0 \) from model (3) was tested by a two-degree-of-freedom Wald \( \chi^2 \)-test, using a custom R script. The scaling factor \( \lambda \) was determined by REML based on model (1), using the rrBLUP package (Endelman 2011), and was the same for all markers tested, as in the EMMAX approach of Kang et al. (2010). Using the same unscaled covariance \( \Sigma(\lambda) \) for \( e \) in single-marker screens and in tests for A × A interactions (as described below) ensured that the test statistics involving a given marker \( j \) in both analyses were asymptotically independent under the assumption of normality for genotype means \( g \) (see Supplemental File S5 for a proof and discussion).

Screens based on LD analyses

In screens based on LD analyses (LD screens), correlations in allelic dosage among markers were tested for exceptionally high values. For a given set of individuals, the LD correlation between markers \( j \) and \( k \), adjusted for population structure and genetic relatedness, was \( r_{jk} \) from the LD model described above; LD correlations were assumed centered around zero so that
\( E[r_{jk}] = 0 \) and \( \text{Var}[r_{jk}] = E[r_{ij}^2] \). In an approach similar, but not identical, to that of Tenesa et al. (2007), who regressed \( r_{ij}^2 \) on physical distance, \( E[r_{ij}^2] \) was modelled as

\[
E[r_{ij}^2] = \max \left( \frac{1}{n}, \frac{1}{a_0 + a_1 d_{ij}} \right),
\]

where \( n = N - \text{rank}(Q) \), with \( N \) the number of individuals in the set considered and \( \text{rank}(Q) = 5 \) (Hotelling 1953); \( d_{ij} \) is the physical distance between markers; \( a_0 \) and \( a_1 \) are regression coefficients from a generalized linear model assuming a Gamma distribution for \( r_{ij}^2 \), fitted on all pairs of markers less than 100 kb apart. On the one hand, \( \frac{1}{n} \) characterized LD due only to sampling variation (Weir and Hill, 1980) for pairs of markers that were either far apart from each other (such that \( \frac{1}{a_0 + a_1 d_{ij}} < \frac{1}{n} \)) or on different chromosomes. On the other hand, \( \frac{1}{a_0 + a_1 d_{ij}} \) characterized LD due to sampling variation and physical linkage. To test for outstandingly high values of LD, the following null distribution was assumed:

\[
\frac{z(r_{jk})^2}{\text{Var}[z(r_{jk})]} \sim \chi_1^2,
\]

where \( z \) denotes the Fisher z-transform \( (z(r_{jk}) = \text{arctanh}(r_{jk})) \), used here for approximate normality of \( z(r_{jk}) \); \( \text{Var}[z(r_{jk})] \) was approximated by first-order Taylor expansion of \( z(r_{jk}) \) about zero, as \( \text{Var}[z(r_{jk})] \approx [z'(0)]^2 \text{Var}[r_{jk}] \) with \( z'(0) = 1 \), so \( \text{Var}[z(r_{jk})] \) was simply approximated by \( E[r_{ij}^2] \). A pair of markers \( j \) and \( k \) was preselected if the \( p \)-value from

\[
\frac{z(r_{jk})^2}{\text{Var}[z(r_{jk})]} \sim \chi_1^2
\]

was lower than a Bonferroni FWER of 0.5, i.e. \( 0.5 / \binom{m}{2} \) (again, a rather high threshold of 0.5 was chosen here for sensitivity of the screen).
Supplemental File S5 – Asymptotic independence of the statistics in single-marker tests and tests on epistatic marker pairs

In this appendix, we will assume that all model matrices involved are of full column rank.

For a given pair of markers under assay and an unscaled covariance matrix $\Sigma$ for random deviations, the two following fits describe the effects associated to each marker separately, with respect to some vector of genotype means $g$.

\[
g = W_1 \beta_1 + \epsilon_1 \quad \text{such that} \quad W_1^\prime \Sigma^{-1} \epsilon_1 = 0 \quad (C1)
\]

\[
g = W_2 \beta_2 + \epsilon_1 \quad \text{such that} \quad W_2^\prime \Sigma^{-1} \epsilon_2 = 0 \quad (C2)
\]

$W_1 = [Q \ X_1]$ and $W_2 = [Q \ X_2]$, with $Q$ reflecting the effects of covariates, $X_1$ and $X_2$ reflecting the effects attributed to markers 1 and 2, respectively, not necessarily only their additive effects. Condition (C1) characterizes $\hat{\beta}_1$ as the best linear unbiased estimator (BLUE) of $\beta_1$ under the following model (M1): $g = W_1 \beta_1 + e_1, E[e_1] = 0, \text{Var}[e_1] = \Sigma \sigma_1^2$; and $\hat{\beta}_1 = (W_1^\prime \Sigma^{-1} W_1)^{-1} W_1^\prime \Sigma^{-1} g$. Similarly, by condition (C2), $\hat{\beta}_2$ is the BLUE of $\beta_2$ under the following model (M2): $g = W_2 \beta_2 + e_2; E[e_2] = 0, \text{Var}[e_2] = \Sigma \sigma_2^2$.

The following fit describes the joint effects of the two markers, potentially including some interactive marker effects through matrix $Z$ and corresponding coefficients $\hat{\epsilon}$, while effects associated to covariates and each marker separately are accounted for through $W = [Q \ X_1 \ X_2]$ and corresponding coefficients $\hat{\delta}$. 
\( \mathbf{g} = \mathbf{W}\hat{\mathbf{\delta}} + \mathbf{Z}\hat{\mathbf{\tau}} + \mathbf{\hat{e}}_3 \) such that

\[
\begin{bmatrix} \mathbf{W}' \\ \mathbf{Z}' \end{bmatrix} \Sigma^{-1} \mathbf{\hat{e}}_3 = \mathbf{0} \quad \text{(C3)}
\]

By condition (C3), \( \hat{\mathbf{\delta}} \) and \( \hat{\mathbf{\tau}} \) are the BLUE of \( \mathbf{\delta} \) and \( \mathbf{\tau} \) under the following model (M3): \( \mathbf{g} = \mathbf{W}\delta + \mathbf{Z}\tau + \mathbf{e}_3; \ E[\mathbf{e}_3] = \mathbf{0}, \ \text{Var}[\mathbf{e}_3] = \mathbf{\Sigma}\mathbf{\sigma}_3^2. \)

In what follows, we will further assume \( \mathbf{e}_3 \sim N(\mathbf{0}, \mathbf{\Sigma}\mathbf{\sigma}_3^2). \)

□ Under model (M3), a Wald \( \chi^2 \)-statistic based on \( \hat{\mathbf{\beta}}_1 \) or \( \hat{\mathbf{\beta}}_2 \) is asymptotically independent from a Wald \( \chi^2 \)-statistic based on \( \hat{\mathbf{\tau}}. \)

Let \( \mathbf{I} \) be the identity matrix and \( \mathbf{R} = \mathbf{I} - \mathbf{W}(\mathbf{W}'\Sigma^{-1}\mathbf{W})^{-1}\mathbf{W}'\Sigma^{-1} \) be the matrix of projection onto the space orthogonal to the column space of \( \mathbf{W}. \)

By condition (C3):

- \( \mathbf{R}\hat{\mathbf{e}}_3 = \hat{\mathbf{e}}_3 \) and \( (\mathbf{R}\mathbf{Z})'\Sigma^{-1}\hat{\mathbf{e}}_3 = \mathbf{0}. \) So \( (\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{R}\hat{\mathbf{e}}_3 = \mathbf{0} \)
- \( \mathbf{R}\mathbf{W} = \mathbf{0}. \) So \( \mathbf{R}\hat{\mathbf{e}}_3 = \mathbf{R}(\mathbf{g} - \mathbf{W}\hat{\mathbf{\delta}} - \mathbf{Z}\hat{\mathbf{\tau}}) = \mathbf{R}(\mathbf{g} - \mathbf{Z}\hat{\mathbf{\tau}}) \)

Therefore:

\[
(\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{R}\mathbf{\hat{\tau}} = (\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{Rg}
\]

Assuming that \( (\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{RZ} \) is full rank, we obtain:

\[
\hat{\mathbf{\tau}} = [(\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{RZ}]^{-1}(\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{Rg}
\]

The covariance between the estimates of interactive marker effects and the estimates of single-marker effects is then:

\[
\text{Cov} \left[ \mathbf{\hat{\tau}}, \mathbf{\hat{\beta}}_1 \right] = [(\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{RZ}]^{-1}(\mathbf{R}\mathbf{Z})'\Sigma^{-1}\mathbf{R}\text{Var}[\mathbf{g}]\Sigma^{-1}\mathbf{W}_1 (\mathbf{W}_1'\Sigma^{-1}\mathbf{W}_1)^{-1}
\]
Besides, under model (M3):

- \( \Sigma^{-1} R \text{Var}[g] \Sigma^{-1} W_1 (W_1 \Sigma^{-1} W_1)^{-1} = \sigma^2 \Sigma^{-1} R W_1 (W_1 \Sigma^{-1} W_1)^{-1} \)
- \( RW_1 = 0 \) (since \( RW = 0 \))

Therefore:

\[
\text{Cov} \left[ \hat{\tau}, \hat{\beta}_1 \right] = 0
\]

Moreover, if \( g \) is normally distributed under model (M3), so are \( \hat{\tau} \) and \( \hat{\beta}_1 \), since they are linear combinations of \( g \). Then, \( \text{Cov} \left[ \hat{\tau}, \hat{\beta}_1 \right] = 0 \) implies that \( \hat{\tau} \) and \( \hat{\beta}_1 \) are independent (Seber and Lee 2003, pp. 24-25). Therefore, under the asymptotic assumption of the Wald \( \chi^2 \)-test (variance component estimates assumed fixed), the test statistics based on \( \hat{\tau} \) and \( \hat{\beta}_1 \) are independent.

Tests based on \( \hat{\beta}_1 \) may be useful to screen for potential epistatic effects, in that, under model (M3), \( E[\hat{\beta}_1] = (W_1 \Sigma^{-1} W_1)^{-1} W_1 \Sigma^{-1} (W \delta + Z \tau) \) and \( W_1 \hat{\beta}_1 \) may therefore partially reflect epistatic effects, by projection of \( Z \tau \) onto the column space of \( W_1 \). However, from the (approximate) independence between test statistics based on \( \hat{\tau} \) and \( \hat{\beta}_1 \), it follows that random variation at \( \hat{\beta}_1 \) (due to estimation error) should not carry over to \( \hat{\tau} \), so that, under the null hypothesis, the occurrence of a false positive at the screening step should be independent from the occurrence of a false positive at the testing step.

Importantly, the results of independence between \( \hat{\tau} \) and \( \hat{\beta}_1 \) required that model (M1) be nested in model (M3). Furthermore, \( \text{Cov} \left[ \hat{\tau}, \hat{\beta}_1 \right] = 0 \) relied on the assumption that the unscaled covariance matrix of random deviations was the same for model (M1) and model (M3): \( \text{Var}[e_1] \) and \( \text{Var}[e_3] \) were defined as proportional to the same matrix \( \Sigma \). In association studies based on
linear mixed models, typically \( \Sigma(\lambda) = \lambda^{-1}XX' + I \), with \( \lambda \) a factor scaling genomic relationships from \( XX' \) relatively to errors, assumed independent. In this study, we followed the EMMAX approach and set \( \lambda \) as its REML estimate under the null model \( g = Q\alpha + e, e \sim N(0, \Sigma(\lambda)) \), in regressions involving \( \hat{\beta}_1 \) and \( \hat{\beta}_2 \) (Kang et al. 2010). So here, the EMMAX approach was useful not only for computational tractability, but also to ensure approximate independence of the statistics from single-marker tests and from epistatic tests.

The asymptotic independence between test statistics based on \( \hat{\tau} \) and \( \hat{\beta}_2 \) and its implications can be shown in a similar way.
Fig. S1 – Density plots of sequencing depth and posterior variance at selected markers, in the northern association panel (NAP) and the southern association panel (SAP). Sequencing depth: number of reads observed at selected SNPs averaged over individuals, by ploidy level. Posterior variance: variance about allelic dosage at selected SNPs averaged over individuals, by ploidy level. Significance was assessed by paired $t$-tests. The insets correspond to a narrower range, between 0 and the 90%-percentile of sequencing depth or posterior variance.
**Fig. S2** – Normal quantile–quantile plots of residuals from the null genomic model in the northern association panel (NAP), conditionally on $Q$ and $X$, standardized as in Santos Nobre and da Motta Singer (2007) (Eq. 2.4). PH: plant height; C: C content; N: N content; Ash: mineral concentration. Filled red circles: presumed outliers in C and Ash (removed from all analyses), corresponding to one same genotype (conditional residuals more than five standard units away from zero). Open red circles: extreme values in PH and C, removed from association analyses (conditional residuals more than four standard units away from zero). Open black circles correspond to the data filtered for association tests (after presumed outliers and extreme values have been removed).
Fig. S3 – Normal quantile–quantile plots of residuals from the null genomic model in the southern association panel (SAP), conditionally on $Q$ and $X$, standardized as in Santos Nobre and da Motta Singer (2007) (Eq. 2.4). PH: plant height; C: C content; N: N content; Ash: mineral concentration.
**Fig. S4** – Linkage disequilibrium in the *P. virgatum* association panels. Correlation in allelic dosage between markers (*r*), adjusted for population structure and relatedness. Markers considered are those with a MAF higher than 0.05 and a residual variance (variance of markers after accounting for population structure) higher than 2(0.02)(0.98) in each ecotype. (a,b) Relationship between *r*² and physical distance in (a) the northern association panel (NAP; 512 individuals) and (b) the southern association panel (SAP; 426 individuals); the blue curves correspond to the expected value of *r*² in each panel, from a Gamma regression fitted on pairs of markers less than 100 kb apart. (c) Consistency in *r* across panels; the orange dashed line represents the simple linear regression of *r* in the SAP on *r* in the NAP; R² is the proportion of variation explained by this regression.
**Fig. S5** – Uniform quantile–quantile plots for marginal effects of 439,170 markers on plant height (PH), C content (C), N content (N), and mineral concentration (Ash) in the northern association panel (NAP). Genomic inflation: ratio of the observed median of Wald $\chi^2$-test statistics over the expected median from a $\chi^2$ distribution with one degree of freedom. Filled blue circles correspond to the untransformed data; open black circles correspond to the quantile-transformed data; green dots correspond to a FDR lower than 0.05. Labels consist of chromosome identifier and within-chromosome position (in Mb) based on *P. virgatum* reference genome version 4.1.
Fig. S6 – Uniform quantile–quantile plots for marker × ecotype interactions of 83,290 markers on plant height (PH), C content (C), N content (N), and mineral concentration (Ash) in the northern association panel (NAP). Genomic inflation: ratio of the observed median of Wald $\chi^2$-test statistics over the expected median from a $\chi^2$ distribution with one degree of freedom. Filled blue circles correspond to the untransformed data; open black circles correspond to the quantile-transformed data; green dots correspond to a FDR lower than 0.05. Labels consist of chromosome identifier and within-chromosome position (in Mb) based on P. virgatum reference genome version 4.1.
Fig. S7 – Normalized expression levels, on a log₂ scale, of transcripts related to genes of interest. Normalized expression levels are based on the gene expression atlas of Zhang et al. (2013). Genes of interest are those associated to markers with significant (a) additive marker effects, (b)
marker × ecotype interactions, and (c) A × A effects. Samples correspond to experiments (e.g. seed germination) and developmental stages (e.g., 24h). Error bars indicate standard deviation across biological replicates. The red dashed line corresponds to the value below which expression levels are essentially null (Zhang et al. 2013). Significance of differences in expression level were inferred from a one-way analysis of variance for log₂(Normalized expression levels) on samples. Letters above bars indicate significance groups based on significant differences at a 5% level, after multiple-testing correction by Tukey’s method. Developmental stages in germination (G1, G2, G3, G4), plant establishment (V1, V3, V5), elongation (E4), flowering (R4, R5) and seed development (S0, S1, S2, S3, S4) refer to stages as described by Moore et al. (1991). DAP: days after pollination.
Table S1 – Genomic correlation between *P. virgatum* association panels

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genomic correlation</th>
<th>p-value (H₀: no correlation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>1.00</td>
<td>0.0026</td>
</tr>
<tr>
<td>C</td>
<td>0.82</td>
<td>0.34</td>
</tr>
<tr>
<td>N</td>
<td>-0.24</td>
<td>0.57</td>
</tr>
<tr>
<td>Ash</td>
<td>0.85</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Trait: plant height (PH); C content (C); N content (N); mineral concentration (Ash). The genomic correlation between panels was based on a multivariate extension of the null genomic model, and was estimated directly by REML. The *p*-value was derived from a likelihood ratio test and tested the hypothesis that there was no consistency in genomic additive effects from the northern association panel (NAP) to the southern association panel (SAP) (genomic correlation set to zero).
Table S2 – Significance of marker effects in the northern association panel (NAP) to the southern association panel (SAP)

<table>
<thead>
<tr>
<th>Marker effect</th>
<th>Trait</th>
<th>Chr.</th>
<th>Position</th>
<th>NAP</th>
<th>Allele frequency</th>
<th>p-value (FDR)</th>
<th>SAP</th>
<th>Allele frequency</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marginal effect</td>
<td>C</td>
<td>1N</td>
<td>70995666</td>
<td>0.38</td>
<td>3.5×10⁻⁸ (0.011)</td>
<td></td>
<td>0.27</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5K</td>
<td>26898353</td>
<td>0.06</td>
<td>5.1×10⁻⁸ (0.011)</td>
<td>&lt;0.01</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5N</td>
<td>10757408</td>
<td>0.05</td>
<td>1.6×10⁻⁷ (0.024)</td>
<td>&lt;0.01</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9N</td>
<td>17707955</td>
<td>0.26</td>
<td>4.4×10⁻⁷ (0.049)</td>
<td>0.17</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>5N</td>
<td>15226177</td>
<td>0.05</td>
<td>1.1×10⁻⁷ (0.047)</td>
<td>15226243</td>
<td>0.02</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Marker-by-ecotype interaction</td>
<td>PH</td>
<td>1N</td>
<td>5781535</td>
<td>0.35</td>
<td>7.9×10⁻⁷ (0.011)</td>
<td>0.28</td>
<td>0.039</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2K</td>
<td>76597647</td>
<td>0.29</td>
<td>5.1×10⁻⁸ (0.0019)</td>
<td>0.30</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4N</td>
<td>55017987</td>
<td>0.08</td>
<td>1.2×10⁻⁶ (0.015)</td>
<td>55015922</td>
<td>0.09</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × A (GO)</td>
<td>C</td>
<td>2K/2N</td>
<td>27354293/877943800,57/0.48</td>
<td>4.0×10⁻⁹ (0.078)</td>
<td>0.47/0.53</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × A (LD)</td>
<td>C</td>
<td>1K/2N</td>
<td>72051660/7693200,0.45/0.69</td>
<td>2.0×10⁻⁷ (0.040)</td>
<td>0.31/0.62</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>1K/2N</td>
<td>72051660/7693200,0.45/0.69</td>
<td>4.1×10⁻⁷ (0.039)</td>
<td>0.31/0.62</td>
<td>0.053</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>4N/9K</td>
<td>48130189/7638971,0.75/0.74</td>
<td>3.6×10⁻⁷ (0.039)</td>
<td>0.74/0.78</td>
<td>0.77</td>
<td></td>
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</tr>
</tbody>
</table>

Markers were deemed to be significant if they had a FDR lower than 0.05 for any given trait, based on association analyses performed in the NAP on untransformed data. A × A: additive-by-additive interaction for marker pairs preselected based on LD screens (LD), or GO screen (GO). Trait: plant height (PH); C content (C); N content (N); mineral concentration (Ash). Chr.: Chromosome. Position: within-chromosome position of the marker selected in the NAP (based on association tests and model selection within chromosome) and selected in the SAP (most significant marker among those within 20 kb of markers selected in the NAP and with r² higher than 0.5); if not specified, positions are the same in the NAP and the SAP. In the NAP, p-values are inferred from a Wald χ²-test; in the SAP, p-values are inferred from a one-sided Wald z-test testing for consistency in effect from the NAP to the SAP. Chromosome identifier and within-chromosome position are based on P. virgatum reference genome version 4.1.
Table S3 – Effects of significant markers in the northern association panel (NAP)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Effect</th>
<th>Coefficient (p-value)</th>
<th>R²LR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>4x</td>
</tr>
<tr>
<td>C</td>
<td>1N/70995666</td>
<td>-1.60 (1.1×10⁻⁷)</td>
<td>-2.14 (5.6×10⁻⁴)</td>
</tr>
<tr>
<td>C</td>
<td>5K/26898353</td>
<td>-2.50 (1.8×10⁻⁷)</td>
<td>-2.78 (2.7×10⁻⁵)</td>
</tr>
<tr>
<td>C</td>
<td>5N/10757408</td>
<td>2.47 (3.3×10⁻⁴)</td>
<td>3.66 (3.8×10⁻⁷)</td>
</tr>
<tr>
<td>C</td>
<td>9N/17707955</td>
<td>-1.37 (5.0×10⁻⁷)</td>
<td>-1.50 (1.7×10⁻⁴)</td>
</tr>
<tr>
<td>C</td>
<td>2K/27354293</td>
<td>3.44 (2.8×10⁻⁴)</td>
<td>4.49 (2.2×10⁻⁴)</td>
</tr>
<tr>
<td></td>
<td>2N/87794380</td>
<td>-0.36 (0.59)</td>
<td>-0.69 (0.34)</td>
</tr>
<tr>
<td></td>
<td>2K/27354293(U)</td>
<td>-0.78 (0.29)</td>
<td>-1.86 (0.072)</td>
</tr>
<tr>
<td></td>
<td>2N/87794380(U)</td>
<td>2.89 (1.6×10⁻¹)</td>
<td>3.59 (2.5×10⁻³)</td>
</tr>
<tr>
<td></td>
<td>2K/27354293 x 2N/87794380</td>
<td>-2.03 (3.8×10⁶)</td>
<td>-2.58 (9.4×10⁵)</td>
</tr>
<tr>
<td>C</td>
<td>1K/72051660</td>
<td>-1.22 (0.22)</td>
<td>-1.27 (0.25)</td>
</tr>
<tr>
<td></td>
<td>2N/7693200</td>
<td>-0.48 (0.54)</td>
<td>-0.61 (0.51)</td>
</tr>
<tr>
<td></td>
<td>1K/72051660(U)</td>
<td>-1.27 (0.24)</td>
<td>-0.74 (0.55)</td>
</tr>
<tr>
<td></td>
<td>2N/7693200(U)</td>
<td>-2.20 (0.018)</td>
<td>-2.07 (0.083)</td>
</tr>
<tr>
<td></td>
<td>1K/72051660 x 2N/7693200</td>
<td>2.15 (1.9×10⁻⁴)</td>
<td>2.28 (2.0×10⁻³)</td>
</tr>
<tr>
<td>PH</td>
<td>1N/5781535</td>
<td>-15.98 (3.5×10⁻⁵)</td>
<td>-15.12 (4.1×10⁻³)</td>
</tr>
<tr>
<td></td>
<td>1N/5781535(U)</td>
<td>20.43 (9.3×10⁻²)</td>
<td>21.66 (3.7×10⁻¹)</td>
</tr>
<tr>
<td>PH</td>
<td>2K/76597647</td>
<td>-19.89 (2.5×10⁻⁸)</td>
<td>-20.19 (3.4×10⁻⁹)</td>
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<td>2K/76597647(U)</td>
<td>21.25 (6.0×10⁻⁸)</td>
<td>22.18 (4.7×10⁻⁸)</td>
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<tr>
<td>PH</td>
<td>4N/55017987</td>
<td>-16.75 (9.5×10⁻⁶)</td>
<td>-16.51 (7.0×10⁻⁶)</td>
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<td>4N/55017987(U)</td>
<td>21.86 (1.4×10⁻⁶)</td>
<td>18.98 (5.6×10⁻⁶)</td>
</tr>
<tr>
<td>Ash</td>
<td>5N/15226177</td>
<td>-5.36 (1.5×10⁻¹)</td>
<td>-6.27 (2.1×10⁻³)</td>
</tr>
<tr>
<td>Ash</td>
<td>1K/72051660</td>
<td>2.41 (0.24)</td>
<td>2.77 (0.20)</td>
</tr>
<tr>
<td></td>
<td>2N/7693200</td>
<td>0.62 (0.71)</td>
<td>0.87 (0.63)</td>
</tr>
<tr>
<td></td>
<td>1K/72051660(U)</td>
<td>2.63 (0.23)</td>
<td>1.81 (0.46)</td>
</tr>
<tr>
<td></td>
<td>2N/7693200(U)</td>
<td>5.07 (8.3×10⁻³)</td>
<td>4.97 (0.037)</td>
</tr>
</tbody>
</table>
Markers were deemed to be significant if they had a FDR lower than 0.05 for any given trait, based on association analyses performed in the NAP on untransformed data. Effect and significance of markers were estimated based on the NAP (All; \( n = 512 \)), tetraploids only (4x; \( n = 274 \)), or octoploids only (8x; \( n = 238 \)). Trait: plant height (PH); C content (C); N content (N); mineral concentration (Ash).

Effect: additive effect (marker name only), upland-specific adjustment on additive marker effect (marker name followed by (U)), additive-by-additive interaction (two marker names). Coefficient: estimated effect in a linear mixed model accounting for population structure and relatedness; \( p \)-values are inferred from a Wald \( \chi^2 \)-test. Empty cells indicate the absence of marker \( \times \) ecotype interaction for 8x individuals: all 8x individuals were upland, so baseline marker effects correspond to effects in the upland ecotype. \( R^2_{LR} \): Proportion of variation explained by the marker based on a maximum-likelihood ratio between the model of marker association and the null genomic model (Magee 1990, Sun et al. 2010). Models were fitted using the rrBLUP package in R (Endelman 2011).

<table>
<thead>
<tr>
<th></th>
<th>( 1K/72051660 \times 2N/7693200 )</th>
<th>( 4N/48130189 )</th>
<th>( 9K/7638971 )</th>
<th>( 4N/48130189(U) )</th>
<th>( 9K/7638971(U) )</th>
<th>( 4N/48130189 \times 9K/7638971 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>-4.29 (4.1\times 10^{-7})</td>
<td>-6.39 (2.8\times 10^{-5})</td>
<td>-6.47 (0.010)</td>
<td>-4.89 (0.026)</td>
<td>0.056</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>-4.45 (3.1\times 10^{-5})</td>
<td>-7.05 (2.7\times 10^{-3})</td>
<td>-7.36 (8.7\times 10^{-5})</td>
<td>-5.84 (3.0\times 10^{-4})</td>
<td>0.42 (0.82)</td>
<td>-0.29 (0.89)</td>
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<tr>
<td></td>
<td>-4.35 (0.023)</td>
<td>-0.26 (0.90)</td>
<td>-0.30 (0.89)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.72 (3.6\times 10^{-1})</td>
<td>5.17 (5.8\times 10^{-5})</td>
<td>3.87 (0.010)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References


Gilmour, A.R., B.J. Gogel, B.R. Cullis, R. Thompson, and D. Butler. 2009. ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead, UK


