

Genotype by Environment analysis using RAP

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Genotype by Environment Analysis using the RAP package

The RAP package is developed as an easy to use package for analysing data of plant breeding experiments with plenty of options for plotting and reporting the results of the analyses.

The package has 3 main components:

- Modeling trial data for single trials and extracting results
- Genotype by Environment (GxE) analysis
- QTL analysis

This vignette deals with the GxE part of the package and describes in detail how to perform the different types of analysis that are available in the package.

The following types of analysis can be done using RAP:

- Best variance-covariance model
 - AMMI Analysis
 - Finlay-Wilkinson Analysis
 - Computation of mega environments
 - Computation of stability measures
-

Data preparation

Just as for the analysis of single field trials the input for GxE analysis in the RAP package is an object of class TD. For a detailed description on how to construct such an object see the vignette Modeling field trials using RAP. For this vignette the TD object created in the final step of this vignette, `TDGxE`, will be used as analysis object.

Best variance-covariance model

The function `gxeVarComp` fits models with different variance-covariance structures to the GxE data and determines the best model for the data. With the default settings for the function `lme4` is used for fitting the models and only a compound symmetry model is fitted. When changing the modeling engine to `asreml` 8 different models are fitted:

- identity
- compound symmetry
- diagonal
- heterogeneous compound symmetry
- outside
- factor analytic with one factor
- factor analytic with two factors
- unstructured

The best model for the data is selected based on either the Akaike Information Criterion (AIC) or the Bayesian Information Criterion (BIC). Which criterion is used is determined by the parameter `criterion` in the function `gxeVarComp`.

Using the TDGxE TD object created in the RAP vignette the function can be used as follows:

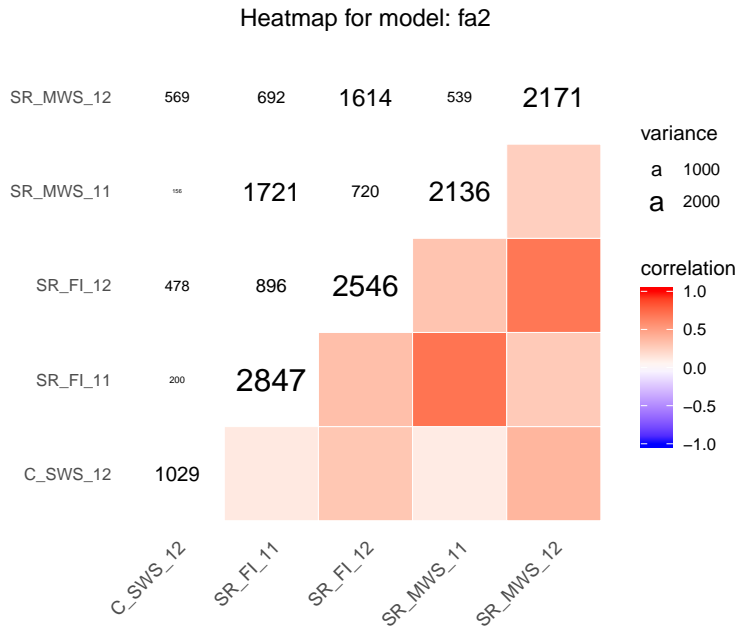
```
## Use lme4 for fitting the models - only compound symmetry.
geVC <- gxeVarComp(TD = TDGxE, trait = "BLUES_GY")
summary(geVC)
#> Best model: cs, based on BIC.
#>           AIC      BIC Deviance NParameters
#> cs 31188.53 31199.65 31184.53           2
```

```
## Use asreml for fitting the models - 8 models fitted.
## Use AIC as criterion for determining the best model.
if (requireNamespace("asreml", quietly = TRUE)) {
  geVC2 <- gxeVarComp(TD = TDGxE, trait = "BLUES_GY", engine = "asreml",
                     criterion = "AIC")
  summary(geVC2)
}
#> Best model: fa2, based on AIC.
#>           AIC      BIC Deviance NParameters
#> fa2      27310.09 27384.80 27282.09           14
#> unstructured 27311.58 27391.63 27281.58           15
#> fa         27518.37 27571.74 27498.37           10
#> outside     27548.83 27580.86 27536.83            6
#> hcs         27639.91 27671.93 27627.91            6
#> cs         27672.67 27683.34 27668.67            2
#> diagonal    27904.72 27931.40 27894.72            5
#> identity    28001.40 28006.74 27999.40            1
```

As becomes clear from the summary the best model based on AIC is the model with a factor analytic variance-covariance structure with two factors. Note that for the both factor analytic models to be fitted a minimum of 5 environments are needed. If the data contains less environments those two models are skipped.

A heat map of the correlation between the environments based on the best fitted model can be plotted.

```
if (requireNamespace("asreml", quietly = TRUE)) {
  plot(geVC2)
}
```



The upper left of the plot displays the variance between environments. Larger values are displayed in a bigger font. In the lower right of the plot correlations between environments are shown. A dark red color indicates a strong positive correlation between environments, a dark blue a strong negative correlation.

A report containing the most important results of the analysis can be made as well.

```
report(geVC2, outfile = "./myReports/varCompReport.pdf")
```

AMMI Analysis

The Additive Main Effects and Multiplicative Interaction (AMMI) model fits a model which involves the Additive Main effects (i.e. genotype and trial) along with the Multiplicative Interaction effects. Then a principal component analysis is done on the residuals (multiplicative interaction). This results in an interaction characterized by Interaction Principal Components (IPCA) enabling simultaneous plotting of genotypes and trials.

The ammi analysis can be performed with the RAP package using the function `gxeAmmi`.

```
## Run gxeAmmi with default settings.
geAm <- gxeAmmi(TD = TDGxE, trait = "BLUES_GY")
summary(geAm)
#> Principal components
#> =====
#>
#> PC1 PC2
#> Standard deviation 1030.58353 715.42427
#> Proportion of Variance 0.49975 0.24083
#> Cumulative Proportion 0.49975 0.74058
#>
#> Anova
#> =====
#>
#> Df Sum Sq Mean Sq F value Pr(>F)
#> Genotype 383 760625194 1985967 3.7378 < 2.2e-16 ***
#> Environment 4 8310680494 2077670123 3910.4240 < 2.2e-16 ***
```

```
#> Interactions      1532  813975825      531316
#> PC1                386  406785224      1053848      3.8030 < 2.2e-16 ***
#> PC2                384  196031610      510499      1.8422  6.41e-13 ***
#> Residuals         762  211158990      277112
#> ---
#> Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#>
#> Environment scores
#> =====
#>                PC1          PC2
#> C_SWS_12    0.2278314 -0.81879355
#> SR_FI_11   -0.5946811  0.16138788
#> SR_FI_12    0.4069946  0.53407849
#> SR_MWS_11  -0.4826721 -0.01143373
#> SR_MWS_12   0.4425272  0.13476091
```

With the default settings in the principal components analysis a maximum of 2 principal components are used. This can be changed using the `nPC` parameter in the function. The number of principal components can never be larger than the number of environments and the number of genotypes in the data. By specifying `nPC = NULL` the algorithm will determine the number of principal components to use using a method of forward selection.

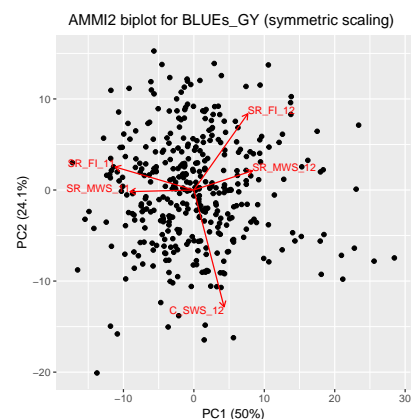
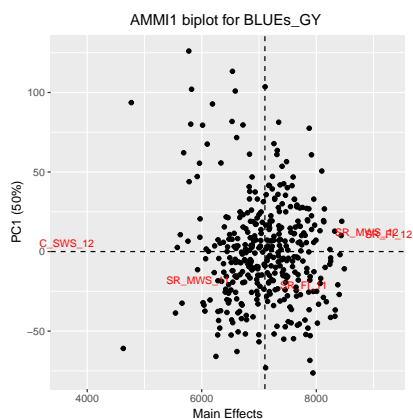
```
## Run gxeAmmi with 3 principal components.
geAm2 <- gxeAmmi(TD = TDGxE, trait = "BLUES_GY", nPC = 3)
summary(geAm2)
#> Principal components
#> =====
#>                PC1          PC2          PC3
#> Standard deviation    1030.58353  715.42427  535.36893
#> Proportion of Variance    0.49975    0.24083    0.13486
#> Cumulative Proportion    0.49975    0.74058    0.87545
#>
#> Anova
#> =====
#>                Df      Sum Sq    Mean Sq    F value    Pr(>F)
#> Genotype         383  760625194    1985967    3.7378 < 2.2e-16 ***
#> Environment        4  8310680494  2077670123  3910.4240 < 2.2e-16 ***
#> Interactions     1532  813975825      531316
#> PC1                386  406785224      1053848    3.9500 < 2.2e-16 ***
#> PC2                384  196031610      510499    1.9134  1.703e-10 ***
#> PC3                382  109775420      287370    1.0771    0.2345
#> Residuals        380  101383571      266799
#> ---
#> Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#>
#> Environment scores
#> =====
#>                PC1          PC2          PC3
#> C_SWS_12    0.2278314 -0.81879355  0.06977221
#> SR_FI_11   -0.5946811  0.16138788  0.64279041
#> SR_FI_12    0.4069946  0.53407849 -0.14397531
#> SR_MWS_11  -0.4826721 -0.01143373 -0.73127110
#> SR_MWS_12   0.4425272  0.13476091  0.16268378
```

```
## Run gxeAmmi. Algorithm determines number of principal components.
geAm3 <- gxeAmmi(TD = TDGxE, trait = "BLUES_GY", nPC = NULL)
summary(geAm3)
#> Principal components
#> =====
#>
#>                PC1      PC2
#> Standard deviation 1030.58353 715.42427
#> Proportion of Variance 0.49975 0.24083
#> Cumulative Proportion 0.49975 0.74058
#>
#> Anova
#> =====
#>
#>          Df      Sum Sq   Mean Sq   F value    Pr(>F)
#> Genotype 383 760625194 1985967 3.7378 < 2.2e-16 ***
#> Environment 4 8310680494 2077670123 3910.4240 < 2.2e-16 ***
#> Interactions 1532 813975825 531316
#> PC1 386 406785224 1053848 3.8030 < 2.2e-16 ***
#> PC2 384 196031610 510499 1.8422 6.41e-13 ***
#> Residuals 762 211158990 277112
#> ---
#> Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#>
#> Environment scores
#> =====
#>
#>                PC1      PC2
#> C_SWS_12 0.2278314 -0.81879355
#> SR_FI_11 -0.5946811 0.16138788
#> SR_FI_12 0.4069946 0.53407849
#> SR_MWS_11 -0.4826721 -0.01143373
#> SR_MWS_12 0.4425272 0.13476091
```

If the data contains a column year it is possible to perform the AMMI analysis per year in the data. This can be done by specifying the parameter `byYear = TRUE`.

The results of an AMMI analysis can be displayed in a biplot. Two types of biplot are available. “AMMI1” plots the main effects against the first principal component. “AMMI2” plots the first against the second principal component.

```
## Create an AMMI1 and AMMI2 biplot.
plot(geAm, scale = 0.5, plotType = "AMMI1")
plot(geAm, scale = 0.5, plotType = "AMMI2")
```



The

AMMI plot function has many options to customize the plot. It is possible to plot different principal components on the axis, group and color genotypes by a variable in the data and use a different scaling factor. Run `help(plot.AMMI)` for more details.

For the AMMI analysis a report can be made as well.

```
report(geAm, outfile = "./myReports/AMMIReport.pdf")
```

Finlay-Wilkinson Analysis

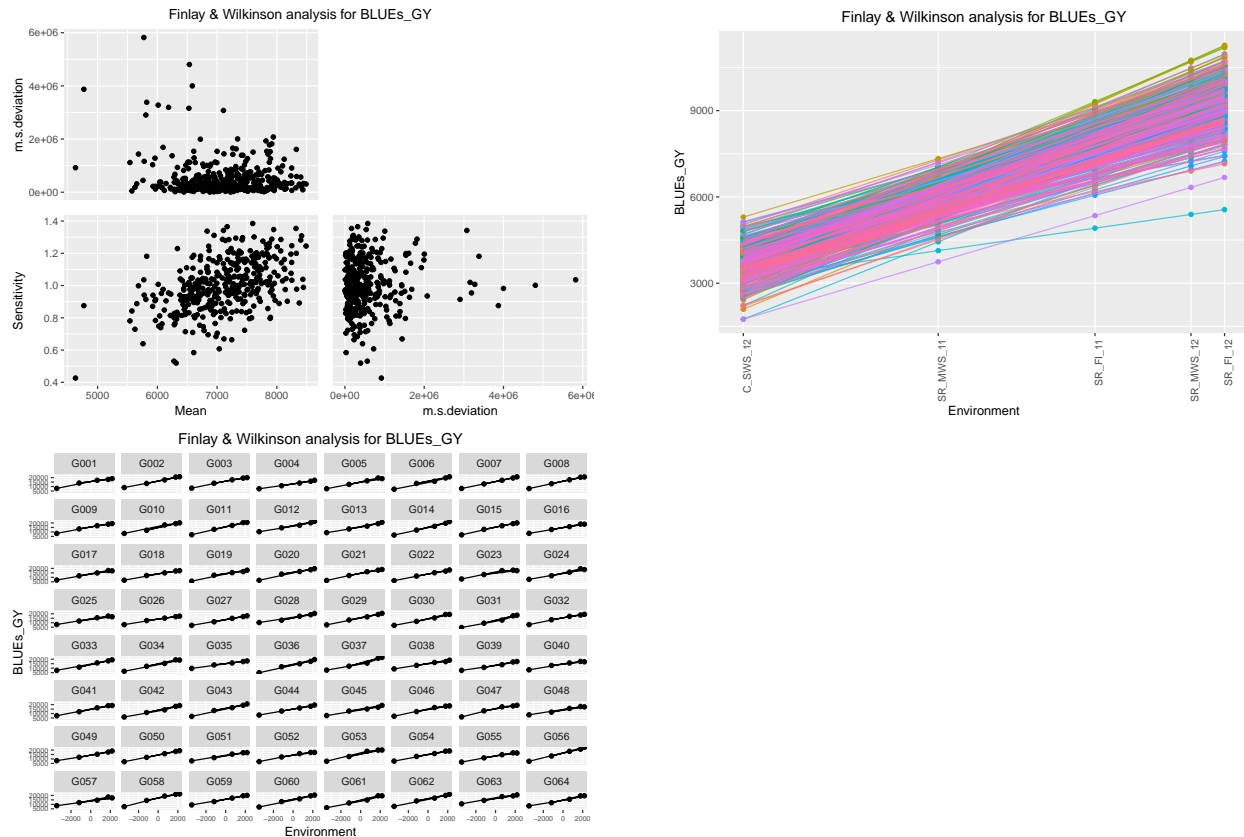
With the Finlay-Wilkinson Analysis (Finlay and Wilkinson 1963) a modified joint regression analysis is used to rank germplasm based on phenotypic stability for each individual trait.

In the RAP package this analysis can be done using the `gxFW` function.

```
geFW <- gxFW(TD = TDGxE, trait = "BLUEs_GY")
summary(geFW)
#> Environmental effects
#> =====
#>      trial      effect      SE      mean rank
#> 1  C_SWS_12 -3450.6949 56.48279 3651.430      5
#> 2  SR_FI_11  661.1322 56.58694 7762.838      3
#> 3  SR_FI_12 2176.7587 56.48279 9279.343      1
#> 4  SR_MWS_11 -1171.7041 56.58694 5930.812      4
#> 5  SR_MWS_12 1784.5081 56.48279 8887.059      2
#>
#> Anova
#> =====
#>
#>              Df      Sum Sq      Mean Sq      F value Pr(>F)
#> genotype      383  760927259    1986755      3.7129 <2e-16 ***
#> trial          4  8308722428   2077180607 3881.8460 <2e-16 ***
#> Sensitivities   383  200189459     522688      0.9768 0.6051
#> Residual      1147  613761116     535101
#> Total         1917 9883600263     5155764
#> ---
#> Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#>
#> Most sensitive genotypes
#> =====
#> genotype      sens  sigmaE  genMean      sigma      mse
#>      G202 0.4261900 0.157044 4630.041 326.7125 920155.42
#>      G229 0.5190070 0.157044 6314.679 326.7125 394503.53
#>      G338 0.5311154 0.157044 6278.680 326.7125 564566.01
#>      G330 0.5840103 0.157044 6609.668 326.7125 29086.74
#>      G285 0.6073710 0.157044 7036.768 326.7125 724189.99
```

Several plots can be made to investigate the output of the analysis.

```
plot(geFW, plotType = "scatter")
plot(geFW, plotType = "line")
plot(geFW, plotType = "trellis")
```



A report can be made as well.

```
report(geFW, outfile = "./myReports/FWReport.pdf")
```

Computation of mega environments

For the computation of mega environments an AMMI model is fitted and then using the fitted values of this model the environments are clustered

Mega environments can be created by two methods. The first method groups environments based on their best performing genotype; i.e. environments that share the same best genotype belong to the same mega environment, regardless whether environments correspond to years or locations.

In the second method genotypes that are above a certain quantile are used to classify locations into mega environments that are consistent across years. In this method, genotypes are scored according to whether they are above the `cutOff` threshold for the genotypic ranking within each location (1 if a genotype is above the `cutOff` and 0 if below). This genotype by location matrix with 1's and 0's is used to calculate the correlation between locations. Then, correlations across years are combined using the method by Charter and Alexander (Charter and Alexander 1993). The combined correlations are used to calculate Euclidean distances for hierarchical clustering. The number of mega environments obtained with the hierarchical clustering procedure is chosen to maximize the correlated response to selection within mega environments, as proposed in Atlin et al (Atlin et al. 2000).

Since the test data doesn't contain information about the year only the first method is available for this data. This is the default setting for the `gxMegaEnv` function.

```
geMegaEnv <- gxMegaEnv(TD = TDGxE, trait = "BLUES_GY")
#> Mega factor      Trial Winning genotype AMMI estimates
#>           1 SR_MWS_12                G037      10863.969
#>           2 SR_FI_12                 G056      11528.407
```

```
#>          3  C_SWS_12          G188      5423.140
#>          4  SR_FI_11          G276      10137.297
#>          4  SR_MWS_11          G276      7953.709
```

As can be seen in the column Mega Factor in the output 4 mega environments have been created. In the environments SR_FI_11 and SR_MWS_11 G276 is the best genotype so those two environments have been clustered together. The other three environments have different winning genotypes and therefore are in their own mega environment.

Computation of stability measures

Different measures of stability can be calculated using the RAP package, the cultivar-superiority measure of Lin & Binns (LIN and BINNS 1988), Shukla's (Shukla 1972) stability variance and Wricke's (Wricke 1962) ecovalence.

The cultivar-superiority measure is the sum of the squares of the difference between genotypic mean in each environment and the mean of the best genotype, divided by twice the number of environments. Genotypes with the smallest values of the superiority tend to be more stable, and closer to the best genotype in each environment.

Shukla's stability variance (static stability) is defined as the variance around the germplasm's phenotypic mean across all environments. This provides a measure of the consistency of the genotype, without accounting for performance.

Wricke's Ecovalence Stability Coefficient is the contribution of each genotype to the GxE sum of squares, in an unweighted analysis of the GxE means. A low value indicates that the genotype responds in a consistent manner to changes in environment; i.e. stable from a dynamic point of view. Like static stability, the Wricke's Ecovalence does not account for genotype performance.

```
geStab <- gxeStability(TD = TDGxE, trait = "BLUEs_GY")
summary(geStab)
#>
#> Cultivar-superiority measure (Top 10% genotypes)
#> genotype superiority mean
#> G202 13494769 4630.041
#> G288 13135607 4770.062
#> G384 9272209 5774.064
#> G249 8821607 5541.442
#> G316 8303387 5625.830
#> G208 8296293 5822.169
#> G260 8266599 5807.864
#> G248 8251252 5573.973
#> G378 8197606 5684.175
#> G383 7950219 5760.066
#> G250 7893958 5655.763
#> G136 7698152 5780.283
#> G360 7549292 6014.688
#> G295 7282660 5920.772
#> G257 7210056 5960.757
#> G205 7009958 6187.658
#> G161 6882322 5967.887
#> G165 6877280 5927.171
#> G254 6797544 6096.553
#> G324 6778109 5971.614
#> G315 6676581 6022.966
```



```

#>      G326      6599884 6056.449
#>      G356      6568367 6078.123
#>      G219      6563032 6038.901
#>      G381      6350470 6536.117
#>      G338      6334648 6278.680
#>      G321      6316695 6067.733
#>      G096      6234851 6116.712
#>      G229      6204870 6314.679
#>      G251      6112043 6247.754
#>      G199      6105092 6145.454
#>      G157      5976124 6585.634
#>      G333      5959314 6218.026
#>      G357      5829326 6294.176
#>      G283      5818191 6528.855
#>      G223      5814390 6284.350
#>      G361      5757641 6225.296
#>      G127      5733376 6284.475
#>      G031      5715564 6336.111
#>
#> Static stability (Top 10% genotypes)
#>   genotype   static   mean
#>      G286 12046595 7106.561
#>      G077 10809302 7596.784
#>      G181 10433051 6833.935
#>      G197 10340757 7915.891
#>      G358 10325838 7169.372
#>      G073 10225251 7947.329
#>      G384 10169164 5774.064
#>      G208 10089354 5822.169
#>      G056 10048045 8320.500
#>      G037 9961493 7876.846
#>      G120 9789337 7312.918
#>      G084 9518877 8068.285
#>      G099 9463566 6949.386
#>      G014 9431445 7619.940
#>      G100 9414558 8414.758
#>      G058 9320748 8400.497
#>      G093 9249250 7427.062
#>      G194 9231666 7343.354
#>      G131 9177058 7139.452
#>      G179 9150819 7198.045
#>      G212 9103521 7418.642
#>      G371 9094483 7090.381
#>      G381 9031031 6536.117
#>      G369 8998574 8326.462
#>      G297 8931974 7831.617
#>      G103 8898290 7766.727
#>      G031 8872464 6336.111
#>      G141 8783816 6609.460
#>      G259 8758228 6720.370
#>      G036 8737823 6859.104
#>      G195 8697237 7234.842
#>      G075 8679875 8006.810

```

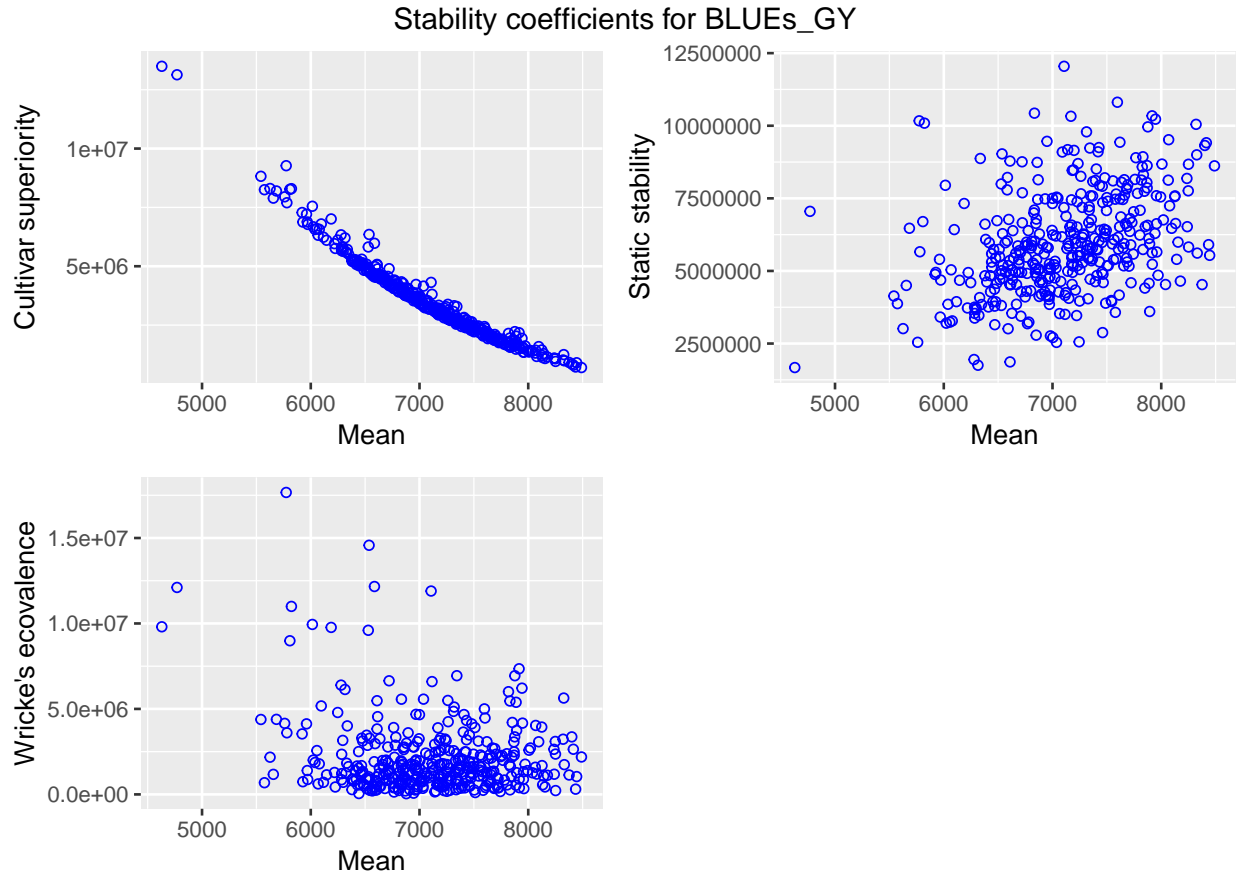
```

#>      G142  8673762 8250.561
#>      G262  8627998 7870.004
#>      G101  8618730 8489.525
#>      G011  8583549 7825.607
#>      G156  8510243 7402.909
#>      G143  8477603 7192.949
#>      G061  8463380 7181.751
#>
#> Wricke's ecovalence (Top 10% genotypes)
#> genotype  wricke      mean
#>      G384 17665165 5774.064
#>      G381 14574450 6536.117
#>      G157 12162796 6585.634
#>      G288 12107556 4770.062
#>      G286 11896559 7106.561
#>      G208 10997293 5822.169
#>      G360  9942805 6014.688
#>      G202  9805416 4630.041
#>      G205  9769627 6187.658
#>      G283  9600957 6528.855
#>      G260  8988071 5807.864
#>      G197  7349575 7915.891
#>      G194  6944368 7343.354
#>      G037  6943038 7876.846
#>      G259  6646124 6720.370
#>      G353  6599333 7115.091
#>      G338  6394171 6278.680
#>      G276  6214758 7942.348
#>      G229  6139238 6314.679
#>      G071  6008682 7818.405
#>      G369  5636616 8326.462
#>      G285  5571807 7036.768
#>      G181  5569426 6833.935
#>      G167  5497294 7260.758
#>      G141  5481074 6609.460
#>      G297  5462534 7831.617
#>      G291  5389608 7888.538
#>      G254  5175351 6096.553
#>      G175  5110657 7319.000
#>      G077  5003208 7596.784
#>      G120  4857177 7312.918
#>      G251  4791634 6247.754
#>      G169  4684561 6967.923
#>      G080  4672020 7409.070
#>      G089  4664193 6999.729
#>      G334  4547866 6615.061
#>      G166  4467591 7602.611
#>      G378  4389954 5684.175
#>      G249  4382411 5541.442

```

Plotting the results yields a scatter plot for each stability measure.

```
plot(geStab)
```



For the computation of stability measures a report can be made.

```
report(geStab, outfile = "./myReports/stabReport.pdf")
```

It is possible to calculate the stability measures based on mega environments instead of regular environments. To do so the parameter `useMegaEnv` has to be set to `TRUE`.

```
## Compute stabilities measures based on mega environments computed in
## previous paragraph.
geStabME <- gxeStability(TD = geMegaEnv, trait = "BLUEs_GY", useMegaEnv = TRUE)
summary(geStabME)
#>
#> Cultivar-superiority measure (Top 10% genotypes)
#> genotype superiority mean
#> G202 16062254 4630.041
#> G288 12951183 4770.062
#> G249 10485808 5541.442
#> G250 9531106 5655.763
#> G248 9465304 5573.973
#> G316 9317957 5625.830
#> G383 9113378 5760.066
#> G384 8779234 5774.064
#> G378 8629384 5684.175
#> G260 8434024 5807.864
#> G136 8397868 5780.283
```

```

#>      G208      8206311 5822.169
#>      G315      8167359 6022.966
#>      G326      8146785 6056.449
#>      G165      8128119 5927.171
#>      G219      8070708 6038.901
#>      G161      7945952 5967.887
#>      G338      7865491 6278.680
#>      G295      7800827 5920.772
#>      G251      7798687 6247.754
#>      G229      7735152 6314.679
#>      G257      7705611 5960.757
#>      G360      7683005 6014.688
#>      G324      7662296 5971.614
#>      G356      7640894 6078.123
#>      G199      7521240 6145.454
#>      G321      7450241 6067.733
#>      G357      7325598 6294.176
#>      G096      7297447 6116.712
#>      G223      7204198 6284.350
#>      G254      7155783 6096.553
#>      G333      7072766 6218.026
#>      G205      6983714 6187.658
#>      G329      6980348 6329.899
#>      G299      6920678 6283.417
#>      G361      6806109 6225.296
#>      G127      6753023 6284.475
#>      G323      6744943 6297.963
#>      G216      6675019 6346.940
#>
#> Static stability (Top 10% genotypes)
#>   genotype   static   mean
#>      G286 13999250 7106.561
#>      G077 13531672 7596.784
#>      G197 13367454 7915.891
#>      G073 12691302 7947.329
#>      G181 12527941 6833.935
#>      G084 12483187 8068.285
#>      G358 12390526 7169.372
#>      G037 12314466 7876.846
#>      G120 12157644 7312.918
#>      G056 12087526 8320.500
#>      G100 11859049 8414.758
#>      G014 11846648 7619.940
#>      G208 11835917 5822.169
#>      G099 11713861 6949.386
#>      G036 11445637 6859.104
#>      G212 11399241 7418.642
#>      G058 11343973 8400.497
#>      G142 11259150 8250.561
#>      G262 11234646 7870.004
#>      G031 11190122 6336.111
#>      G143 11079405 7192.949
#>      G101 10973261 8489.525

```

```

#>      G093 10905123 7427.062
#>      G297 10889964 7831.617
#>      G061 10850468 7181.751
#>      G194 10823502 7343.354
#>      G156 10741754 7402.909
#>      G141 10692245 6609.460
#>      G011 10690663 7825.607
#>      G075 10685906 8006.810
#>      G103 10671127 7766.727
#>      G384 10632688 5774.064
#>      G371 10582157 7090.381
#>      G006 10556652 7691.924
#>      G259 10552739 6720.370
#>      G375 10506227 7634.404
#>      G131 10502525 7139.452
#>      G198 10434018 7556.159
#>      G167 10359247 7260.758
#>
#> Wricke's ecovalence (Top 10% genotypes)
#>      genotype      wricke      mean
#>      G384 12366447 5774.064
#>      G381 10236181 6536.117
#>      G202 9024066 4630.041
#>      G288 8961553 4770.062
#>      G286 8802312 7106.561
#>      G208 7854699 5822.169
#>      G157 7377751 6585.634
#>      G205 6494081 6187.658
#>      G260 6334611 5807.864
#>      G283 6327283 6528.855
#>      G360 6279887 6014.688
#>      G229 5407739 6314.679
#>      G338 5228157 6278.680
#>      G297 5206988 7831.617
#>      G353 5197017 7115.091
#>      G285 5127803 7036.768
#>      G037 5105810 7876.846
#>      G071 5034271 7818.405
#>      G194 5008186 7343.354
#>      G259 4665244 6720.370
#>      G197 4561397 7915.891
#>      G077 4517946 7596.784
#>      G181 4478829 6833.935
#>      G276 4373825 7942.348
#>      G383 3987184 5760.066
#>      G141 3951390 6609.460
#>      G120 3885420 7312.918
#>      G291 3713240 7888.538
#>      G073 3710574 7947.329
#>      G254 3643464 6096.553
#>      G080 3551743 7409.070
#>      G251 3531617 6247.754
#>      G169 3466748 6967.923

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#>      G084  3425281 8068.285
#>      G167  3397592 7260.758
#>      G369  3387634 8326.462
#>      G334  3376417 6615.061
#>      G330  3235845 6609.668
#>      G187  3216586 7480.441
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