Case Study : Augmented Block Design. Analysis with Fixed Model

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CASE STUDY PRESENTATION

A toy dataset : three blocks, 4 checks and 8 new varieties

PREPARATION OF THE WORKING INTERFACE IN R

```
### I. Set working directory  ####
# On RStudio: tab 'Session'-> Set Working Directory -> Choose Directory.
# Choose the directory containing the Alpha_latticefile and the associated R script.

### II. Possibly, installation of new R packages needed for the analysis on RStudio:
# Click on the 'Packages' tab in the bottom-right window of R Studio interface->'Install Packages'
# Comment #1: R package installation requires a connection to internet
# Comment #2: Once packages have been installed,
# no need to re-install them again when you close-open again RStudio.

### III. Initialisation of the working space
# To erase all graphs
graphics.off()
# To erase objects from the working space - Clean up of the memory
rm(list = ls())


# this is a trick to detect which folder contains the R script and the Alpha_lattice
main_dir <- dirname(rstudioapi::getSourceEditorContext()$path)
setwd(main_dir)
```

LOADING REQUIRED METHODS FOR ANALYSIS

```
library(agricolae)
library(emmeans)
library(ggplot2) ; library(gridExtra)

# constraints on effects for ANOVA
options(contrasts=c("contr.sum", "contr.poly"))
```

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ANALYSIS OF THE CASE STUDY

The case study will ba analysed using a classical method based on fixed model (GLM)

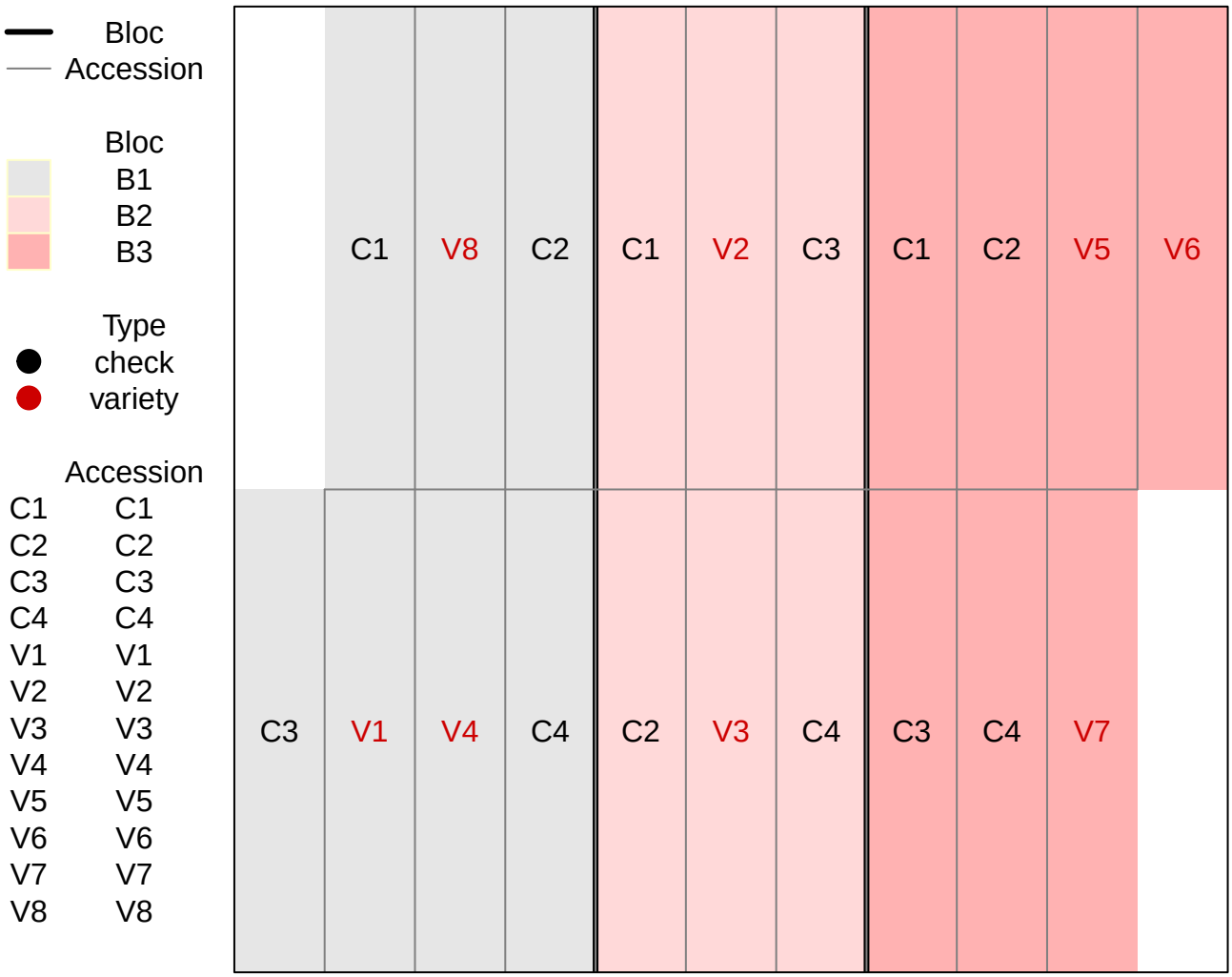
```
## ### the data, with map of the field
Produc <- read.table("AugmentedBlockDesign1b.csv", sep = ";", dec = ".", header = TRUE)
str(Produc)
```

```
## 'data.frame':    20 obs. of  6 variables:
##  $ Accession: chr  "C1" "C1" "C1" "C2" ...
##  $ Type     : chr  "check" "check" "check" "check" ...
##  $ Bloc     : chr  "B1" "B2" "B3" "B1" ...
##  $ weight   : int  10 8 6 3 5 6 10 9 8 15 ...
##  $ x        : int  2 5 8 4 5 9 1 7 8 4 ...
##  $ y        : int  2 2 2 2 1 2 1 2 1 1 ...
```

```
### the trial ?
with(Produc,
     table(Accession,Bloc))
```

```
##          Bloc
## Accession B1 B2 B3
##        C1  1  1  1
##        C2  1  1  1
##        C3  1  1  1
##        C4  1  1  1
##        V1  1  0  0
##        V2  0  1  0
##        V3  0  1  0
##        V4  1  0  0
##        V5  0  0  1
##        V6  0  0  1
##        V7  0  0  1
##        V8  1  0  0
```

```
## visualize the field:
library(desplot)
x11(width = 7, height = 5)
desplot(Bloc ~ x + y, Produc,
        col =  Type, text = Accession, cex = 1,
        out1 = Bloc, out2 = Accession,
        out2.gpar=list(col = "gray50", lwd = 1, lty = 1),
        main = "Augmented Block Design - Example 1")
```




```
##############################################
##############################################  Analysis 'by hand' using a fixed model  ####################
##############################################

## type I SS
Ana1a <- aov(weight ~ Bloc + Accession , data = Produc)  # accessions 'corrected for the bloc effect'
summary(Ana1a)
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Bloc         2   41.8   20.89   5.531 0.04349 *
## Accession   11 1632.1  148.37  39.275 0.00011 ***
## Residuals    6   22.7    3.78
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Ana1b <- aov(weight ~ Accession + Bloc , data = Produc)  # blocks 'corrected' for genotype effect
summary(Ana1b)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## Accession   11 1673.2  152.11  40.265 0.000102 ***
## Bloc         2    0.7    0.33   0.088 0.916711
## Residuals    6   22.7    3.78
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## the two ANOVA are different because some combinations are lacking



###################### Analysis of the checks only ######################
# this is a simple RCBD

## SS of accessions corrected for block effect
AnaChecks <- aov(weight ~ Bloc + Accession , data = subset(Produc, subset = Type == "check"))
summary(AnaChecks)
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Bloc         2   0.67    0.33   0.088 0.91671
## Accession    3 191.33   63.78  16.882 0.00249 **
## Residuals    6  22.67    3.78
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
###################### Phenotypic values of new entries, "corrected for the block effect"
```

the *block effect* for correction is estimated in the ANOVA of the checks

```
## What are the block effects ?
model.tables(AnaChecks)
```

```
## Tables of effects
##
##  Bloc
## Bloc
##      B1      B2      B3
##  0.1667 -0.3333  0.1667
##
##  Accession
## Accession
##     C1     C2     C3     C4
## -1.333 -4.667 -0.333  6.333
```

```
model.tables(AnaChecks)$tables$Bloc
```

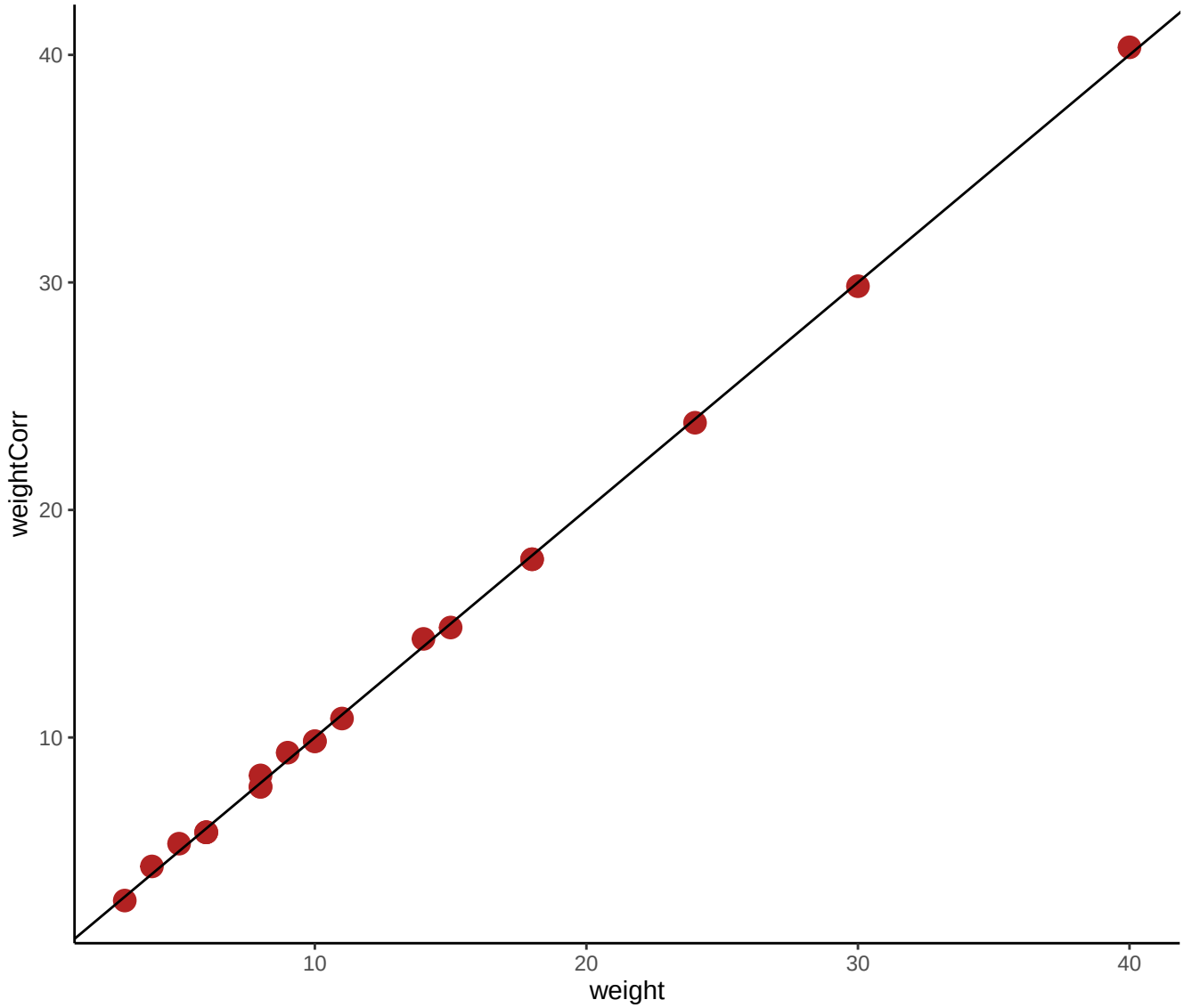
```
## Bloc
##         B1         B2         B3
##  0.1666667 -0.3333333  0.1666667
```

```
### Syntax to correct the phenotypic values of the 'new entries"
Produc$EffetBloc <-sapply(Produc$Bloc, switch,                          ## use of the swith() fnction
                          B1=model.tables(AnaChecks)$tables$Bloc[1],    ## the Bi block effect is taken fro
                          B2=model.tables(AnaChecks)$tables$Bloc[2],    ## the results of ANOVA table
                          B3=model.tables(AnaChecks)$tables$Bloc[3]
                          )

## create the vector of all data "corrected for the block effect"
Produc$weightCorr <- Produc$weight - Produc$EffetBloc

Produc
```

```
##    Accession    Type Bloc weight  x y  EffetBloc weightCorr
## 1         C1   check   B1     10  2 2  0.1666667   9.833333
## 2         C1   check   B2      8  5 2 -0.3333333   8.333333
## 3         C1   check   B3      6  8 2  0.1666667   5.833333
## 4         C2   check   B1      3  4 2  0.1666667   2.833333
## 5         C2   check   B2      5  5 1 -0.3333333   5.333333
## 6         C2   check   B3      6  9 2  0.1666667   5.833333
```

```
## 7         C3   check   B1     10  1 1  0.1666667   9.833333
## 8         C3   check   B2      9  7 2 -0.3333333   9.333333
## 9         C3   check   B3      8  8 1  0.1666667   7.833333
## 10        C4   check   B1     15  4 1  0.1666667  14.833333
## 11        C4   check   B2     14  7 1 -0.3333333  14.333333
## 12        C4   check   B3     18  9 1  0.1666667  17.833333
## 13        V1 variety   B1      6  2 1  0.1666667   5.833333
## 14        V2 variety   B2     40  6 2 -0.3333333  40.333333
## 15        V3 variety   B2      4  6 1 -0.3333333   4.333333
## 16        V4 variety   B1     24  3 1  0.1666667  23.833333
## 17        V5 variety   B3     11 10 2  0.1666667  10.833333
## 18        V6 variety   B3     18 11 2  0.1666667  17.833333
## 19        V7 variety   B3      8 10 1  0.1666667   7.833333
## 20        V8 variety   B1     30  3 2  0.1666667  29.833333
```

```
x11()
(gr1 <- ggplot(Produc) + aes(x = weight, y = weightCorr) +
                geom_point(color = 'firebrick', cex = 4) +
                geom_abline(slope = 1, intercept = 0) +
                theme_classic()
        )
```




```
#########################   to verify / validate :
```

```
## CAUTION : This verification works ONLY gor 'checks' beacuse the design is balanced for checks
## so the SS of accessions is independnat from the SS of block: we and do the ANOVA in both direction
## and it shall produce the SAME results

Valid1 <- aov(weightCorr ~ Bloc + Accession, data = subset(Produc,subset = Type == "check"))
summary(Valid1)
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Bloc         2   0.00    0.00    0.00 1.00000
## Accession    3 191.33   63.78   16.88 0.00249 **
## Residuals    6  22.67    3.78
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Valid2 <- aov(weightCorr ~ Accession + Bloc, data = subset(Produc,subset = Type == "check"))
summary(Valid2)
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Accession    3 191.33   63.78   16.88 0.00249 **
## Bloc         2   0.00    0.00    0.00 1.00000
## Residuals    6  22.67    3.78
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Are the results expected ?

```
###################### the above procedure can be simply done by computing "marginal means"
########### also called 'lsmeans'

emmeans(Ana1a, ~ Accession)
```

```
##  Accession emmean   SE df lower.CL upper.CL
##  C1          8.00 1.12  6    5.254    10.75
##  C2          4.67 1.12  6    1.921     7.41
##  C3          9.00 1.12  6    6.254    11.75
##  C4         15.67 1.12  6   12.921    18.41
##  V1          5.83 2.10  6    0.696    10.97
##  V2         40.33 2.10  6   35.196    45.47
##  V3          4.33 2.10  6   -0.804     9.47
##  V4         23.83 2.10  6   18.696    28.97
##  V5         10.83 2.10  6    5.696    15.97
##  V6         17.83 2.10  6   12.696    22.97
##  V7          7.83 2.10  6    2.696    12.97
##  V8         29.83 2.10  6   24.696    34.97
##
## Results are averaged over the levels of: Bloc
## Confidence level used: 0.95
```

```
###################### whaht accessions differ from checks ?
################### débobinage : quelles sont les accessions différentes des checks ?

## just use the formulas of the slides:
c <- 4
r <- 3
ddl <- (r-1)*(c-1)
MSE <- summary(Ana1a)[[1]]$'Mean Sq'[3]  # 3rd term of 'Mean Sq' column of the summary table of ANOVA

# SE of the difference between 2 adj means of selections in different blocks (cf dias)
Sv <- sqrt(2*(c+1)*MSE/c)

#vSE of the difference between adjusted selection mean and check. c'est la quantité utile à connaitre !
```

```
Svc <- sqrt(((r+1)*(c+1)*MSE)/(r*c))

# We are , for example, interested to those corrected vaues that outperform the checks.
## the CI is given following a t distribution

# critical value of t:
qt(0.95,ddl)
```

```
## [1] 1.94318
```

```
## numerical value of Least Significant Interval:
LSI <- qt(0.95,ddl) * Svc
LSI
```

```
## [1] 4.87591
```

```
# so any new entry that outperforms the 'best check' by this quantity is significantly different from it.
Produc
```

```
##    Accession    Type Bloc weight  x y  EffetBloc weightCorr
## 1         C1   check   B1     10  2 2  0.1666667   9.833333
## 2         C1   check   B2      8  5 2 -0.3333333   8.333333
## 3         C1   check   B3      6  8 2  0.1666667   5.833333
## 4         C2   check   B1      3  4 2  0.1666667   2.833333
## 5         C2   check   B2      5  5 1 -0.3333333   5.333333
## 6         C2   check   B3      6  9 2  0.1666667   5.833333
## 7         C3   check   B1     10  1 1  0.1666667   9.833333
## 8         C3   check   B2      9  7 2 -0.3333333   9.333333
## 9         C3   check   B3      8  8 1  0.1666667   7.833333
## 10        C4   check   B1     15  4 1  0.1666667  14.833333
## 11        C4   check   B2     14  7 1 -0.3333333  14.333333
## 12        C4   check   B3     18  9 1  0.1666667  17.833333
## 13        V1 variety   B1      6  2 1  0.1666667   5.833333
## 14        V2 variety   B2     40  6 2 -0.3333333  40.333333
## 15        V3 variety   B2      4  6 1 -0.3333333   4.333333
## 16        V4 variety   B1     24  3 1  0.1666667  23.833333
## 17        V5 variety   B3     11 10 2  0.1666667  10.833333
## 18        V6 variety   B3     18 11 2  0.1666667  17.833333
## 19        V7 variety   B3      8 10 1  0.1666667   7.833333
## 20        V8 variety   B1     30  3 2  0.1666667  29.833333
```

So, what is the list of the new entreis that outperfom the best check ?

```
###########################################
###########################################  Analysis using agricolae  ##################################
###########################################

## another elegant way to see the design:
with(Produc,
by(Accession, Bloc, as.character)
)
```

```
## Bloc: B1
## [1] "C1" "C2" "C3" "C4" "V1" "V4" "V8"
## ------------------------------------------------------------
## Bloc: B2
## [1] "C1" "C2" "C3" "C4" "V2" "V3"
## ------------------------------------------------------------
## Bloc: B3
## [1] "C1" "C2" "C3" "C4" "V5" "V6" "V7"
```

```r
## the agricolae::DAU() fits augmented design

modelDAU1 <- DAU.test(Produc$Bloc, Produc$Accession, Produc$weight,
                      method = "lsd", group = TRUE,
                      console = TRUE)
```

```
##
## ANALYSIS DAU:  Produc$weight
## Class level information
##
## Block:  B1 B2 B3
## Trt  :  C1 C2 C3 C4 V1 V2 V3 V4 V5 V6 V7 V8
##
## Number of observations:  20
##
## ANOVA, Treatment Adjusted
## Analysis of Variance Table
##
## Response: Produc$weight
##                            Df  Sum Sq Mean Sq F value    Pr(>F)
## block.unadj                 2   41.79  20.894
## trt.adj                    11 1632.10 148.372  39.275 0.0001096 ***
## Control                     3  191.33  63.778  16.882 0.0024938 **
## Control + control.VS.aug.  8 1440.76 180.095  47.672 7.176e-05 ***
## Residuals                   6   22.67   3.778
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ANOVA, Block Adjusted
## Analysis of Variance Table
##
## Response: Produc$weight
##                       Df  Sum Sq Mean Sq F value    Pr(>F)
## trt.unadj             11 1673.22  152.11
## block.adj              2    0.67    0.33  0.0882 0.9167114
## Control                3  191.33   63.78 16.8824 0.0024938 **
## Augmented              7 1151.88  164.55 43.5583 0.0001001 ***
## Control vs augmented   1  330.01  330.01 87.3551 8.504e-05 ***
## Residuals              6   22.67    3.78
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## coefficient of variation: 15.4 %
## Produc$weight Means: 12.65
##
## Critical Differences (Between)
##                                                 Std Error Diff.
## Two Control Treatments                                 1.586984
## Two Augmented Treatments (Same Block)                  2.748737
## Two Augmented Treatments(Different Blocks)             3.073181
## A Augmented Treatment and A Control Treatment          2.380476
##
##
## Treatments with the same letter are not significantly different.
##
##    Produc$weight groups
## V2     40.333333      a
## V8     29.833333      b
## V4     23.833333     bc
```

```
## V6     17.833333     cd
## C4     15.666667     de
## V5     10.833333     ef
## C3      9.000000      f
## C1      8.000000     fg
## V7      7.833333     fg
## V1      5.833333     fg
## C2      4.666667      g
## V3      4.333333      g
##
## Comparison between treatments means
##
## <<< to see the objects: comparison and means  >>>
```

```
## The table "ANOVA, Treatment Adjusted" is the same as Ana1a ( Bloc + Accessions)
## the table "ANOVA, Block Adjusted" is the same as Ana1b (Accessions + Bloc)


## il y a une petite diff numérique sur Svc entre agricolae et calculs manuels

options(digits = 2)
modelDAU1$means
```

```
##    Produc.weight std r Min Max  Q25 Q50  Q75 mean.adj  SE block
## C1           8.0 2.0 3   6  10  7.0   8  9.0      8.0 1.1
## C2           4.7 1.5 3   3   6  4.0   5  5.5      4.7 1.1
## C3           9.0 1.0 3   8  10  8.5   9  9.5      9.0 1.1
## C4          15.7 2.1 3  14  18 14.5  15 16.5     15.7 1.1
## V1           6.0  NA 1   6   6  6.0   6  6.0      5.8 1.9    B1
## V2          40.0  NA 1  40  40 40.0  40 40.0     40.3 1.9    B2
## V3           4.0  NA 1   4   4  4.0   4  4.0      4.3 1.9    B2
## V4          24.0  NA 1  24  24 24.0  24 24.0     23.8 1.9    B1
## V5          11.0  NA 1  11  11 11.0  11 11.0     10.8 1.9    B3
## V6          18.0  NA 1  18  18 18.0  18 18.0     17.8 1.9    B3
## V7           8.0  NA 1   8   8  8.0   8  8.0      7.8 1.9    B3
## V8          30.0  NA 1  30  30 30.0  30 30.0     29.8 1.9    B1
```

```
### see lsmeans and weightCorr to compare


## If one wants pairwise comapsrisons détail : si on veut les comparaisons deux à deux, et non pas les gro
modelDAU2 <- DAU.test(Produc$Bloc, Produc$Accession, Produc$weight,
                      method="lsd", group = FALSE,
                      console = TRUE)
```

```
##
## ANALYSIS DAU:  Produc$weight
## Class level information
##
## Block:  B1 B2 B3
## Trt  :  C1 C2 C3 C4 V1 V2 V3 V4 V5 V6 V7 V8
##
## Number of observations:  20
##
## ANOVA, Treatment Adjusted
## Analysis of Variance Table
##
## Response: Produc$weight
##                            Df Sum Sq Mean Sq F value  Pr(>F)
## block.unadj                 2     42    20.9
## trt.adj                    11   1632   148.4    39.3 0.00011 ***
```

```
## Control                    3    191    63.8    16.9 0.00249 **
## Control + control.VS.aug.  8   1441   180.1    47.7 7.2e-05 ***
## Residuals                  6     23     3.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## ANOVA, Block Adjusted
## Analysis of Variance Table
##
## Response: Produc$weight
##                      Df Sum Sq Mean Sq F value  Pr(>F)
## trt.unadj            11   1673     152
## block.adj             2      1       0    0.09  0.9167
## Control               3    191      64   16.88  0.0025 **
## Augmented             7   1152     165   43.56  0.0001 ***
## Control vs augmented  1    330     330   87.36 8.5e-05 ***
## Residuals             6     23       4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## coefficient of variation: 15 %
## Produc$weight Means: 13
##
## Critical Differences (Between)
##                                                 Std Error Diff.
## Two Control Treatments                                      1.6
## Two Augmented Treatments (Same Block)                       2.7
## Two Augmented Treatments(Different Blocks)                  3.1
## A Augmented Treatment and A Control Treatment               2.4
##
##
## Comparison between treatments means
##
## <<< to see the objects: comparison and means  >>>
```

```
head(modelDAU2$comparison, 12)
```

```
##         Difference pvalue sig.
## C1 - C2       3.33 0.0804    .
## C1 - C3      -1.00 0.5518
## C1 - C4      -7.67 0.0030   **
## C1 - V1       2.17 0.3978
## C1 - V2     -32.33 0.0000  ***
## C1 - V3       3.67 0.1744
## C1 - V4     -15.83 0.0006  ***
## C1 - V5      -2.83 0.2790
## C1 - V6      -9.83 0.0062   **
## C1 - V7       0.17 0.9464
## C1 - V8     -21.83 0.0000  ***
## C2 - C3      -4.33 0.0342    *
```

```
## en conclusion : agricolae est utile et juste pour l'analyse des plans en blocs augmentés.
## Il utilise un modèle fixe d'ANOVA.
## on peut raffiner un peu mieux que agricolae.



###################
#####################################  Analysis using plantbreeding
###################
```

```
install.packages("plantbreeding", repos="http://R-Forge.R-project.org")  ## unclear if maintained
## requires reshape

library(plantbreeding)
Ana3 <- aug.rcb(dataframe = Produc, genotypes = "Accession", block = "Bloc", yvar = "weight")
```

```
## Phenotypes and adjusted values :
##
##    Accession    Type Bloc weight  x y EffetBloc weightCorr yvar.adj
## 13        V1 variety   B1      6  2 1      0.17        5.8      5.8
## 14        V2 variety   B2     40  6 2     -0.33       40.3     40.3
## 15        V3 variety   B2      4  6 1     -0.33        4.3      4.3
## 16        V4 variety   B1     24  3 1      0.17       23.8     23.8
## 17        V5 variety   B3     11 10 2      0.17       10.8     10.8
## 18        V6 variety   B3     18 11 2      0.17       17.8     17.8
## 19        V7 variety   B3      8 10 1      0.17        7.8      7.8
## 20        V8 variety   B1     30  3 2      0.17       29.8     29.8
```

```
## Standard error of  different comparisions
##
## Difference between check means:  1.6
##
## Difference adjusted yield of two varities in same block :  2.7
##
## Difference between two varieties in different blocks:  3.1
##
## Difference between two varieties and a check mean:  2.5
```

```
# analysis of variance
Ana3$anova
```

```
##           Df Sum Sq Mean Sq F value Pr(>F)
## Genotypes  3  191.3    63.8   16.88 0.0025 **
## Block      2    0.7     0.3    0.09 0.9167
## Residual   6   22.7     3.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## compare with Ana2 and modelDAU1


Ana3$adjusted_values # yield observed and expected value table
```

```
##    Accession    Type Bloc weight  x y EffetBloc weightCorr yvar.adj
## 13        V1 variety   B1      6  2 1      0.17        5.8      5.8
## 14        V2 variety   B2     40  6 2     -0.33       40.3     40.3
## 15        V3 variety   B2      4  6 1     -0.33        4.3      4.3
## 16        V4 variety   B1     24  3 1      0.17       23.8     23.8
## 17        V5 variety   B3     11 10 2      0.17       10.8     10.8
## 18        V6 variety   B3     18 11 2      0.17       17.8     17.8
## 19        V7 variety   B3      8 10 1      0.17        7.8      7.8
## 20        V8 variety   B1     30  3 2      0.17       29.8     29.8
```

```
## same as agricolae. Does not provide for checks.

str(Ana3)
```

```
## List of 6
##  $ anova          :Classes 'anova' and 'data.frame': 3 obs. of  5 variables:
##   ..$ Df     : int [1:3] 3 2 6
##   ..$ Sum Sq : num [1:3] 191.333 0.667 22.667
```

```
##   ..$ Mean Sq: num [1:3] 63.778 0.333 3.778
##   ..$ F value: num [1:3] 16.8824 0.0882 NA
##   ..$ Pr(>F) : num [1:3] 0.00249 0.91671 NA
##  $ adjusted_values:'data.frame': 8 obs. of  9 variables:
##   ..$ Accession : Factor w/ 12 levels "C1","C2","C3",..: 5 6 7 8 9 10 11 12
##   ..$ Type      : chr [1:8] "variety" "variety" "variety" "variety" ...
##   ..$ Bloc      : Factor w/ 3 levels "B1","B2","B3": 1 2 2 1 3 3 3 1
##   ..$ weight    : int [1:8] 6 40 4 24 11 18 8 30
##   ..$ x         : int [1:8] 2 6 6 3 10 11 10 3
##   ..$ y         : int [1:8] 1 2 1 1 2 2 1 2
##   ..$ EffetBloc : num [1:8] 0.167 -0.333 -0.333 0.167 0.167 ...
##   ..$ weightCorr: num [1:8] 5.83 40.33 4.33 23.83 10.83 ...
##   ..$ yvar.adj  : num [1:8] 5.83 40.33 4.33 23.83 10.83 ...
##  $ se_check       : num 1.59
##  $ se_within      : num 2.75
##  $ se_diff        : num 3.07
##  $ se_geno        : num 2.51
```

```
## Ana3$se_geno (Difference between two varieties /entries and a check mean) equates Svc , as expected
##  approximation in agricolae ?
```