Two-way split-plot design

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2023-11-17

Two-way ANOVA & pairwise comparison post hoc tests in a split-plot design.

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```
# (install &) load packages
pacman::p_load(
  conflicted,
  desplot,
  emmeans,
  ggtext,
  lme4,
  lmerTest,
  MetBrewer,
  multcomp,
  multcompView,
  tidyverse)

# handle function conflicts
conflicts_prefer(dplyr::filter)
```

```
conflicts_prefer(dplyr::select)
conflicts_prefer(lmerTest::lmer)
```

1 Data

This dataset was originally published in Gomez and Gomez (1984) from a yield (kg/ha) trial with 4 genotypes (`G`) and 6 nitrogen levels (`N`), leading to 24 treatment level combinations. The data set here has 3 complete replicates (`rep`) and is laid out as a split-plot design.

1.1 Import

```
# data is available online:
path <- "https://raw.githubusercontent.com/SchmidtPaul/dsfair_quarto/master/data/Gomez&Gom
```

```
dat <- read_csv(path) # use path from above
dat
```

```
# A tibble: 72 x 7
   yield   row   col rep   mainplot G     N
   <dbl> <dbl> <dbl> <chr> <chr>    <chr> <chr>
 1  4520     4     1 rep1  mp01     Simba Goomba
 2  5598     2     2 rep1  mp02     Simba Koopa
 3  6192     1     3 rep1  mp03     Simba Toad
 4  8542     2     4 rep1  mp04     Simba Peach
 5  5806     2     5 rep1  mp05     Simba Diddy
 6  7470     1     6 rep1  mp06     Simba Yoshi
 7  4034     2     1 rep1  mp01     Nala  Goomba
 8  6682     4     2 rep1  mp02     Nala  Koopa
 9  6869     3     3 rep1  mp03     Nala  Toad
10  6318     4     4 rep1  mp04     Nala  Peach
# i 62 more rows
```

1.2 Format

Before anything, the columns `rep`, `N` and `G` should be encoded as factors, since R by default encoded them as character.

```
dat <- dat %>%
  mutate(across(c(rep:N), ~ as.factor(.x)))
```

1.3 Explore

We make use of `dlookr::describe()` to conveniently obtain descriptive summary tables. Here, we get can summarize per nitrogen level, per genotype and also per nitrogen-genotype-combination.

```
dat %>%
  group_by(N) %>%
  dlookr::describe(yield) %>%
  select(2:sd) %>%
  arrange(desc(mean))
```

```
# A tibble: 6 x 5
  N          n    na  mean    sd
  <fct>  <int> <int> <dbl> <dbl>
1 Diddy     12     0 5866.  832.
2 Toad      12     0 5864. 1434.
3 Yoshi     12     0 5812  2349.
4 Peach     12     0 5797. 2660.
5 Koopa     12     0 5478.  657.
6 Goomba    12     0 4054.  672.
```

```
dat %>%
  group_by(G) %>%
  dlookr::describe(yield) %>%
  select(2:sd) %>%
  arrange(desc(mean))
```

```
# A tibble: 4 x 5
  G         n    na  mean    sd
  <fct> <int> <int> <dbl> <dbl>
1 Simba    18     0 6554. 1475.
2 Nala     18     0 6156. 1078.
3 Timon    18     0 5563. 1269.
4 Pumba    18     0 3642. 1434.
```

```
dat %>%
  group_by(N, G) %>%
  dlookr::describe(yield) %>%
  select(2:sd) %>%
  arrange(desc(mean)) %>%
  print(n=Inf)
```

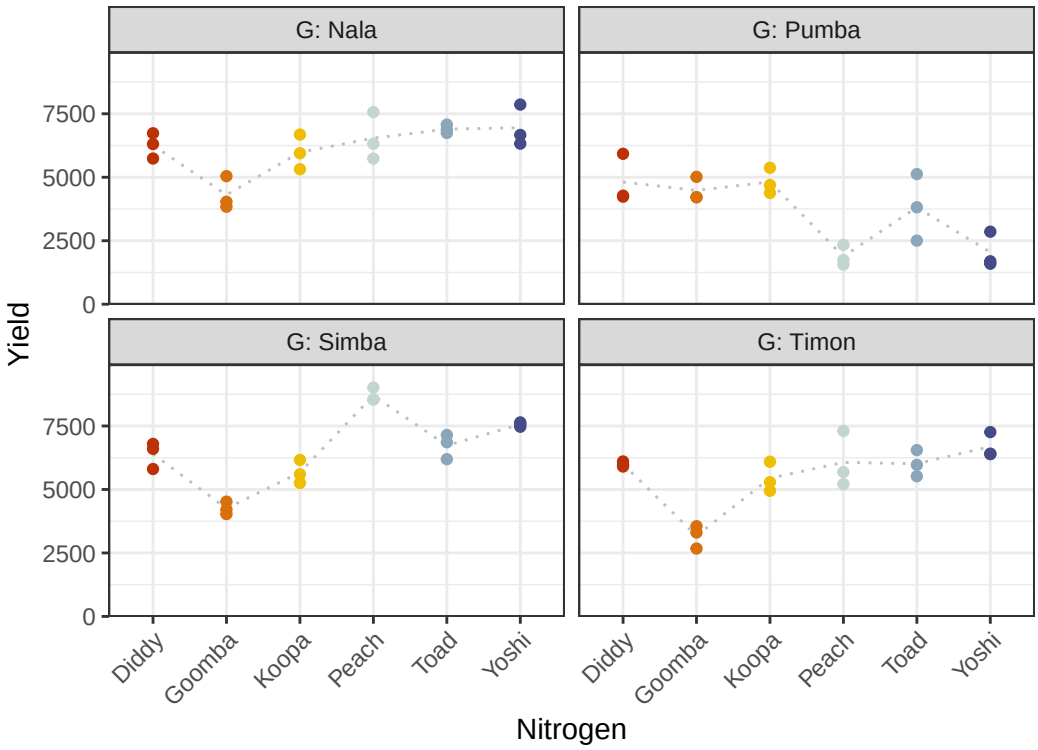
```
# A tibble: 24 x 6
   N      G         n    na  mean     sd
   <fct>  <fct> <int> <int> <dbl>  <dbl>
 1 Peach  Simba     3     0 8701.  270.
 2 Yoshi  Simba     3     0 7563.   86.9
 3 Yoshi  Nala      3     0 6951.  808.
 4 Toad   Nala      3     0 6895   166.
 5 Toad   Simba     3     0 6733.  490.
 6 Yoshi  Timon     3     0 6687.  496.
 7 Peach  Nala      3     0 6540.  936.
 8 Diddy  Simba     3     0 6400   523.
 9 Diddy  Nala      3     0 6259   499.
10 Peach  Timon     3     0 6065. 1097.
11 Toad   Timon     3     0 6014   515.
12 Diddy  Timon     3     0 5994   101.
13 Koopa  Nala      3     0 5982   684.
14 Koopa  Simba     3     0 5672   458.
15 Koopa  Timon     3     0 5443.  589.
16 Koopa  Pumba     3     0 4816   506.
17 Diddy  Pumba     3     0 4812   963.
18 Goomba Pumba     3     0 4481.  463.
19 Goomba Nala      3     0 4306   646.
20 Goomba Simba     3     0 4253.  248.
21 Toad   Pumba     3     0 3816  1311.
22 Goomba Timon     3     0 3177.  453.
23 Yoshi  Pumba     3     0 2047.  703.
24 Peach  Pumba     3     0 1881.  407.
```

Additionally, we can decide to plot our data. One way to deal with the combination of two factors would be to use panels/facets in ggplot2.

Note that we here define a custom set of colors for the Nitrogen levels that will be used throughout this chapter.

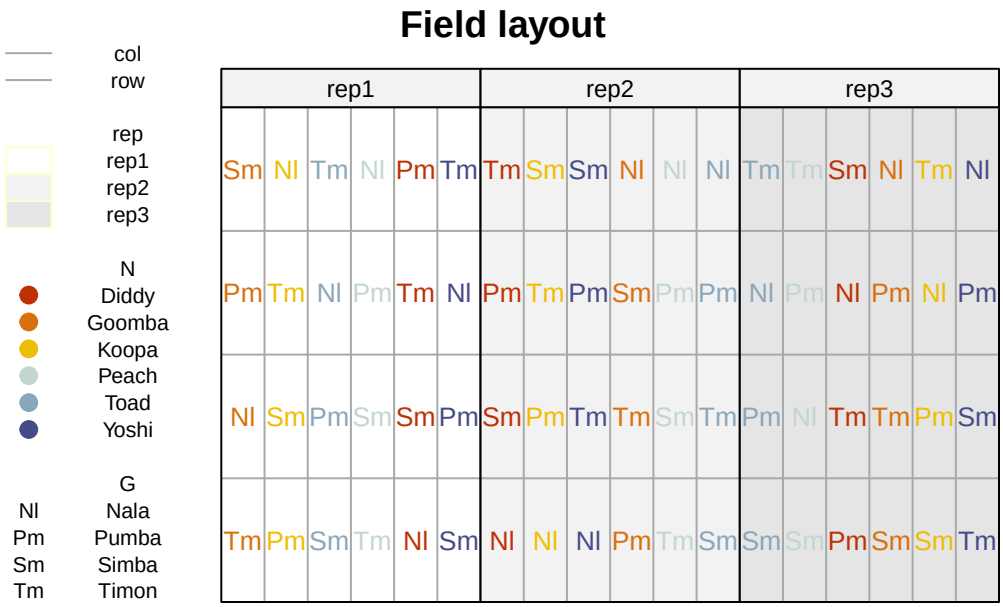
```r
Ncolors <- met.brewer("VanGogh2", 6) %>%
  as.vector() %>%
  set_names(levels(dat$N))
```

```r
ggplot(data = dat) +
  aes(y = yield, x = N, color = N) +
  facet_wrap(~G, labeller = label_both) +
  stat_summary(
    fun = mean,
    colour = "grey",
    geom = "line",
    linetype = "dotted",
    group = 1
  ) +
  geom_point() +
  scale_x_discrete(
    name = "Nitrogen"
  ) +
  scale_y_continuous(
    name = "Yield",
    limits = c(0, NA),
    expand = expansion(mult = c(0, 0.1))
  ) +
  scale_color_manual(
    values = Ncolors,
    guide = "none"
  ) +
  theme_bw() +
  theme(axis.text.x = element_text(
    angle = 45,
    hjust = 1,
    vjust = 1
  ))
```

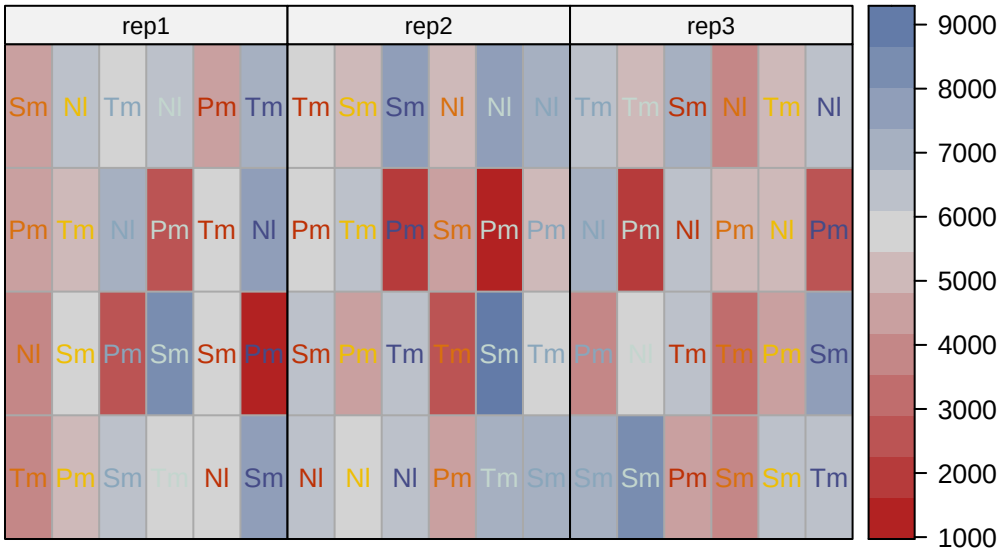


Finally, since this is an experiment that was laid with a certain experimental design (= a split-plot design) - it makes sense to also get a field plan. This can be done via `desplot()` from {desplot}.

```
desplot(
  data = dat,
  form = rep ~ col + row | rep, # fill color per rep, headers per rep
  col.regions = c("white", "grey95", "grey90"),
  text = G, # genotype names per plot
  cex = 0.8, # genotype names: font size
  shorten = "abb", # genotype names: abbreviate
  col = N, # color of genotype names for each N-level
  col.text = Ncolors, # use custom colors from above
  out1 = col, out1.gpar = list(col = "darkgrey"), # lines between columns
  out2 = row, out2.gpar = list(col = "darkgrey"), # lines between rows
  main = "Field layout", # plot title
  show.key = TRUE, # show legend
  key.cex = 0.7 # legend font size
  )
```

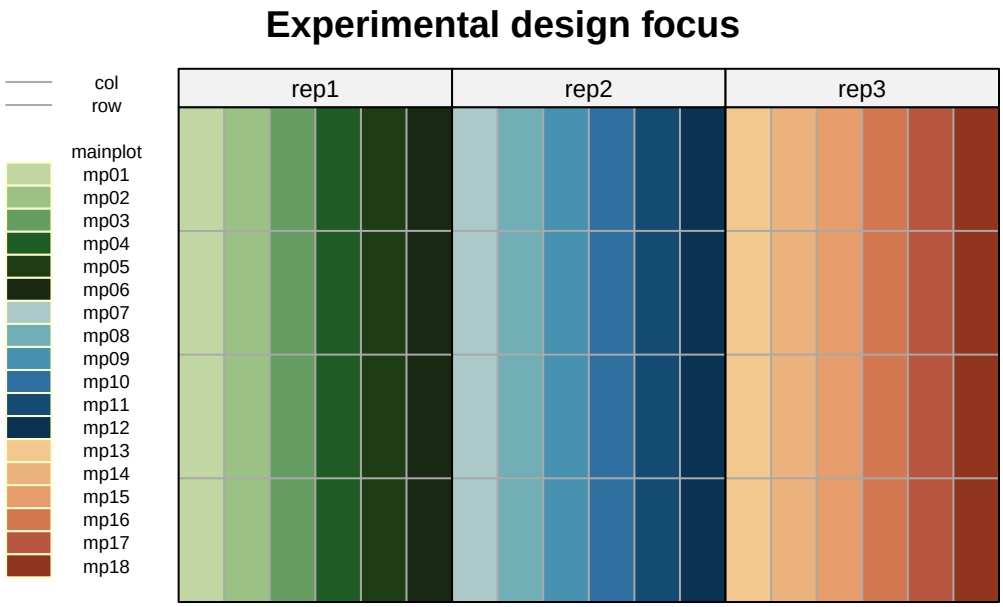



```
desplot(
  data = dat,
  form = yield ~ col + row | rep, # fill color per rep, headers per rep
  text = G, # genotype names per plot
  cex = 0.8, # genotype names: font size
  shorten = "abb", # genotype names: abbreviate
  col  = N, # color of genotype names for each N-level
  col.text = Ncolors, # use custom colors from above
  out1 = col, out1.gpar = list(col = "darkgrey"), # lines between columns
  out2 = row, out2.gpar = list(col = "darkgrey"), # lines between rows
  main = "Yield per plot", # plot title
  show.key = FALSE # show legend
  )
```




```
mainplotcolors <- c(met.brewer("VanGogh3", 6),
                    met.brewer("Hokusai2", 6),
                    met.brewer("OKeeffe2", 6)) %>%
  as.vector() %>%
  set_names(levels(dat$mainplot))

desplot(
  data = dat,
  form = mainplot ~ col + row | rep, # fill color per rep, headers per rep
  col.regions = mainplotcolors,
  out1 = col, out1.gpar = list(col = "darkgrey"), # lines between columns
  out2 = row, out2.gpar = list(col = "darkgrey"), # lines between rows
  main = "Experimental design focus", # plot title
  show.key = TRUE, # don't show legend
  key.cex = 0.6
  )
```



2 Model

Finally, we can decide to fit a linear model with yield as the response variable. In this example it makes sense to mentally group the effects in our model as either *design effects* or *treatment effects*. The treatments here are the genotypes `G` and the nitrogen levels `N` which we will include in the model as main effects, but also via their interaction effect `N:G`. Regarding the design, the model needs to contain a block (`rep`) effect representing the three complete blocks. Additionally, there should also be random effects for the 18 mainplots, since they represent additional randomization units.

```
mod <- lmer(yield ~ G + N + G:N +
              rep + (1 | rep:mainplot),
            data = dat)
```

⚠ Model assumptions met? (click to show)

It would be at this moment (i.e. after fitting the model and before running the ANOVA), that you should check whether the model assumptions are met. Find out more in the summary article "Model Diagnostics"

3 ANOVA

Based on our model, we can then conduct an ANOVA:

```
ANOVA <- anova(mod)
ANOVA
```

```
Type III Analysis of Variance Table with Satterthwaite's method
      Sum Sq  Mean Sq NumDF DenDF F value    Pr(>F)
G   89885051 29961684     3    36 85.7416 < 2.2e-16 ***
N   19192886  3838577     5    10 10.9849 0.0008277 ***
rep   683088   341544     2    10  0.9774 0.4095330
G:N 69378044  4625203    15    36 13.2360 2.078e-10 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Accordingly, the ANOVA's F-test found the nitrogen-genotype-interaction to be statistically significant ($p < .001$***).

4 Mean comparison

Besides an ANOVA, one may also want to compare adjusted yield means between cultivars via post hoc tests (t-test, Tukey test etc.). Especially because of the results of this ANOVA, we should compare means for all `N:G` interactions and **not** for the `N` and/or `G` main effects. When doing so, we still have multiple options to choose from. I here decide to compare all genotype means per nitrogen

```
mean_comp <- mod %>%
  emmeans(specs = ~ N|G) %>% # adj. mean per cultivar
  cld(adjust = "Tukey", Letters = letters) # compact letter display (CLD)

mean_comp
```

```
G = Nala:
 N      emmean  SE   df lower.CL upper.CL .group
 Goomba   4306 366 41.9     3297     5315  a
 Koopa    5982 366 41.9     4973     6991   b
 Diddy    6259 366 41.9     5250     7268   b
 Peach    6540 366 41.9     5531     7550   b
 Toad     6895 366 41.9     5886     7904   b
```

```
 Yoshi    6951 366 41.9     5941     7960   b

G = Pumba:
 N      emmean  SE   df lower.CL upper.CL .group
 Peach    1881 366 41.9      871     2890  a
 Yoshi    2047 366 41.9     1037     3056  a
 Toad     3816 366 41.9     2807     4825   b
 Goomba   4481 366 41.9     3472     5491   b
 Diddy    4812 366 41.9     3803     5821   b
 Koopa    4816 366 41.9     3807     5825   b

G = Simba:
 N      emmean  SE   df lower.CL upper.CL .group
 Goomba   4253 366 41.9     3243     5262  a
 Koopa    5672 366 41.9     4663     6681  ab
 Diddy    6400 366 41.9     5391     7409   bc
 Toad     6733 366 41.9     5723     7742   bc
 Yoshi    7563 366 41.9     6554     8573    cd
 Peach    8701 366 41.9     7691     9710     d

G = Timon:
 N      emmean  SE   df lower.CL upper.CL .group
 Goomba   3177 366 41.9     2168     4187  a
 Koopa    5443 366 41.9     4433     6452   b
 Diddy    5994 366 41.9     4985     7003   b
 Toad     6014 366 41.9     5005     7023   b
 Peach    6065 366 41.9     5056     7075   b
 Yoshi    6687 366 41.9     5678     7697   b

Results are averaged over the levels of: rep
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95
Conf-level adjustment: sidak method for 6 estimates
P value adjustment: tukey method for comparing a family of 6 estimates
significance level used: alpha = 0.05
NOTE: If two or more means share the same grouping symbol,
      then we cannot show them to be different.
      But we also did not show them to be the same.
```

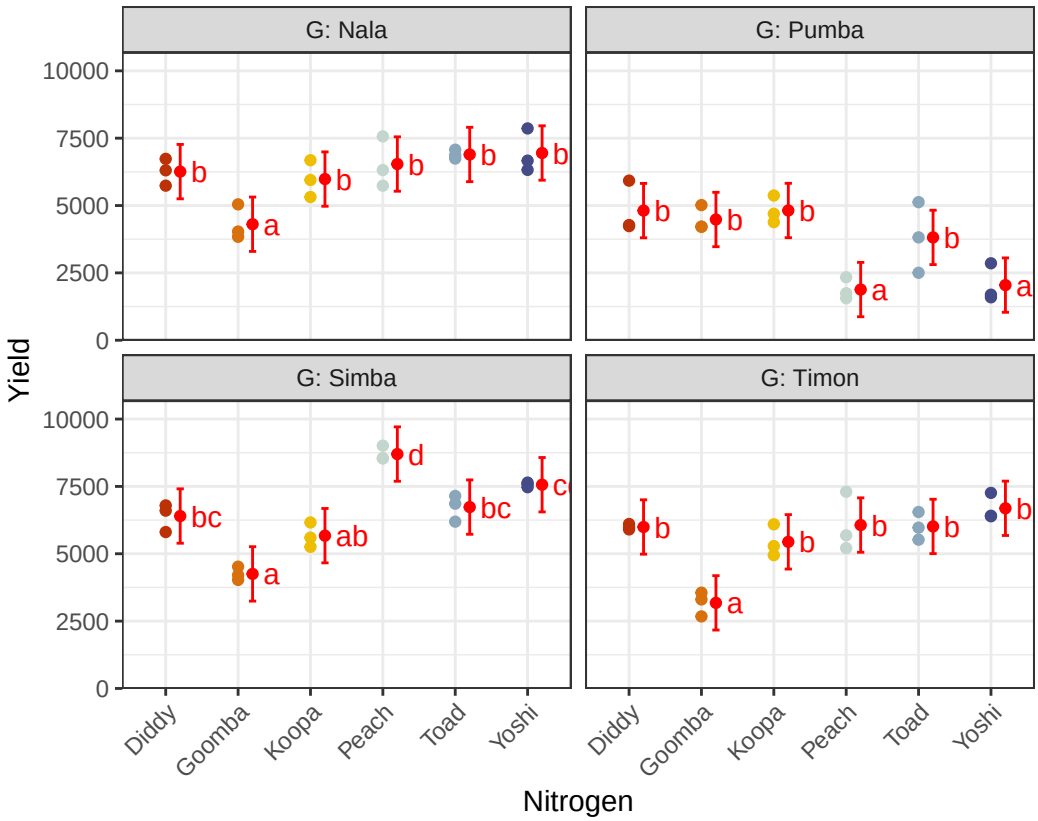
Note that if you would like to see the underlying individual contrasts/differences between adjusted means, simply add `details = TRUE` to the `cld()` statement. Furthermore, check out the Summary Article "Compact Letter Display".

Finally, we can create a plot that displays both the raw data and the results, *i.e.* the comparisons of the adjusted means that are based on the linear model.

```
my_caption <- "The four facettes represent genotypes Simba, Nala, Timon and Pumba. Black d

ggplot() +
  facet_wrap(~G, labeller = label_both) + # facette per G level
  aes(x = N) +
  # black dots representing the raw data
  geom_point(
    data = dat,
    aes(y = yield, color = N)
  ) +
  # red dots representing the adjusted means
  geom_point(
    data = mean_comp,
    aes(y = emmean),
    color = "red",
    position = position_nudge(x = 0.2)
  ) +
  # red error bars representing the confidence limits of the adjusted means
  geom_errorbar(
    data = mean_comp,
    aes(ymin = lower.CL, ymax = upper.CL),
    color = "red",
    width = 0.1,
    position = position_nudge(x = 0.2)
  ) +
  # red letters
  geom_text(
    data = mean_comp,
    aes(y = emmean, label = str_trim(.group)),
    color = "red",
    position = position_nudge(x = 0.35),
    hjust = 0
  ) +
  scale_x_discrete(
    name = "Nitrogen"
  ) +
  scale_y_continuous(
    name = "Yield",
    limits = c(0, NA),
```

```
  expand = expansion(mult = c(0, 0.1))
) +
scale_color_manual(
  values = Ncolors,
  guide = "none"
) +
theme_bw() +
labs(caption = my_caption) +
theme(
  plot.caption = element_textbox_simple(margin = margin(t = 5)),
  plot.caption.position = "plot",
  axis.text.x = element_text(
    angle = 45,
    hjust = 1,
    vjust = 1
  )
)
```



The four facettes represent genotypes Simba, Nala, Timon and Pumba. Black dots represent raw data. Red dots and error bars represent adjusted means with 95% confidence limits per cultivar. For each genotype separately, means followed by a common letter are not significantly different according to the Tukey−test.

Gomez, Kwanchai A, and Arturo A Gomez. 1984. *Statistical Procedures for Agricultural Research*. 2nd ed. An International Rice Research Institute Book. Nashville, TN: John Wiley & Sons.