Two-way ANOVA with interaction in R

Jeevan Koshy

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**Question**

*In Maize seed germination experiment, eight different type of seed treatments (g1, g2, g3, g4, g5, g6, g7, g8) and two different moisture levels (m1, m2) compared for seed germination. Carry out the analysis of variance and test for the significance difference among seed treatments, moisture levels and their interaction at 1% level.*

**Aim**

**Factor 1**

H0: There is no significant difference between the means of the different seed treatments.

HI: There is a significant difference between atleast one pair of means of the different seed treatments.

**Factor 2**

H0: There is no significant difference between the means of the different moisture levels.

H1: There is a significant difference between atleast one pair of means of the different moisture levels.

**Interaction**

H0: There is no significant difference between the means of the interaction effect of the two factors.

H1: There is a significant difference between atleast one pair of means of the interaction effect of the two factors.

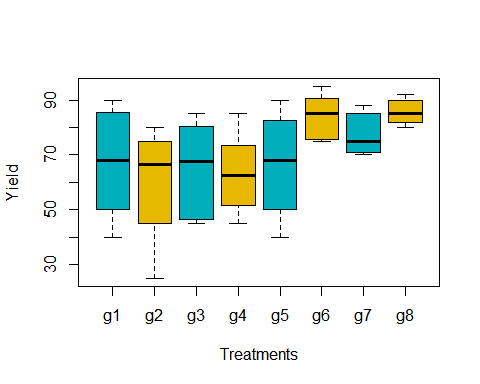
**Alpha level** = 0.01

**Procedure**

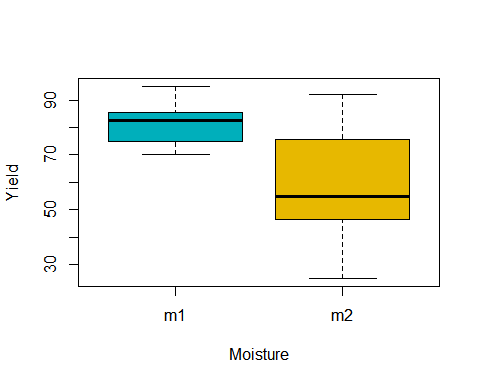
maize<-read.table(file = "clipboard",header = TRUE)  
str(maize)

## 'data.frame': 64 obs. of 5 variables:  
## $ Yield : int 85 80 80 85 90 90 85 90 50 50 ...  
## $ Treatments : Factor w/ 8 levels "g1","g2","g3",..: 1 2 3 4 5 6 7 8 1 2 ...  
## $ Moisture : Factor w/ 2 levels "m1","m2": 1 1 1 1 1 1 1 1 2 2 ...  
## $ Repetition : Factor w/ 4 levels "r1","r2","r3",..: 1 1 1 1 1 1 1 1 1 1 ...  
## $ Interaction: Factor w/ 16 levels "m1g1","m1g2",..: 1 2 3 4 5 6 7 8 9 10 ...

boxplot(Yield~Treatments, data = maize, xlab = "Treatments", ylab = "Yield", col = c("#00AFBB", "#E7B800"))



boxplot(Yield~Moisture, data = maize, xlab = "Moisture", ylab = "Yield", col = c("#00AFBB", "#E7B800"))



boxplot(Yield~Interaction, data = maize, xlab = "Treatment\*Moisture", ylab = "Yield",col = c("#00AFBB", "#E7B800"))  
model<-aov(Yield~Treatments+Moisture+Treatments\*Moisture, data = maize)  
summary(model)

## Df Sum Sq Mean Sq F value Pr(>F)   
## Treatments 7 5530 790 21.48 8.45e-13 \*\*\*  
## Moisture 1 7526 7526 204.66 < 2e-16 \*\*\*  
## Treatments:Moisture 7 3997 571 15.53 1.97e-10 \*\*\*  
## Residuals 48 1765 37   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

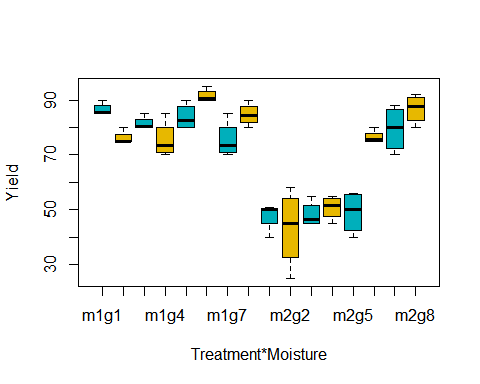
library(lsmeans)

## Warning: package 'lsmeans' was built under R version 3.5.3

## Loading required package: emmeans

## Warning: package 'emmeans' was built under R version 3.5.3

## The 'lsmeans' package is now basically a front end for 'emmeans'.  
## Users are encouraged to switch the rest of the way.  
## See help('transition') for more information, including how to  
## convert old 'lsmeans' objects and scripts to work with 'emmeans'.



lm1<-lm(Yield~Treatments+Moisture+Treatments\*Moisture, data=maize)   
lsm1<-lsmeans(lm1,"Treatments")

## NOTE: Results may be misleading due to involvement in interactions

pairs(lsm1)

## contrast estimate SE df t.ratio p.value  
## g1 - g2 7.38 3.03 48 2.432 0.2497   
## g1 - g3 2.25 3.03 48 0.742 0.9951   
## g1 - g4 4.00 3.03 48 1.319 0.8870   
## g1 - g5 0.75 3.03 48 0.247 1.0000   
## g1 - g6 -16.88 3.03 48 -5.566 <.0001   
## g1 - g7 -10.38 3.03 48 -3.422 0.0259   
## g1 - g8 -18.62 3.03 48 -6.143 <.0001   
## g2 - g3 -5.12 3.03 48 -1.690 0.6932   
## g2 - g4 -3.38 3.03 48 -1.113 0.9508   
## g2 - g5 -6.62 3.03 48 -2.185 0.3786   
## g2 - g6 -24.25 3.03 48 -7.998 <.0001   
## g2 - g7 -17.75 3.03 48 -5.854 <.0001   
## g2 - g8 -26.00 3.03 48 -8.575 <.0001   
## g3 - g4 1.75 3.03 48 0.577 0.9990   
## g3 - g5 -1.50 3.03 48 -0.495 0.9996   
## g3 - g6 -19.12 3.03 48 -6.308 <.0001   
## g3 - g7 -12.62 3.03 48 -4.164 0.0030   
## g3 - g8 -20.88 3.03 48 -6.885 <.0001   
## g4 - g5 -3.25 3.03 48 -1.072 0.9596   
## g4 - g6 -20.88 3.03 48 -6.885 <.0001   
## g4 - g7 -14.38 3.03 48 -4.741 0.0005   
## g4 - g8 -22.62 3.03 48 -7.462 <.0001   
## g5 - g6 -17.62 3.03 48 -5.813 <.0001   
## g5 - g7 -11.12 3.03 48 -3.669 0.0131   
## g5 - g8 -19.38 3.03 48 -6.390 <.0001   
## g6 - g7 6.50 3.03 48 2.144 0.4030   
## g6 - g8 -1.75 3.03 48 -0.577 0.9990   
## g7 - g8 -8.25 3.03 48 -2.721 0.1412   
##   
## Results are averaged over the levels of: Moisture   
## P value adjustment: tukey method for comparing a family of 8 estimates

lsm2<-lsmeans(lm1,"Moisture")

## NOTE: Results may be misleading due to involvement in interactions

pairs(lsm2)

## contrast estimate SE df t.ratio p.value  
## m1 - m2 21.7 1.52 48 14.306 <.0001   
##   
## Results are averaged over the levels of: Treatments

lsm3<-lsmeans(lm1,~Treatments:Moisture)  
pairs(lsm3)

## contrast estimate SE df t.ratio p.value  
## g1,m1 - g2,m1 10.25 4.29 48 2.390 0.5632   
## g1,m1 - g3,m1 5.00 4.29 48 1.166 0.9981   
## g1,m1 - g4,m1 11.00 4.29 48 2.565 0.4447   
## g1,m1 - g5,m1 2.75 4.29 48 0.641 1.0000   
## g1,m1 - g6,m1 -5.00 4.29 48 -1.166 0.9981   
## g1,m1 - g7,m1 11.00 4.29 48 2.565 0.4447   
## g1,m1 - g8,m1 1.75 4.29 48 0.408 1.0000   
## g1,m1 - g1,m2 38.75 4.29 48 9.037 <.0001   
## g1,m1 - g2,m2 43.25 4.29 48 10.087 <.0001   
## g1,m1 - g3,m2 38.25 4.29 48 8.921 <.0001   
## g1,m1 - g4,m2 35.75 4.29 48 8.338 <.0001   
## g1,m1 - g5,m2 37.50 4.29 48 8.746 <.0001   
## g1,m1 - g6,m2 10.00 4.29 48 2.332 0.6034   
## g1,m1 - g7,m2 7.00 4.29 48 1.633 0.9537   
## g1,m1 - g8,m2 -0.25 4.29 48 -0.058 1.0000   
## g2,m1 - g3,m1 -5.25 4.29 48 -1.224 0.9968   
## g2,m1 - g4,m1 0.75 4.29 48 0.175 1.0000   
## g2,m1 - g5,m1 -7.50 4.29 48 -1.749 0.9219   
## g2,m1 - g6,m1 -15.25 4.29 48 -3.557 0.0578   
## g2,m1 - g7,m1 0.75 4.29 48 0.175 1.0000   
## g2,m1 - g8,m1 -8.50 4.29 48 -1.982 0.8227   
## g2,m1 - g1,m2 28.50 4.29 48 6.647 <.0001   
## g2,m1 - g2,m2 33.00 4.29 48 7.696 <.0001   
## g2,m1 - g3,m2 28.00 4.29 48 6.530 <.0001   
## g2,m1 - g4,m2 25.50 4.29 48 5.947 <.0001   
## g2,m1 - g5,m2 27.25 4.29 48 6.355 <.0001   
## g2,m1 - g6,m2 -0.25 4.29 48 -0.058 1.0000   
## g2,m1 - g7,m2 -3.25 4.29 48 -0.758 1.0000   
## g2,m1 - g8,m2 -10.50 4.29 48 -2.449 0.5231   
## g3,m1 - g4,m1 6.00 4.29 48 1.399 0.9878   
## g3,m1 - g5,m1 -2.25 4.29 48 -0.525 1.0000   
## g3,m1 - g6,m1 -10.00 4.29 48 -2.332 0.6034   
## g3,m1 - g7,m1 6.00 4.29 48 1.399 0.9878   
## g3,m1 - g8,m1 -3.25 4.29 48 -0.758 1.0000   
## g3,m1 - g1,m2 33.75 4.29 48 7.871 <.0001   
## g3,m1 - g2,m2 38.25 4.29 48 8.921 <.0001   
## g3,m1 - g3,m2 33.25 4.29 48 7.755 <.0001   
## g3,m1 - g4,m2 30.75 4.29 48 7.171 <.0001   
## g3,m1 - g5,m2 32.50 4.29 48 7.580 <.0001   
## g3,m1 - g6,m2 5.00 4.29 48 1.166 0.9981   
## g3,m1 - g7,m2 2.00 4.29 48 0.466 1.0000   
## g3,m1 - g8,m2 -5.25 4.29 48 -1.224 0.9968   
## g4,m1 - g5,m1 -8.25 4.29 48 -1.924 0.8519   
## g4,m1 - g6,m1 -16.00 4.29 48 -3.731 0.0366   
## g4,m1 - g7,m1 0.00 4.29 48 0.000 1.0000   
## g4,m1 - g8,m1 -9.25 4.29 48 -2.157 0.7203   
## g4,m1 - g1,m2 27.75 4.29 48 6.472 <.0001   
## g4,m1 - g2,m2 32.25 4.29 48 7.521 <.0001   
## g4,m1 - g3,m2 27.25 4.29 48 6.355 <.0001   
## g4,m1 - g4,m2 24.75 4.29 48 5.772 0.0001   
## g4,m1 - g5,m2 26.50 4.29 48 6.180 <.0001   
## g4,m1 - g6,m2 -1.00 4.29 48 -0.233 1.0000   
## g4,m1 - g7,m2 -4.00 4.29 48 -0.933 0.9999   
## g4,m1 - g8,m2 -11.25 4.29 48 -2.624 0.4072   
## g5,m1 - g6,m1 -7.75 4.29 48 -1.807 0.9016   
## g5,m1 - g7,m1 8.25 4.29 48 1.924 0.8519   
## g5,m1 - g8,m1 -1.00 4.29 48 -0.233 1.0000   
## g5,m1 - g1,m2 36.00 4.29 48 8.396 <.0001   
## g5,m1 - g2,m2 40.50 4.29 48 9.445 <.0001   
## g5,m1 - g3,m2 35.50 4.29 48 8.279 <.0001   
## g5,m1 - g4,m2 33.00 4.29 48 7.696 <.0001   
## g5,m1 - g5,m2 34.75 4.29 48 8.104 <.0001   
## g5,m1 - g6,m2 7.25 4.29 48 1.691 0.9392   
## g5,m1 - g7,m2 4.25 4.29 48 0.991 0.9997   
## g5,m1 - g8,m2 -3.00 4.29 48 -0.700 1.0000   
## g6,m1 - g7,m1 16.00 4.29 48 3.731 0.0366   
## g6,m1 - g8,m1 6.75 4.29 48 1.574 0.9655   
## g6,m1 - g1,m2 43.75 4.29 48 10.203 <.0001   
## g6,m1 - g2,m2 48.25 4.29 48 11.253 <.0001   
## g6,m1 - g3,m2 43.25 4.29 48 10.087 <.0001   
## g6,m1 - g4,m2 40.75 4.29 48 9.504 <.0001   
## g6,m1 - g5,m2 42.50 4.29 48 9.912 <.0001   
## g6,m1 - g6,m2 15.00 4.29 48 3.498 0.0669   
## g6,m1 - g7,m2 12.00 4.29 48 2.799 0.3042   
## g6,m1 - g8,m2 4.75 4.29 48 1.108 0.9989   
## g7,m1 - g8,m1 -9.25 4.29 48 -2.157 0.7203   
## g7,m1 - g1,m2 27.75 4.29 48 6.472 <.0001   
## g7,m1 - g2,m2 32.25 4.29 48 7.521 <.0001   
## g7,m1 - g3,m2 27.25 4.29 48 6.355 <.0001   
## g7,m1 - g4,m2 24.75 4.29 48 5.772 0.0001   
## g7,m1 - g5,m2 26.50 4.29 48 6.180 <.0001   
## g7,m1 - g6,m2 -1.00 4.29 48 -0.233 1.0000   
## g7,m1 - g7,m2 -4.00 4.29 48 -0.933 0.9999   
## g7,m1 - g8,m2 -11.25 4.29 48 -2.624 0.4072   
## g8,m1 - g1,m2 37.00 4.29 48 8.629 <.0001   
## g8,m1 - g2,m2 41.50 4.29 48 9.679 <.0001   
## g8,m1 - g3,m2 36.50 4.29 48 8.512 <.0001   
## g8,m1 - g4,m2 34.00 4.29 48 7.929 <.0001   
## g8,m1 - g5,m2 35.75 4.29 48 8.338 <.0001   
## g8,m1 - g6,m2 8.25 4.29 48 1.924 0.8519   
## g8,m1 - g7,m2 5.25 4.29 48 1.224 0.9968   
## g8,m1 - g8,m2 -2.00 4.29 48 -0.466 1.0000   
## g1,m2 - g2,m2 4.50 4.29 48 1.049 0.9994   
## g1,m2 - g3,m2 -0.50 4.29 48 -0.117 1.0000   
## g1,m2 - g4,m2 -3.00 4.29 48 -0.700 1.0000   
## g1,m2 - g5,m2 -1.25 4.29 48 -0.292 1.0000   
## g1,m2 - g6,m2 -28.75 4.29 48 -6.705 <.0001   
## g1,m2 - g7,m2 -31.75 4.29 48 -7.405 <.0001   
## g1,m2 - g8,m2 -39.00 4.29 48 -9.096 <.0001   
## g2,m2 - g3,m2 -5.00 4.29 48 -1.166 0.9981   
## g2,m2 - g4,m2 -7.50 4.29 48 -1.749 0.9219   
## g2,m2 - g5,m2 -5.75 4.29 48 -1.341 0.9919   
## g2,m2 - g6,m2 -33.25 4.29 48 -7.755 <.0001   
## g2,m2 - g7,m2 -36.25 4.29 48 -8.454 <.0001   
## g2,m2 - g8,m2 -43.50 4.29 48 -10.145 <.0001   
## g3,m2 - g4,m2 -2.50 4.29 48 -0.583 1.0000   
## g3,m2 - g5,m2 -0.75 4.29 48 -0.175 1.0000   
## g3,m2 - g6,m2 -28.25 4.29 48 -6.588 <.0001   
## g3,m2 - g7,m2 -31.25 4.29 48 -7.288 <.0001   
## g3,m2 - g8,m2 -38.50 4.29 48 -8.979 <.0001   
## g4,m2 - g5,m2 1.75 4.29 48 0.408 1.0000   
## g4,m2 - g6,m2 -25.75 4.29 48 -6.005 <.0001   
## g4,m2 - g7,m2 -28.75 4.29 48 -6.705 <.0001   
## g4,m2 - g8,m2 -36.00 4.29 48 -8.396 <.0001   
## g5,m2 - g6,m2 -27.50 4.29 48 -6.414 <.0001   
## g5,m2 - g7,m2 -30.50 4.29 48 -7.113 <.0001   
## g5,m2 - g8,m2 -37.75 4.29 48 -8.804 <.0001   
## g6,m2 - g7,m2 -3.00 4.29 48 -0.700 1.0000   
## g6,m2 - g8,m2 -10.25 4.29 48 -2.390 0.5632   
## g7,m2 - g8,m2 -7.25 4.29 48 -1.691 0.9392   
##   
## P value adjustment: tukey method for comparing a family of 16 estimates

library(multcompView)

## Warning: package 'multcompView' was built under R version 3.5.3

CLD(lsm1,Letters = "abcde",alpha=0.01)

## Warning: 'CLD' will be deprecated. Its use is discouraged.  
## See '? CLD' for an explanation. Use 'pwpp' or 'multcomp::cld' instead.

## Treatments lsmean SE df lower.CL upper.CL .group  
## g2 59.8 2.14 48 55.4 64.1 a   
## g4 63.1 2.14 48 58.8 67.4 a   
## g3 64.9 2.14 48 60.6 69.2 a   
## g5 66.4 2.14 48 62.1 70.7 a   
## g1 67.1 2.14 48 62.8 71.4 a   
## g7 77.5 2.14 48 73.2 81.8 b   
## g6 84.0 2.14 48 79.7 88.3 b   
## g8 85.8 2.14 48 81.4 90.1 b   
##   
## Results are averaged over the levels of: Moisture   
## Confidence level used: 0.99   
## P value adjustment: tukey method for comparing a family of 8 estimates   
## significance level used: alpha = 0.01

CLD(lsm2,Letters = "abcdefgh",alpha=0.01)

## Warning: 'CLD' will be deprecated. Its use is discouraged.  
## See '? CLD' for an explanation. Use 'pwpp' or 'multcomp::cld' instead.

## Moisture lsmean SE df lower.CL upper.CL .group  
## m2 60.2 1.07 48 58.1 62.4 a   
## m1 81.9 1.07 48 79.8 84.1 b   
##   
## Results are averaged over the levels of: Treatments   
## Confidence level used: 0.99   
## significance level used: alpha = 0.01

CLD(lsm3,Letters = "abcdefgh",alpha=0.01)

## Warning: 'CLD' will be deprecated. Its use is discouraged.  
## See '? CLD' for an explanation. Use 'pwpp' or 'multcomp::cld' instead.

## Treatments Moisture lsmean SE df lower.CL upper.CL .group  
## g2 m2 43.2 3.03 48 37.2 49.3 a   
## g1 m2 47.8 3.03 48 41.7 53.8 a   
## g3 m2 48.2 3.03 48 42.2 54.3 a   
## g5 m2 49.0 3.03 48 42.9 55.1 a   
## g4 m2 50.8 3.03 48 44.7 56.8 a   
## g4 m1 75.5 3.03 48 69.4 81.6 b   
## g7 m1 75.5 3.03 48 69.4 81.6 b   
## g2 m1 76.2 3.03 48 70.2 82.3 bc   
## g6 m2 76.5 3.03 48 70.4 82.6 bc   
## g7 m2 79.5 3.03 48 73.4 85.6 bc   
## g3 m1 81.5 3.03 48 75.4 87.6 bc   
## g5 m1 83.8 3.03 48 77.7 89.8 bc   
## g8 m1 84.8 3.03 48 78.7 90.8 bc   
## g1 m1 86.5 3.03 48 80.4 92.6 bc   
## g8 m2 86.8 3.03 48 80.7 92.8 bc   
## g6 m1 91.5 3.03 48 85.4 97.6 c   
##   
## Confidence level used: 0.99   
## P value adjustment: tukey method for comparing a family of 16 estimates   
## significance level used: alpha = 0.01

**Conclusion**

1. The calculated probability value for the first factor is less than 0.01(8.45e-13). Therefore, the null hypothesis is rejected. Hence, we can conclude that there is a significant difference between atleast one pair of means of the different seed treatments. The results of the post hoc test indicates that treatments g1, g2, g3, g4 and g5 are the same significantly and g6, g7, g8 are the same significantly. Either one of the treatments from the two sets can be chosen.
2. The calculated probability value for the second factor is less than 0.01(8.45e-13). Therefore, the null hypothesis is rejected. Hence, we can conclude that there is a significant difference between atleast one pair of means of the different moisture levels. The results of the post hoc test indicates that moisture levels m1 and m2 are significantly different and either one of the moisture levels can be chosen.
3. The calculated probability value for the interaction factor is less than 0.01(8.45e-13). Therefore, the null hypothesis is rejected. Hence, we can conclude that there is a significant difference between atleast one pair of means of the different interaction effects for the two factors. The results of the post hoc test indicates that the interaction effect of treatment g6 and moisture level m1 is significantly the best for the purpose of seed germination.