Two-way ANOVA with interaction in R

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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.

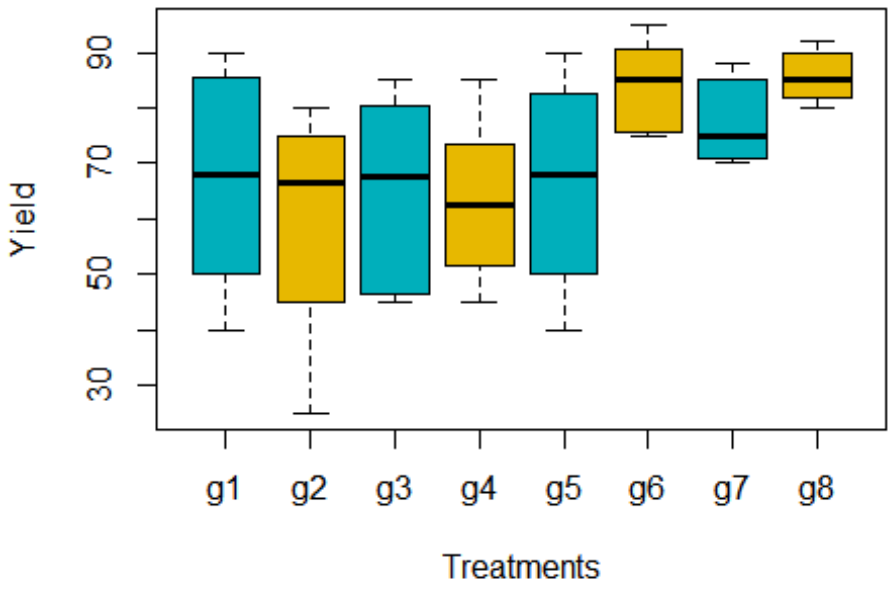
Alpha level = 0.01

Procedure

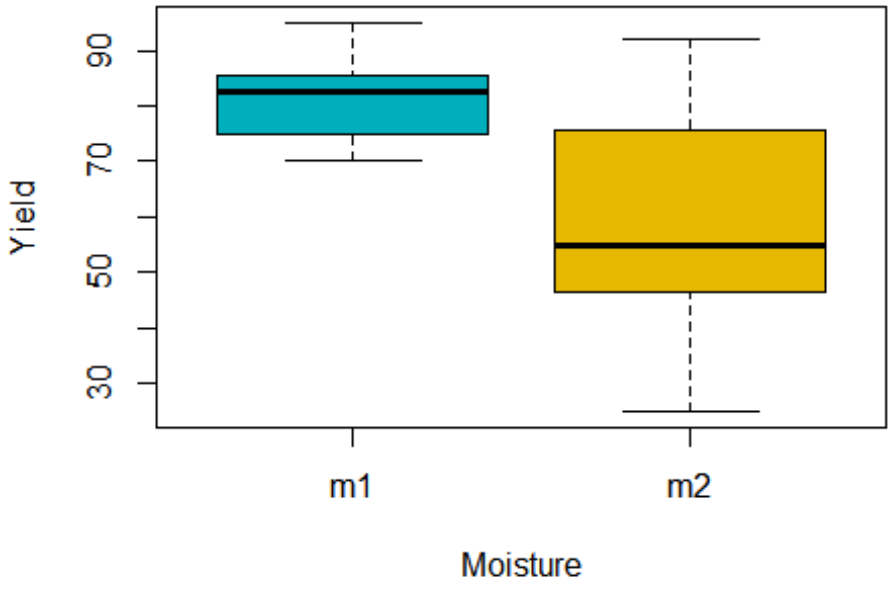
```
maize<-read.table(file = "clipboard",header = TRUE)
str(maize)
```

```
## '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
```

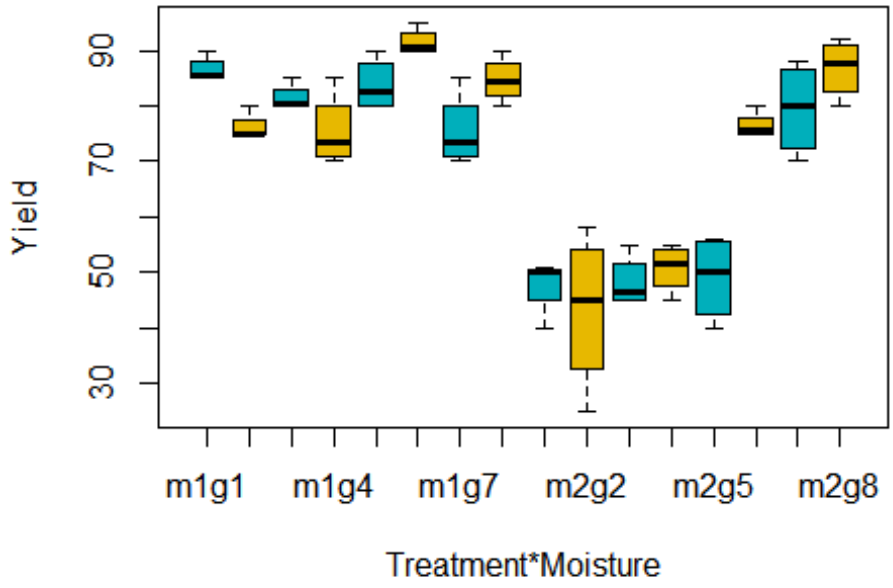
```
library(lsmeans)
```

```
## 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.