

Most common statistical tests in R (Part-1)

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All about multiple comparison of means from RAW data to publication ready graphs in R.

Install and library (load) the required packages

- Install the packages

```
install.packages("ggpubr")
install.packages("ggplot2")
install.packages("dplyr")
install.packages("ggpubfigs")
```

or install ggpubfigs like this:

```
install.packages("devtools")
library(devtools)
devtools::install_github("JLSteenwyk/ggpubfigs")
```

- Load required packages

```
library(ggpubr)
```

```
## Loading required package: ggplot2
```

```
library(ggplot2)
library(dplyr)
```

```
##
```

```
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
```

```
##
```

```
## filter, lag
```

```
## The following objects are masked from 'package:base':
```

```
##
```

```
## intersect, setdiff, setequal, union
```

```
library(ggpubfigs)
```

```
##
```

```
## Attaching package: 'ggpubfigs'
```

```
## The following object is masked from 'package:ggplot2':
```

```
##
```

```
##     theme_grey
```

One way ANOVA-test:

Demo data set:

We will use PlantGrowth (a builtin R data set) for this example.

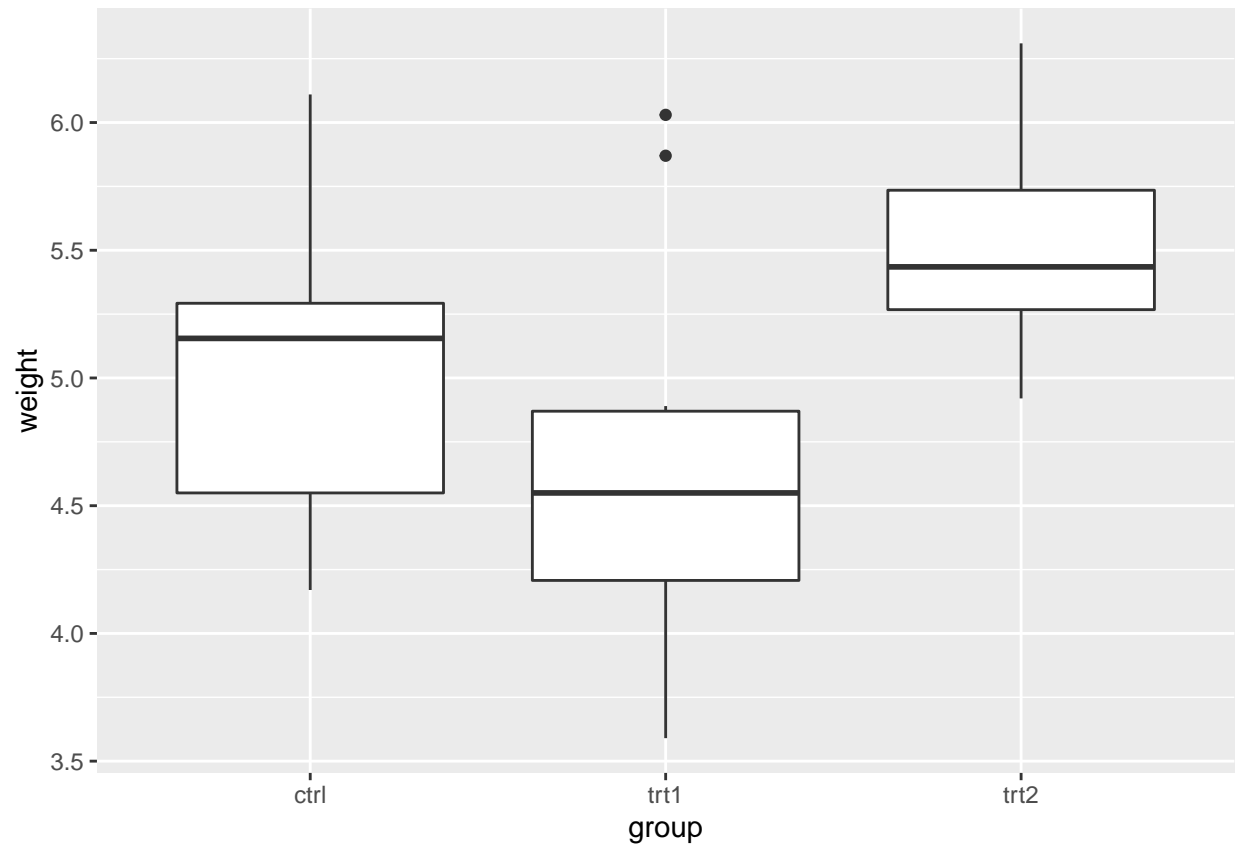
```
data("PlantGrowth")  
head(PlantGrowth)
```

```
##   weight group  
## 1   4.17  ctrl  
## 2   5.58  ctrl  
## 3   5.18  ctrl  
## 4   6.11  ctrl  
## 5   4.50  ctrl  
## 6   4.61  ctrl
```

```
View(PlantGrowth)
```

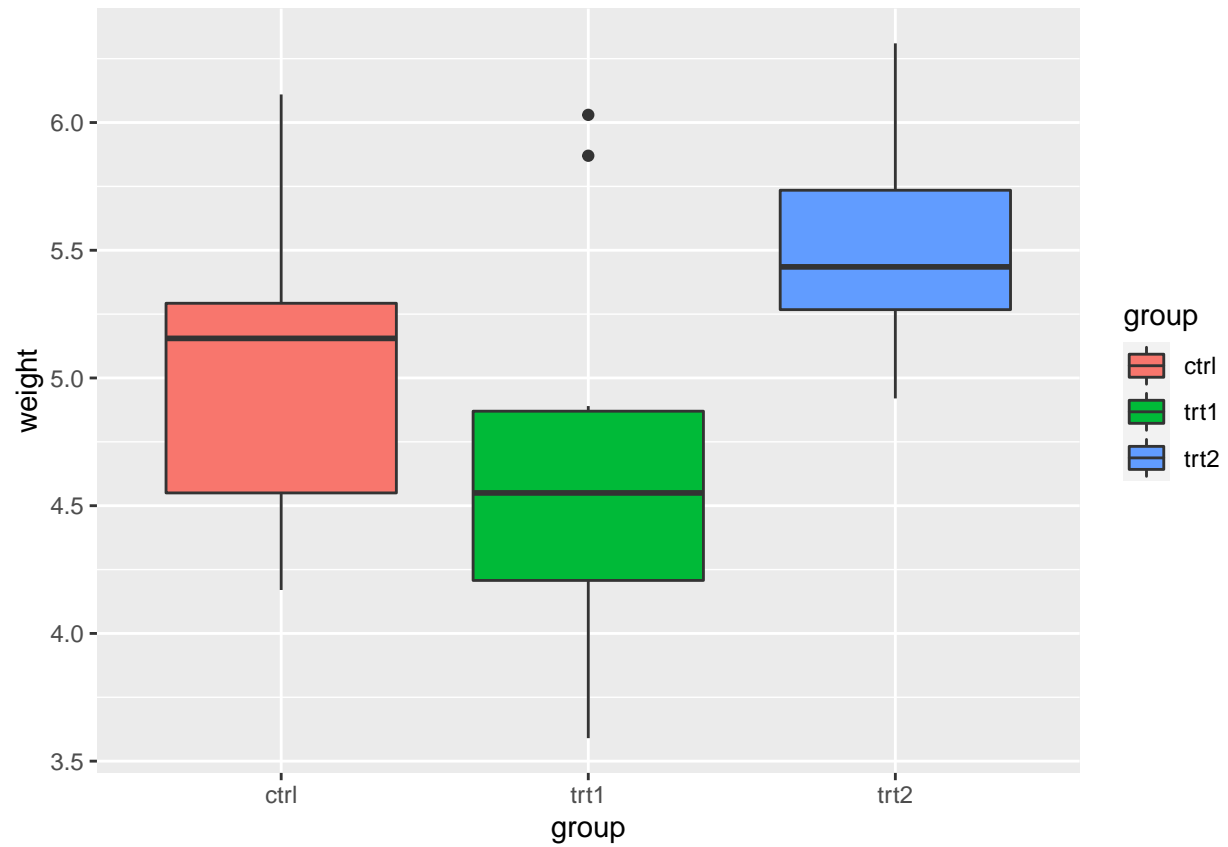
make a simple boxplot

```
ggplot(data = PlantGrowth, aes(x= group, y=weight))+  
  geom_boxplot()
```



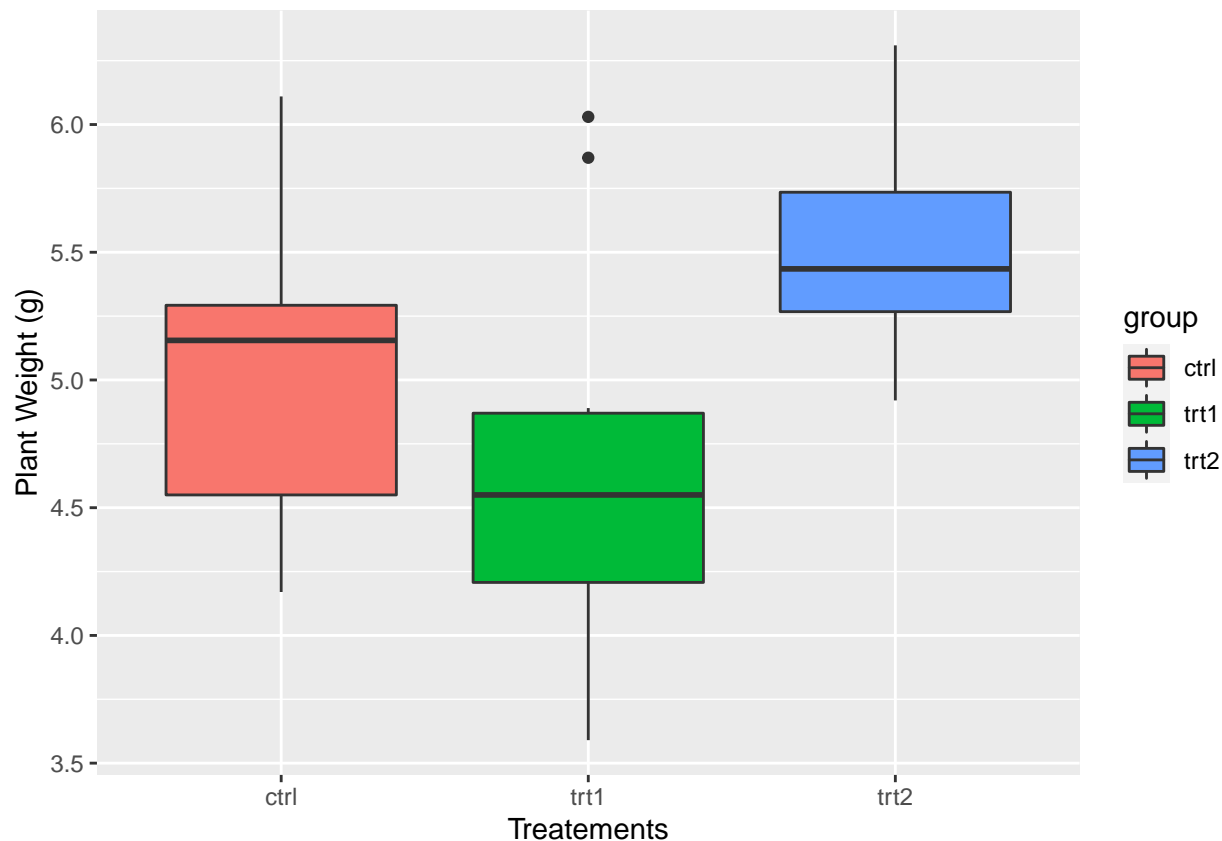
add color (fill) argument to boxplot

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+  
  geom_boxplot()
```



Change the axis labels

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+  
  geom_boxplot()+  
  labs(x="Treatements", y="Plant Weight (g)")
```



one-way ANOVA test calculations

Two important R functions `aov()` and `summary.aov()` can be used to see the outcomes of one-way ANOVA model

```
# Analysis of variance calculation
model_aov <- aov(weight ~ group, data = PlantGrowth)
# Sumamry to view the output
summary(model_aov)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## group          2  3.766   1.8832   4.846 0.0159 *
## Residuals     27 10.492   0.3886
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

$Pr(>F)$ corresponding to the p-value of the test `###` Interpretation of the one-way ANOVA results p-value less than 0.05 shows that model is significant, we can conclude that there are significant differences between the groups (treatments) highlighted with “*” in the summary of the one-way ANOVA model.

Multiple pairwise comparison (TUKEY HSD test):

As the ANOVA test is significant, we can compute Tukey HSD (Tukey Honest Significant Differences, R function: `TukeyHSD()`) for performing multiple pairwise-comparison between the means of groups.

We can now compute the Tukey HSD test by using the following function of R:

```
TukeyHSD(model_aov)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = weight ~ group, data = PlantGrowth)
##
## $group
##          diff          lwr          upr      p adj
## trt1-ctrl -0.371 -1.0622161 0.3202161 0.3908711
## trt2-ctrl  0.494 -0.1972161 1.1852161 0.1979960
## trt2-trt1  0.865  0.1737839 1.5562161 0.0120064
```

- **diff:** difference between means of the two groups
- **lwr,upr:** the lower and the upper end point of the confidence interval at 95% (default)
- **p adj:** p-value after adjustment for the multiple comparisons. It can be seen from the output, that only the difference between trt2 and trt1 is significant with an adjusted p-value of 0.012, which is less than 0.05.

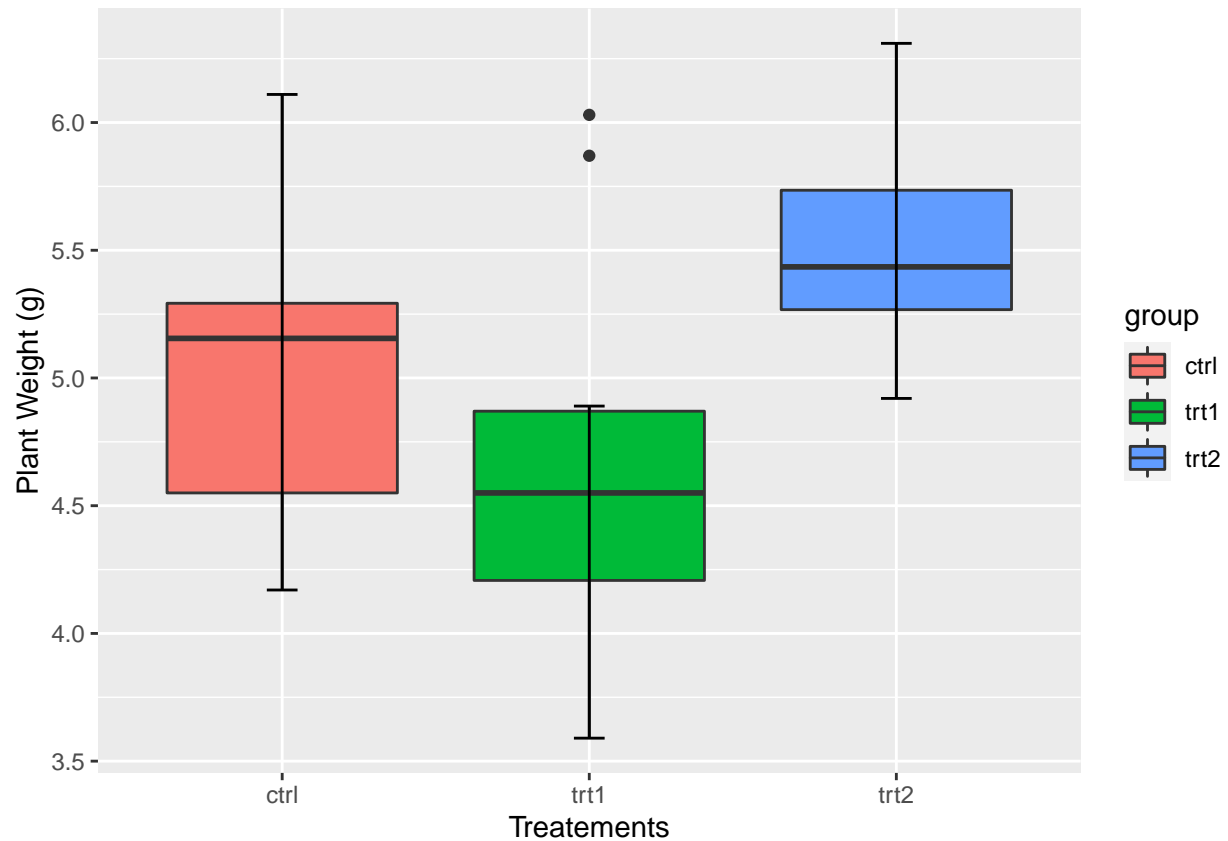
Visualize and specify the comparisons we want without computing tests:

Define the comparisons in an object

```
my_comparisons <- list( c("ctrl", "trt1"), c("ctrl", "trt2"), c("trt1", "trt2"))
```

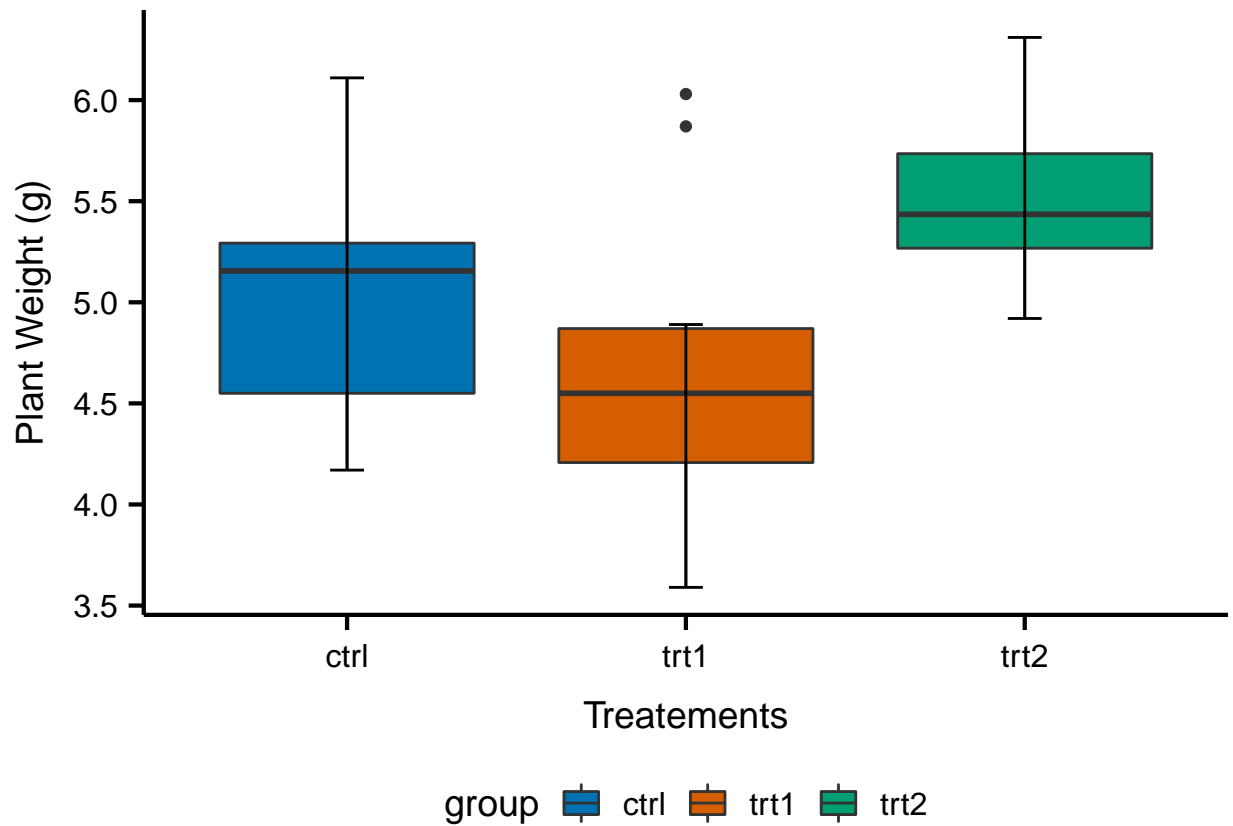
make a boxplot with errorbar caps

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatements", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)
```



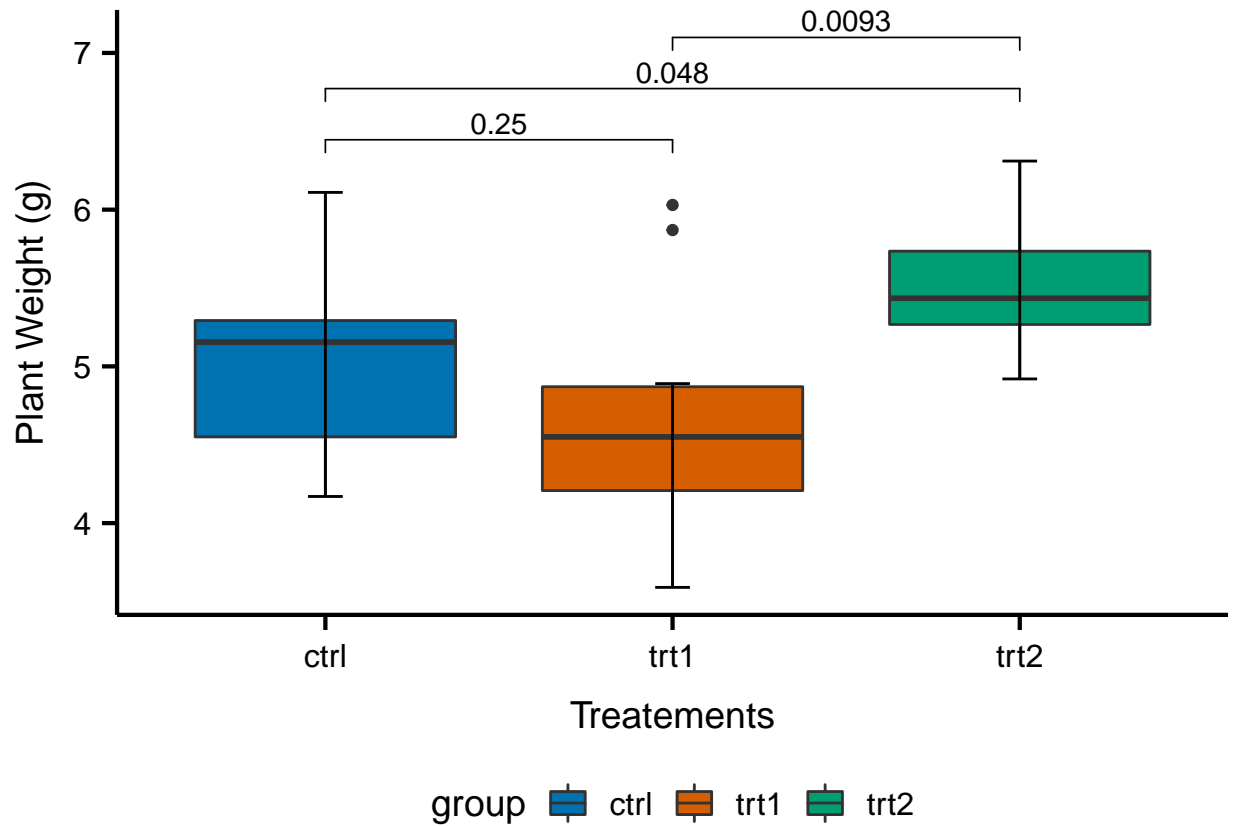
publication ready graphs can be made using ggpubfig package in the following way:

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatements", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)+
  scale_fill_manual(values = friendly_pal("ito_seven")) + theme_simple()
```



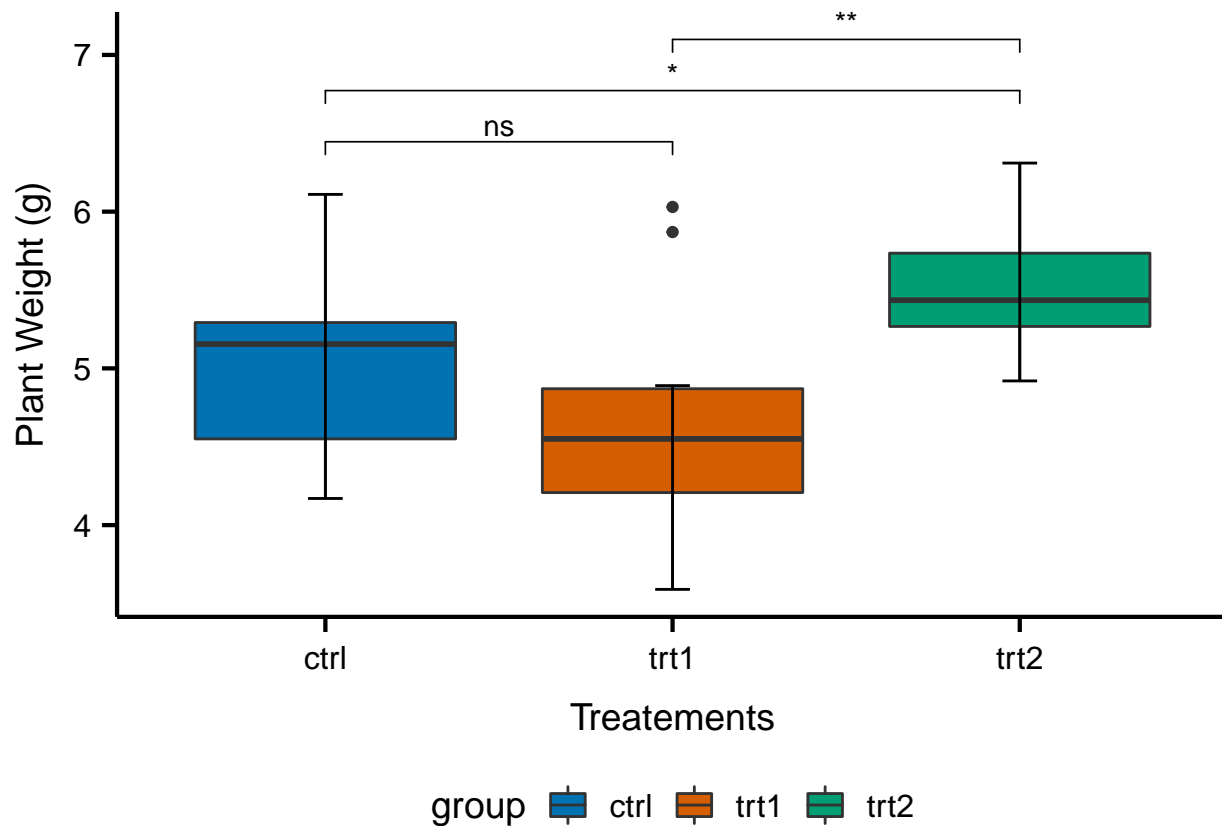
show significant differences of our comparisons on boxplot

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatements", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)+
  scale_fill_manual(values = friendly_pal("ito_seven")) + theme_simple()+
  stat_compare_means(method = "t.test", comparisons = my_comparisons)
```

- show significant levels instead of using p-values

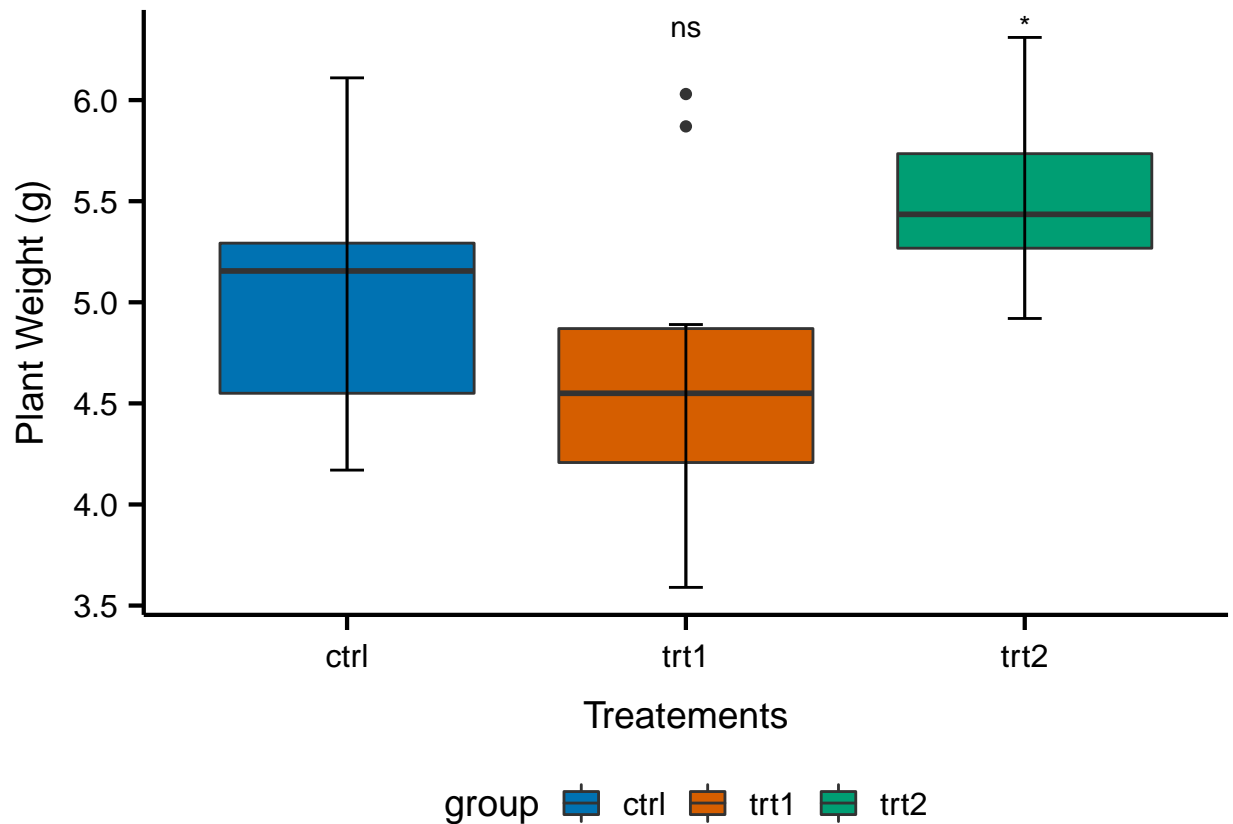
```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatements", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)+
  scale_fill_manual(values = friendly_pal("ito_seven")) + theme_simple()+
  stat_compare_means(method = "t.test", comparisons = my_comparisons, label = "p.signif")
```



Pairwise comparison against a reference

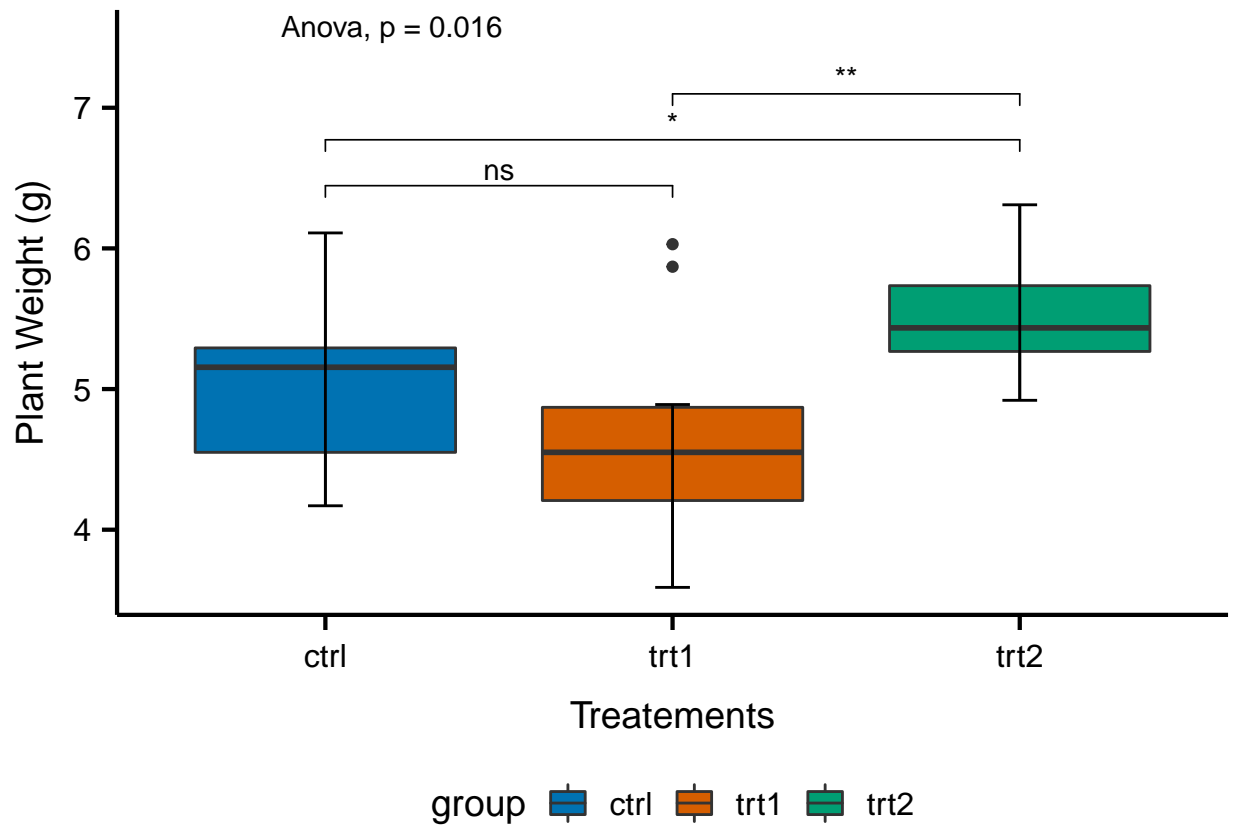
- Show significant differences based on a reference treatment, in this case our reference group treatment will be ctrl

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatments", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)+
  scale_fill_manual(values = friendly_pal("ito_seven")) +theme_simple()+
  stat_compare_means(method = "t.test", ref.group = "ctrl", label = "p.signif")
```

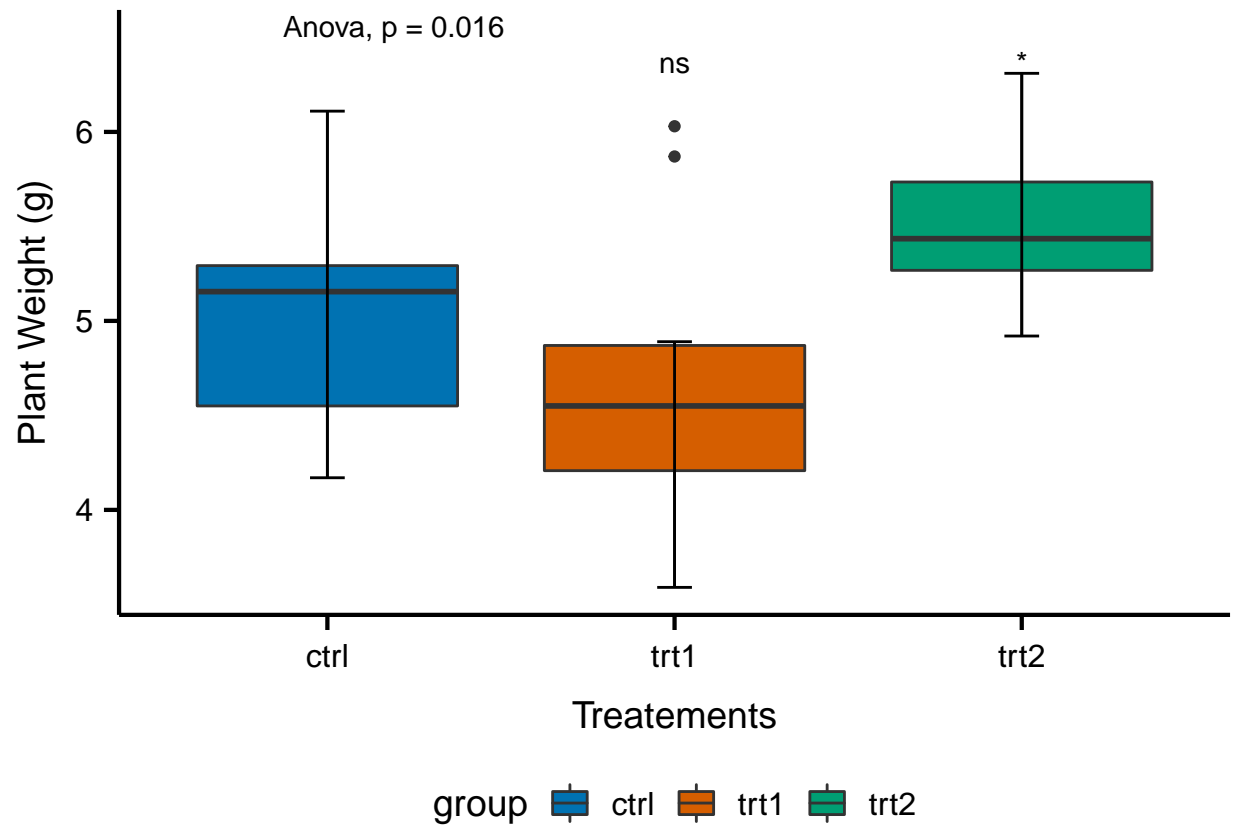


**show ANOVA results

```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatements", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)+
  scale_fill_manual(values = friendly_pal("ito_seven")) +theme_simple()+
  stat_compare_means(method = "t.test", comparisons = my_comparisons, label = "p.signif")+
  # Pairwise comparison against a reference
  stat_compare_means(method = "anova", label.y = 7.5)
```



```
ggplot(data = PlantGrowth, aes(x= group, y=weight, fill=group))+
  geom_boxplot()+
  labs(x="Treatements", y="Plant Weight (g)")+
  stat_boxplot(geom = 'errorbar', width=0.1)+
  scale_fill_manual(values = friendly_pal("ito_seven")) + theme_simple()+
  stat_compare_means(method = "t.test", ref.group = "ctrl", label = "p.signif")+
  stat_compare_means(method = "anova", label.y = 6.5)
```



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Best regards,

Dr Muhammad Aammar Tufail