Making a Box_plot with Lettering

1- Install the required packages by using install.packages(““package_name);for ggpubfig use this: (devtools::install_github(”JLSteenwyk/ggpubfigs”) and the library these:

```
library(readxl)
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v ggplot2 3.3.5     v purrr   0.3.4
## v tibble  3.1.6     v dplyr   1.0.7
## v tidyr   1.1.4     v stringr 1.4.0
## v readr   2.1.0     v forcats 0.5.1

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(agricolae)
library(devtools)
```

```
## Loading required package: usethis
```

```
library(ggpubfigs)
```

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

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

2- set your r directory in a folder where your data is present, (Ctrl+shift+h)

3- import your dataset into R

```
inp_data <- read_excel("rice_growth.xlsx",
                       col_types = c("text", "text", "numeric"))
print(inp_data)
```

```
## # A tibble: 21 x 3
##    inoculation variable          value
##    <chr>       <chr>             <dbl>
##  1 Ctrl        Shoot Length (cm)   4.7
##  2 T-1         Shoot Length (cm)   7.7
##  3 T-2         Shoot Length (cm)  12.9
##  4 T-3         Shoot Length (cm)  14.4
##  5 T-4         Shoot Length (cm)  13.5
##  6 T-5         Shoot Length (cm)  10.8
##  7 T-6         Shoot Length (cm)   7.9
##  8 Ctrl        Shoot Length (cm)   6.2
##  9 T-1         Shoot Length (cm)   3.5
## 10 T-2         Shoot Length (cm)  16.4
## # ... with 11 more rows
```

4- Statitical analysis of your data to get lettering. Here I have used LSD.test to calculate the multiple comparison of means on my data sets:

```
value_max = inp_data %>% group_by(inoculation) %>% summarize(max_value = max(value))
lsd=LSD.test(aov(value ~ inoculation, data=inp_data), trt = "inoculation", group = T)
sig.letters <- lsd$groups[order(row.names(lsd$groups)), ]
```

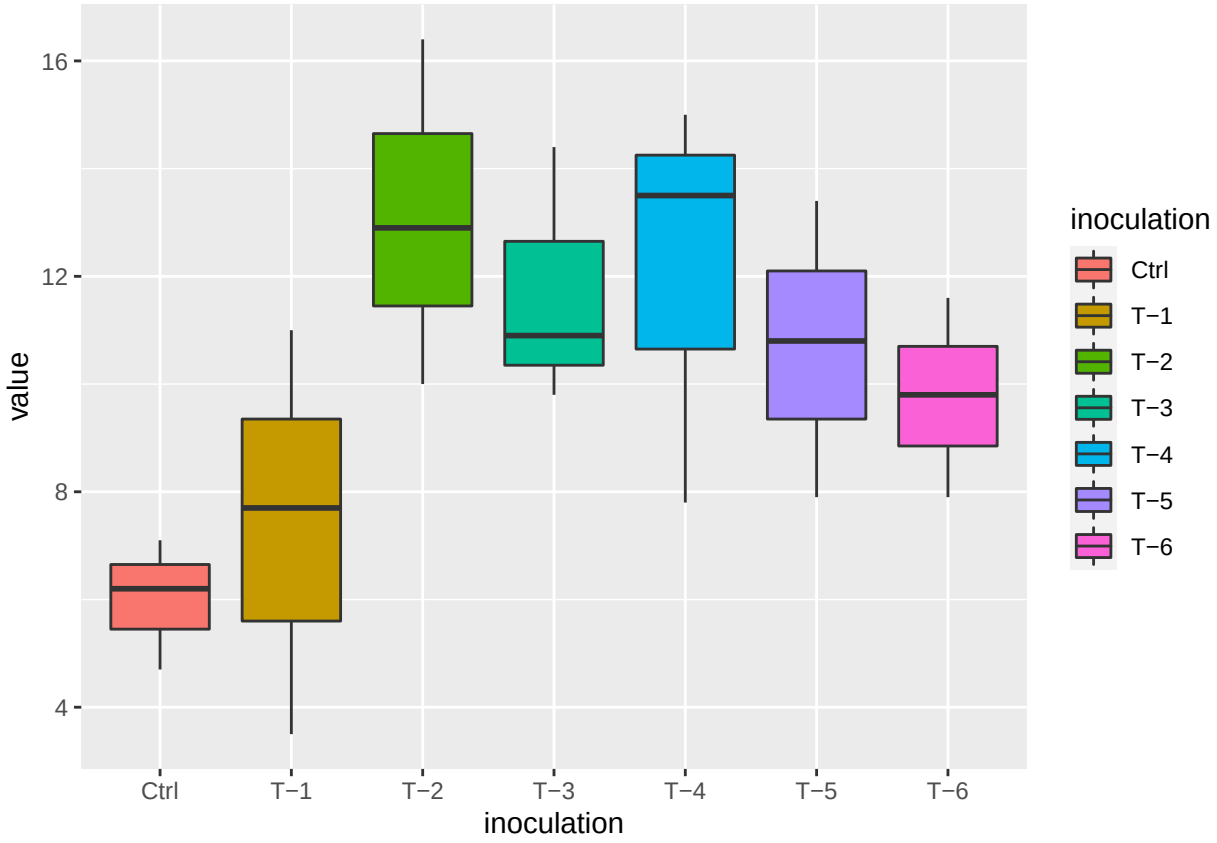
This will also give you the significant letters along the group of treatments (inoculation).

5- you can also change the order of your treatments to appear on the x-axis:

```
inp_data$inoculation <- factor(inp_data$inoculation, levels = c("Ctrl", "T-1", "T-2",
                                                                "T-3", "T-4", "T-5",
                                                                "T-6"))
```

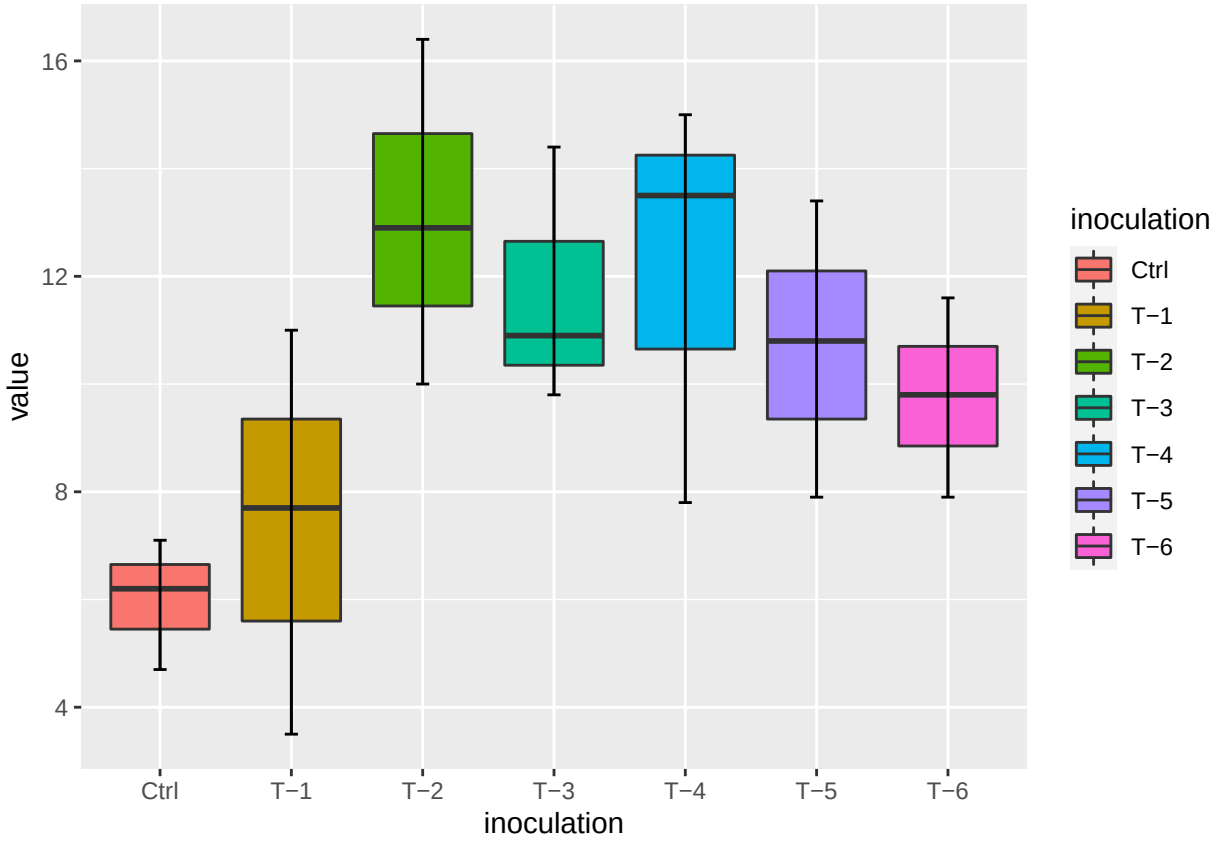
6- Plot the simple boxplot using ggplot2

```
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()
```



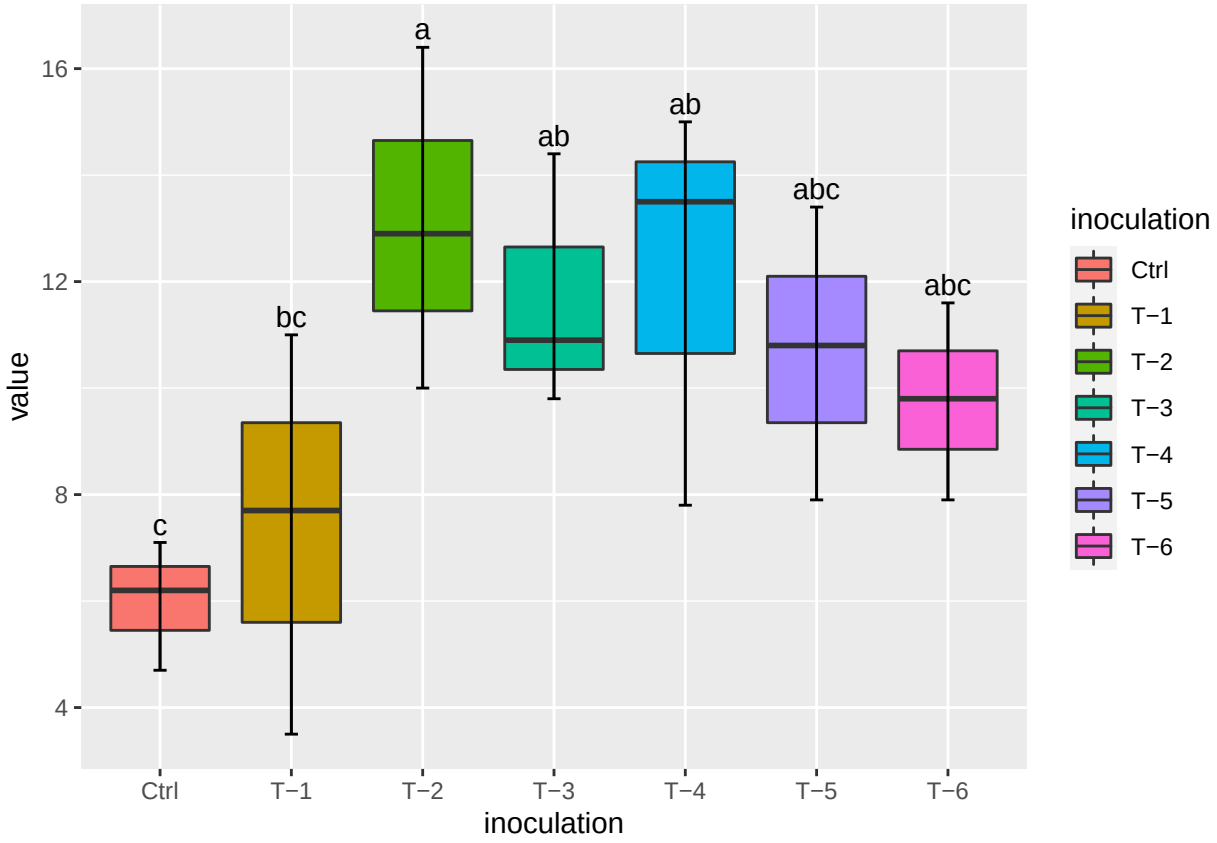
7- Adding caps to the error bars:

```
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()+
  stat_boxplot(geom = 'errorbar', width = 0.1)
```



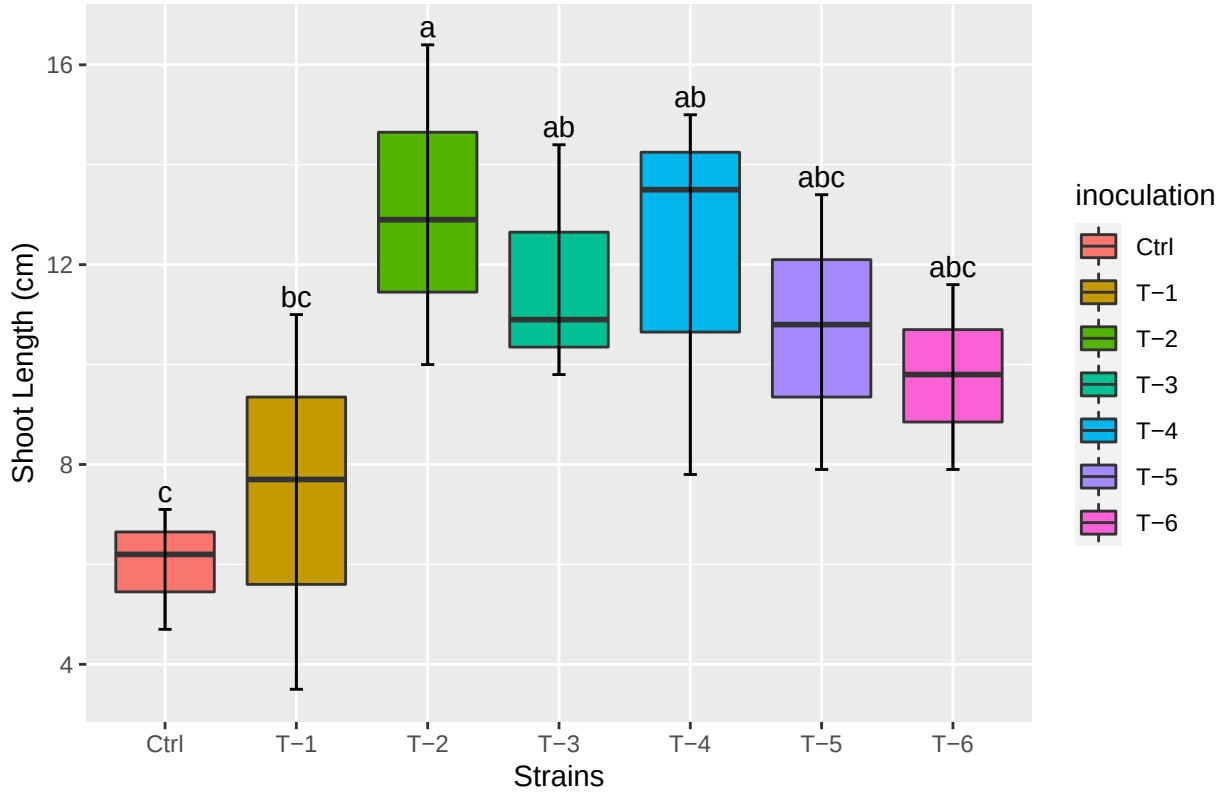
8- Adding significant letters got in step 4

```
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()+stat_boxplot(geom = 'errorbar', width = 0.1)+
  geom_text(data = value_max, aes(x=inoculation, y = 0.15 + max_value,
                                  label = sig.letters$groups), vjust=0)
```



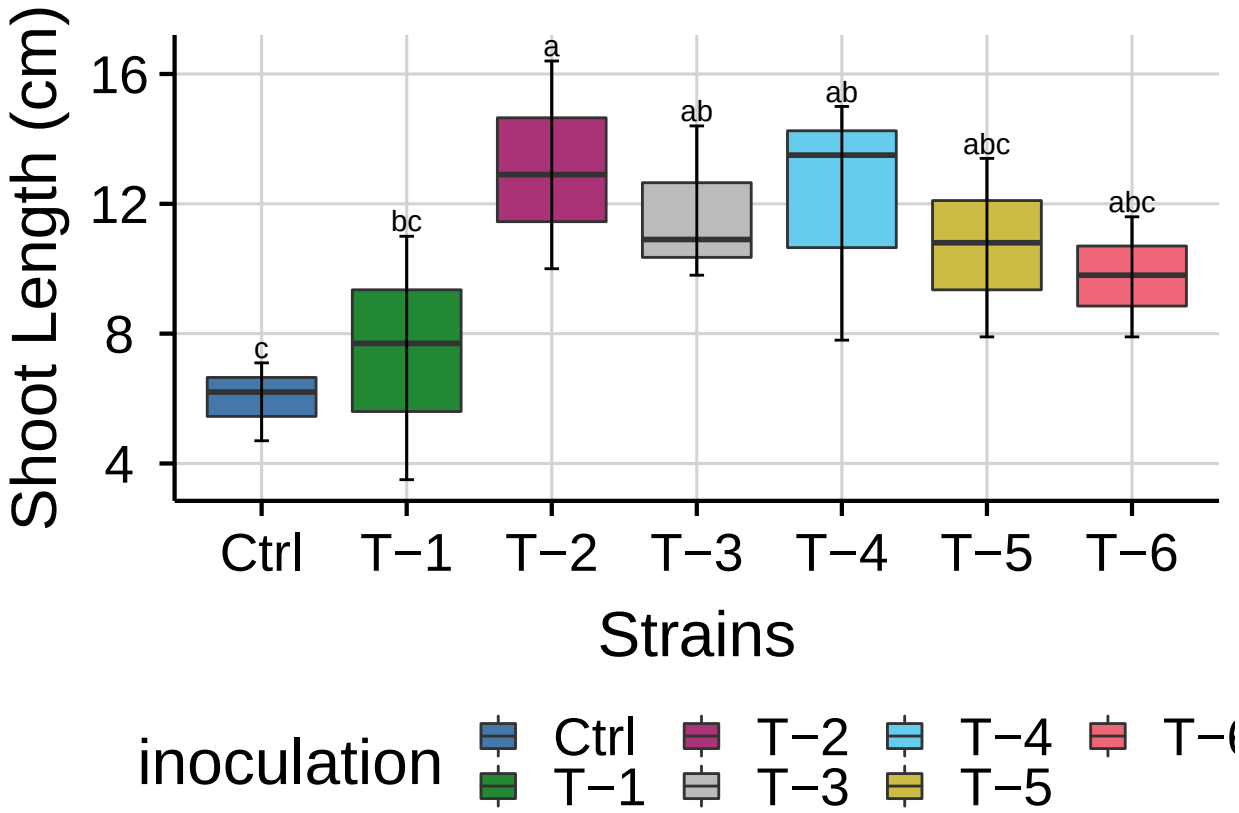
9- Adding axis labels:

```
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()+stat_boxplot(geom = 'errorbar', width = 0.1)+
  geom_text(data = value_max, aes(x=inoculation, y = 0.15 + max_value,
                                  label = sig.letters$groups), vjust=0)+
  ggtitle("") + xlab("Strains") + ylab("Shoot Length (cm)")
```



10- publication ready theme using ggpubfig package:

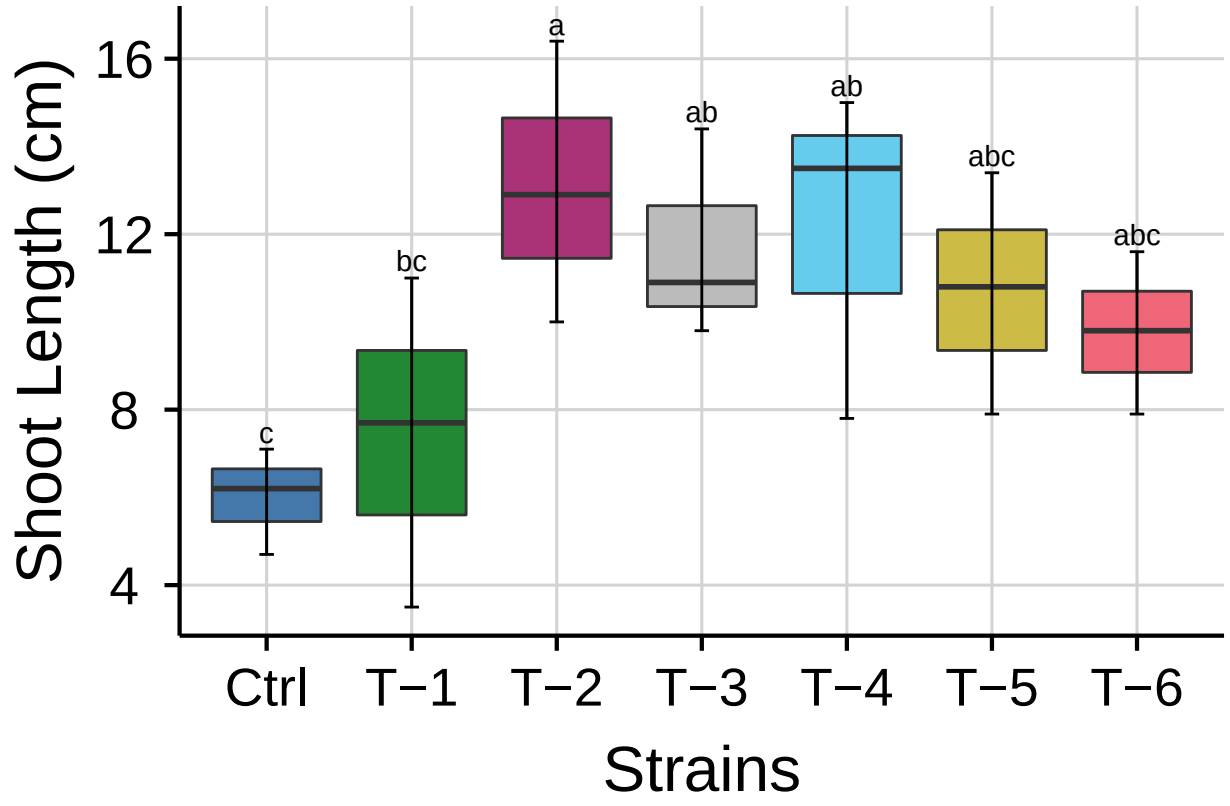
```
library(ggpubfigs)
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()+stat_boxplot(geom = 'errorbar', width = 0.1)+
  geom_text(data = value_max, aes(x=inoculation, y = 0.15 + max_value,
                                  label = sig.letters$groups), vjust=0)+
  ggtitle("") + xlab("Strains") + ylab("Shoot Length (cm)")+
  scale_fill_manual(values = friendly_pal("bright_seven")) + theme_big_grid()
```



You can explore more themes and figure types in the followoing website for ggpubfig: https://github.com/JLSteenwyk/ggpubfigs/blob/master/README.md#install

11- Removing legends:

```
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()+stat_boxplot(geom = 'errorbar', width = 0.1)+
  geom_text(data = value_max, aes(x=inoculation, y = 0.15 + max_value,
                                  label = sig.letters$groups), vjust=0)+
  ggtitle("") + xlab("Strains") + ylab("Shoot Length (cm)")+
  scale_fill_manual(values = friendly_pal("bright_seven")) + theme_big_grid()+
  theme(legend.position = "none")
```

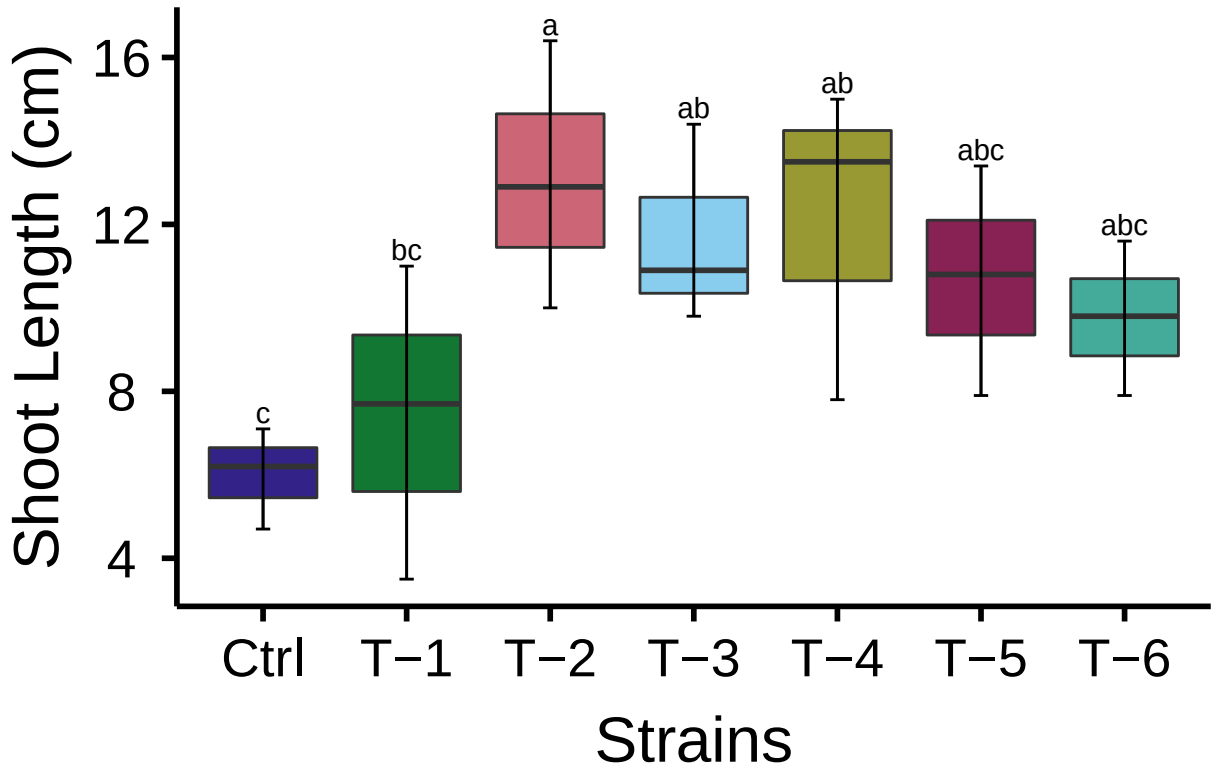


12- Saving high quality plots as tiff file in your working directory choosen in step-2

ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+ geom_boxplot()+stat_boxplot(geom = 'errorbar', width = 0.1)+ geom_text(data = value_max, aes(x=inoculation, y = 0.15 + max_value, label = sig.letters$groups), vjust=0)+ ggtitle("") + xlab("Strains") + ylab("Shoot Length (cm)")+ scale_fill_manual(values = friendly_pal("bright_seven")) + theme_big_grid()+ theme(legend.position = "none")+ ggsave("shoot_length.tiff", units="in", width=8, height=8, dpi=300, compression = 'lzw')

13- Change the theme:

```
ggplot(data = inp_data, aes(x = inoculation, y = value, fill=inoculation))+
  geom_boxplot()+stat_boxplot(geom = 'errorbar', width = 0.1)+
  geom_text(data = value_max, aes(x=inoculation, y = 0.15 + max_value,
                                  label = sig.letters$groups), vjust=0)+
  ggtitle("") + xlab("Strains") + ylab("Shoot Length (cm)")+
  scale_fill_manual(values = friendly_pal("muted_nine")) + theme_big_simple()+
  theme(legend.position = "none")
```


Shoot Length (cm)
16
12
8
4
a
ab
ab
abc
abc
bc
c
Ctrl
T−1
T−2
T−3
T−4
T−5
T−6
Strains