```r
setwd("/Users/francescadagostini/desktop")
data<-read.table("SilicaData.csv",sep=",",header=TRUE)

library(ggplot2)
library(tidyverse)
library(vegan)
library(ggplot2)
library(ggrepel)
library(scales)
library(ggpubr)

data$Specie_Trt<-factor(data$Specie_Trt,levels=c("S_WS","S_WW","FM_WS",
"FM_WW","PM_WS","PM_WW"),
              labels=c("Sorghum WS","Sorghum WW", "FingerM WS", "FingerM WW","PearlM
WS","PearlM WW"))
data$Specie<-factor(data$Specie, levels=c("FM","PM","S"),
              labels=c("Finger millet","Pearl millet","Sorghum"))
data$Trt<-factor(data$Trt, levels=c("WS","WW"),
              labels=c("Water stress","Well watered"))
data$Genotype<-factor(data$Genotype, levels=c("IE2511","IE3476","IE4450",
"IE4456","IP9859", "IP18019","IP13327","IP2367","IP18021","IS11061",
"IS35215","IS23075","IS38025","IS35216"),
labels=c("FM1","FM2","FM6","FM7","PM2","PM7","PM1","PM5","PM9","S5","S8","S2","S6",
"S9"))
data$Origin<-factor(data$Origin, levels=c("Ethiopia","Pakistan","Sudan"),
              labels=c("Ethiopia landraces","Pakistan landraces","Sudan landraces"))

###SHAPIRO TEST&LAVENE

shapiro.test(data$TotSilica)

leveneTest(TotSilica~Species,data=data)

###BOXPLOT

boxplot1<-ggplot(data,aes(x=Genotype,y=TE_Plt,fill=Trt))+
  geom_boxplot()+
  facet_wrap(~Origin,scales="free_x")+
  labs(title="",
       x="",
       y="Transpiration efficiency (g/L)")+
  stat_summary(fun="mean",aes(group=Trt),position=position_dodge(0.75),
              geom="point",shape=23,size=3,fill="white")
jpeg("TranspirationEfficiency.jpeg",width=2500,height=2000,res=300)
boxplot1+theme_bw()+scale_fill_brewer(palette="BuPu")+
  theme(legend.title=element_blank(),legend.position="bottom",
       text=element_text(size=15),axis.title=element_text(size=13),
       axis.text.x=element_text(angle=90,vjust=0.6))
dev.off()

boxplot2<-ggplot(data,aes(x=Specie,y=SilicaPercentage,fill=Trt))+
  geom_boxplot()+
  labs(title="",
       x="",
       y="Silica extracted %")+
  stat_summary(fun="mean",geom="point",shape=23,size=3,fill="white")
jpeg("Silica.jpeg",width=2500,height=2000,res=300)
boxplot2+theme_bw()+scale_fill_brewer(palette="BuPu")+
```

```
        theme(legend.title=element_blank(),legend.position="bottom",
               text=element_text(size=15),axis.title=element_text(size=13),
               axis.text.x=element_text(angle=90, vjust=0.6))
dev.off()

###ANOVA

aov_silica<-aov(data$SilicaPercentage~data$Genotype)
TukeyHSD(aov_silica)

###LINEAR REGRESSION TOTAL

Regre<-ggplot(data=data,aes(x = TWU_Plt,y=SilicaPercentage))+
  facet_wrap(~Specie,scales="free_x",nrow=1)+
  labs(title="",
       x="Total water transpired (L)",
       y="Silica extracted %")+
  geom_point(aes(color=Trt))+
  geom_smooth(method="lm", color="black",size=0.5,se=TRUE)+
  stat_cor(label.y=15)+
  jpeg("Regression_TWU.jpeg",width=2500,height=2000,res=300)
Regre+theme_bw()+scale_color_brewer(palette="Paired")+
  theme(legend.title=element_blank(),legend.position="bottom", aspect.ratio=1,
        text=element_text(size=15),axis.title=element_text(size=13))
dev.off()

###LINEAR REGRESSION SPECIES

Regre<-ggplot(data=data,aes(x =TWU_Plt,y=SilicaPercentage))+
  facet_wrap(~Genotype,scales="free_x",nrow= 1) +
  labs(title="",
       x="Total water transpired (L)",
       y="Silica extracted %") +
  geom_smooth(method="lm",color="black",size=0.5,se=TRUE)+
  jpeg("Regression_TWU_Sorghum.jpeg",width=2500,height=2000,res=300)
Regre+theme_bw()+scale_color_brewer(palette="Paired")+
theme(legend.title=element_blank(),legend.position="bottom",aspect.ratio=1,
        text=element_text(size=15),axis.title=element_text(size=13))
dev.off()

###LINEAR MODEL
model1<-lm(data$SilicaPercentage~data$TWT)
summary(model1)

model2<-lm(data$SilicaPercentage~data$Specie)
summary(model2)

model3<-lm(data$SilicaPercentage~data$Genotype)
summary(model3)

model4<-lm(data$SilicaPercentage~data$TWT+data$Genotype)
summary(model4)

model5<-lm(data$SilicaPercentage~data$TWT*data$Genotype)
summary(model5)

anova(model1,model2,model3,model4,model5)
```

```r
###RDA
dataset<-read.table("SilicaData.csv",sep=",",header=TRUE)

X<-dataset[,c(7,8, 10, 13, 16:22)]
X<-na.omit(X)

myrda<-rda(X[,-c(1:3)]~X$Trt+Condition(X$Genotype+X$Specie),scale=TRUE,
na.action=na.exclude)

sc<-scores(myrda,display=c("species","sites","bp","cn"),scaling=3,choices=c(1:7))

A<-as.data.frame(sc$sites)
B<-as.data.frame(sc$species)
C<-as.data.frame(sc$biplot)
D<-as.data.frame(sc$centroids)

round((myrda$CA$ei/sum(myrda$CA$ei))*100,2)

plotmyrda<-ggplot(data=A,aes(x=RDA1,y=PC1))+
  geom_vline(xintercept=0,colour='gray70',linetype='dashed')+

  # Sites (samples)
  geom_point(aes(color=factor(X$Trt),shape=factor(X$Specie)),size=2)+

  # Species (variables)
  geom_point(data=B,colour='black',size=3)+
  geom_text_repel(data=B,aes(fontface='bold',label=rownames(B)),colour='black',
size=5)+

  # Biplot (predictors)
  geom_segment(data=C,aes(x=0,y=0,xend=RDA1,yend=PC1),
arrow=arrow(length=unit(0.1,'inches')),size=0.5,color='gray50')+

geom_text(data=C,aes(x=RDA1,y=PC1,label=rownames(C),fontface='bold'),color='gray50'
,vjust=0,nudge_y=0.1,size=8)+

  # Aesthetics
  theme_bw()+
  theme(legend.background=element_rect(size=0.5,linetype='solid',colour='gray24'))+
  theme(legend.position="bottom")+
  theme(legend.title=element_blank())+
  labs(x=paste('PC1 (', '36.10', '%)',sep=''),
       y=paste('PC2 (', '18.85', '%)',sep=''))+
  scale_color_manual(labels=c("Water stress","Well
watered"),values=c("cadetblue3","deepskyblue4"))+
  scale_shape_manual(labels=c('Finger millet','Pearl
millet','Sorghum'),values=c(15,19,17))+
  ggtitle("RDA")+
  theme(plot.title=element_text(hjust=0.5,size=10,face="bold"))

plotmyrda

# Save the plot
ggsave(filename="RDA_Condition(SpeGeno).jpeg",plot=plotmyrda,dpi="print",units="in"
,width=8,height=8)
```