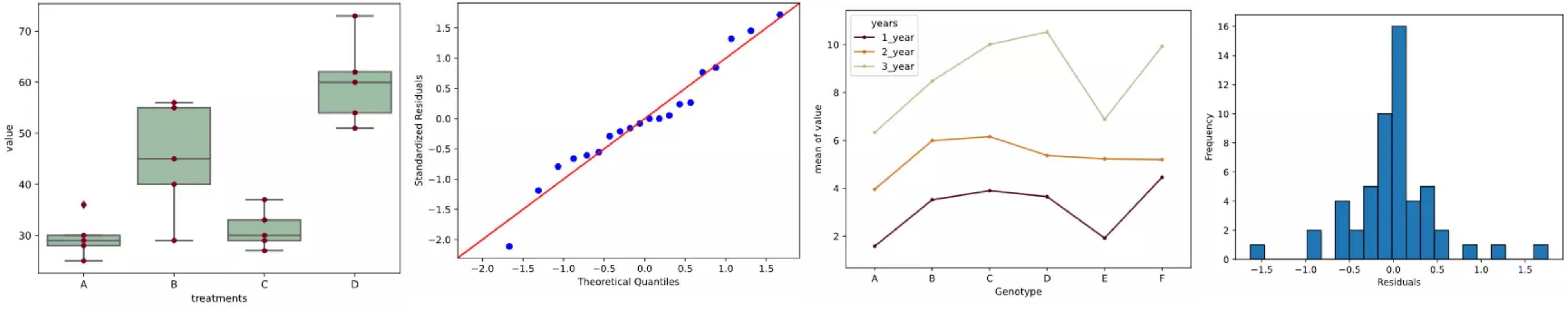
ANOVA using Python (with examples)

Renesh Bedre 15 minute read [Follow @renbedre](https://twitter.com/renbedre?ref_src=twsrc%5Etfw) (https://twitter.com/renbedre?ref_src=twsrc%5Etfw)



What is ANOVA (ANalysis Of VAriance)?

- ANOVA test used to compare the means of more than 2 groups (t-test can be used to compare 2 groups)
- Groups mean differences inferred by analyzing variances
- ANOVA uses variance-based *F* test to check the group mean equality. Sometimes, ANOVA *F* test is also called omnibus test as it tests non-specific null hypothesis i.e. all group means are equal
- Main types: One-way (one factor) and two-way (two factors) ANOVA (factor is an independent variable)

Note: In ANOVA, group, factors, and independent variables are similar terms

ANOVA Hypotheses

- *Null hypothesis*: Groups means are equal (no variation in means of groups)
  H_0: $\mu_1=\mu_2=\ldots=\mu_p$
- *Alternative hypothesis*: At least, one group mean is different from other groups
  H_1: All μ are not equal

Learn more about hypothesis testing and interpretation (https://www.reneshbedre.com/blog/hypothesis-testing.html)

ANOVA Assumptions

- Residuals (experimental error) are normally distributed (Shapiro-Wilks Test)
- Homogeneity of variances (variances are equal between treatment groups) (Levene’s or Bartlett’s Test)
- Observations are sampled independently from each other

How ANOVA works?

- Check sample sizes: equal number of observation in each group
- Calculate Mean Square for each group (MS) (SS of group/level-1); level-1 is a degree of freedom (df) for a group
- Calculate Mean Square error (MSE) (SS error/df of residuals)
- Calculate *F* value (MS of group/MSE)

One-way (one factor) ANOVA with Python

ANOVA effect model, table, and formula

$$y_{ik} = \mu + \alpha_i + \epsilon_{ik}$$

$$SS_T = SS_B + SS_E$$

Where, y_{ik} = k^{th} observation of i^{th} level of groups,
μ = overall population mean (unknown) ,
α_i = Main effect for groups (deviation from the μ) ,
ϵ_{ik} = Error,
i = levels for groups (i = 1,2...,p) ,
k = Observations or replicates for each group (k = 1,2...,r) ,

Source of variation	**degree of freedom (Df)**	**Sum of squares (SS)**	**Mean square (MS)**	***F* value**	**Significance**
Group (between)	Df_B = p-1	SS_B	$MS_B = SS_B/Df_B$	MS_B/MS_E	*p* value
Residuals or error (within)	Df_E = p(r-1)	SS_E	$MS_E = SS_E/Df_E$		
Total	Df_T = pr-1	SS_T			

Where, $SS_B = \sum_i p_i(\bar{y_{i.}} - \bar{y_{..}})^2$,

$$SS_E = \sum_{ik}(y_{ik} - \bar{y_{i.}})^2,$$

$$SS_T = SS_B + SS_E = \sum_{ik}(y_{ik} - \bar{y_{..}})^2,$$

ANOVA example

Example data for one-way ANOVA analysis tutorial, dataset (https://www.reneshbedre.com/assets/posts/anova/onewayanova.txt)

A	B	C	D
25	45	30	54
30	55	29	60
28	29	33	51
36	56	37	62
29	40	27	73

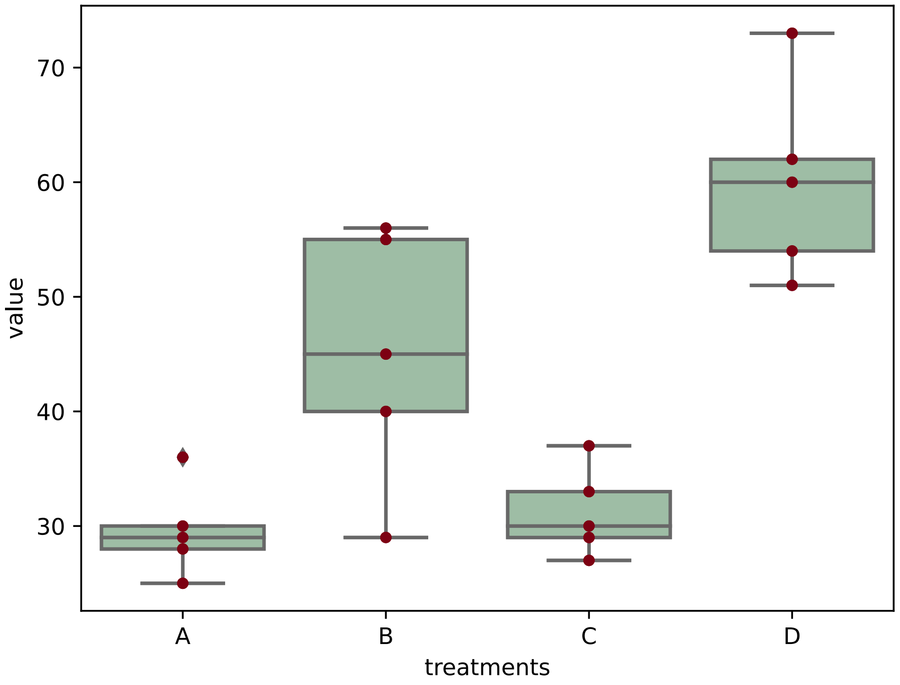
Here, there are four treatments (A, B, C, and D), which are groups for ANOVA analysis. Treatments are independent variable and termed as factor. As there are four types of treatments, treatment factor has four levels.

For this experimental design, there is only factor (treatments) or independent variable to evaluate, and therefore, one-way ANOVA method is suitable for analysis.

Note: If you have your own dataset, you should import it as pandas dataframe. Learn how to import data using pandas (https://www.reneshbedre.com/blog/import-data-pandas.html)

```
# I am using Python 3
# load packages
import pandas as pd
# load data file
df = pd.read_csv("https://reneshbedre.github.io/assets/posts/anova/onewayanova.txt", sep="\t")
# reshape the d dataframe suitable for statsmodels package
df_melt = pd.melt(df.reset_index(), id_vars=['index'], value_vars=['A', 'B', 'C', 'D'])
# replace column names
df_melt.columns = ['index', 'treatments', 'value']

# generate a boxplot to see the data distribution by treatments. Using boxplot, we can
# easily detect the differences between different treatments
import matplotlib.pyplot as plt
import seaborn as sns
ax = sns.boxplot(x='treatments', y='value', data=df_melt, color='#99c2a2')
ax = sns.swarmplot(x="treatments", y="value", data=df_melt, color='#7d0013')
plt.show()
```


value
70
60
50
40
30
A
B
C
D
treatments

```
# load packages
import scipy.stats as stats
# stats f_oneway functions takes the groups as input and returns ANOVA F and p value
fvalue, pvalue = stats.f_oneway(df['A'], df['B'], df['C'], df['D'])
print(fvalue, pvalue)
# 17.492810457516338 2.639241146210922e-05

# get ANOVA table as R like output
import statsmodels.api as sm
from statsmodels.formula.api import ols

# Ordinary Least Squares (OLS) model
model = ols('value ~ C(treatments)', data=df_melt).fit()
anova_table = sm.stats.anova_lm(model, typ=2)
anova_table
# output (ANOVA F and p value)
                sum_sq    df         F    PR(>F)
C(treatments)  3010.95   3.0  17.49281  0.000026
Residual        918.00  16.0       NaN       NaN

# ANOVA table using bioinfokit v1.0.3 or later (it uses wrapper script for anova_lm)
from bioinfokit.analys import stat
res = stat()
res.anova_stat(df=df_melt, res_var='value', anova_model='value ~ C(treatments)')
res.anova_summary
# output (ANOVA F and p value)
                 df   sum_sq   mean_sq         F    PR(>F)
C(treatments)   3.0  3010.95  1003.650  17.49281  0.000026
Residual       16.0   918.00    57.375       NaN       NaN

# note: if the data is balanced (equal sample size for each group), Type 1, 2, and 3 sums of squares
# (typ parameter) will produce similar results.
```

Interpretation

The *p* value obtained from ANOVA analysis is significant (*p* < 0.05), and therefore, we conclude that there are significant differences among treatments.

> **Note**: If you have unbalanced (unequal sample size for each group) data, you can perform similar steps as described for one-way ANOVA with balanced design (equal sample size for each group).

From ANOVA analysis, we know that treatment differences are statistically significant, but ANOVA does not tell which treatments are significantly different from each other. To know the pairs of significant different treatments, we will perform multiple pairwise comparison (**post hoc comparison**) analysis for all unplanned comparison using **Tukey’s honestly significantly differenced (HSD)** test.

Tukey’s HSD test accounts for multiple comparisons and corrects for family-wise error rate (FWER) (https://www.reneshbedre.com/blog/multiple-hypothesis-testing-corrections.html) (inflated type I error)

> **Tukey and Tukey-kramer formula**,
>
> Tukey's HSD (When equal sample size in each group),
>
> $$HSD = q_{A,\alpha,dof}\sqrt{\frac{MS_E}{n}}$$
>
> Tukey-Kramer method (When unequal sample size in each group),
>
> $$HSD = q_{A,\alpha,dof}\sqrt{\frac{MS_E}{2}(\frac{1}{n_i}+\frac{1}{n_j})}$$
>
> Where,

$q_{A,\alpha,dof}$ = studentized range statistic with A number of groups, α significance level (0.05 or 0.01), and dof degrees of freedom
MS_E = mean square error from ANOVA
n = sample size in each group (when the sample size is equal in two comparing groups)
n_i, n_j = sample size in group i and j (when the sample size is unequal in two comparing groups)

Alternatively, Scheffe’s method is completely coherent with ANOVA and considered as more appropriate post hoc test for significant ANOVA for all unplanned comparisons. However, it is highly conservative than other post hoc tests.

```
# we will use bioinfokit (v1.0.3 or later) for performing tukey HSD test
# check documentation here https://github.com/reneshbedre/bioinfokit
from bioinfokit.analys import stat
# perform multiple pairwise comparison (Tukey's HSD)
# unequal sample size data, tukey_hsd uses Tukey-Kramer test
res = stat()
res.tukey_hsd(df=df_melt, res_var='value', xfac_var='treatments', anova_model='value ~ C(treatments)')
res.tukey_summary
# output
  group1 group2  Diff      Lower      Upper   q-value   p-value
0      A      B  15.4   1.692871  29.107129  4.546156  0.025070
1      A      C   1.6 -12.107129  15.307129  0.472328  0.900000
2      A      D  30.4  16.692871  44.107129  8.974231  0.001000
3      B      C  13.8   0.092871  27.507129  4.073828  0.048178
4      B      D  15.0   1.292871  28.707129  4.428074  0.029578
5      C      D  28.8  15.092871  42.507129  8.501903  0.001000

# Note: p-value 0.001 from tukey_hsd output should be interpreted as <=0.001
```

Above results from Tukey’s HSD suggests that except A-C, all other pairwise comparisons for treatments rejects null hypothesis (*p* < 0.05) and indicates statistical significant differences.

Note: Tukey's HSD test is conservative and increases the critical value to control the experimentwise type I error rate. If you have a large number of comparisons (say > 10 or 20) to make using Tukey's test, there may be chances that you may not get significant results for all or expected pairs. If you are interested in only specific or few comparisons and you won't find significant differences using Tukey's test, you may split the data for specific comparisons or use the *t*-test

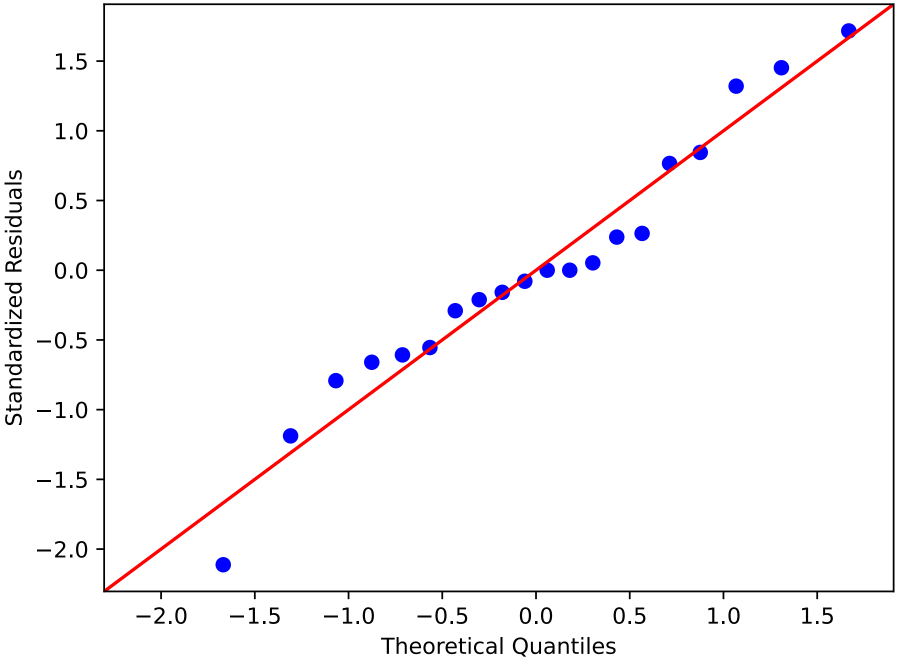
Test ANOVA assumptions

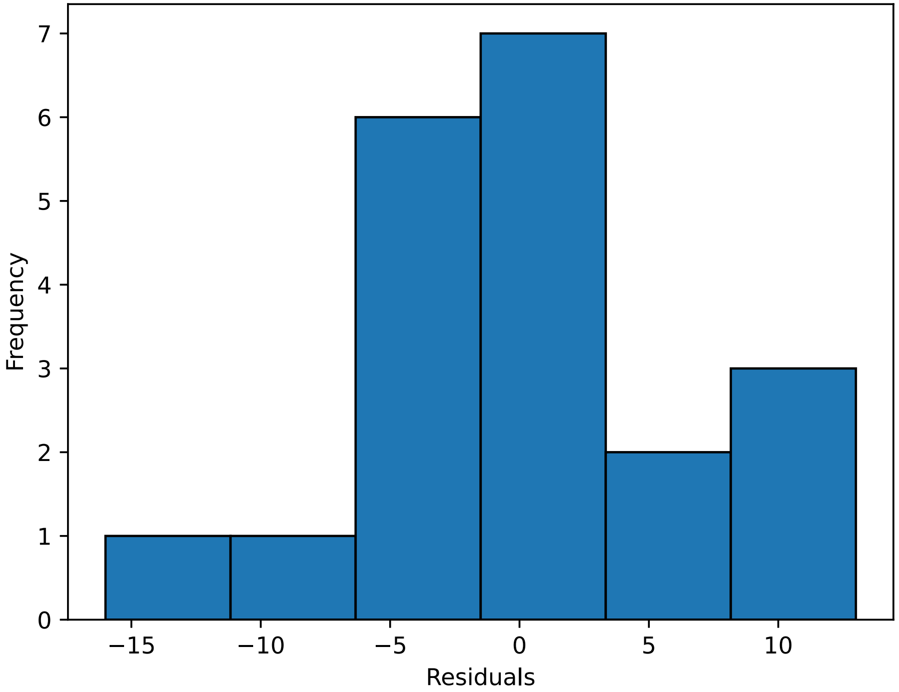
- ANOVA assumptions can be checked using test statistics (e.g. Shapiro-Wilk, Bartlett's, Levene's test) and the visual approaches such as residual plots (e.g. QQ-plots) and histograms.
- The visual approaches perform better than statistical tests. For example, the Shapiro-Wilk test has low power for small sample size data and deviates significantly from normality for large sample sizes.

Now, I will generate QQ-plot from standardized residuals (outliers can be easily detected from standardized residuals than normal residuals)

```
# QQ-plot
import statsmodels.api as sm
import matplotlib.pyplot as plt
# res.anova_std_residuals are standardized residuals obtained from ANOVA (check above)
sm.qqplot(res.anova_std_residuals, line='45')
plt.xlabel("Theoretical Quantiles")
plt.ylabel("Standardized Residuals")
plt.show()

# histogram
plt.hist(res.anova_model_out.resid, bins='auto', histtype='bar', ec='k')
plt.xlabel("Residuals")
plt.ylabel('Frequency')
plt.show()
```


Standardized Residuals
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0
−2.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
Theoretical Quantiles


Frequency
7
6
5
4
3
2
1
0
−15
−10
−5
0
5
10
Residuals

As the standardized residuals lie around the 45-degree line, it suggests that the residuals are approximately normally distributed

In the histogram, the distribution looks approximately normal and suggests that residuals are approximately normally distributed

Shapiro-Wilk test can be used to check the **normal distribution of residuals**. *Null hypothesis*: data is drawn from normal distribution.

```
# load packages
import scipy.stats as stats
w, pvalue = stats.shapiro(model.resid)
print(w, pvalue)
# 0.9685019850730896 0.7229772806167603
```

As the *p* value is non significant, we fail to reject null hypothesis and conclude that data is drawn from normal distribution.

As the data is drawn from normal distribution, use Bartlett’s test to check the **Homogeneity of variances**. *Null hypothesis*: samples from populations have equal variances.

```
# load packages
import scipy.stats as stats
w, pvalue = stats.bartlett(df['A'], df['B'], df['C'], df['D'])
print(w, pvalue)
5.687843565012841 0.1278253399753447

# if you have a stacked table, you can use bioinfokit v1.0.3 or later for the bartlett's test
from bioinfokit.analys import stat
res = stat()
res.bartlett(df=df_melt, res_var='value', xfac_var='treatments')
res.bartlett_summary
# output
                 Parameter   Value
0      Test statistics (T)  5.6878
1  Degrees of freedom (Df)  3.0000
2                  p value  0.1278
```

As the *p* value (0.12) is non significant, we fail to reject null hypothesis and conclude that treatments have equal variances.

Levene's test can be used to check the Homogeneity of variances when the data is not drawn from normal distribution.

```
# if you have a stacked table, you can use bioinfokit v1.0.3 or later for the Levene's test
from bioinfokit.analys import stat
res = stat()
res.levene(df=df_melt, res_var='value', xfac_var='treatments')
res.levene_summary
# output
                 Parameter   Value
0      Test statistics (W)  1.9220
1  Degrees of freedom (Df)  3.0000
2                  p value  0.1667
```

Two-way (two factor) ANOVA (factorial design) with Python

Example data for two-way ANOVA analysis tutorial, dataset (https://www.reneshbedre.com/assets/posts/anova/twowayanova.txt)

From dataset, there are two factors (independent variables) viz. genotypes and yield in years. Genotypes and years has five and three levels respectively (see one-way ANOVA to know factors and levels).

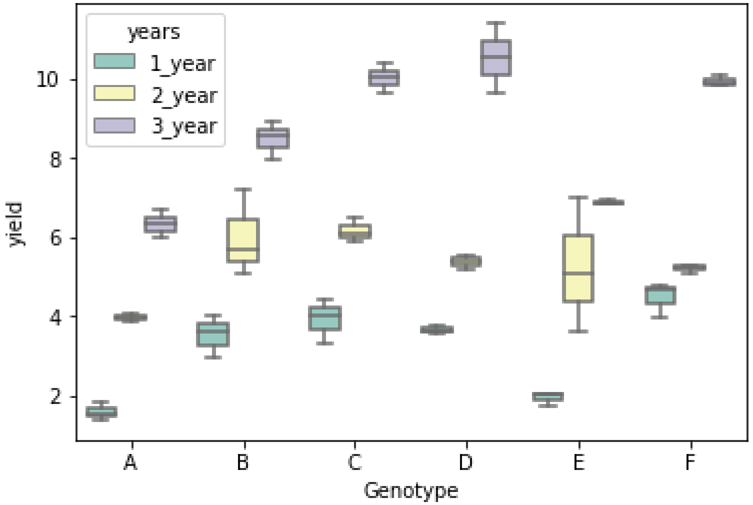
For this experimental design, there are two factors to evaluate, and therefore, two-way ANOVA method is suitable for analysis. Here, using two-way ANOVA, we can simultaneously evaluate how type of genotype and years affects the yields of plants. If you apply one-way ANOVA here, you can able to evaluate only one factor at a time.

From two-way ANOVA, we can tests three hypotheses 1) effect of genotype on yield 2) effect of time (years) on yield, and 3) effect of genotype and time (years) interactions on yield

Note: If you have your own dataset, you should import it as pandas dataframe. Learn how to import data using pandas (https://www.reneshbedre.com/blog/import-data-pandas.html)

```
# load packages
import pandas as pd
import seaborn as sns
# load data file
d = pd.read_csv("https://reneshbedre.github.io/assets/posts/anova/twowayanova.txt", sep="\t")
# reshape the d dataframe suitable for statsmodels package
# you do not need to reshape if your data is already in stacked format. Compare d and d_melt tables for detail
# understanding
d_melt = pd.melt(d, id_vars=['Genotype'], value_vars=['1_year', '2_year', '3_year'])
# replace column names
d_melt.columns = ['Genotype', 'years', 'value']
d_melt.head()
# output
  Genotype   years  value
0        A  1_year   1.53
1        A  1_year   1.83
2        A  1_year   1.38
3        B  1_year   3.60
4        B  1_year   2.94

# generate a boxplot to see the data distribution by genotypes and years. Using boxplot, we can easily detect the
# differences between different groups
sns.boxplot(x="Genotype", y="value", hue="years", data=d_melt, palette="Set3")
```


years
1_year
2_year
3_year
yield
10
8
6
4
2
A
B
C
D
E
F
Genotype

```
# load packages
import statsmodels.api as sm
from statsmodels.formula.api import ols
# Ordinary Least Squares (OLS) model
# C(Genotype):C(years) represent interaction term
model = ols('value ~ C(Genotype) + C(years) + C(Genotype):C(years)', data=d_melt).fit()
anova_table = sm.stats.anova_lm(model, typ=2)
anova_table
# output
                          sum_sq    df           F        PR(>F)
C(Genotype)            58.551733   5.0   32.748581  1.931655e-12
C(years)              278.925633   2.0  390.014868  4.006243e-25
C(Genotype):C(years)   17.122967  10.0    4.788525  2.230094e-04
Residual               12.873000  36.0         NaN           NaN

# ANOVA table using bioinfokit v1.0.3 or later (it uses wrapper script for anova_lm)
from bioinfokit.analys import stat
res = stat()
res.anova_stat(df=d_melt, res_var='value', anova_model='value~C(Genotype)+C(years)+C(Genotype):C(years)')
res.anova_summary
# output
                        df      sum_sq     mean_sq           F        PR(>F)
C(Genotype)            5.0   58.551733   11.710347   32.748581  1.931655e-12
C(years)               2.0  278.925633  139.462817  390.014868  4.006243e-25
C(Genotype):C(years)  10.0   17.122967    1.712297    4.788525  2.230094e-04
Residual              36.0   12.873000    0.357583         NaN           NaN
```

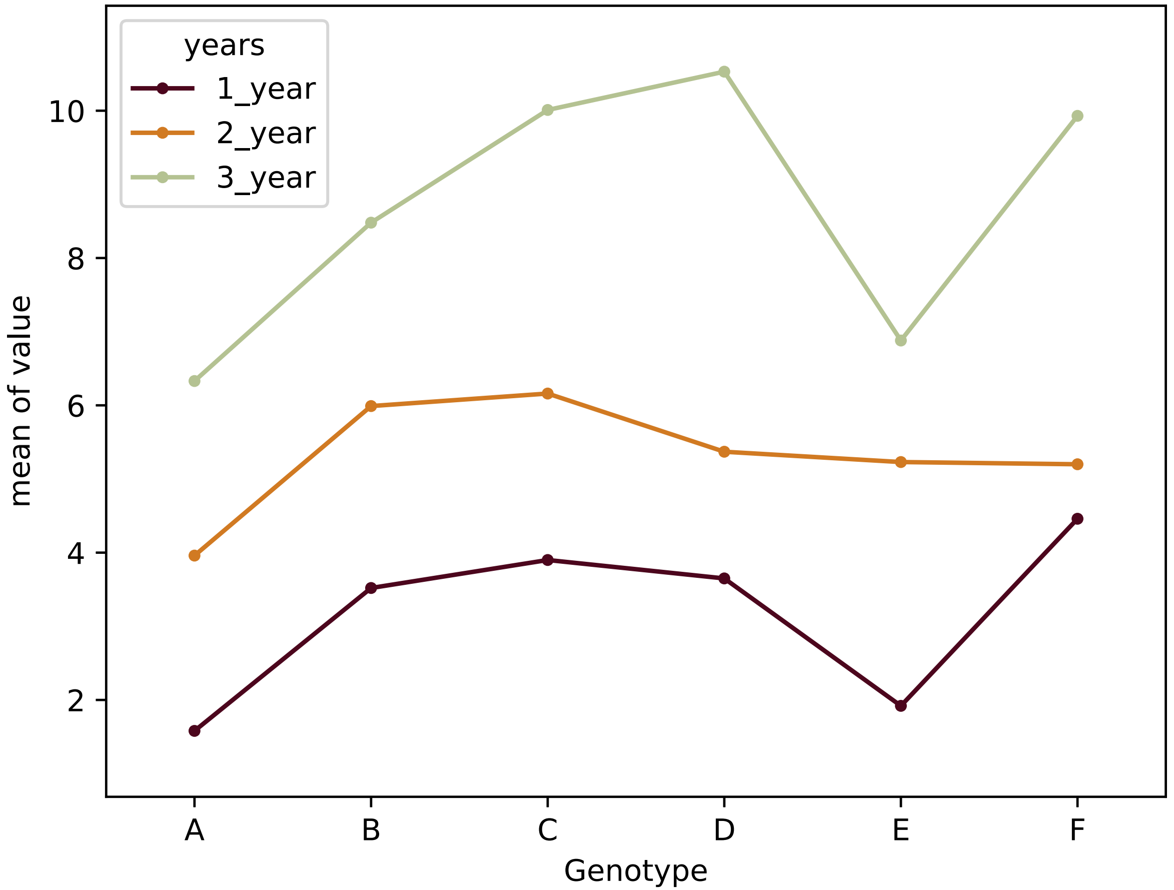
Note: If you have unbalanced (unequal sample size for each group) data, you can perform similar steps as described for two-way ANOVA with the balanced design but set $typ = 3$. Type 3 sums of squares (SS) is recommended for an unbalanced design for multifactorial ANOVA.

Interpretation

The *p* value obtained from ANOVA analysis for genotype, years, and interaction are statistically significant (*p*<0.05). We conclude that type of genotype significantly affects the yield outcome, time (years) significantly affects the yield outcome, and interaction of both genotype and time (years) significantly affects the yield outcome.

As the interaction is significant, let’s visualize the interaction plot (also called profile plot) for interaction effects,

```
from statsmodels.graphics.factorplots import interaction_plot
import matplotlib.pyplot as plt
fig = interaction_plot(x=d_melt['Genotype'], trace=d_melt['years'], response=d_melt['value'],
    colors=['#4c061d','#d17a22', '#b4c292'])
plt.show()
```



- The interaction plot helps to visualize the means of the response of the two factors (Genotype and years) on one graph. Generally, the X-axis should have a factor with more levels.
- From the interaction plot, the interaction effect is significant between the Genotype and years because three lines are not parallel (roughly parallel factor lines indicate no interaction - additive model). This interaction is also called ordinal interaction as the lines do not cross each other.
- For a more reliable conclusion of the interaction plot, it should be verified with the *F* test for interaction

Multiple pairwise comparisons (Post-hoc test)

Now, we know that genotype and time (years) differences are statistically significant, but ANOVA does not tell which genotype and time (years) are significantly different from each other. To know the pairs of significant different genotype and time (years), perform multiple pairwise comparison (**Post-hoc comparison**) analysis using **Tukey’s HSD** test.

```
# we will use bioinfokit (v1.0.3 or later) for performing tukey HSD test
# check documentation here https://github.com/reneshbedre/bioinfokit
from bioinfokit.analys import stat
# perform multiple pairwise comparison (Tukey HSD)
# unequal sample size data, tukey_hsd uses Tukey-Kramer test
res = stat()
# for main effect Genotype
res.tukey_hsd(df=d_melt, res_var='value', xfac_var='Genotype', anova_model='value~C(Genotype)+C(years)+C(Genotype):C(years)')
res.tukey_summary
# output
   group1 group2      Diff     Lower     Upper    q-value   p-value
0       A      B  2.040000  1.191912  2.888088  10.234409  0.001000
1       A      C  2.733333  1.885245  3.581421  13.712771  0.001000
2       A      D  2.560000  1.711912  3.408088  12.843180  0.001000
3       A      E  0.720000 -0.128088  1.568088   3.612145  0.135306
4       A      F  2.573333  1.725245  3.421421  12.910072  0.001000
5       B      C  0.693333 -0.154755  1.541421   3.478361  0.163609
6       B      D  0.520000 -0.328088  1.368088   2.608771  0.453066
7       B      E  1.320000  0.471912  2.168088   6.622265  0.001000
8       B      F  0.533333 -0.314755  1.381421   2.675663  0.425189
9       C      D  0.173333 -0.674755  1.021421   0.869590  0.900000
10      C      E  2.013333  1.165245  2.861421  10.100626  0.001000
11      C      F  0.160000 -0.688088  1.008088   0.802699  0.900000
12      D      E  1.840000  0.991912  2.688088   9.231036  0.001000
13      D      F  0.013333 -0.834755  0.861421   0.066892  0.900000
14      E      F  1.853333  1.005245  2.701421   9.297928  0.001000

# Note: p-value 0.001 from tukey_hsd output should be interpreted as <=0.001

# for main effect years
res.tukey_hsd(df=d_melt, res_var='value', xfac_var='years', anova_model='value ~ C(Genotype) + C(years) + C(Genotype):C(years)')
res.tukey_summary
# output
```

```
   group1  group2      Diff     Lower     Upper    q-value  p-value
0  1_year  2_year  2.146667  1.659513  2.633821  15.230432    0.001
1  1_year  3_year  5.521667  5.034513  6.008821  39.175794    0.001
2  2_year  3_year  3.375000  2.887846  3.862154  23.945361    0.001

# for interaction effect between genotype and years
res.tukey_hsd(df=d_melt, res_var='value', xfac_var=['Genotype','years'], anova_model='value ~ C(Genotype) + C(years) +
C(Genotype):C(years)')
res.tukey_summary.head()
# output
        group1       group2  Diff     Lower     Upper    q-value   p-value
0  (A, 1_year)  (A, 2_year)  2.38  0.548861  4.211139   6.893646  0.002439
1  (A, 1_year)  (A, 3_year)  4.75  2.918861  6.581139  13.758326  0.001000
2  (A, 1_year)  (B, 1_year)  1.94  0.108861  3.771139   5.619190  0.028673
3  (A, 1_year)  (B, 2_year)  4.41  2.578861  6.241139  12.773520  0.001000
4  (A, 1_year)  (B, 3_year)  6.90  5.068861  8.731139  19.985779  0.001000
```

Test ANOVA assumptions

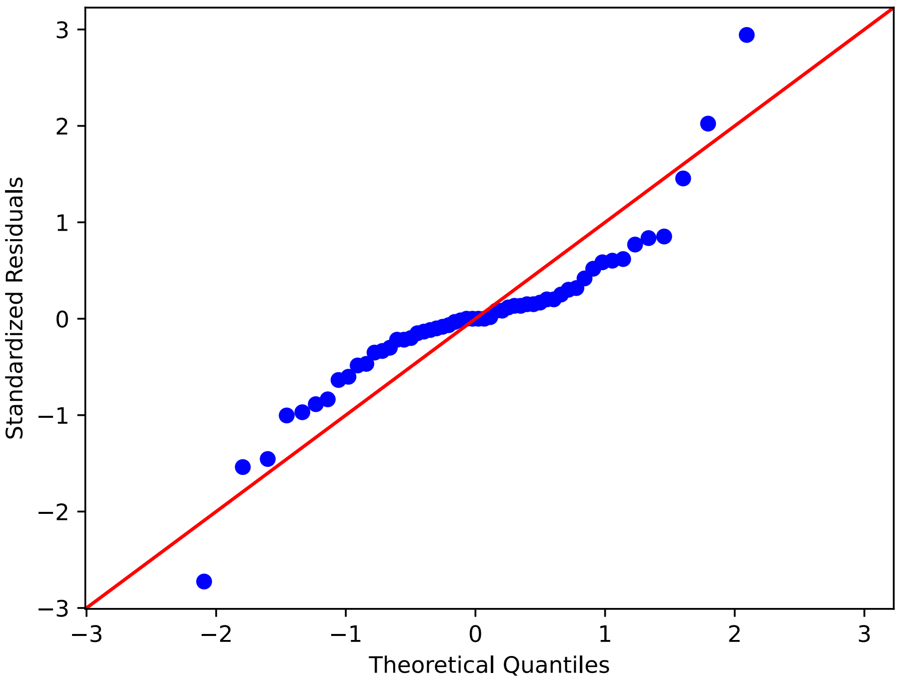
Similar to one-way ANOVA, you can use visual approaches, **Bartlett’s** or **Levene’s**, and **Shapiro-Wilk test** to validate the assumptions for homogeneity of variances and normal distribution of residuals.

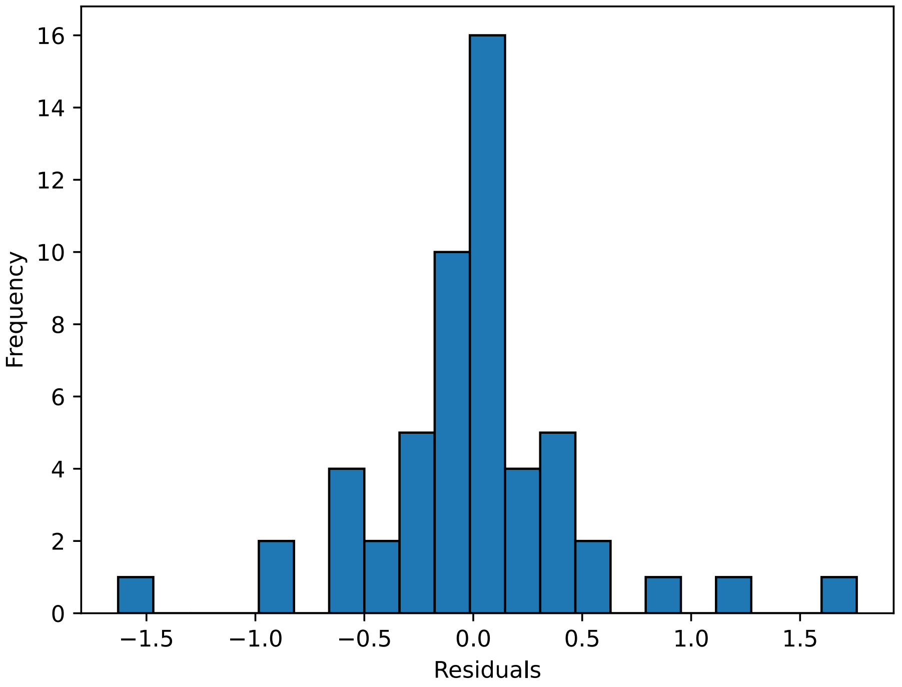
```
# QQ-plot
import statsmodels.api as sm
import matplotlib.pyplot as plt
# res.anova_std_residuals are standardized residuals obtained from two-way ANOVA (check above)
sm.qqplot(res.anova_std_residuals, line='45')
plt.xlabel("Theoretical Quantiles")
plt.ylabel("Standardized Residuals")
plt.show()

# histogram
plt.hist(res.anova_model_out.resid, bins='auto', histtype='bar', ec='k')
plt.xlabel("Residuals")
plt.ylabel('Frequency')
plt.show()

# Shapiro-Wilk test
import scipy.stats as stats
w, pvalue = stats.shapiro(res.anova_model_out.resid)
print(w, pvalue)
0.8978844881057739 0.00023986754240468144
```

Even though we rejected the Shapiro-Wilk test statistics ($p < 0.05$), we should further look for the residual plots and histograms. In the residual plot, standardized residuals lie around the 45-degree line, it suggests that the residuals are approximately normally distributed. Besides, the histogram shows the approximately normal distribution of residuals.


Standardized Residuals
3
2
1
0
−1
−2
−3
−3
−2
−1
0
1
2
3
Theoretical Quantiles


Frequency
16
14
12
10
8
6
4
2
0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
Residuals

> **Note**: The ANOVA model is remarkably robust to the violation of normality assumption, which means that it will have a non-significant effect on Type I error rate and *p* values will remain reliable as long as there are no outliers

We will use Levene's test to check the assumption of homogeneity of variances,

```
# if you have  astacked table, you can use bioinfokit v1.0.3 or later for the Levene's test
from bioinfokit.analys import stat
res = stat()
res.levene(df=d_melt, res_var='value', xfac_var=['Genotype', 'years'])
res.levene_summary
# output
                 Parameter    Value
0      Test statistics (W)   1.6849
1  Degrees of freedom (Df)  17.0000
2                  p value   0.0927
```

As the *p* value (0.09) is non-significant, we fail to reject the null hypothesis and conclude that treatments have equal variances.

References

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- Mangiafico, S.S. 2015. An R Companion for the Handbook of Biological Statistics, version 1.3.2.
- Knief U, Forstmeier W. Violating the normality assumption may be the lesser of two evils. bioRxiv. 2018 Jan 1:498931.
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- Ruxton GD, Beauchamp G. Time for some a priori thinking about post hoc testing. Behavioral ecology. 2008 May 1;19(3):690-3.

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Tags: Statistics

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