

Running title: yield and industrial parameters of oats

Keywords: oats, fungicide application, de-hulling index, yield attributes, quality parameters, multivariate techniques, plant stature.

25 **Abbreviations**

26 PCA-Principal component analysis; PLS-DA –Partial least squares discriminant analysis; OLS-R –
27 Ordinary least squares regression, LV-latent variables, VIP-variable importance projections, AIC-
28 akaike information criterion.

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30

31 **1. Introduction**

32 Oats (*Avena sativa* L.) are an important spring cereals (Sánchez-Martín et al. 2014; Soltani et al.
33 2012). They are fast growing and produces significant amount of fresh fodder within short period
34 (60-70 days) with adequate nutritional value (Bilal et al. 2016). Independent of the end use,
35 quality requirements are of great importance. The identification of agronomic traits that meet
36 the demand of farmers, industry quality and final consumers, and their incorporation into elite
37 oat germplasm are crucial to the development of successful new oat cultivars. Some of the most
38 important traits selected by the Brazilian Oat Breeding Program include wide adaptation, high
39 grain yield and grain quality, short plant cycle, reduced plant height, lodging resistance, frost
40 tolerance, aluminum tolerance, and genetic resistance to the main diseases, such as crown and
41 stem rust, leaf spot, fusarium, and barley yellow dwarf virus (Nava et al. 2016). In this research
42 the use of a mixture of fungicides claimed to have growth retardant effect is explored aiming a
43 better assessment of yield attributes and quality parameters of oats.

44

45 Triazole fungicides are one of the top ten classes of current-use pesticides and have higher
46 consumption as compared to other fungicides available worldwide (Zhou et al. 2016). They inhibit
47 the biosynthesis of fungal ergosterol (Song & Nes, 2007) and provide a broad spectrum of
48 activities (Yang et al. 2014) such as plant growth regulators (Yang et al. 2014). The mechanism of
49 regulatory effect appears to be that they shift the phytohormone balance in plant tissues and

50 inhibit the biosynthesis of gibberellic acid, which leads to a transient raise in abscisic acid content
 51 in plants (Yang et al. 2014) Tebuconazole (TEB) is one of a common triazole fungicide that has
 52 been extensively used in grains, vegetables and fruits for the control of plant pathogenic fungi
 53 (Yang et al. 2014; Marin et al. 2013; Patyal et al. 2013). Additionally, the strobilurins are a new
 54 class of fungicidal compounds and among them is the recently introduced trifloxystrobin
 55 (Reuveni, 2000; Diamantopoulou et al. 2006) that is highly effective against several plant
 56 pathogens (Veloukas et al. 2007; Bubici et al. 2006; Pérez et al. 2002). Mode of action of the
 57 strobilurins towards pathogenic fungi was found to block the mitochondrial respiratory electron
 58 transfer chain between cytochrome b and cytochrome c1 at the ubiquinol oxidizing site (Qo) of
 59 the complex III, ultimately leading to losses of ATP synthesis and inhibition of cellular respiration,
 60 which is also deemed to contribute its broad-spectrum antifungal activity (Cao et al. 2015).
 61 Triazoles and strobilurins have been reported to have secondary properties (e.g., growth
 62 regulator) and have been used to prevent the plant lodging (Matysiak & Kaczmarek, 2013). From
 63 a biochemical point of view, the properties of some triazoles are due to their dual effect on
 64 plants: inhibition of the biosynthesis of gibberellins in the plant (retardant properties), and
 65 inhibition of the biosynthesis of sterols (fungicide properties - Matysiak & Kaczmarek, 2013). As
 66 growth regulators their act not only as chemicals that modulate plant growth but they also act as
 67 signal molecules under various biotic and abiotic stresses (Spence & Bais, 2015). As growth
 68 retardants, triazoles and strobilurins are applied in agronomic and horticultural crops to reduce
 69 unwanted longitudinal shoot growth without lowering plant productivity. Most growth retardants
 70 act by inhibiting gibberellin (GA) biosynthesis (Rademacher, 2000). Currently, it is a common
 71 practice in crop protection to apply multiple pesticide mixtures instead of individual pesticides.
 72 This form of pesticide application likely results in the combined effect (Wang et al. 2016). In this
 73 research the retardant effect of the fungicides is explored in oat cultivars aiming to ameliorate
 74 the lodging index and thereby improve yield and quality requirements of oats. Multivariate

75 techniques were used to assess yield attributes and quality requirements of oat cultivars
76 subjected to two different treatments (without and with fungicide application)

77 **2. Materials and Methods**

78 *2.1. On farm experiments*

79 Subsequent on farm experiments were conducted in Lages (Santa Catarina State, Southern Brazil,
80 on an experimental research field of the Santa Catarina State University) from 2012 to 2015, in
81 completely randomized block design (CRBD) with split plots with and without aerial fungicide
82 application of a mixture of tebuconazole (triazole group) (150 g/Ha) and trifloxystrobin
83 (strobilurin group) (56 g/Ha). All other agronomic practices (such as soil fertilization, weed and
84 disease control) were performed following the Brazilian commission for oat research
85 recommendations ("Comissão Brasileira de Pesquisas em Aveia"-2006). Each plot (useful area)
86 consisted of 5 central lines of 5m of length spaced at 0.2m with 3 repetitions. A mechanized
87 harvest of the oat grains was done for subsequent laboratory analyses. A total of 21 oat cultivars
88 were evaluated during four subsequent years (2012 to 2015).

89

90 *2.2. Yield components analysis*

91 Yield attributes such as plant stature (cm), grain yield (Kg/Ha) and weight of 1000 grains and sieve
92 yield (>2mm) were evaluated at each experiment (2012-2015). Other yield attributes were
93 evaluated previously (unpublished data from 2007 to 2010).

94

95 *2.2.1 Plant stature (cm):*

96 Evaluated on plants at growth stage GS91 (pre-harvest maturity) by measuring 10 plants in a
97 random points in each plot by the use of a ruler. The measurements were done from the soil
98 surface until the apical spikelet located on the top of the panicle and values expressed as mean of
99 10 plants according to equation 1.

$$PS (cm) = \frac{\sum_{n=1}^{n=10} ps}{N} \quad (\text{Eq. 1})$$

Where PS is the plant stature in cm; ps-individual plant stature, N is the total number of plants evaluated in each plot (10).

2.2.2 Sieve yield (> 2 mm)

Refers to the percentage of grains with transverse diameter greater than 2 mm, This was evaluated after harvest, by weighing a sample of 250g and then submitted the sieve with regular stirring during a minute; in a sieve of 40 cm x 30 cm dimensions, rectangular, containing holes (sieve) of 2 mm x 20 mm. This process was done twice. The result was expressed by the fraction between the grain weight retained on the sieve and the initial weight (250 g) as represented in the equation 2.

$$Sy (\%) = \frac{RGW}{IGW} * 100 \quad (\text{Eq. 2})$$

Where Sy is the sieve yield (>2mm); RGW is the retained grain weight in a sieve and IGW is the initial grain weight (250g for this experiment).

2.2.3 Yield (Kg/Ha):

The grain yield was measured from each experimental plot (4 central linear meters of each plot, spaced at 0.2m, in a useful area of 2.4m²). The yield was then converted to hectare after weight correction using a standard moisture content of 13% according to equation 3.

$$119 \quad \left[yield = \frac{ww * \left(\frac{100 - rm}{100 - 13} \right)}{2.4 * 10000} \right] \quad (Eq. 3)$$

120 Where: ww – wet weight (Kg) and rm is the real weight moisture (%).

121 2.2.4 Weight of 1000 grains (Wg):

122 A sample of a thousand grains', obtained from each experimental plot were electronically
123 counted (Sanick ESC 2011) and weighted using a precision balance and expressed in grams (g).

124

125 2.3 Quality parameters

126 Quality requirement parameters of the grains independent of end use such as lodging index,
127 weight of hectoliter (Kg/100L), and the de-hulling index were evaluated.

128

129 2.3.1 The lodging index

130 Estimated visually and expressed as a percentage, taking into account the angle formed in the
131 vertical position of the plant stem in relation to the ground. 5m² of area was used. The
132 methodology of Moes and Stobbe (1991) was used and the lodging index expressed according to
133 the equation 4.

$$134 \quad [Li(\%) = I * A * 2] \quad (Eq.4)$$

135 Where (Li) is the lodging index; (I) -the degree of inclination of plants, ranging from 0 to 5, where
136 0 represents absence of inclination (all plants in a vertical position), while 5 indicates that all
137 plants are completely bedridden (horizontally); (A) represents the area with lodged plants,
138 ranging from 0 to 10, where 0 corresponds to the absence of lodged plants in the plot, and 10 all
139 plants lodged.

140

141 2.3.2 Weight of hectoliter (Kg/100L):

142 The weight of hectoliter was measured according to the Brazilian official wheat grain quality roles
143 (Brazil, Ministry of Agriculture, Livestock and supply, Normative role 38, from November 30 of
144 2010), using an hectoliter weight analyzer (Dalle-Molle, model T40EL, 0.25L of capacity). Briefly,
145 grain samples from each experimental plot were placed in the analyzer for volume determination
146 and then weighed. The hectoliter weight (HW) was then expressed according to equation 5.

147
$$[HW = W_v * 0.4]_{(Eq. 5)}$$

148 HW is the hectoliter weight; Wv represent the weight of the sample after volume determination in
149 analyzer and 0.4 is a calibration coefficient of the equipment.

150

151 2.3.4 De-hulling index (%):

152 A sample of 1000 grains was weighed, de-hulled manually and then weighed again. The de-hulling
153 index expressed as in equation 6.

154
$$\left[DHI (\%) = \frac{W_{1000g}}{wdh} * 100 \right]_{(Eq. 6)}$$

155 DHI is the de-hulling index, W1000g the weight of 1000 grains before de-hulling and wdh is the weight of
156 the sample after de-hulling.

157

158 *2.4 Data mining and Statistics*

159 Data from each year were summarized, subjected to normality test, homogeneity of variance
160 test, analysis of variance using a split plot design and where differences were found, Tukey HSD

($P < 0.05$) was used to test if the differences are statistically significant. Non-supervised multivariate analysis (principal component analysis-PCA) was also applied aiming to observe similarities between the tested cultivars and find variables main related to the similarities. Ordinary least squares regression models (OLS-R) were also applied aiming to find the importance of each variable and those mostly affected the cultivars and quality parameters. A second approach (supervised techniques) namely partial least squares discriminant analysis (PLS-DA) aiming to better classify the samples and find the main variables related to quality parameters were used for dimensionality reduction and find the between and within class variance of the dataset. All statistical analyses were done in R software (R core team, 2016, version 3.3.3) using functions, R packages and scripts produced by our research group. A report is also available as supplementary data for data reproducibility.

3. Results and discussions

Results of the analysis of variance (ANOVA) and multiple comparison tests (Tukey test, 5% of probability) of the four field experiments (2012 to 2015) showed to exist differences between cultivars and the effect of fungicide application regarding the de-hulling index (DHI), hectoliter weight (HW), loading index (Li), weight of 1000 grains (Wg), plant stature (PS), sieve yield (Sy) and grain yield (Yield- see supplementary report for ANOVA and multiple comparison tests). Statistically significant higher value of DHI was found for cultivar URS-BRAVA and the lower value observed for URS-FAPA-SLAVA (Tukey test, $p < 0.05$). Cultivars URS-GURIA and URS-TARIMBA presented higher loading index and lower indices were observed for IPR-AFRODITE, URS-TAURA and UPFA-OURO. Regarding the plant stature, cultivars URS21 and URS-GURIA presented plants with superior stature and URS-TAURA with lower stature. The sieve yield was higher in UPFPS-FARROUPILHA, URS-TORENA, URS-TAURA, URS-CORONA and URS-GUAPA and lower in the cultivar URS-FAPA-SLAVA. The weight of 1000 grains was found to be higher in the cultivars

FAEM006 and UPFPS-FARROUPILHA and lower in cultivars URS-FAPA-SLAVA, BARBARASUL, BRISASUL and URS-ESTAMPA. Finally, the grain yield was higher in UPFA-OURO and IPR-AFRODITE and lower in URS-ESTAMPA and FAEM5-CHIARASUL. Regarding the fungicide effect in all parameters evaluated, it was observed that fungicide application improved all parameters. Interestingly, the lodging index was higher without fungicide application (see supplementary report).

Previous studies (Bilal et al. 2012; Iannucci et al. 2011; Lodhi et al. 2009) have reported that cultivars have significant differences in plant height and variations are due to genetic make-up and can also be affected by nitrogen. According to Brunava et al. (2015), higher yield was found in plants with lower plant height. This parameter can be affected by agronomic management practices such as seeding rates, chemical seed treatments and foliar fungicide. In a research of Mourtzins and co-workers (2015), seeding rate and seed treatments did not show effect on oat yield, plant height and lodging index, but foliar fungicide application was considered mainly if disease is expected and improved the grain yield (Mourtzinis et al. 2015; Oliveira et al 2012). Higher values of the weight of 1000 grains were correlated with dough periods, good processing quality and are dependent of cultivar (Brunava et al. 2015).

Follmann and co-workers (2016) studying the genetic progress of oat cultivars with fungicide application reported an annual genetic progress in yield in 1% with fungicide and 0.08% without fungicide application. They reported that Fungicide application is feasible method. Fungicide application also improved the hectoliter weight. The hectoliter weight (HW) is a measure of the bulk density of the grain and is indicator of grain quality and millability. To ensure effective milling, the HW should be above 50 Kg.h⁻¹ for oats, as consequence, grains with high HW commands a higher price per ton, but the measured value is a combination of grain characteristics including friction, grain shape and polydispersity (Follmann et al 2016). HW was

claimed to be influenced by moisture during storage and nitrogen adubation (Gutkoski et al 2006; Kolchinski & Schuch, 2003). Emvula (2012) reported that HW can be affected plant stress, soil fertility, and environmental conditions, i.e., anything that impacts the movement of nutrients to the kernel during grain filling.

De-hulling index is another important characteristic in oat grains. High hull content of oats limits use as on farm-feed. The oat hull is mainly fibre, hemicellulose, cellulose and lignin. There are low levels of protein, fats, starch and water-soluble carbohydrates. As a result, digestibility of oat hull is low. For these reasons, high hull content of oat groat is a major determinant of decreasing nutritional quality, which is important if oat is used for feed. Reduction of the proportion of hulls in grains evidently results in marked increase in metabolized energy content when it's used in animal feed (Biel et al. 2014).

Lodging in small grains such as oat is a problem of considerable importance. Its effects on yield, HW, seed quality and other characteristics have been reported. The need for lodging resistant cultivars is becoming more urgent. Lodging was reported to be affected by environmental conditions attending the development of the plant as well as genetic make-up of the plant itself (Hancock & Smith, 1963). Lodging will always have a negative effect on yield. Yield losses in lodged crops come as a result of poor grain filling, head loss and bird damage (Hancock & Smith, 1963). Lodging alters the plant's growth and development, affecting flowering and interfering with photosynthesis and carbohydrate movement within the plant. It can interfere with plant's ability to extract nutrients and moisture from the soil, resulting in incomplete grain fill and smaller kernels. This can give yield losses of up to 40% depending on the severity and timing of the lodging (Hancock & Smith, 1963). In this sense of ideas, fungicide application can be an alternative to reduce lodging in oat plants.

Aiming to better understand if there is an association of all variables studied, a Pearson correlation was performed (Table 1). Interestingly, fungicide application was positively correlated with all variables except for lodging index where a negative association was found, that prompt us to speculate that the application of fungicide reduce the lodging of oat plants. Lodging index was negatively associated with sieve yield, weight of 1000 grains and yield, important parameters in oat industry.

Looking to find the important variables in our dataset and dimensionality reduction, a second approach (non-supervised (principal component analysis -PCA, hierarchical cluster analysis -HCA) and supervised multivariate techniques (partial least squares discriminant analysis -PLS-DA, ordinary least squares regression -OLS-R)) was applied. When PCA was applied to the dataset (Figure 1A) an interesting insight was observed. A good separation from cultivars where fungicide was applied and not applied was clearly observed (Figure 1A) except for cultivars URS-TORENA, URS-BARAVA, URS-CORONA, IPR-AFRODITE who showed to be similar with those where fungicide was applied (Figure 1A). A similar trend was also found for URS-FAPA-SLAVA (without fungicide application) who showed to be similar with BRISASUL and URS-TAURA (with fungicide application). The total variance explained by PCA was 66.05%, being 45.50 and 20.55% for component 1(PC1) and 2 (PC2) respectively. The loading values are also presented in the Figure 1B. The important variables that classified samples can be observed in squared loadings (Figure 1B) and were the grain yield, de-hulling, hectoliter, sieve yield and plant stature. Plant stature and the lodging index highly affected cultivars without fungicide application. Fungicide application mostly affected the yield, de-hulling index, hectoliter weight and the sieve yield. Interestingly, our findings prompt us to state that taller plant stature observed without fungicide lead to high lodging index for the same plants. Sample clustering in PC1 were duo hectoliter weight, de-hulling index, sieve yield and grain yield (see supplementary report for contribution of the

variables) and in PC2 due to lodging index, plant stature and yield. In general manner, the variables hectoliter weight, yield, lodging index and de-hulling most contributed to the data variability. The scores of PCA indicated that 09 cultivars highly contributed to the variability (URS-FAPA-SLAVA, URS-TARIMBA, URS-ESTAMPA, URS-GURIA, URS-TAURA, BARBARASUL, UPFA-OURO, BRISASUL and IPR-AFRODITE). A hierarchical cluster dendrogram (HCA) is also presented in the Figure 2. As it can be observed, a clear separation of the cultivars was found. 3 different groups can be observed in the HCA. URS-FAPA-SLAVA (without fungicide application) grouped alone. Samples where fungicide was applied grouped together except of a simple misclassification for UPSPF-FARROUPILHA and URS-BRAVA. HCA analysis reinforced the results previously observed in PCA analysis that lead us to state that the application of fungicide have a role in agronomic and quality parameters of oat cultivars evaluated.

The second approach applied in data screening and finding important variables was the use of regression models, at this time, an ordinary least square regression (OLS-R). The influence of all variables was measured. Firstly in the model 1 was tested the influence of the cultivars and in the model 2 was tested the effect fungicide application on quality parameters (hectoliter weight, de-huuling index and lodging index). Ordinary least squares (OLS) or linear least squares is a method for estimating the unknown parameters in a linear regression model, with the goal of minimizing the differences between the observed responses in some arbitrary dataset and the responses predicted by the linear approximation of the data. The resulting estimator can be expressed by a simple formula, especially in the case of a single regressor on the right-hand side. The results from OLS-R models are summarized in the Table 2 together with the akaike information criterion (AIC) and the variance explained by each model are presented. Looking for the second model (with better AIC), quality parameters were positively influenced by the plant stature. Without fungicide application, sieve yield, weight of 1000 grains and yield are affected.

Finally, the dataset was also subjected to partial least squares discriminant analysis (PLS-DA). Partial least square (PLS) is a versatile algorithm which can be used to predict either continuous or discrete/categorical variables. Classification with PLS is termed PLS-DA, where the DA stands for discriminant analysis. The PLS-DA algorithm has many favorable properties for dealing with multivariate data; one of the most important of which is how variable collinearity is dealt with, and the model's ability to rank variables' predictive capacities within a multivariate context. PLS-DA is performed in order to sharpen the separation between groups of observations, by hopefully rotating PCA (Principal Components Analysis) components such that a maximum separation among classes is obtained, and to understand which variables carry the class separating information. Results from PLS-DA analysis are presented in the Figure 3. The accuracy of the model, the error rate or misclassification error is also provided. The loadings, scores and confusion matrix are provided in the supplementary report. PLS-DA model was satisfactory in classifying samples, a good separation from samples with and without fungicide was also found. The total variability captured was 62.02%, being 49.41% and 12.61% for latent variable (LV) 1 and 2 respectively. Cultivar URS-FAPA-SLAVA separated alone. Besides, URS-BRAVA, UPFPS-FARROUPILHA, IPR-AFRODITE and URS-CORONA (without fungicide) clustered with those where fungicide application was performed. An error rate of 11.90% was found for our model. The circle of correlations (Figure 3B) clearly indicates that cultivars without fungicide application were mostly affected by the lodging index and those with fungicide application by yield, de-hulling index and hectoliter weight. The loadings indicated that the first latent variable (LV1) was negatively influenced by all variables except the lodging index. LV2 was positively affected by lodging index and the grain yield. PLS-DA was the best technique in classifying samples beside the lower variability captured than PCA.

4. Conclusions

Fungicide application affects the plant stature and lodging index in oat cultivars. The lodging index is negatively related to plant stature, and consequently influences the grain yield, the

hectoliter weight and the sieve yield. Quality parameters of oats (hectoliter, de-hulling and lodging indices) are negatively affected by plant stature and positively by fungicide application. Multivariate techniques (PCA, PLS-DA, OLS-R) classified samples according to fungicide application and were capable in finding the important variables that affect yield attributes and quality parameters and can be applied in a broad range of agricultural systems for predicting important aspects that affect plant growth and development.

5. Supplementary data

A report in html format is provided for data reproducibility. Detailed statistical analysis not shown in the manuscript can be found here.

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416 Table and Figure captions

417 Table 1: Pearson correlation matrices between all variables studied in the datasets. The
418 significance level for each correlation is also provided.

419

420 **Table 2:** Results of OLS-regression. Values in the table represent the coefficients of the ordinary
421 least square regression models using a generalized linear model (glm) and standard errors for
422 each coefficient or independent variable in parenthesis. The Akaike Information Criterion (AIC)
423 for each model is also provided.

424

425 **Figure 1:** (A) Score plots and (B) squared loadings of the principal component analysis (PCA) of oat
426 cultivars studied.

427 **Figure 2:** Hierarchical cluster dendrogram of all cultivars studied showing sample similarities and
428 dissimilarities. A cophenetic correlation of 92% was found

429 **Figure 3:** (A) Score plot of partial least squares discriminant analysis oat cultivars dataset and (B) the circle
430 of correlations showing the variables most correlated with and without fungicide application

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Tables and Figures

Table 1: Pearson correlations of all variables studied

	HW	Li	PS	Sy	Wg	Yield	Application
HW							
Li	-0.19* * * *						
PS	0.18* * * *	0.47* * * *					
Sy	0.38* * * *	-0.16* * * *	0.16* * * *				
Wg	0.41* * * *	-0.33* * * *	0.06	0.29* * * *			
Yield	0.47* * * *	-0.34* * * *	-0.18* * * *	0.09	0.26* * * *		
Application	0.30* * * *	-0.06	0.06	0.14* *	0.07	0.40* * * *	
Cult	0.00	0.08	0.02	0.00	-0.07	-0.07	0.00

Significance levels: $p < .0001$ '****'; $p < .001$ '***', $p < .01$ '**', $p < .05$ '*'

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Table 2: Results of OLS Regression

	<i>Dependent variable:</i>	
	Cult (1)	HW + DHI + Li (2)
DHI	0.06 (0.05)	
HW	0.05 (0.09)	
Li	0.01 (0.01)	
PS	-0.02 (0.03)	1.56*** (0.12)
Sy	0.01 (0.04)	-0.42** (0.20)
Wg	-0.06 (0.06)	-1.69*** (0.28)
Yield	-0.0004 (0.0003)	-0.004*** (0.002)
Application	0.09 (0.61)	4.93 (3.17)
Cult		0.38 (0.24)
Constant	8.90** (4.31)	97.71*** (20.38)
Observations	504	504
Akaike Inf. Crit.	3,256.78	4,935.05

Note: * p<0.1; ** p<0.05; *** p<0.01

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Figure 1A

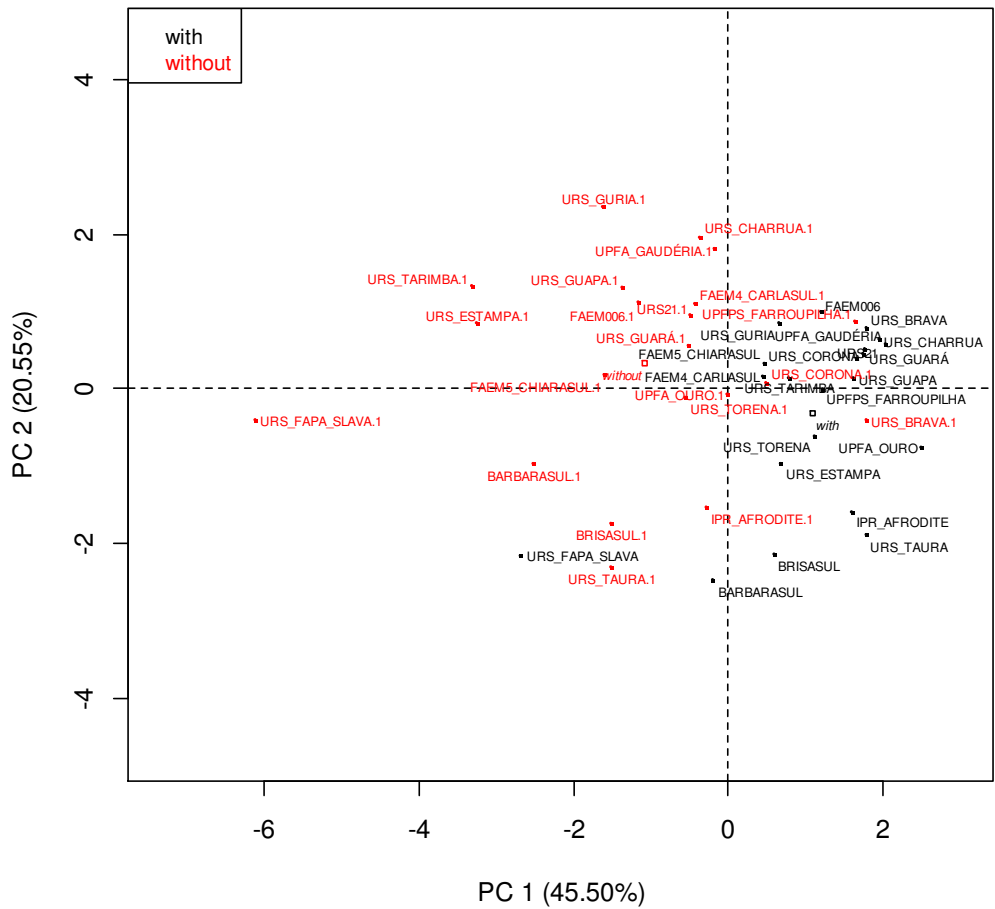
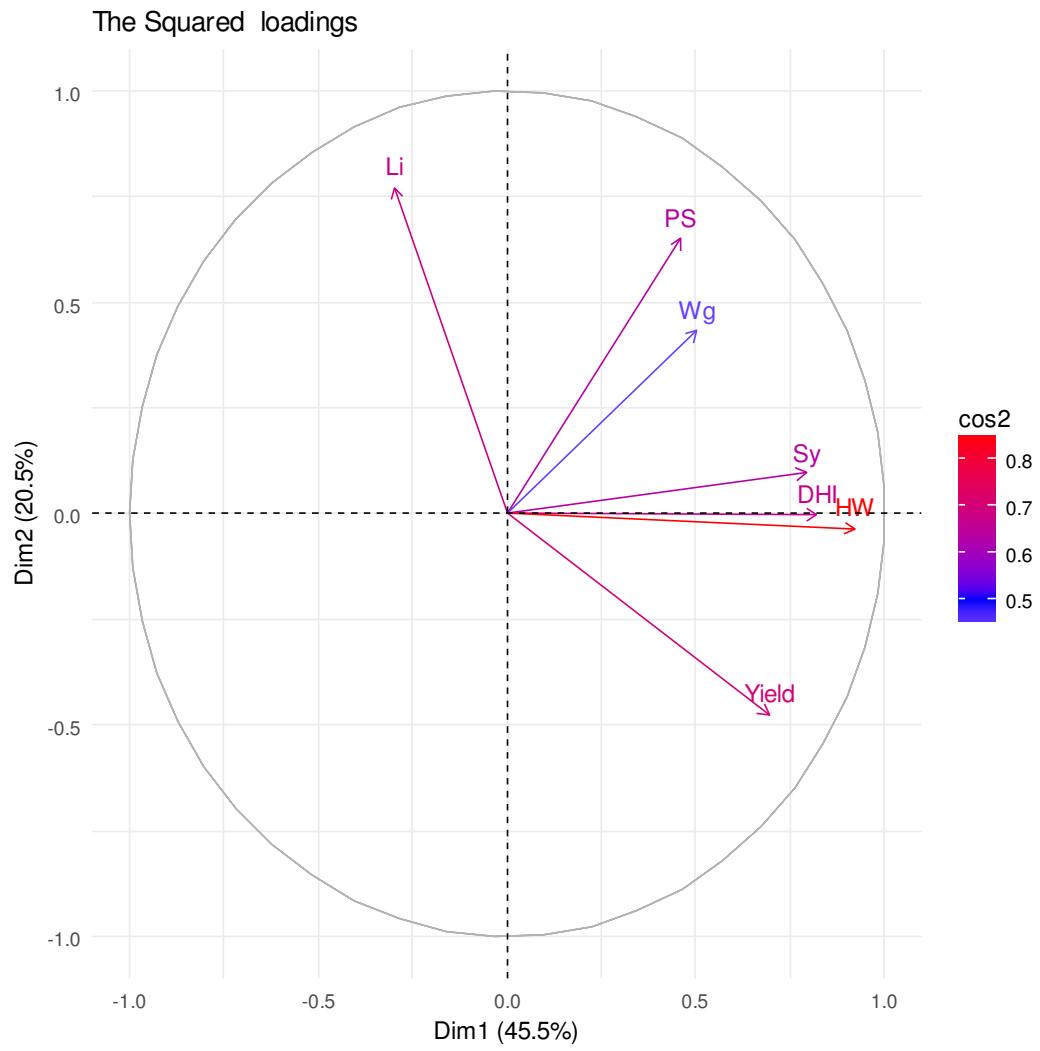


Figure 1B

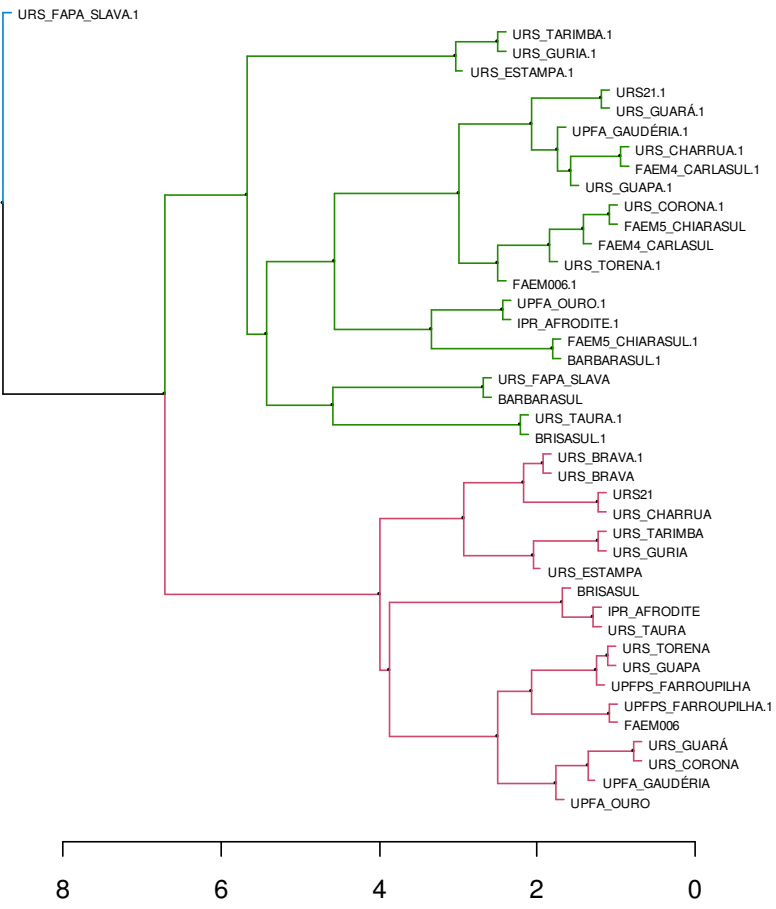


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484 Figure 2



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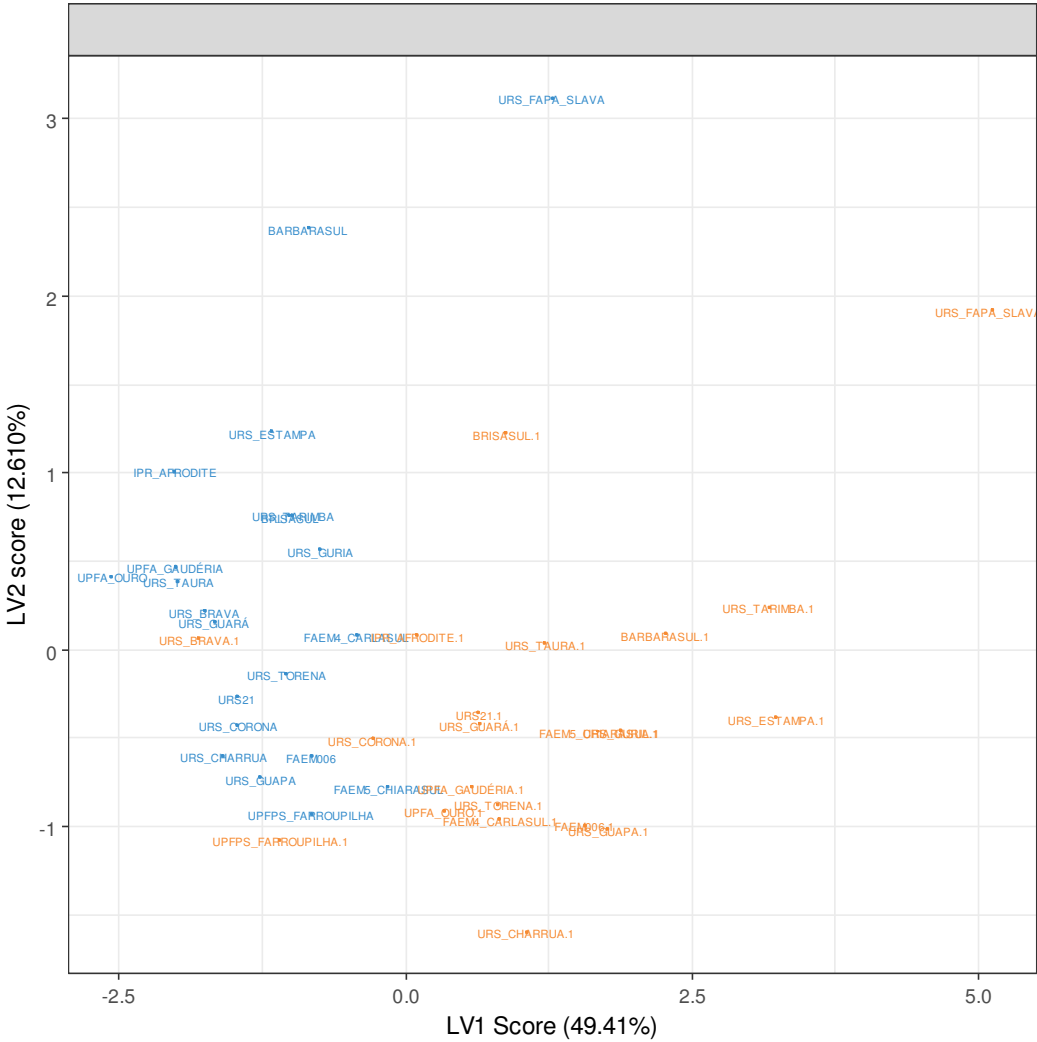
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493 Figure 3a



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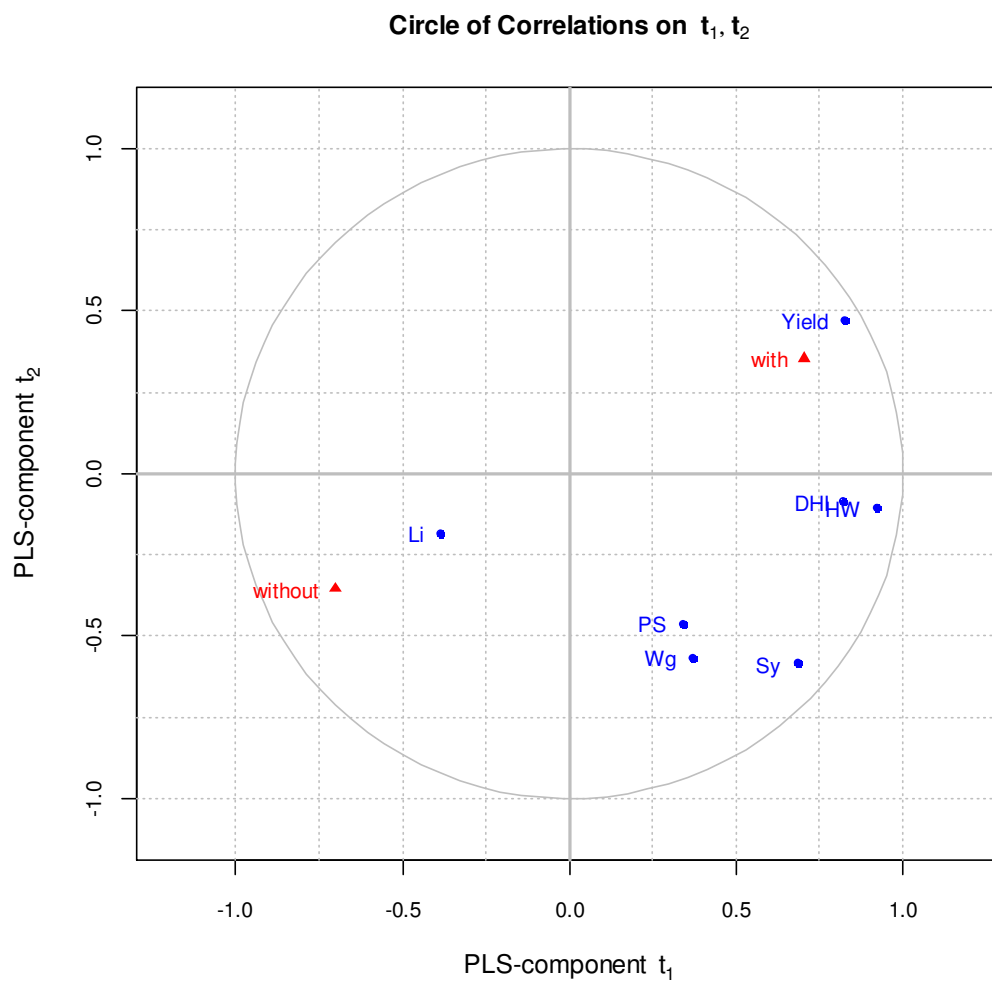
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502 Figure 3B



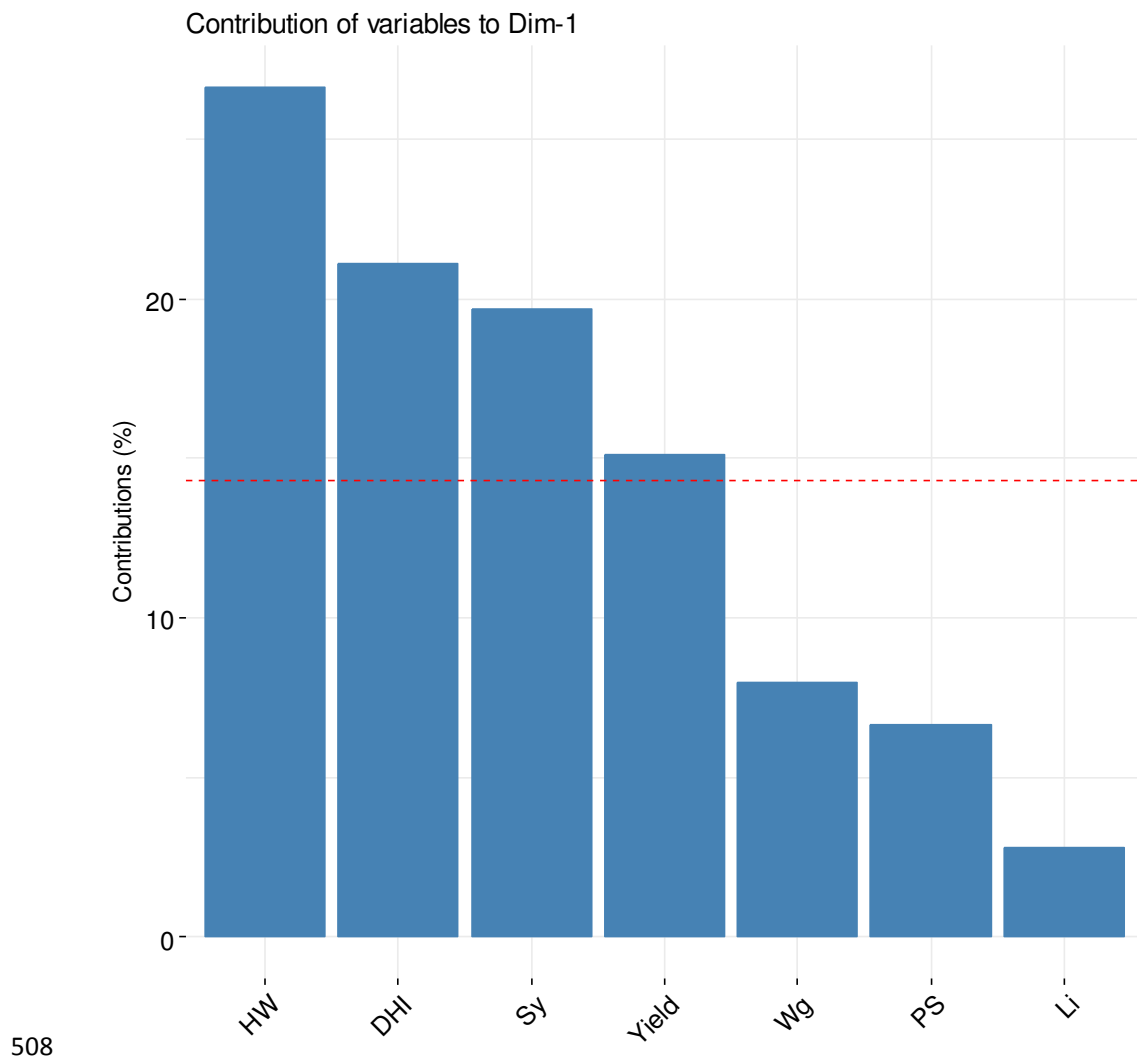
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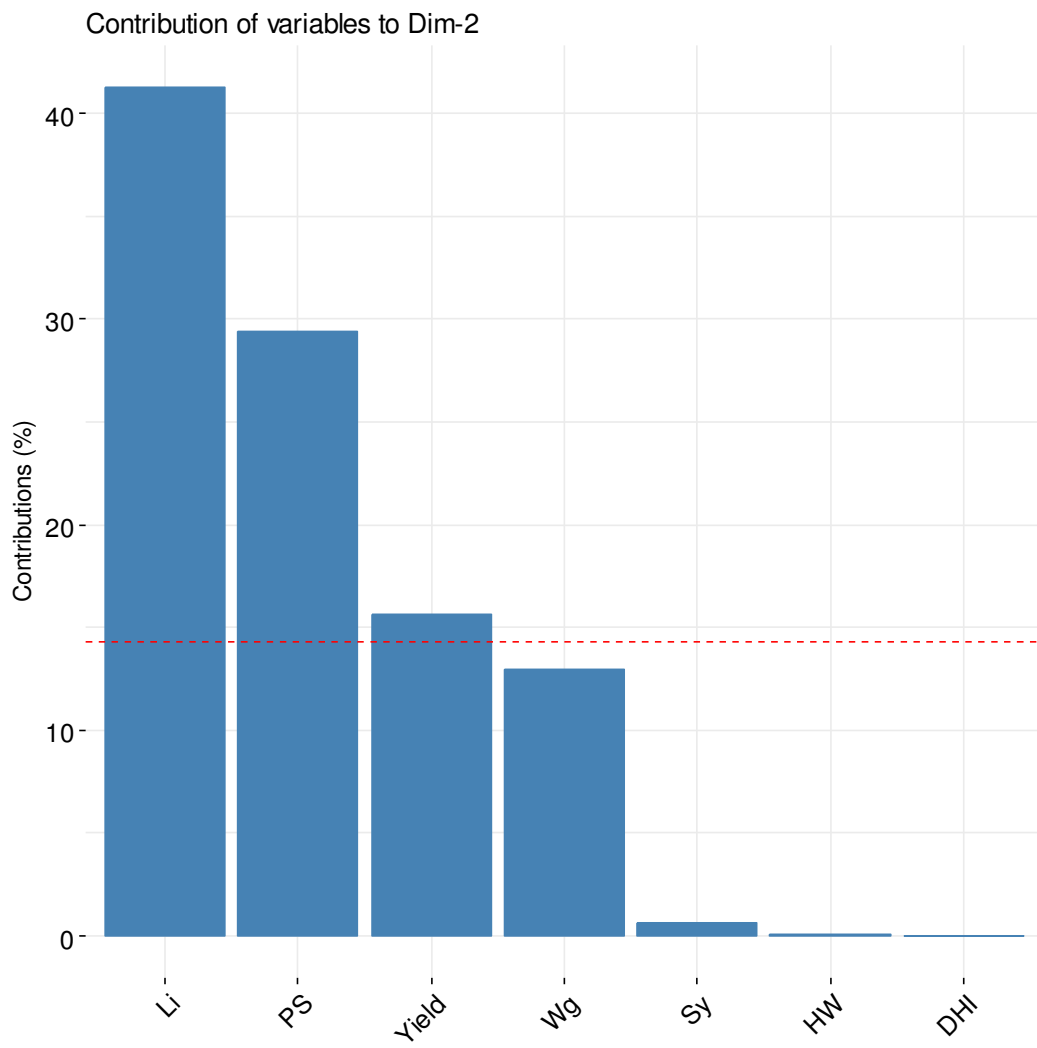
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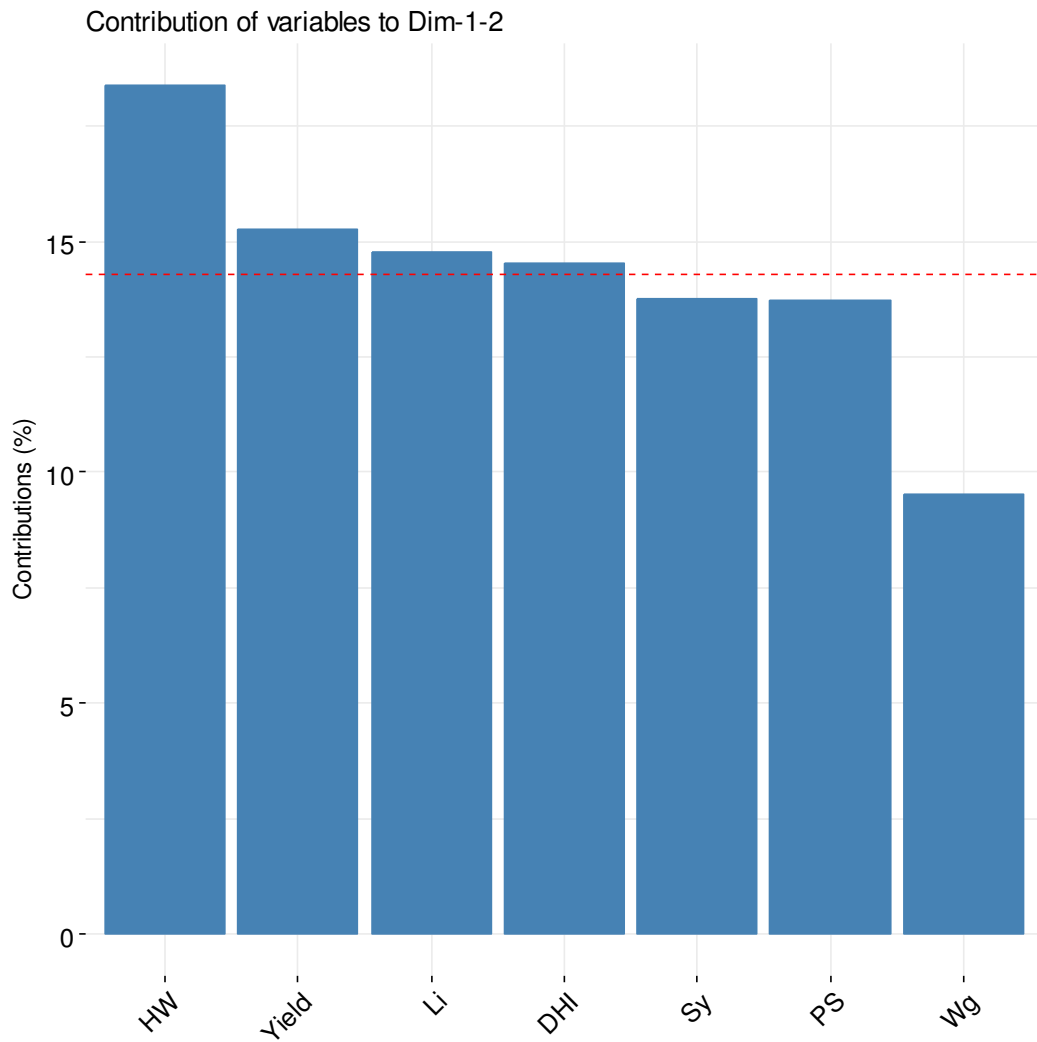
506 **Supplementary data**

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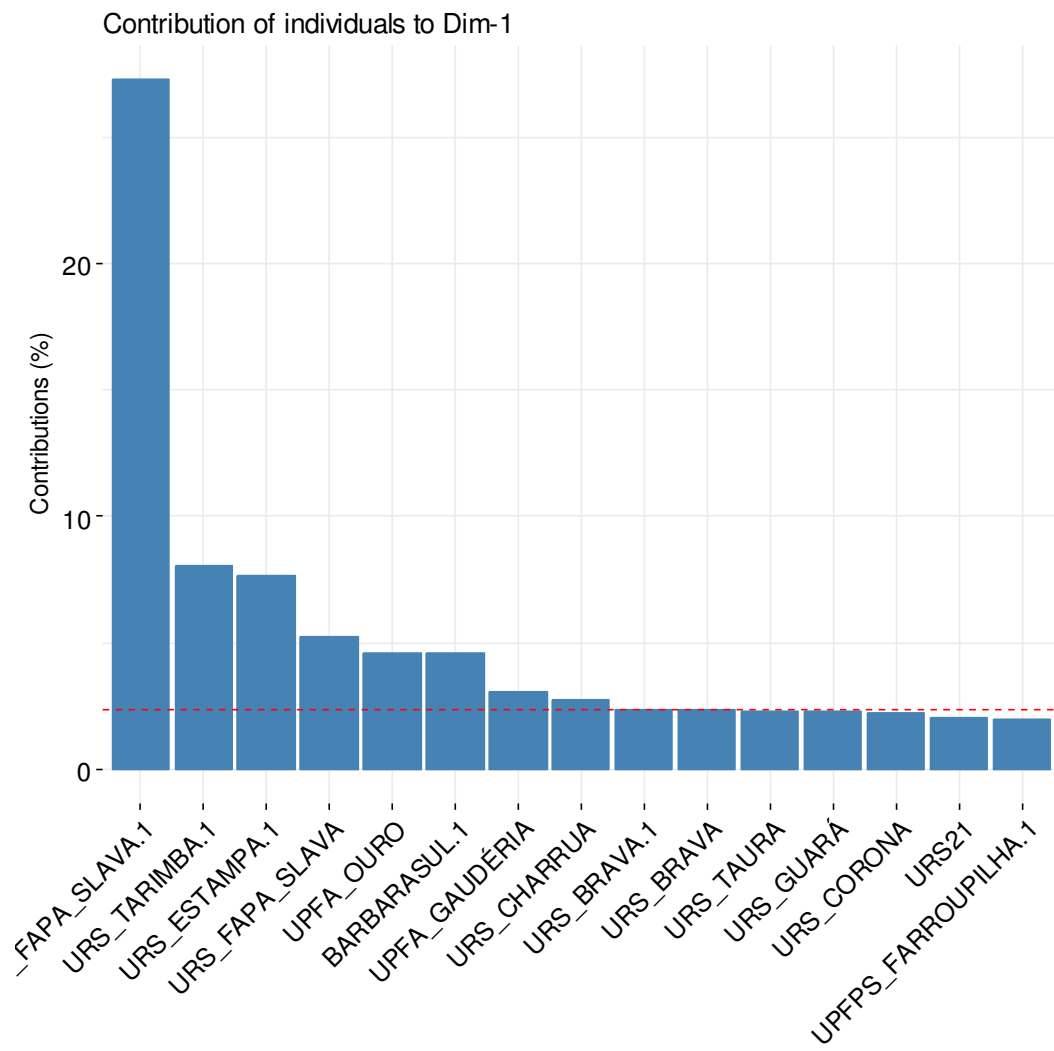




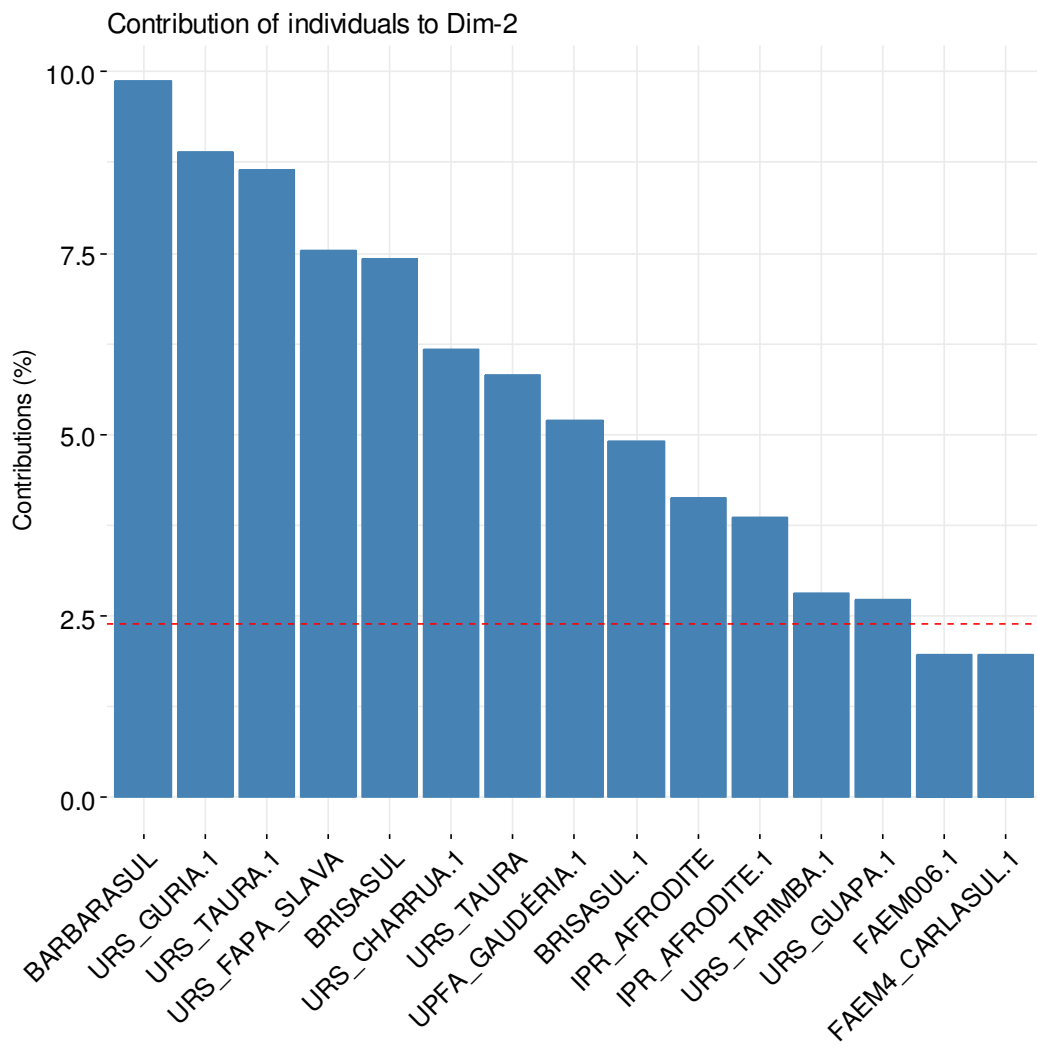
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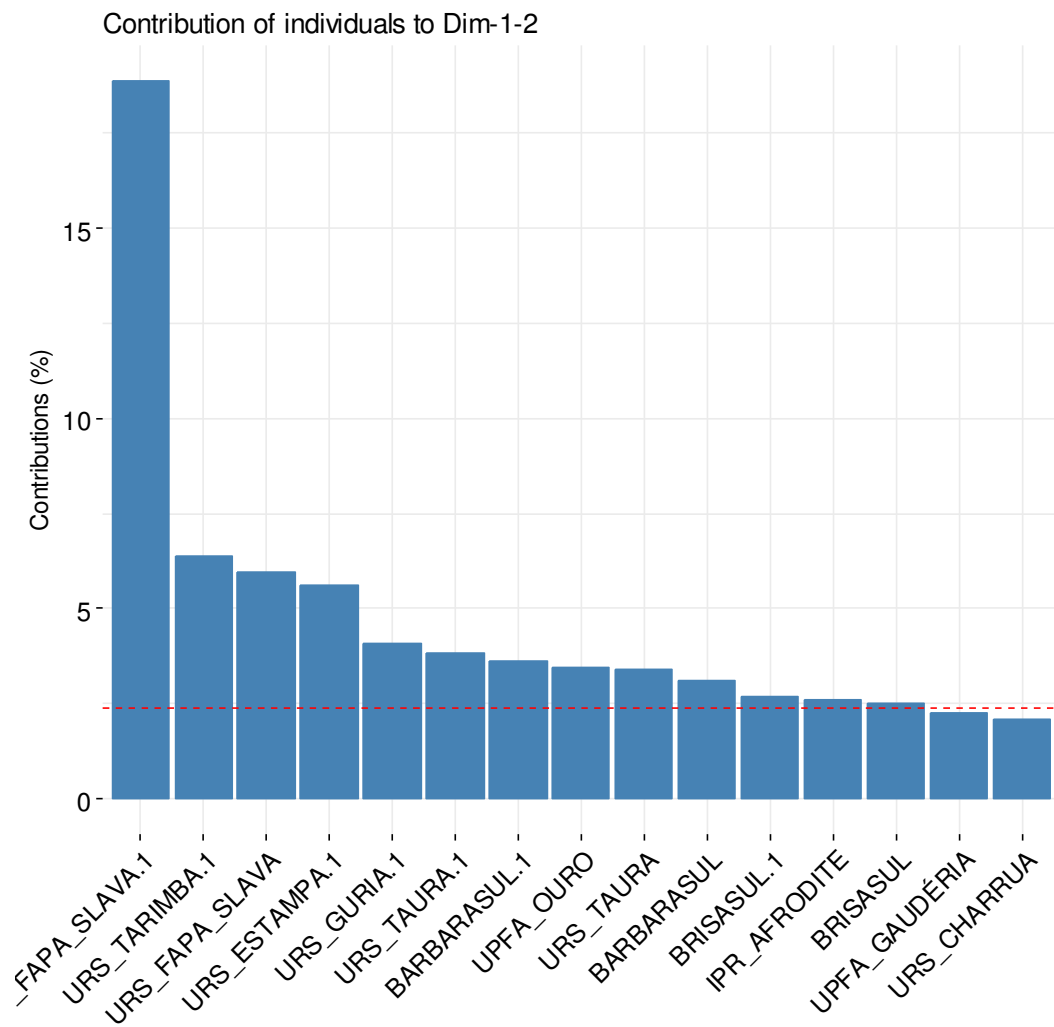
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515 PLS-DA

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