Journal of Agricultural Science and Technology B 7 (2017) 86-99
doi: 10.17265/2161-6264/2017.02.003

Discriminating Important Agronomic and Industrial Parameters of White Oat Cultivars Treated with Fungicide Based on SIMCA Algorithm

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Abstract: Selection of effective agronomic and industrial parameters of oat cultivars is a decisive step in oat breeding programs for development of new oat and elite cultivars. In this study, a new approach was utilized to distinguish the most informative agronomic and industrial parameters that are most affected with fungicide application in oat cultivars. Four subsequent field experiments from 2007 to 2010 were conducted in completely randomized block design (CRBD) with split plots. Total nine oat cultivars with or without fungicide application were evaluated for plant height, sieve yield, grain yield, lodging index, weight of hectoliter and de-hulling index. Soft independent modeling of class analogy (SIMCA) was conducted as one-class and multi-classes models to identify important variables that can be used to discriminate samples. Results showed that SIMCA was effective, and lodging index, de-hulling index, sieve yield, plant height and grain yield were most affected oat parameters. Therefore, SIMCA algorithm can be used to easily discriminate some agronomic and quality parameters of oats.

Key words: Oat cultivars, SIMCA modeling, non-supervised techniques, fungicide application, growth, yield, principal component analysis, hierarchical cluster analysis, model classification.

1. Introduction

Soft independent modeling of class analogies (SIMCA) is a very useful technique for classifying high-dimensional observations, because it incorporates principal component analysis (PCA) for dimension reduction [1]. As PCA is applied to each group separately, SIMCA provides additional information on the different groups, such as the relevance of the different variables and measures of separation. In contrast with this approach, one can also apply PCA once to the full set of observations, and then continue the analysis by performing a classification rule for low-dimensional data [1]. PCA is mainly used as an overall dimension reduction technique and hierarchical cluster analysis (HCA) tries to find groups containing similar objects. If additionally more information is wanted about the individual group structures, the SIMCA strategy is preferred.

The general idea is to create a PCA model using data for samples/objects belonging to a class and classify new objects based on how good the model can fit them. The decision is made using two residual distances—Q (squared residual distance) and T_2 (score distance) [2]. The classification performance is assessed using true/false positives and negatives, showing the ability of a classification model to recognize class members (sensitivity or true positive rate) and how good the model is for identifying

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strangers (specificity or true negative rate) [2]. SIMCA has been used to discriminate adulterated and unadulterated Fourier transform infrared spectroscopy (FT-IR) minced meat samples [3], for screening Brazilian gasoline quality [4], for rapid and precise identification of herbal medicines [5, 6], for discrimination of wood pellet quality [7] and detection of insect infested tomatoes [8]. Implementations of these multivariate tools in classifying oat cultivars are scarce in the literature.

White oats (*Avena sativa* L.) are important spring cereals [9]. They are fast growing and produce significant amount of fresh fodder within short period (60-70 d) with adequate nutritional value [10]. The identification of oat agronomic traits that meet the demand of farmers, industry quality and final consumers, and their incorporation into elite oat germplasm are crucial to the development of successful new oat cultivars.

In Southern Brazil, due to the highly complex and diverse pathogen populations, severe epidemics occur every year [11]. Crown rust is a major oat disease, occurring yearly in all oat-producing regions in Brazil [11], and yield losses caused by the disease can reach 50% [11, 12]. In addition, crown rust can reduce the grain quality, which negatively affects the industrialization and commercialization of oat grain [12]. The primary means of controlling crown rust have been through genetic resistance, although in most cases, resistance has been quickly overcome by the pathogen [13].

Triazole fungicides are one of the top 10 classes of current-use pesticides and have higher consumption as compared to other fungicides available worldwide [14]. Tebuconazole is one of a common triazole fungicide that has been extensively used in grains, vegetables, fruits and oats for the control of plant pathogenic fungi [15-17]. Additionally, the strobilurins are a new class of fungicidal compounds and among them is the recently introduced trifloxystrobin [18, 19] which has been also used in oat cultivars. It is a common practice in crop protection to apply multiple pesticide mixtures instead of individual pesticides. This form of pesticide application likely results in the combined effect in oat cultivars. In this research, the use of fungicides is explored using non-supervised multivariate analysis (PCA and HCA) and SIMCA techniques, aiming to find important variables that are most affected by fungicide application on oat cultivars and analyze the effect of fungicide application on agronomic and industrial quality parameters of oats. The results can be used to build fast models for discriminating new oat cultivars in breeding programs and other agricultural sectors.

2. Materials and Methods

2.1 On Farm Experiments

Subsequent on-farm experiments were conducted in Lages (Santa Catarina State, Southern Brazil), on an experimental research field of the Santa Catarina State University from 2007 to 2010, in completely randomized block design (CRBD) with split plots. A total of six plots was used, where in three plots, aerial fungicide application of a mixture of tebuconazole (triazole group) (150 g/ha) and trifloxystrobin (strobilurin group) (56 g/ha) was applied and other three plots without fungicide application. The fungicide treatment was carried out in the emergence of the earliest rust-leaf pustules on the most susceptible cultivar. The fungicide was applied in the dose of 0.75 L/ha in a volume of spray mixture of 200 L/ha. All other agronomic practices, such as soil fertilization, weed and disease control, were performed following the Brazilian commission for oat research recommendations [20]. Each plot (useful area) consisted of five central lines of 5 m length spaced at 0.2 m and 0.4 m between plots. The density used was of 300 seeds/m^2. A mechanized harvest of the oat grains was done for subsequent laboratory analyses. A total of nine oat cultivars (ALBASUL, URS21, URS22, URS-GUAPA, UPF15, UPF16, UPF18, UFRGS14 and UFRGS19) were evaluated during four subsequent years.

2.2 Yield Components Analysis

Yield attributes, such as plant stature (PS), grain yield, weight of 1,000 grains (Wg) and sieve yield (Sy > 2 mm) were evaluated at each experiment (2007-2010).

2.2.1 PS (cm)

PS of plants was evaluated at growth stage GS91 (pre-harvest maturity) by measuring 10 pegged plants in a random points in each plot by the use of a ruler. The measurements were done from the soil surface until the apical spikelet located on the top of the panicle, and values were expressed as mean of 10 plants according to Eq. (1):

$$\mathrm{PS\,(cm)} = \frac{\sum_{n=1}^{n=10} \mathrm{PS}_n}{N} \quad (1)$$

where, PS is the mean height of 10 plants; PS_n is the individual plant height and N is the total number of plants evaluated in each plot (N = 10).

2.2.2 Sy (%)

Sy refers to the percentage of grains with transverse diameter greater than 2 mm. This was evaluated after harvest, by weighing a sample of 250 g and then submitted the sieve with regular stirring during 1 min, in a sieve of 40 cm × 30 cm dimensions, rectangular, containing holes (sieve) of 2 mm × 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 Eq. (2):

$$\mathrm{Sy\,(\%)} = \frac{\mathrm{RGW}}{\mathrm{IGW}} \times 100 \quad (2)$$

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

2.2.3 Yield (kg/ha)

Grain yield was measured from each experimental plot (four central linear meters of each plot, spaced at 0.2 m, in a useful area of 2.4 m^2). The yield was then converted to hectare after weight correction using a standard moisture content of 13% according to Eq. (3):

$$\text{Grain yield (kg/ha)} = \frac{\mathrm{WW} \times \left(\frac{100\% - \mathrm{RM}\,\%}{100\% - 13\%}\right)}{2.4 \times 10,000} \quad (3)$$

where, WW is wet weight (kg) and RM is the real weight moisture (%).

2.2.4 Wg (g)

A sample of 1,000 grains obtained from each experimental plot were electronically counted (Sanick ESC 2011) and weighted using a precision balance and expressed in grams (g).

2.3 Quality Parameters

Quality parameters of the grains independent of end use, such as lodging index (LI), weight of hectoliter (HW) (kg/100 L) and de-hulling index (DHI), were evaluated as described below:

2.3.1 LI (%)

LI was estimated visually and expressed as a percentage, taking into account the angle formed in the vertical position of the plant stem in relation to the ground. Area of 5 m^2 was used for determination of LI. LI was calculated according to the methodology of Moes and Stobbe [21] and expressed as Eq. (4):

$$\mathrm{LI\,(\%)} = I \times A \times 2 \quad (4)$$

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

2.3.2 HW (kg/100 L)

HW was measured according to the Brazilian official wheat grain quality roles [22], using a hectoliter weight analyzer (Dalle-Molle, model T40EL, 0.25 L of capacity). Briefly, grain samples from each experimental plot were placed in the analyzer for volume determination and then weighed. HW was then expressed as Eq. (5):

$$\text{HW (kg/100 L)} = \text{Wv} \times 0.4 \quad (5)$$

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

2.3.3 DHI (%)

A sample of 1,000 grains was weighed, de-hulled manually and then weighed again. DHI was expressed as in Eq. (6):

$$\text{DHI (\%)} = \frac{\text{Wg}}{\text{Wdh}} \times 100 \quad (6)$$

where, DHI is the de-hulling index, Wg is the weight of 1,000 grains before de-hulling and Wdh is the weight of the sample after de-hulling.

2.4 Statistics Analysis

Data from four years were summarized and subjected to normality test, homogeneity of variance test and analysis of variance (ANOVA) using a split plot design. Tukey's honest significant difference (HSD) (P < 0.05) was used to test if the differences are statistically significant. Non-supervised multivariate analysis (PCA and HCA) were firstly applied to the all dataset to observe similarities between the tested cultivars and find variables main related to the similarities. Secondly, SIMCA (one-class and multi class model classification) was applied to find the importance of each variable in each object and those mostly discriminating agronomic and quality characteristics of oats. All statistical analyses were done in R software [23].

3. Results and Discussion

3.1 ANOVA

Results of ANOVA and multiple comparison tests (Tukey's test, 5% of probability) of the four field experiments (2007-2010) showed the differences in fungicide application, regarding DHI, HW, PS, Wg and yield. Significant statistical differences in cultivars (P < 0.05) were only observed in PS and Wg parameters. PS was significantly higher in cultivars UPF18 and UPF15 and lower in ALBASUL cultivar. All other cultivars were similar (P < 0.005). The Wg was higher in UFRGS14 and lower in ALBASUL. Similarly as observed in PS, all other cultivars did not show significant statistical differences.

It has previously been reported that cultivars have significant differences in PS [24, 25], and variations are due to genetic make-up and can also be affected by nitrogen [26]. The PS in this study ranged from 70 cm to 130 cm, and interestingly, the similar values have been reported by Brazilian research group [27]. Cultivar and growing season have also been reported to affect the PS [28, 29]. According to Brunava et al. [28], higher yield was found in plants with lower PS. PS can be affected by agronomic management practices, such as seeding rates, chemical seed treatments and foliar fungicide. In the research of Mourtzinis et al. [30], seeding rate and seed treatments did not show effect on oat yield, PS and LI, but foliar fungicide application was considered mainly if disease is expected and improved the grain yield [30, 31].

The Wg parameter ranged from 20 g to 50 g. Values in the similar range have been previously reported by Mourtzinis et al. [30] and Hisir et al. [32]. Higher values of Wg were correlated with dough periods, good processing quality and dependent of cultivar [28]. Siloriya et al. [33], Carvalho et al. [34] and Troup [35] also reported Wg values in the range of 36-39, 26-31 and 31-34 g, respectively. Breeders are constantly working towards a better plant ideotype, focusing on yield, disease resistance and grain quality [33].

Fungicide application significantly improved the DHI, HW, Wg, LI and yield of oat grains (P < 0.05, Tukey's test). Follmann et al. [36] studied the genetic progress of oat cultivars with fungicide application and 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 HW. As reported by Wychowaniec et al. [37], HW is a measure of the bulk density of the grain and is indicator of grain quality and millability. To ensure effective milling, HW should be above 50 kg/100 L 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 [36]. Similar values in HW were also reported in other literatures [30, 31, 34-38]. HW was claimed to be influenced by moisture during storage and nitrogen adubation [39, 40]. Emvula [41] reported that HW can be affected by plant stress, soil fertility and environmental conditions, i.e., anything that impacts the movement of nutrients to the kernel during grain filling.

DHI is another important characteristic in oat grains. As reported by Peltonen-Sainio et al. [38] and Biel et al. [42], 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 is used in animal feed. DHI in this research ranged from 30% to 80%, similar with that reported by Carvalho et al. [34], but higher than that found by Peltonen-Sainio [38].

LI in this research ranged from 0% to 80%. 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 [43]. The effects of shade, temperature and soil fertility can be a consequence of great variations found in this research. Hancock and Smith [43] related lodging to water content of tillers, length and weight of internodes, nodding angles, culm diameter and wall thickness. Besides, the results in this study showed that fungicide application can ameliorate LI by reducing it. 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 [43]. 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 [43]. In this sense of ideas, fungicide application can be an alternative to reduce lodging in oat plants.

3.2 PCA and HCA

When data were subjected to non-supervised multivariate statistical techniques (PCA and HCA), a clear separation of samples applied with fungicide and those without fungicide was found (Fig. 1). The total variance captured by the first two components (PCs) was 63.2%, being 43.7% and 19.5% for PC1 and PC2, respectively. PC1 was highly influenced by HW and the yield of oat grains and PC2 by Sy and PS (Fig. 2). Those four variables mostly contributed to the data variability of PCA model, taking into account all dataset. The scores of PCA indicated that cultivars UPF18, UPF15 and UFRGS14 (with fungicide) and URS21, URS-GUAPA and UFRGS19 (without fungicide) highly contributed to the variability in PC1. PC2 was mostly influenced by URS22, ALBASUL (with fungicide) and UPF18, ALBASUL (without fungicide).

The HCA of the same dataset confirmed the sample clustering found by PCA (Fig. 3). The cophenetic correlation of sample clustering was 0.85. HCA performed a better classification of the samples, except for UFRGS14 (without fungicide) which showed similarity with those where fungicide was applied (Fig. 3).

3.3 SIMCA

Finally, after non-supervised data analysis on all dataset, SIMCA was performed. One-class model and multi-class model classification were done to find good model with good accuracy, sensitivity, specificity, orthogonal and score distances. SIMCA was performed in a first instance taking cultivars as factor or class matrix (nine classes) and then fungicide as class matrix (two classes—with and without fungicide). Results of SIMCA are summarized in the Table 1, where the performance of class model is tested.

Interestingly, the ability of each model to recognize class members (sensitivity) was higher (1) for all models. Besides, the model of class URS-GUAPA was good in identifying strangers (higher specificity), and the model of class UFRGS19 showed lower quality in identifying strangers (Table 1). A detailed analysis of these two classes is presented in Figs. 4-7,

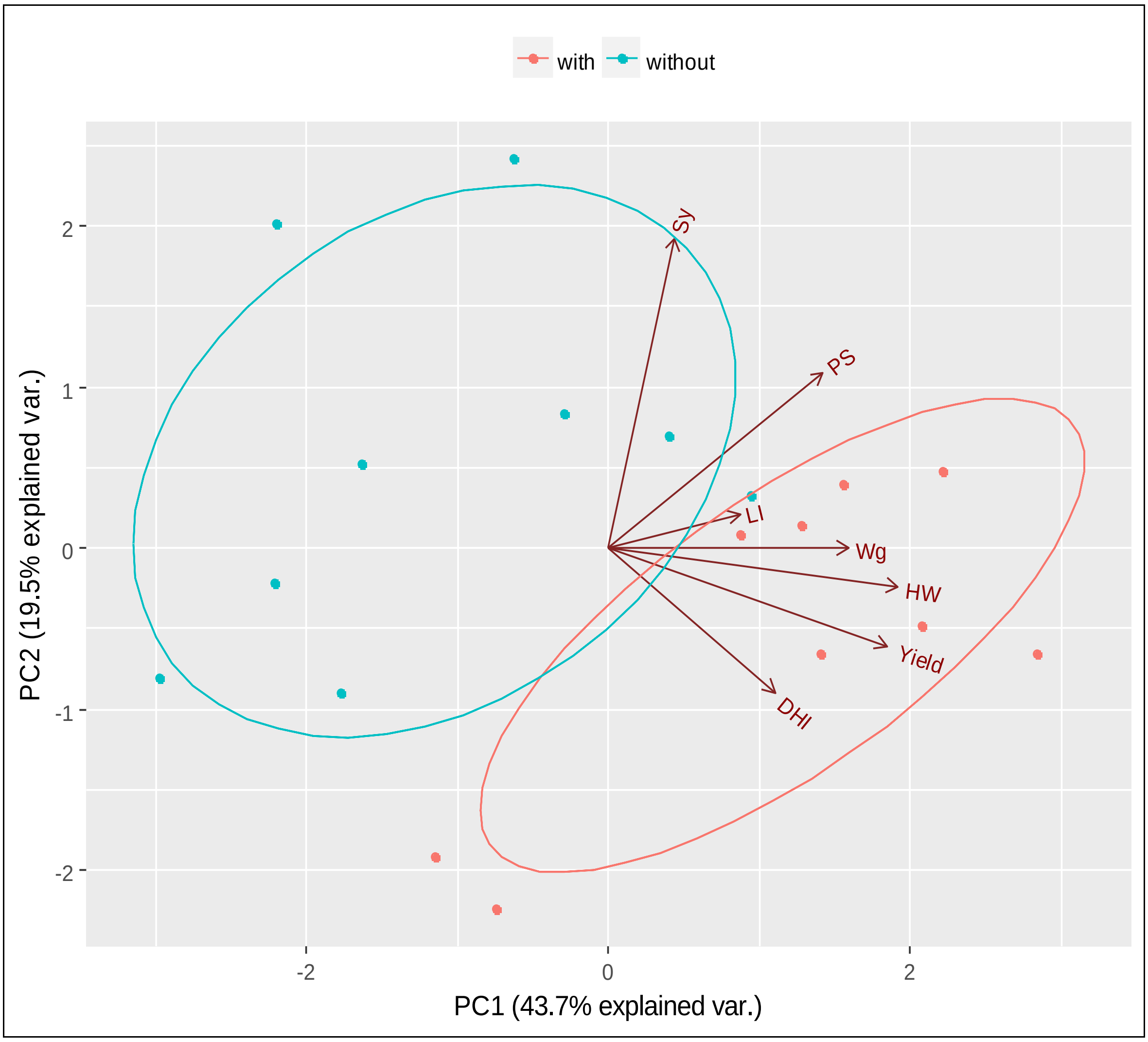

Fig. 1 Score plot of PC analysis of all dataset, showing the separation of samples with or without fungicide application.

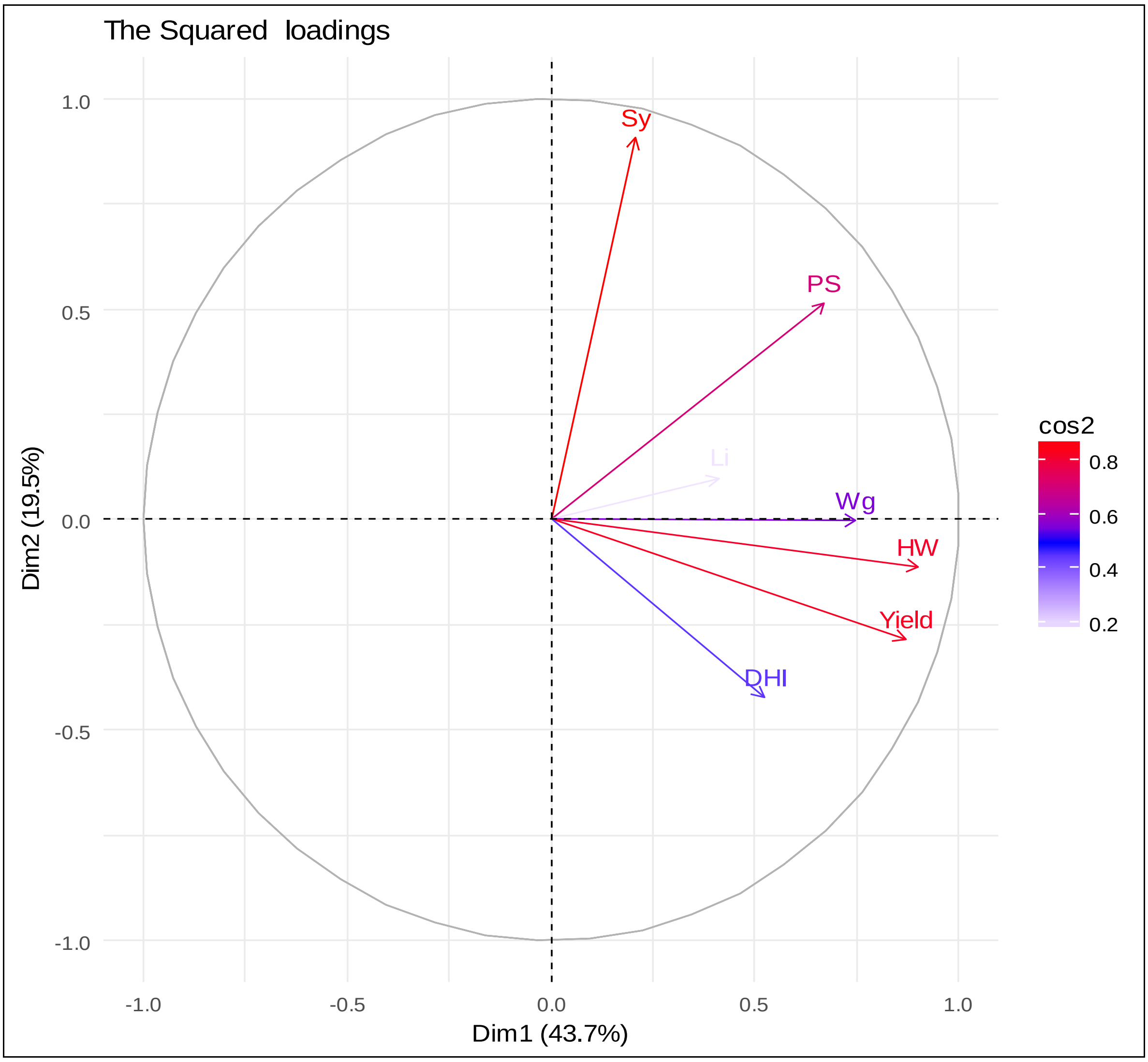

Fig. 2 The squared loadings showing the variables that most contributed to data variability in PC1 and PC2, respectively.

respectively. The model of URS-GUAPA was capable in identifying the true negative observation in the classification (Table 2).

One class analysis of each model was capable in finding the important variables affected by the fungicide application in each oat cultivar and in a general manner. Yield, Sy and PS was highly affected in class ALBASUL, while Li, Sy, HW and DHI in classes UFRGS14, UFRGS19, UPF15 and UPF16. The variables most affected in the class UPF18 were Sy, Li, PS, yield and Wg. URS-GUAPA was affected regarding the HW, yield and Sy. For class URS21, the variables yield, Sy, PS and HW were affected. Finally, URS22 was affected in Sy, HW, yield and Li variables. In general, the application of fungicide influenced the LI, DHI, HW, Sy, PS and grain yield. Using Hotelling's T_2 and Q residual values, SIMCA algorithm effectively identified variables that are most affected by fungicide application and found similar cultivars. Such effectiveness has been previously reported by Mireei et al. [8] and Yang et al. [15] in their research with herbal medicines and infested tomatoes, respectively. The method provided a flexible modeling of oat dataset. T_2 and Q residuals were useful indicators in the SIMCA models for evaluating differences between the samples.

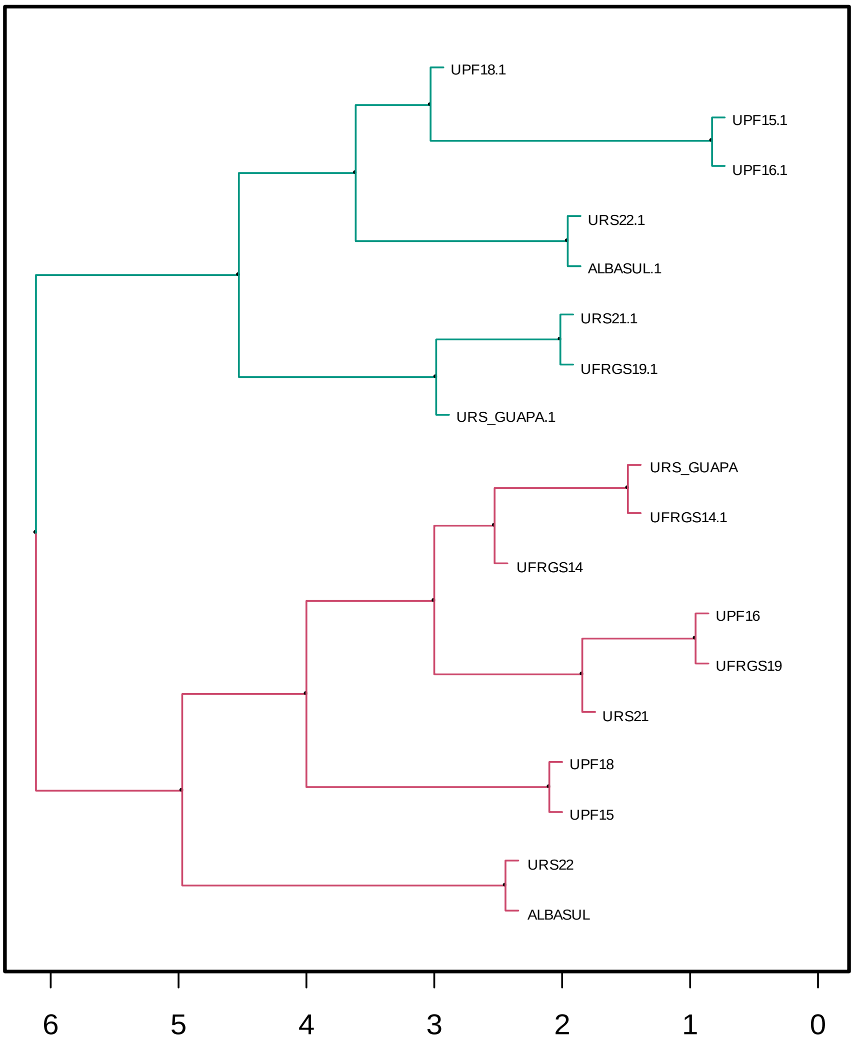

Fig. 3 HCA dendrogram of all dataset with cophenetic correlation of 85% showing the sample clustering.
These samples in red are with fungicide application and those in green (those with the number 1 after the name) without fungicide application.
A sample mismatch can be also observed in the HCA.

Table 1 Results of SIMCA multiple classes' classification showing the performance of each class model.

Class*	Specificity	Sensitivity	MRP	PC1	PC2	PC3	CV
ALBASUL	0.1979167	1	0.7129630	44.60	24.71	19.99	89.30
UFRGS14	0.1666667	1	0.7407407	36.81	29.38	19.23	85.42
UFRGS19	0.0937500	1	0.8055556	39.07	25.92	20.25	85.24
UPF15	0.1041667	1	0.7962963	39.84	30.36	15.50	85.70
UPF16	0.2500000	1	0.6666667	39.54	27.69	15.69	82.92
UPF18	0.1458333	1	0.7592593	35.73	31.16	17.25	84.14
URS_GUAPA	0.4687500	1	0.4722222	42.52	26.68	16.82	86.02
URS21	0.2395833	1	0.6759259	45.19	26.81	18.00	90.00
URS22	0.1666667	1	0.7407407	42.80	23.29	16.32	82.41
Total	0.2037037	1	0.7078189				

MRP: misclassified rate of prediction; PC: principal component; CV: cumulative variance.
* Significance level at 0.01.

Table 2 Detailed analysis of the performance of one-class model of UFRGS19 and URS-GUAPA.

Class	TP	FP	TN	FN
UFRGS19				
Comp1	12	94	2	0
Comp2	12	95	1	0
Comp3	12	94	2	0
URS-GUAPA				
Comp1	9	80	16	3
Comp2	8	74	22	4
Comp3	7	50	46	5

TP: number of true positives; FP: number of false positives; FN: number of false negatives; TN: number of true negatives identified by each model.

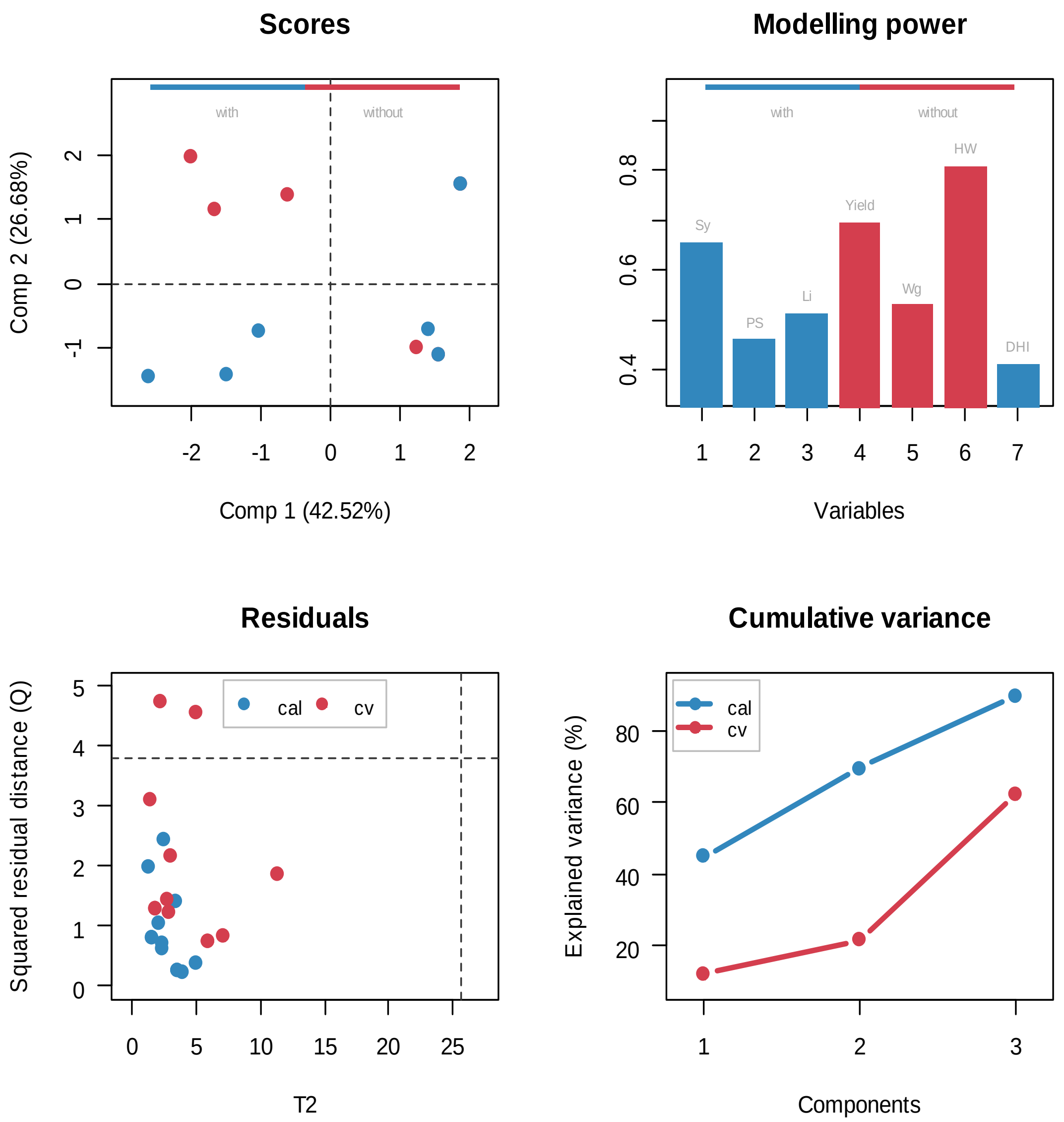

Fig. 4 Detailed one-class model classification of SIMCA for cultivar URS-GUAPA, showing the scores of PCA, the modeling power of the variables, the residuals (Q and T_2), the variance explained the first two components.

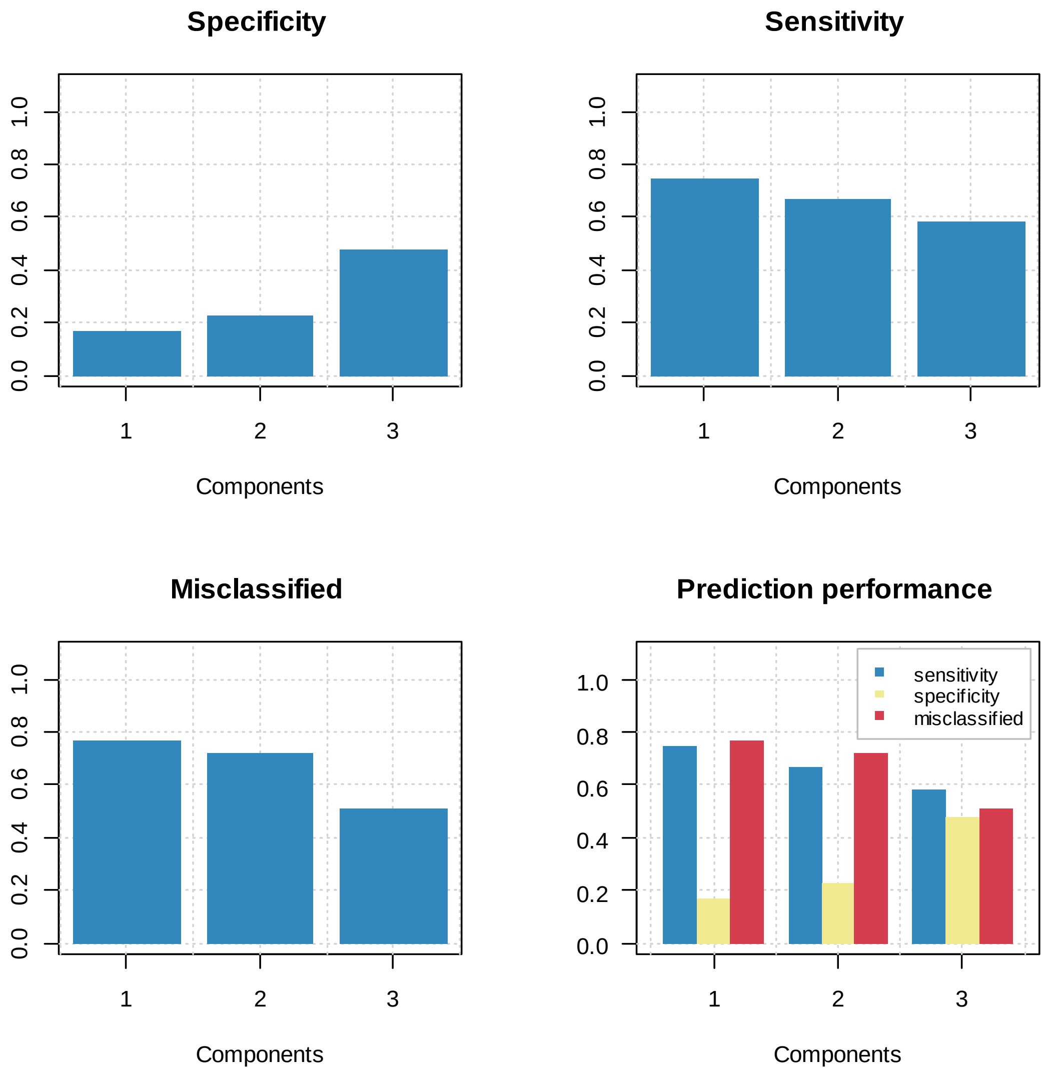

Fig. 5 The specificity, sensitivity, misclassified samples and the prediction performance of each component.

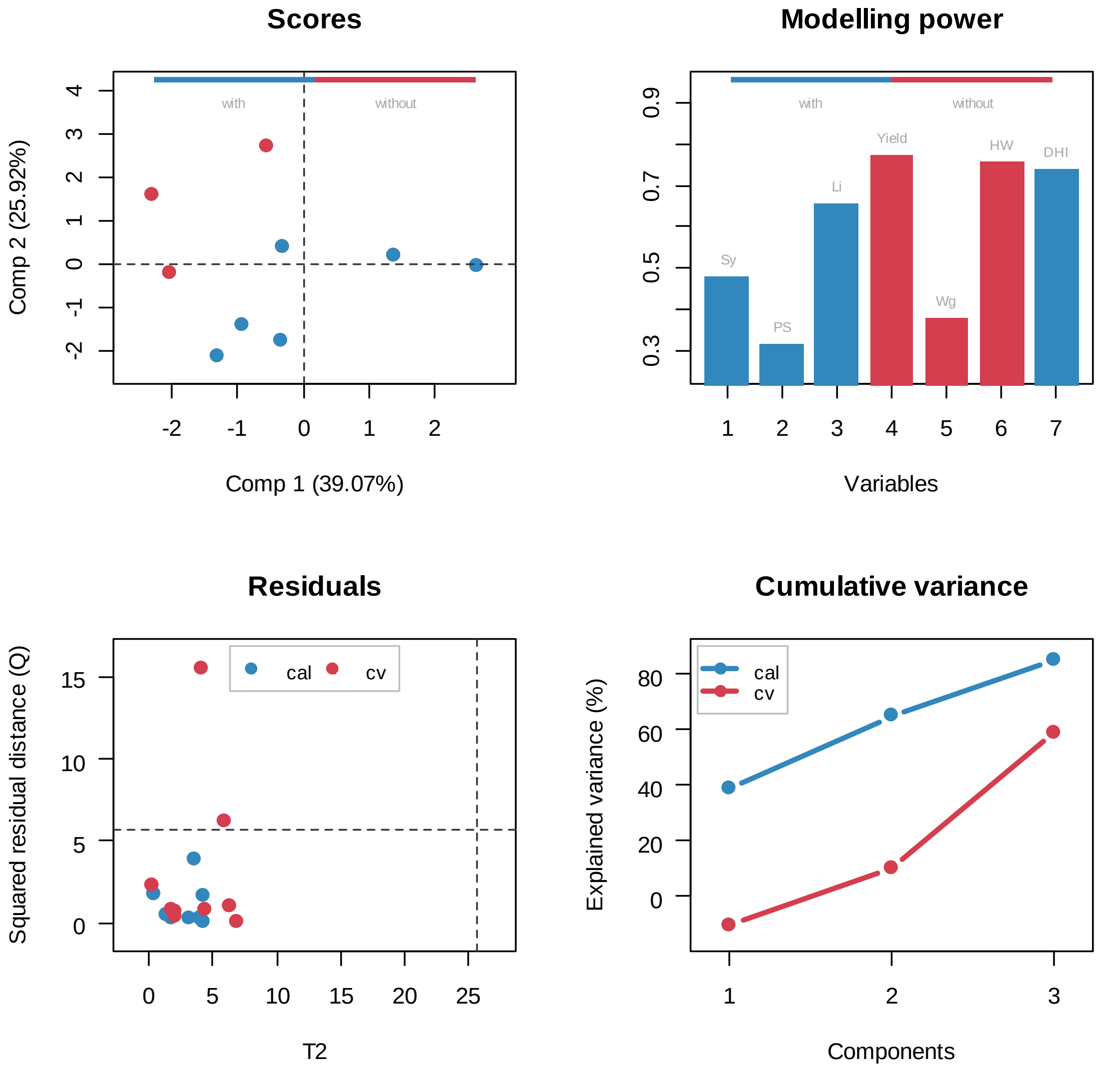

Fig. 6 Detailed one-class model classification of SIMCA for cultivar UFRGS19 showing the scores of PCA, the modeling power of the variables, the residuals (*Q* and T_2), the variance explained the first two components.

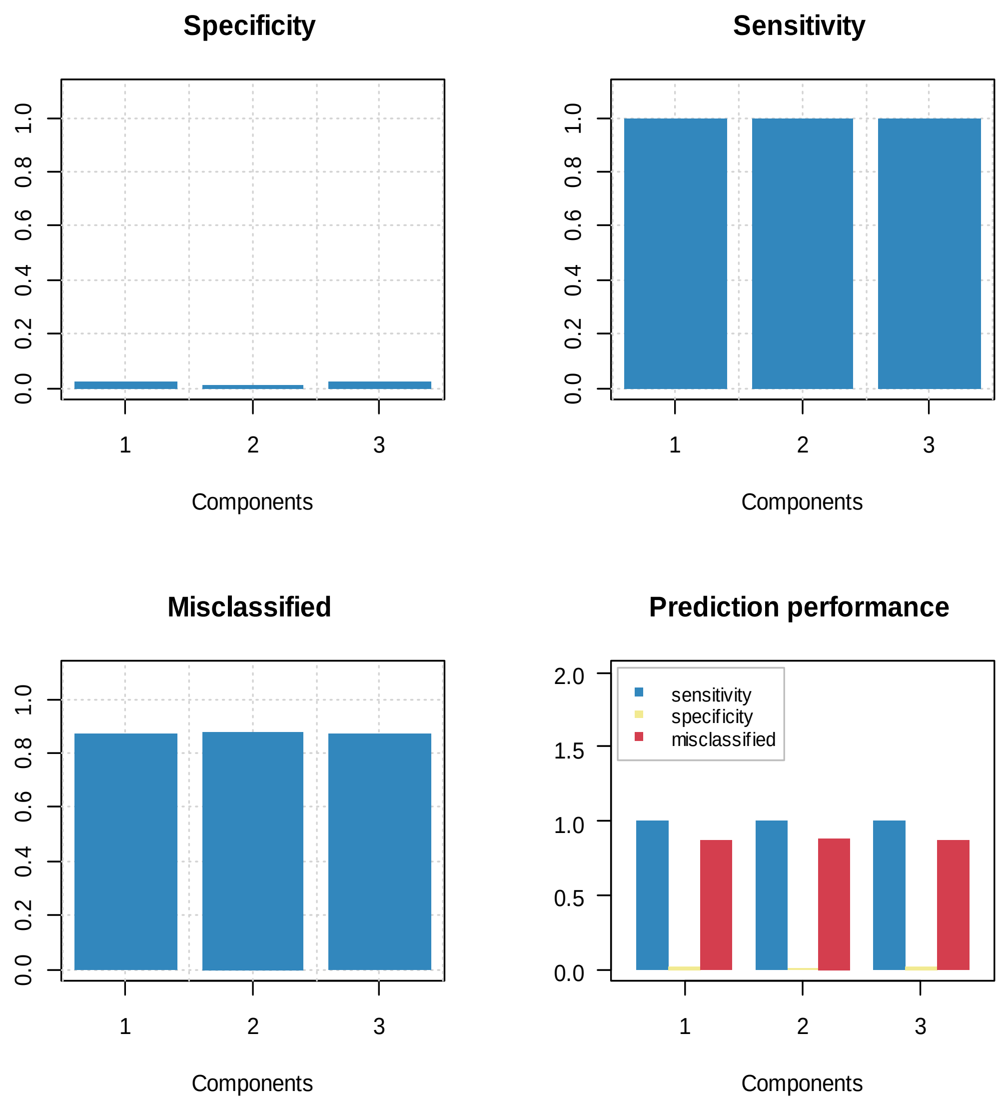

Fig. 7 The specificity, sensitivity, misclassified samples and the prediction performance of each component.

4. Conclusions

In this study, the feasibility of using SIMCA analysis combined with PCA and HCA to find the informative agronomic and industrial parameters of fungicide applied oat cultivars was demonstrated. Fungicide application in oat cultivars affected the LI, HW, Sy, PS and grain yield, which are important parameters in several breeding and agricultural programs. SIMCA algorithm can be used to easily discriminate important variables in many other programs.

Acknowledgments

Technical assistance of under-graduated students of Laboratory of Crop Plants, Department of Agronomy, Santa Catarina State University is to be acknowledged. Also thank CAPES-Brazil/CNPq-Brazil for providing fellowships and the programs PROAP/CAPES, PROMOP/UDESC and FAPESC for financial assistance.

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