Associations between agronomic performance and grain chemical traits in oat

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ABSTRACT
Great progress has already been achieved in breeding white oats (Avena sativa L.). However, breeders of this species need to make available to the market higher yielding and higher grain quality cultivars. Therefore, it is necessary to optimize the strategies of selection of superior genotypes. The present work aimed to estimate the correlation between traits related to agronomic performance and traits related to chemical composition of white oat grains, seeking to add understanding for the selection of higher yielding genotypes with high nutritional quality. Thirty families from the cross between the cultivars Albasul and UPF 15 were used, and 31 families from the cross between the cultivars IAC 7 and UFRGS 19. Six traits related to agronomic performance and five traits corresponding to the chemical composition of the grains were evaluated. To assess the relationship between the different traits evaluated, simple correlation and canonical correlation analyzes were performed. The results demonstrate the existence of simple correlations between grain chemical constituents and traits related to agronomic performance. The pair-by-pair relationship between traits of these two groups was also observed, but the environmental action strongly interfered with these correlations. The canonical correlation analysis allowed the verification of dependence between the evaluated trait groups where genotypes with higher number of grains per panicle, high mass of one thousand grains, with lower stature and lower number of spikelets, will have lower lipid and total fiber content, but with higher protein contents.

Highlighted Conclusions
1. There is a relationship of dependence between the agronomic performance trait group and the oat grain chemical constituent group.
2. Indirect selection of grains with higher nutritional quality and high agronomic performance will be feasible providing there is a strict control of environmental conditions.
3. Selections that seek superiority in all traits studied will not be possible, given the presence of negative correlations.

INTRODUCTION
For many years, white oat (Avena sativa L.) breeding programs have selected genotypes based on agronomic trait performance, prioritizing higher yield and pathogen resistance (Peterson et al. 2005; Martínez et al. 2010). Gains were obtained, since in 1961 the world yield of this cereal was ca. 1.3 tons per hectare and in 2017 this average yield exceeded 2.5 tons per hectare (FAOSTAT 2019).

Nutritional and industrial quality have also become important attributes in the selection of oat genotypes (Frey and Holland 1999, Cervantes-Martínez et al. 2001, Chernyshova et al. 2007, Crestani et al. 2012, Sikora et al. 2013, Hawerroth et al. 2015, Silveira et al. 2016a,b, Verdi et al. 2019). The proper constitution of this cereal meets the growing demand for functional foods. The grains of this species have high protein quality, lipid content with predominance of unsaturated fatty acids and adequate carbohydrate content. Also, a high proportion of dietary fibers, especially β-glucans, as well as components with antioxidant properties (Crestani et al. 2012, Marshall et al. 2013, Sikora et al. 2013). For the human diet there is a preference for grains with lower levels of saturated fatty acids, rich in protein and fiber, especially β-glucans related to lowering cholesterol, blood sugar and weight (Peterson et al. 2005, Daou and Zhang 2012).
The estimation of correlations between traits is an analysis that allows to verify the existence of linear relationship between two variables. This information is very important, especially when aiming at indirect gains, for traits with low heritability or difficult to measure, or to infer whether selection over one trait will influence the performance of another trait throughout selection cycles (Cruz et al. 2012). The correlation between the chemical constituents of oat grains and agronomic performance traits, which are already routinely estimated in breeding programs of this species, would facilitate the selection of genotypes that aggregate high yield and high nutritional quality.

An even broader strategy is canonical correlation analysis, which allows one to estimate the existence and intensity of the relationship between groups of variables, composed of linear combinations of the various traits that constitute them (Cruz et al. 2012). This technique has been shown to be appropriate for estimating relationships between trait groups in different species such as tomato, potato, castor, passion fruit, beans, corn, rice, soybeans and oats (Coimbra et al. 2000, Viana et al. 2003, Zeng 2005, Lorenzetti et al. 2006, Rigão et al. 2009, Santos et al. 2010, Brum et al. 2011, Souza et al. 2012, 2015, Ceccon et al. 2015, Alves et al. 2017, Pereira et al. 2017). Therefore, this study aimed to estimate the correlation between traits related to agronomic performance and traits related to chemical composition of white oat grains, seeking to aid the selection of higher yielding genotypes with high nutritional quality.

MATERIAL AND METHODS

In this study, 61 F₂ oat families, grown in an experimental area of the Plant Genomics and Breeding Center, Eliseu Maciel School of Agronomy of the Federal University of Pelotas - Pelotas / RS, in 2013. Of these, 30 families originated from the crossing between cultivars Albasul and UPF 15, and 31 families originated from the cross between the cultivars IAC 7 and UFRGS 19 were analyzed.

A randomized complete block design with two replications was used. Within each block, a row from each family was sown. This row contained ten plants, from which three central plants were selected for evaluation. The spacing used was 0.3 m between rows and 0.2 m between plants.

Soil tillage in the experimental area followed the recommendations of the Brazilian Oat Research Commission (CBPA 2006), and acidity and macronutrient fertilization (NPK) corrections were performed as required by the soil chemical quality analysis in order to supply the crop demands. When required, tebuconazole fungicide was applied at a dosage of 0.75 L ha⁻¹ (Folicur CE - Bayer Crop Science Ltda., Sao Paulo, Brazil).

The traits related to agronomic performance were: height (HGT - cm), number of panicles per plant (NP), number of spikelets per panicle (NS), panicle mass (PM - g), number of grains per panicle (NG), and mass of one thousand grains (MTG - g). The evaluated traits, corresponding to the chemical composition of white oat grains were: protein content (PROT - g 100 g⁻¹), lipid content (LIP - g 100 g⁻¹), total dietary fiber (FT - g 100 g⁻¹), β-glucan (BG - g 100 g⁻¹) and carbohydrate content (TC - g 100 g⁻¹) which was calculated by difference. For the chemical quality analysis of the grains the proximal infrared reflectance spectrophotometry (NIRS) technique was adopted. In view of this method the grain samples were manually peeled in sufficient quantity to total 7 g of ground material. The grains were ground in a Willey mill (Marconi, Piracicaba, Brazil), with a 0.5 mm sieve opening. The ground material was analyzed in NIRS, Perstorp Analytical, model 5000 (Maryland, USA), at the Food Research Center (Cepa), University of Passo Fundo (UPF). The calibration curves for the determination of the chemical quality traits of white oat grains in NIRS were constructed by Cepa Physicochemical Laboratory using the “New Infra soft International Software” program, by analyzing 100 samples of white oats performed according to the methodologies recommended by AOAC (1997) and AACC (1999). NIRS readings were taken in triplicate and results expressed in g 100 g⁻¹ on a dry basis.

First, an analysis of variance was performed to confirm whether there were significant differences between families, so that they better represented the performance of different oat genotypes. Then, descriptive measures were obtained between families (means, minimum value, maximum value and coefficients of variation). To evaluate the relationship between the different traits evaluated, simple correlation and canonical correlation analyzes were performed. The significance of simple correlations was tested by the t-statistic. The canonical correlations were estimated between the group consisting of the traits related to agronomic performance (EST, NP, NS, NG and MTG) and the group consisting of traits related to the chemical quality of grains (PROT, LIP, FT and BG), according to the procedures described by Cruz et al. (2012).

The null hypothesis: (H0: r₁ = r₂ = .... = rn = 0, s = min (p, q)) for the canonical correlations were tested using the chi-square test:

\[ X^2 = - \frac{n-1}{2} (p + q + 3) \ln \pi (1-r^2) \] . Analyzes were performed using the software GENES (Cruz 2013).
RESULTS AND DISCUSSION

Preliminarily, a multicollinearity analysis was performed between the traits. Considering that the presence of collinearity, resulting from high estimates of phenotypic or genotypic correlations between the studied traits, produces different effects on canonical procedures and makes the results inaccurate, the carbohydrate content and panicle mass were withdrawn from the analysis.

Table 1 shows the mean, minimum and maximum values obtained for all traits evaluated in the 61 oat families, as well as the indication of significant differences (detected by analysis of variance) and the coefficient of variation (CV).

| Traits | Mean | Minimum | Maximum | CV% |
|--------|------|---------|---------|-----|
| PROT   | 17.17* | 13.11   | 28.68   | 8.23 |
| LIP    | 5.79*  | 4.63    | 9.22    | 7.56 |
| FT     | 8.79*  | 7.23    | 9.45    | 2.39 |
| BG     | 5.41*  | 2.50    | 8.89    | 21.58|
| HGT    | 79.91* | 57.67   | 104.00  | 11.16|
| NP     | 5.38*  | 2.33    | 11.33   | 24.42|
| NS     | 26.63* | 13.75   | 51.27   | 19.63|
| NG     | 48.94* | 24.74   | 89.81   | 19.56|
| MTG    | 31.77* | 18.31   | 66.21   | 15.03|

* significant difference in the analysis of variance (F test, p ≤ 0.05). PROT - protein content, LIP - lipid content, FT - total dietary fiber, BG - β-glucans, HGT - stature, NP - number of panicles per plant, NS - number of spikelets per panicle, NG - number of grains per panicle and MTG - mass of one thousand grains.

The traits related to the chemical quality of the grains presented the lowest CV values, except for BG content. Among the traits related to agronomic performance the lowest CV was observed for HGT. The phenotypic variability between the families was verified and the correlation coefficients could be estimated with the proper representation of the association between the traits, since the lack of variation in one of the traits could cause indeterminacy in the correlation estimates.

Table 2 shows the phenotypic, genotypic and environmental correlation coefficients obtained among the agronomic performance traits. The phenotypic correlations between HGT and NP, HGT and NS, NP and MTG and NS and NG were significant and positive. These responses are in agreement with results observed in previous work with different oat genotypes (Dumlupinar et al. 2012, Ahmed et al. 2013, Vaisi and Golparvar 2013, Zaheri et al. 2013, Krishna et al. 2014). Predominantly the genetic correlations between these traits had greater magnitude, confirming these associations and indicating the possibility of a more consistent association with the improvement of environmental conditions.

| Traits | rp | rG | re |
|--------|----|----|----|
| HGT x NP | 0.3806* | 0.7304* | 0.3625* |
| HGT x NS  | 0.3760* | 0.5826* | 0.1008 |
| HGT x NG  | 0.2114 | 0.3980* | 0.2135 |
| HGT x MTG | 0.2411 | 0.9882* | -0.0333|
| NP x NS  | 0.0564 | 0.1216 | -0.0179 |
| NP x NG  | -0.0260 | 0.0347 | -0.1157 |
| NP x MTG | 0.2607* | 0.3927* | -0.0483|
| NS x NG  | 0.7247* | 0.6923* | 0.7806* |
| NS x MTG | -0.1297 | -0.0461 | -0.3768* |
| NG x MTG | -0.4279* | -0.4620* | -0.3859* |

* significant at p ≤ 0.05 by the t-test. HGT - stature, NP - number of panicles per plant, NS - number of spikelets per panicle, NG - number of grains per panicle and MTG - mass of one thousand grains.
The genotypic correlation between HGT and NG and between HGT and MTG was significant. However, the phenotypic correlation was not observed due to a strong influence of the environment. In the analysis of ten different F₂ oat populations, the correlation between height and mass of one thousand grains was significant, but with low magnitudes, in five populations (Lorencetti et al. 2006), suggesting that for some genetic combinations this correlation is fixed. Nevertheless, they are highly influenced by the environment.

Between NG and MTG, the correlation was significant and negative, justifiable due to competition for photoassimilates and the need for compensation between yield components. This result is in agreement with those previously obtained by other authors, who also evaluated the presence of correlations in fixed genotypes and segregating oat populations (Lorencetti et al. 2006, Vaisi and Golparvar 2013, Zaheri et al. 2013).

The correlations between the chemical constituents of the grains are presented in Table 3. Considerable influence of environment (environment correlation) on the manifestation of these correlations was observed. Indeed, some authors have pointed out that these constituents are greatly influenced by environmental factors (Holthaus et al. 1996, Saastamoinen 1998, Doehlert et al. 2001, Martinez et al. 2010, Hawerroth et al. 2013).

The phenotypic correlation between PROT and LIP was significant, however the causes of this correlation were environmental effects, with no genetic correlation, indicating that it was not an inheritable correlation. This correlation was previously observed in a study with different oat genotypes (Hawerroth et al. 2015). Although significant, it presented low magnitude. Our previous work evaluating different Brazilian white oat genotypes, also did not confirm this correlation (Silveira et al. 2016a). Between PROT and FT, there was a positive phenotypic correlation, which is an association by genetic causes, which would present greater magnitude with the reduction of environmental interference. Similar result has already been found in experiments with segregating oat genotypes (Crestani et al. 2012, Hawerroth et al. 2015, Silveira et al. 2016a). It is a correlation of interest for the oat's destination for human consumption, enabling the selection of genotypes with high protein content and dietary fiber.

The correlation between PROT and BG was significant, but of low magnitude. This correlation would be of interest to breeders, however there is no consensus in the literature, some reports of correlation (Cervantes-Martinez et al. 2002, Hang et al. 2007, Sari and Ünay 2017) and others showing the lack of correlation (Hawerroth et al. 2015, Silveira et al. 2016a, Yan et al. 2016). These studies evaluated different genotypes and the contrasting results express the variability for these characters. It was also possible to verify an inheritable correlation between LIP and BG. This correlation was negative, as previously observed (Hawerroth et al. 2015), indicating that the increase in the content of one of these characters will be detrimental to the other. This information is important, since for human food an increase in fiber content and a reduction in lipid content are sought (Cervantes-Martinez et al. 2002, Peterson et al. 2005). Yan et al. (2016) also observed this correlation in Canadian oat genotypes, but this correlation was positive. These facts suggest that genomic regions responsible for these traits (QTLs - Quantitative traits loci) are close, on the same chromosome, maintaining associations between these traits, however there is genetic variability. Predictably, the BG, an integral part of the TF, were positively correlated with this character.

Table 3. Coefficients of phenotypic ($r_p$), genotypic ($r_g$) and environmental ($r_e$) correlations between traits related to chemical quality of white oat grains, evaluated in 61 families in the F₂ generation.

| Traits         | $r_p$     | $r_g$     | $r_e$     |
|----------------|-----------|-----------|-----------|
| PROT x LIP     | 0.2523*   | 0.1787    | 0.3854*   |
| PROT x FT      | 0.2977*   | 0.6183*   | 0.0064    |
| PROT x BG      | 0.2851*   | 0.2732*   | 0.3054*   |
| LIP x FT       | -0.1832   | 0.0419    | -0.4350*  |
| LIP x BG       | -0.4780*  | -0.5229*  | -0.4123*  |
| FT x BG        | 0.4855*   | 0.4716*   | 0.5189*   |

* significant at p $\leq$ 0.05 by the t-test. PROT - protein content, LIP - lipid content, FT - total dietary fiber, BG - β-glucans.

Table 4 shows the correlation values between agronomic performance traits and characters related to chemical grain quality. Among the twenty associations tested, ten genotypic correlations were significant. However, only three phenotypic correlations were significant, being positive between FT and height and between FT and NP and negative between LIP and MTG, all with low magnitudes. This fact indicates the environmental interference on the correlations. However, the influence of the environment did not occur in a linear way in favoring or harming the traits pairwise, since no environmental correlation was significant.
A better control of the environmental conditions would allow a better expression of the genetic potential of the evaluated genetic constitutions, and could isolate some deviations provided by environmental action.

In this work significant genotypic correlations were observed between HGT and PROT (negative) and between HGT and LIP (positive). These correlations have been reported in maize (Okporie and Oselebe 2007). The correlation between height and the evaluated chemical constituents would allow a practical indirect selection before the end of the crop cycle. Perhaps the implementation of an experiment in a greenhouse can have higher control of environmental conditions and enable the observation of phenotypic correlations. The correlation between β-glucan content and one thousand grain mass was observed in a previous study (Sari and Ünay 2017). In the work reported here, the correlation between these traits was only genotypic, again attributing to environmental effects the possible cause of this fact.

Considering the canonical correlation analysis, it was observed that only the first canonical pair presented significant correlation (p ≤ 0.05), by the χ² test (Table 5).

The significance of this correlation indicates that Group I, formed by the characters related to the chemical quality of grains, and Group II, which includes the characters related to agronomic performance, are not independent. As previously observed in a study with corn (Alves et al. 2016), a canonical correlation analysis pointed out associations between traits related to agronomic performance and nutritional traits.

The coefficients of the first canonical pair indicate that genotypes with higher number of grains per panicle, high mass of one thousand grains, smaller stature and smaller number of spikelets, will present lower lipid and total fiber content, but with higher protein content. The content of β-glucans was found to have little influence on intergroup relationships. The correlations observed in table 4 were reaffirmed by the canonical correlation analysis, highlighting the negative correlation between LIP and MTG. The increase in MTG is targeted by industries and has been shown to correlate with grain yield in some oat genotypes (Lorencetti et al. 2006, Redaelli et al. 2008). Low lipid content in oat grains intended for human consumption is also required, with levels below 8% suitable for industry (Yan et al. 2016), thus confirming this correlation is in the interest of breeders of this species. The negative associations observed demonstrate the impossibility of obtaining superiority in all evaluated characters, however, associations of interest were observed. The lack of correlation between BG and other traits of agronomic interest

### Table 4. Phenotypic (r_p), genotypic (r_g) and environmental (r_e) correlation coefficients between traits related to agronomic performance and traits related to chemical quality of white oat grains, evaluated in 61 families in the F_2 generation.

| Traits        | r_p     | r_g     | r_e     |
|---------------|---------|---------|---------|
| PROT x HGT    | -0.1701 | -0.7741*| -0.0007 |
| PROT x NP     | -0.0121 | -0.1364 | 0.1762  |
| PROT x NS     | -0.0786 | -0.2657*| 0.1730  |
| PROT x NG     | -0.2438 | -0.4005*| 0.0300  |
| PROT x MTG    | 0.0113  | 0.0128  | 0.0081  |
| LIP x HGT     | -0.0098 | 0.4308* | -0.1823 |
| LIP x NP      | -0.0295 | -0.0757 | 0.0408  |
| LIP x NS      | 0.2488  | 0.3311* | 0.1425  |
| LIP x NG      | 0.1684  | 0.2510  | 0.0230  |
| LIP x MTG     | -0.3263*| -0.4280*| -0.0337 |
| FT x HGT      | 0.3306* | 0.8751* | 0.0331  |
| FT x NP       | 0.2852* | 0.5870* | 0.0446  |
| FT x NS       | 0.1748  | 0.3967* | 0.0124  |
| FT x NG       | -0.0675 | -0.2256 | 0.0796  |
| FT x MTG      | 0.0313  | 0.0557  | 0.0018  |
| BG x HGT      | 0.0707  | -0.0221 | 0.1192  |
| BG x NP       | -0.0196 | -0.0849 | 0.0650  |
| BG x NS       | 0.0244  | 0.0559  | -0.0121 |
| BG x NG       | -0.0959 | -0.1962 | 0.0537  |
| BG x MTG      | 0.1539  | 0.2589* | -0.1099 |

* significant at p < 0.05 by the t-test. PROT - protein content, LIP - lipid content, FT - total dietary fiber, BG - β-glucans, HGT - height, NP - number of panicles per plant, NS - number of spikelets per panicle, NG - number of grains per panicle and MTG - mass of one thousand grains.

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does not facilitate indirect selection for this character, however, does not make it impossible to obtain productive genotypes with high content of these fibers.

| Traits | 1<sup>st</sup> | 2<sup>nd</sup> | 3<sup>rd</sup> | 4<sup>th</sup> |
|--------|-------------|-------------|-------------|-------------|
| PROT   | -0.4647     | 0.9930      | 0.4478      | -0.1717     |
| LIP    | 0.8401      | -0.6080     | 0.7255      | 0.2166      |
| FT     | 0.9062      | 0.4010      | -0.5901     | -0.1699     |
| BG     | 0.1034      | -0.5456     | 0.3925      | 1.2032      |
| HGT    | 0.4746      | -0.2354     | -0.6846     | 0.2544      |
| NP     | 0.3124      | 0.4299      | -0.2653     | -0.7012     |
| NS     | 0.9011      | 0.4334      | 0.7801      | 0.7332      |
| NG     | -0.7985     | -1.1918     | -0.5880     | -0.4861     |
| MTG    | -0.7789     | -0.1261     | -0.4645     | 0.5717      |
| r      | 0.66        | 0.36        | 0.35        | 0.19        |
| $\chi^2$ | 47.64* | 16.68 | 9.01 | 2.02 |

* Significant (p ≤ 0.05) by the chi-square test ($\chi^2$). PROT - protein content, LIP - lipid content, FT - total dietary fiber, BG - β-glucans, HGT - height, NP - number of panicles per plant, NS - number of spikelets per panicle, NG - number of grains per panicle and MTG - mass of one thousand grains.

It can be concluded that there is a dependency relationship between the agronomic performance character group and the oat grain chemical constituent group, enabling the indirect selection of grains with higher nutritional quality, however, a strict control of environmental conditions is necessary. The presence of negative correlations makes it difficult to select genotypes that are superior in all characters evaluated in this study.

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