# Description of a pure seed fraction of oat through usual evaluations and radiographic images[1]

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Abstract – The objective of this work was to evaluate the width and length incidence in a single seed fraction of oat [*Avena sativa* (L.)] cv. Cristal. The seeds were selected by a mechanical divider and by hand, and their correspondence to radiographic images in seeds with glumes and their caryopses. The width and length of the seeds with glumes and their caryopses were measured with electronic calliper, and their weight, with precision balance. Radiographic images of seeds with glumes were taken with an X-ray experimental equipment. The analyst selected seeds with glumes by the width and by the length previously determined and so with more weight, than that obtained by hand selection was slightly narrower, larger and lighter. The presence of the glumes masked the caryopses real dimensions (width and length), and conduced the analyst to select seeds that differed more by the width than by the length. The radiographic images showed the presence, or not, of caryopses inside the seed and its real dimensions. The mechanical partition method for seeds showed to be more efficient because the analyst subjectivity was not considered when the selection upon its dimensions was done. The X-ray analysis was a useful tool that complements the pure seed fraction selection as another factor of seed quality.

Index terms: *Avena sativa*, agronomic characters, X-rays, seed quality.

**Descrição de uma fração pura de sementes de aveia por meio de avaliações usuais e por imagens radiográficas**

Resumo – O objetivo deste trabalho foi avaliar a incidência da largura e do comprimento numa única fração de sementes de aveia [*Avena sativa* (L.)] cv. Cristal. Essa fração de sementes foi selecionada por um divisor mecânico e seleção manual e sua correspondência com imagens radiográficas em sementes com glumas e seus cariopses. Foram medidos a largura e o comprimento das sementes com glumas e seus cariopses com um calibrador eletrônico, e seu peso, com o emprego de uma balança de precisão. As imagens radiográficas das sementes com glumas foram tomadas com um equipamento experimental de raios X. O analista escolheu sementes com glumas de largura e comprimento previamente determinado, e, portanto, com peso maior do que o das obtidas pela separação manual, as quais foram menos largas, mais compridas e mais leves. A presença das glumas mascarou as dimensões reais dos cariopses (largura e comprimento), conduzindo o analista a selecionar sementes que diferiram mais pela largura que pelo comprimento. As imagens radiográficas mostraram a presença, ou não, dos cariopses no interior das sementes e suas reais dimensões. Os métodos de seleção mecânica de sementes mostraram ser os mais eficientes, pois não foi considerada a subjetividade do analista quando a seleção foi feita por suas dimensões. As análises por raios X foram um instrumento útil que complementou a seleção da fração pura como outro fator da qualidade da semente.

Termos para indexação: *Avena sativa*, características agronômicas, raios X, qualidade da semente.

## Introduction

Oat seed quality is of great importance to rapid crop establishment due to the fact that during sowing time (autumn and spring) there are many weed seeds species that compete with the crop for water and nutrients at the first stages of crop development.

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With respect to the germination, large spring oat seeds tend to perform better behaviour than small ones (Naylor, 1993; Bockus & Shroyer,1996; Lopez Castaneda et al., 1996; Nedel et al., 1996). Chastain et al. (1995a, 1995b), working with winter wheat and barley concluded that large seeds germinated faster and emerged rapidly under field conditions. Other authors found positive correlation between seed size and oat yields (Marshall & Sorrells,1992 cited by Guberac et al., 1999; Gan & Stobbe,1995, 1996). In other species such as wheat and barley, results relative to the embryo length and seedling rooting favoured large seeds as compared to small seeds (Kolak, 1994 cited by Guberac et al., 1999).

In order to establish the seed value for sowing, a series of related quality analysis like the purity analysis must be done. In this type of analysis, the pure seed fraction which is representative of the working sample received must be separated. The submitted sample shall be repeatedly divided to attain the weight to be used for the working sample. To perform the purity analysis, the analyst must take care, in order to prevent a subjective selection of the seeds that favours some particular character with detriment to others. If this happen, the result will not correspond to the representative sample of the original lot and will not be correct. For the genus *Avena*, the pure seed fraction includes a number of structures that not only contain an individual fertile flower but also multiple seed units, referred to the conjunction of a fertile flower joined to one or more sterile or fertile flowers of different length (International Seed Testing Association, 1996).

The identification process of this fraction implies that the analyst shall use a particular criterion which is supported on the basis of seed visible characteristics and with mechanical aid, or using pressure, provided that this does not produce damage for the future germination (International Seed Testing Association, 1996).

The seed selection by simple sight observation according to external characteristics is not sufficient because this does not allow to determine the actual dimension of the caryopsis inside the flower. Neither is possible to make precise reference about the caryopsis presence and the embryonic structure integrity nor aspects related to possible internal infestations by insects, all factors that may have decisive influence on germination.

The objective of this work was to evaluate the incidence of width and length dimensions of the oat simple seed fraction (with glumes and their caryopses) on the seed weight, selected by a mechanical divider and by hand, and its correspondence with radiographic images.

## Material and Methods

This work was performed in the Seed Laboratory of the Oliveros Agricultural Experimental Station (E.E.A. Oliveros - INTA) 33º South latitude, 61º West longitude, Santa Fe, Argentina.

A sample of *Avena sativa* (L.) cv. Cristal seeds was used, from which two fractions were obtained using two partition methods: cone mechanical divider and hand selection. The seed sample was poured into a mechanical divider composed of two inverted cones (one into the other) and disposed in a vertical position. The seeds falled in the inner surface of the upper cone and slid on the outer surface of the lower cone which ends in a quarterly divided recipient. The fraction obtained by hand was formed choosing at simple sight a large and wide seed which was used as a pattern to select the other seeds based on the width (3 mm) and length (11 mm) with an average variation of ±0.5 mm.

The sizes (width and length) were measured on seeds with glumes using an electronic calliper (Acme Inc.) and their individual weight (mg) was measured with a precision analytic balance (Mettler H 80) 0.0001 sensibility. Then, lemma and palea were taken off the seeds and the same variables were measured on the caryopsis. Individual radiographic images of seeds with glumes were taken using an experimental X-ray equipment. The seeds were positioned at a distance of 13 cm from the transmitter source, at 10 milliamperes and 35 kilovolts, and for an exposure time of 0.40 seconds. A specific software recorded the radiographic images captured by a digital sensor.

The data were analyzed through a factorial 2x2 (two methods of seed selection: by cone mechanical divider and by hand x two types of seeds: with glumes and their caryopses only).

One hundred replicates of one seed per treatment were used, in a completely randomized design. The means were compared by the F test and Duncan test at 5% level of probability. Correlation and multiple regressions were estimated and simple correlation coefficients (r) and determination coefficients ($R^2$) were calculated. The statistical program MSTAT-C (Michigan State University, 1989) was used.

The individual radiographic images of the seeds with glumes were studied and compared with the analyzed variables from both seed fractions.

## Results and Discussion

The variance analysis for seeds with glumes and their caryopses evaluation partitioned by cone mechanical divider and by hand, showed significant interaction. The evaluation of the seed width between the partition methods showed wider seeds with glumes when the selection was done by hand by the analyst (Table 1). However, the mechanical partition method separated wider caryopses than the ones obtained by hand. Seeds width with lemma and palea were higher than the caryopses width as regard to the glumes presence.

According to the length variable there was significant interaction between seeds with glumes and their caryopses. Seeds with lemma and palea presented significant differences and larger seeds were favoured when mechanical divider was used. Nevertheless, the caryopses were larger when the partition was done by hand. The palea and lemma presence has an important incidence upon seed size producing a mask of the actual width and length of the caryopses being more pronounced for the length.

There were significant differences for the weight variable between both partition methods showing that seeds with glumes and their caryopses, selected by hand, had more weight. The glume presence explains the higher weight of the seeds with glumes than those with caryopses for both partition methods.

The analyst naturally selected seeds with lemma and palea by the width than by the length previously determined and so with more weight, meanwhile seeds obtained by mechanical divider showed to be slightly narrower, larger and more lighter than that obtained by hand selection.

Independently of the partition method used, seeds with lemma and palea were greater (size and weight) than the caryopses.

In both partition methods seeds with glumes and their caryopses inside, the width presented high significative correlation with weight (Table 2). Besides, caryopses length had a significant correlation with their weight in both fractions of seeds. Seeds with glumes showed significant correlation between the variables length and weight; however, determination coefficients only explained about 4% of the incidence of one variable on the another.

The multiple linear regression model that was used to estimate the width and length incidence on seeds for each case, its significance and the correlation and multiple determination coefficients (Table 3). Then, a sequential analysis was done, showing the signification of each variable to the mathematical model and the partial determination coefficients ($R^2$ y $x_i/x_j$) in order to evaluate the contribution of the width to the seed weight when the length is present in the equation and the contribution of the length when the width is present in the equation (Table 4).

The determination coefficient put in evidence that in seeds with glumes selected by both methods, the contribution of the width variable was highly significative when the variable length was present in the regression equation. However, the contribution of the variable length was not significative when the variable width was present in the equation.

In caryopses of seeds obtained with mechanical divider or by hand, both variables, width and length, had highly significative contribution to the weight. The fraction of seeds selected by mechanical divider

**Table 1.** Width (mm), length (mm) and weight (mm) of oat seeds with and without glumes, separated by mechanical divider and hand selected[(1)].

| Partition seed methods | With glumes | Caryopses | Means |
|---|---|---|---|
| | Seed width (mm) | | |
| Mechanical divider | 2.788bA | 2.626aB | 2.528 |
| Selected by hand | 2.865aA | 2.393bB | 2.629 |
| Means | 2.827 | 2.331 | |
| | Seed length (mm) | | |
| Mechanical divider | 12.164aA | 7.868bB | 10.016 |
| Selected by hand | 11.219bA | 8.356aB | 9.787 |
| Means | 11.692 | 8.112 | |
| | Seed weight (mg) | | |
| Mechanical divider | 35.339bA | 24.169bB | 29.754 |
| Selected by hand | 39.335aA | 27.616aB | 33.485 |
| Means | 37.347 | 25.892 | |

[(1)]Values in the same column followed by the same small letter and values in the same line followed by the same capital letter do not differ at 5% of probability (F test); the coefficients of variations were, 10.21%, 9.53% and 24.34% for seed width, length and weight, respectively.

showed similar contributions to the weight (13.5 and 16.7%). On the other hand, the fraction of seeds selected by hand revealed more difference between $R^2$ values, enhancing the contribution of the variable width on the weight (13 and 62.8%). The reason for this fact could be the analyst's tendency to select wide seeds and not large seeds because the glumes presence masked the actual size of the caryopses. The width contribution of seeds with glumes is significative for both fractions, but there is more contribution of the weight when seeds were selected by mechanical divider (62%) (Table 4).

X-ray radiographic images taken with the experimental equipment allowed the verification of the presence or absence of caryopsis inside the seed and its actual size, the central depth and air spaces surrounding the caryopsis (Figure 1). These spaces were considered as a part of the seeds when length and width were measured. It was possible to take measures on the radiographic images through the digital software and they were compared with the same measures taken with the electronic calliper.

X-radiography was a simple, rapid and non-destructive method for analyzing the internal morphology of seeds, and allowed to determine if they were empty or filled.

According to the results obtained in this work, the importance of the use of mechanical divider for the selection of the working sample from the original one to use it for different analysis can be emphasized. Even though this method does not show an absolute advantage for filled seeds selection, they contribute to prevent the analyst's subjectivity. This was revealed by the results obtained from the selection by hand done by the analyst who unconsciously chosed the widest seed as a pattern.

The analyst's procedure was based on the supposition that the caryopsis size may be like the glume size; but in this work, it was concluded that this is not always true. The fact has great importance if it is taken into account that other researchers (Chastain et al, 1995a) had concluded that size has great influence for rapid germination and establishment of oat seeds. Moreover, the results showed more incidence

**Table 2.** Simple correlation (r) and determination ($R^2$) coefficients of oat seeds with glumes and their caryopses divided by mechanical divider and selected by the analyst by hand(1).

| Partition seed methods | | | Width | | | | Length | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | With glumes | | Caryopses | | With glumes | | Caryopses | |
| | | | r | $R^2$ | r | $R^2$ | r | $R^2$ | r | $R^2$ |
| | | | Mechanical divider | | | | | | | |
| Mechanical divider | Length | With glumes | 0.25** | 6.25 | - | - | - | - | - | - |
| | | Caryopses | - | - | 0.60** | 36.00 | - | - | - | - |
| | Weight | With glumes | 0.80** | 64.00 | - | - | 0.21* | 4.41 | - | - |
| | | Caryopses | - | - | 0.62** | 37.20 | - | - | 0.60** | 36.00 |
| | | | Selected by hand | | | | | | | |
| Selected by hand | Length | With glumes | 0.23* | 5.29 | - | - | - | - | - | - |
| | | Caryopses | - | | 0.41** | 16.80 | - | - | - | - |
| | Weight | With glumes | 0.58** | 33.64 | - | - | 0.21* | 4.41 | - | - |
| | | Caryopses | - | - | 0.83** | 68.89 | - | - | 0.52** | 27.04 |

(1)Determination coefficients (%). * and **Significant at 5% and 1% of probability, respectively.

**Table 3.** Multiple linear regression model, correlation (r) and determination ($R^2$) coefficients of oat seeds, with glumes and their caryopses, separated by mechanical divider and selected by the analyst by hand.

| Partition seed methods | Multiple linear regression model(1) ($Y = a + b_1x_1 + b_2x_2$) | r | $R^2$ |
|---|---|---|---|
| Seeds with glumes separated by mechanical divider | $Y = -42.275039 + 27.859x_1 - 0.0054827x_2$ | 0.80** | 0.64 |
| Caryopses from seeds separated by mechanical divider | $Y = -18.44513 + 9.2414x_1 + 2.7516x_2$ | 0.68** | 0.47 |
| Seeds with glumes separated by hand | $Y = -48.5209 + 21.920x_1 + 2.2360x_2$ | 0.58** | 0.34 |
| Caryopses from seeds separated by hand | $Y = -31.73274 + 17.472x_1 + 2.0980x_2$ | 0.85** | 0.73 |

(1)Y: seed weight; $x_1$: seed width; $x_2$: seed length. **Significant at 1% of probability.

**Table 4.** Partial determination ($R^2$) coefficients and significance of the length contribution to weight (mg) when the oat seed width (mm) is present in the regression equation and width (mm) contribution to weight (mg) when the length is present in the regression equation.

| Variable | Partition seed methods | | | |
|---|---|---|---|---|
| | Mechanical divider | | Selected by hand | |
| | With glumes (%) | Caryopses (%) | With glumes (%) | Caryopses (%) |
| $R^2$ $(y.x_2/x_1)^{(1)}$ | $0.02^{ns}$ | 13.50** | $1.00^{ns}$ | 13.00** |
| $R^2$ $(y.x_1/x_2)^{(1)}$ | 62.00** | 16.70** | 30.80** | 62.80** |

$^{(1)}x_1$: seed width; $x_2$: seed length. $^{ns}$Non-significant. **Significant at 1% of probability.

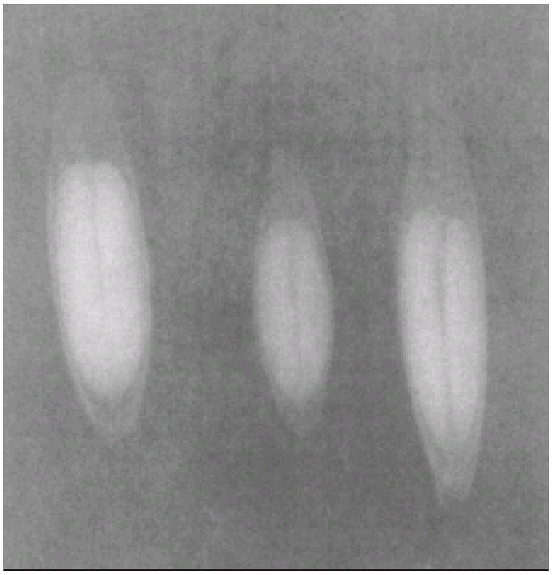

**Figure 1.** Radiographic image to *Avena sativa* (L.) cv. Cristal seeds.

of the variable width on the seed weight. This observation would be helpful to take into account that the seed selection shall be done first by its width during the classification process.

## Conclusions

1. The selection of pure oat seed by external morphologic characters such as width and length require the use of seed mechanical dividers.
2. X-ray technology is a powerful tool as a complement for rapid and efficient selection of pure seed fraction.

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