Kernel Size Variation in Naked Oat

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ABSTRACT

Kernel size in naked oat (Avena sativa L.) is of interest because of the importance of groat size in determining flake size in “old-fashioned” style oat flakes. Here we compared naked oat kernel size distributions with those of hulled oat (Avena sativa L.). Most hulled oat spikelets have two kernels, where the primary kernel is distinctly larger than the secondary kernel. Although naked oat panicle architecture was similar to that of hulled oat, naked oat had a distinctive multiflorous spikelet architecture that contained from one to six kernels. Because single and double-kernel spikelets were most abundant in naked oat, the mean kernel number per spikelet did not differ between hulled oat and most naked genotypes. Like hulled oat, naked oat kernel size decreased with increasing kernel order. All hulled oat and most naked genotypes had size distributions departing from normality, but some naked genotypes had normal kernel size distributions. Variances of naked oat kernel size were much smaller than those of hulled genotypes, indicating a greater degree of kernel sizes uniformity in naked genotypes. However, the increased variation in the hulled oat kernel size appeared to be due to the presence of the hull and hulled oat groats had about the same size variation as did naked oat groats.

Naked oat, also called hull-less oat, are oat that can thresh free of the hulls during harvest. The spikelets of naked oat are distinctively different morphologically from hulled oat and this unique spikelet is referred to as a multiflorous spikelet (Valentine, 1995) or for extreme multiflorous cases, a “chevron-type” spikelet (Burrows, 1986). Whereas, hulled oat spikelets may contain 1 to 3 (rarely 4) florets, naked oat spikelets may contain up to 12 florets (Burrows, 1986; Valentine, 1995). This trait appears to be controlled primarily by a single dominant gene, whose expression may be modified by several other genes (Valentine, 1995).

Studies on oat kernel size uniformity have established that the order of kernels in the hulled oat spikelet has a profound effect on the kernel size. It is well established that the primary kernel of the oat spikelet is the largest in the spikelet and that kernel size decreases with increasing order in the spikelet (Berry, 1920; Doehlert et al., 2002; Hutchinson et al., 1952; Youngs and Shands, 1974), where tertiary kernels are usually too small for many commercial applications. Because naked oat spikelets can contain so many more kernels per spikelet than hulled oat, determination of how kernel size was influenced by kernel order in naked oat became of interest. Although oat kernel size is primarily of interest because of dehulling conditions, persistent interest in the use of naked oat for human food, since it would not require dehulling, stimulates interest in the influence of the multiflorous spikelet on kernel size variation. But groat size is also important for the manufacture of “old fashioned” oat flakes, because the size of the groat determines the size of the flake. Because large flakes are desirable for “old fashioned” style oat flakes, it is of interest to determine groat size in naked oat, relative to hulled oat to determine their suitability for this application.

Size distributions of oat usually depart significantly from normality and tend to more closely resemble bimodal or multimodal distributions. Bimodal distributions are probably derived from subpopulations derived from primary and secondary kernels from double-kernel spikelets (Doehlert et al., 2002, 2004; Takeda and Frey, 1980). Analyses have indicated that double-kernel spikelets account for about 80% of the oat spikelets. Environments that generate higher frequencies of triple-kernel spikelets tend to generate populations that are less bimodal, according to bimodal analysis (Doehlert et al., 2004). Because the long-term goal of this research is to help develop strategies for developing oat cultivars with improved kernel size uniformity, the question as to how the multifloret panicle affected kernel size distributions became intriguing.

In this study, we grew 10 genotypes of naked oat in three environments in a single year experiment in North Dakota, USA, and analyzed their panicle and spikelet structures by hand dissection. We also analyzed grain size distributions of grain harvested from the same plots by digital image analysis and tested for normality and for bimodal distributions. Our objective for this study was to describe naked oat panicle and spikelet structure and to describe the observed variation in naked oat kernel size in comparison with hulled oat and their groat.

MATERIALS AND METHODS

Plant Material

Ten genotypes of naked oat and two genotypes of hulled oat were grown in replicated field plots. The naked genotypes were the cultivars Buff, Paul, and Stark, and the breeding lines ND000149, ND000576, ND000725, ND000844, ND001304, and ND910932. AC Assiniboia and Morton, both hulled cultivars, were grown as hulled reference genotypes. The experiments were grown at three environments in North Dakota (Carrington, Casselton, and Fargo) in the year 2002 growing season with three replicate plots per location. The soil type at Carrington is Heindahl (coarse, loamy, mixed Udic Haploborolls) and Emrick loams (coarse, loamy, Pachic

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The soil at Casselton is characterized as a Perella-Bearden silty clay loam complex. Perella is a fine-silty, mixed, frigid Typic Haplaquoll; Bearden is a fine-silty, frigid Aeric Calciaquoll. The soil at Fargo is a Fargo silty clay, a fine, montmorillonitic, frigid Vertic Haplaquoll. A seeding rate of $2.47 \times 10^6$ kernels ha$^{-1}$ was used for all experiments. Herbicide treatments consisted of pre-emergence application of 3.93 kg ha$^{-1}$ propachlor (2-chloro-N-isopropylacetanilide) and post-emergence application at the 3-leaf stage with a tank mix of 0.14 kg ha$^{-1}$ thifensulfuron [3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoylsulfamoyl] thiophene-2-carboxylic acid], 0.07 kg ha$^{-1}$ tribenuron {2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]benzoic acid}, and 0.14 kg ha$^{-1}$ clopyralid (3,6-dichloropyridine-2-carboxylic acid). Experimental units consisted of four rows spaced 0.3 m apart and 2.4 m long. The two center rows were harvested with a two-row binder and threshed with a plot thresher. The harvested grain was cleaned with a Clipper (Bluffton, IN) Model 400 Office Tester and Cleaner fitted with a 4.75-3.19-mm oblong hole sieve and with aspiration adjusted so that kernels containing a groat were not removed. The sieve allowed all grain to pass through. Ten panicles were selected at random from bundles for panicle analysis.

**Panicle Analysis**

Panicle and spikelet structures were analyzed by hand dissection. Spikelets from all whorls on each panicle were pooled. Number of whorls per panicle was recorded. Initially all spikelets were stripped from the panicle and were sorted according to the number of kernels each contained. Then spikelets were dissected and for each spikelet type, kernels were sorted according to their order, and their mass determined. From this data, whorls per panicle, spikelets per panicle, kernels per panicle, grain mass per panicle, mean mass per kernel, spikelet type frequencies, and mean masses of different order kernels from each spikelet type was recorded. Calculation of these values is described in detail in Doehlert et al. (2002).

**Digital Image Analysis**

Digital image analysis was used to measure dimensions of large numbers (sample of 300–500) of naked and hulled oat. The protocols used were largely identical to those described by Doehlert et al. (2004), except a digital camera was used instead of an analog (film) camera. Thus, 5 megapixel images were downloaded directly to photo editing software. Images were analyzed by the same Amphion (Amerinex Applied Imaging, Amherst, MA) software described in Doehlert et al. (2004) to measure length, width, and image area. Summary statistical analysis generated image area means and image area variances for each experimental plot.

**Experimental Design and Statistical Analysis**

Field plots were arranged in a random complete block design with three replicates. In the analysis of variance, genotypes were considered fixed and environments were considered random. The genotype × environment interaction was used as the error term for testing the genotype effect and the residual error was used to test the genotype × environment interaction. Mean separation was determined by the least significant difference (LSD). Normality of distributions was tested on grain harvested from each individual plot with the Wilk-Shapiro test. These statistical analyses, as well as calculation of frequency distributions, means and variances were performed with the Statistix statistical software package (Analytical Software, Tallahassee, FL). Bimodal analysis was performed by a program written by J.-L. Jannink (Iowa State University) as described in Doehlert et al. (2004).

**RESULTS**

Spikelets of naked genotypes had a distinctly different appearance (Fig. 1), consistent with the multiflorous spikelet with loosely adhering lemma and palea, as described by Burrows (1986). Differences in naked oat spikelet size were evident according to genotype. Whereas many genotypes had relatively small spikelets (small multiflorous spikelets) others, such as ND000237, had relatively large spikelets.

Analysis of panicle architecture indicated some interesting differences among genotypes. Analysis of variance (not shown) indicated significant environmental effects, genotype effects, and significant genotype × environment interaction for whorls per panicle, spikelets...
Genotype One Two Three Four Five Six kernels per spikelet

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Whorls per panicle</th>
<th>Spikelets per panicle</th>
<th>Kernels per panicle</th>
<th>Grain mass per panicle</th>
<th>Mean mass per kernel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buff</td>
<td>3.2 C</td>
<td>15.1 DE</td>
<td>29.8 B-D</td>
<td>709 BC</td>
<td>24.6 D-F</td>
</tr>
<tr>
<td>Paul</td>
<td>3.7 A</td>
<td>22.1 AB</td>
<td>40.0 AB</td>
<td>953 AB</td>
<td>24.8 D-F</td>
</tr>
<tr>
<td>ND000149</td>
<td>3.3 BC</td>
<td>13.2 D</td>
<td>19.3 D</td>
<td>485 C</td>
<td>26.4 C-E</td>
</tr>
<tr>
<td>ND000237</td>
<td>3.1 C</td>
<td>16.1 C-E</td>
<td>42.7 A</td>
<td>776 B</td>
<td>18.4 G</td>
</tr>
<tr>
<td>ND000576</td>
<td>3.6 A</td>
<td>18.9 B-D</td>
<td>35.1 A-C</td>
<td>795 B</td>
<td>23.4 EF</td>
</tr>
<tr>
<td>ND000725</td>
<td>3.7 A</td>
<td>17.2 C-E</td>
<td>26.7 CD</td>
<td>734 BC</td>
<td>27.6 A-D</td>
</tr>
<tr>
<td>ND000844</td>
<td>3.6 A</td>
<td>16.4 C-E</td>
<td>26.3 CD</td>
<td>806 B</td>
<td>30.7 AB</td>
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<tr>
<td>ND001304</td>
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<td>18.0 B-D</td>
<td>36.0 A-C</td>
<td>794 B</td>
<td>22.1 FG</td>
</tr>
<tr>
<td>ND910932</td>
<td>3.1 C</td>
<td>16.5 C-E</td>
<td>30.0 BC</td>
<td>787 B</td>
<td>26.4 C-E</td>
</tr>
<tr>
<td>Stark</td>
<td>3.7 A</td>
<td>20.1 BC</td>
<td>42.6 A</td>
<td>1178 A</td>
<td>29.1 A-C</td>
</tr>
<tr>
<td>AC Assiniboia</td>
<td>3.2 C</td>
<td>19.3 B-D</td>
<td>34.9 A-C</td>
<td>1097 A</td>
<td>31.6 A</td>
</tr>
<tr>
<td>Morton</td>
<td>3.5 AB</td>
<td>25.3 A</td>
<td>42.0 A</td>
<td>1104 B</td>
<td>27.5 B-D</td>
</tr>
</tbody>
</table>

† Values in the same column with the same letter do not differ significantly at P < 0.05 (mean separation LSD).

Greater grain mass per panicle than all naked genotypes, except for Stark. AC Assiniboia also had larger kernels than most genotypes (including Morton) but did not differ significantly in mass from Stark, ND000725, or ND000844. Morton kernels were only significantly larger than two naked genotypes, ND001304 and ND000237. This is interesting in that the kernel mass of the hulled genotypes included the hull, whereas the kernel mass of the naked genotypes was the groat only.

Large differences in the numbers of kernels per spikelet were observed between hulled and naked genotypes. Naked genotypes primarily had one- and two-kernel spikelets, although unlike hulled oat, many naked genotypes had higher frequencies of single-kernel spikelets than double-kernel spikelets. The hulled genotypes had more double-kernel spikelets than any type (Table 2). Hulled genotypes had relatively high frequencies of single-kernel spikelets, and low frequencies of triple-kernel spikelets. Most naked genotypes, but not all, had significantly higher frequencies of triple-kernel spikelets than hulled genotypes. All naked genotypes had some four-kernel spikelets, whereas the hulled genotypes had none. However, only some of the naked genotypes had five- and six-kernel spikelets, and these were present at relatively low frequencies compared with other spikelet types. One genotype, ND000237, had particularly high frequencies of five- and six-kernel spikelets. This genotype also had relatively large spikelets, as pictured in Fig. 1.

Kernel size decreased with increasing order within naked oat spikelets (Fig. 2). However, there was significant diversity in the nature of this progression. In some genotypes, the mass of the primary kernel of the spikelet increased with the total number of kernels in the spikelet, as observed with ND910932 and ND000237. Six of the 10 naked genotypes followed this pattern. However, there was little difference in the mass of primary kernels in any spikelet type in the naked genotypes Stark, Buff, Paul, and ND001304 (see Fig. 2 for Stark data, others not shown).

Digital image analysis indicated that the hulled oat kernels were mostly larger in linear dimensions than the naked oat (Tables 3 and 4). AC Assiniboia kernels were larger than all other genotypes tested (including...
the hulled cultivar Morton). Kernels from the hulled cultivar Morton were larger than kernels from all naked genotypes, except for Stark. The variances of the image areas of hulled oat kernels were, in general, much larger than those of the naked genotypes. The area variance of AC Assiniboia was greater than those of all other genotypes. The area variance of Morton was greater than the area variances of all naked genotypes, except for Stark and ND000725. However, the image area variances of many naked genotypes were only about a third of Morton and 20% of AC Assiniboia, indicating that most naked genotypes had much greater uniformity in kernel size than did the hulled kernels.

Tests for normality of distributions (Table 3) indicated that none of the hulled genotypes had normally distributed kernel sizes, although six of the 10 naked genotypes had size distributions that did not depart significantly from normality. Tests for bimodality conflicted somewhat with this result, in that it indicated that size distributions of all genotypes could be better described by a bimodal model than by a normal distribution. The bimodality coefficient of Morton was higher than all other genotypes, indicating its size distributions were most distinctly bimodal. The bimodality coefficient of AC Assiniboia was less than Morton and did not differ significantly from most naked genotypes. Some naked genotypes, such as Paul, ND000844, and ND910923 had significantly lower bimodality coefficients and were only slightly higher than the threshold to indicate a significant level of bimodality. There was little variation in the Prob1 value, which indicates the probability of a kernel to be in the putative subpopulation 1 (with smaller kernel size) of the bimodal distribution. These values were close to 0.5, indicating that the two subpopulations contained about the same number of kernels in each respective postulated subpopulation.

Graphical depictions of kernel size distributions for AC Assiniboia, Stark, Paul, and Buff oat grown at Carrington (Fig. 3) and Fargo (Fig. 4) are provided so that the differences in these size distributions can be better visualized. These genotypes were selected to be representative of all of the genotypes studied. Other genotypes had similar distributions as shown. At both these locations, AC Assiniboia had a much greater range of sizes than that of any of the naked genotypes. AC Assiniboia distributions had a distinct bimodal appearance, whereas the naked genotypes appeared more normally distributed. Finally, it is visually obvious that the mean sizes of the naked genotypes were all less than that of the hulled cultivars. Size distributions at Casselton were similar to the patterns observed at Carrington (Fig. 3) and Fargo (Fig. 4), but mean kernel sizes were smaller at Casselton (data not shown).

To determine how groats from hulled cultivars compared with naked oat, samples of AC Assiniboia and Morton from these same experimental plots were de-
hulled and their size distributions determined by digital image analysis. Mean image area of groats from AC Assiniboia and Morton were found to be close in size to naked oat groats (Table 4). Analysis of variance (not shown) indicated that AC Assiniboia groats did not differ in image area from any naked oat groat, except Stark, which were larger than AC Assiniboia. The mean image area variances of AC Assiniboia and Morton groats were not significantly different from those of many of the naked genotypes. Only Stark and ND000725 had image area variances significantly larger than those of Morton and AC Assiniboia groats (analysis of variance not shown). The differences in size between oat and groats were primarily due to differences in length, although groat widths were also significantly smaller than whole oat widths (Table 4).

A graphical depiction of size distributions of Morton whole oat and groats grown at Carrington and at Fargo allows for expansion of these observations (Fig. 5). Morton whole oat distributions for kernel image area are seen as being distinctly bimodal. The groat image areas from Fargo also appeared to be bimodal, but the range and mean sizes of the kernels are significantly decreased from the whole oat analysis. Morton groats from Carrington were less distinctly bimodal in their distribution but did not appear distinctly normal in their distribution either. It appears that oat groats from hulled oat are more bimodal in their distribution than naked oat and that they are about as uniform in size as most naked oat cultivars, judging from the variance of their image areas (see Tables 3 and 4).

**DISCUSSION**

Naked oat spikelets differ from hulled oat spikelets in their gross morphology (Fig. 1) as well as in the number of kernels they contained (Table 2). Hulled oat studied here had up to three kernels per spikelet, whereas naked oat spikelets contained as many as six kernels. It is interesting that many naked oat genotypes, which are known for their multiflorous spikelets (Valentine, 1995), had frequencies of single-kernel spikelets greater than that of the hulled oat cultivars. Most naked oat genotypes had higher frequencies of single-kernel spikelets than any other spikelet type. In contrast, hulled oat had higher frequencies of double-kernel spikelets than any other spikelet type. Spikelets with more than four kernels were relatively infrequent among naked genotypes, and many genotypes had no spikelets with five or six kernels. Thus, naked oat with 1.8 kernels per spikelet differed little from hulled genotypes with 1.7 kernels per spikelet. In contrast, Peltonen-Sainio (1994) reported that naked oat had 2.5 kernels per spikelet, whereas hulled oat had only 1.9 kernels per spikelet, which was a significant difference.

Within spikelets, kernel size decreased with increasing order in all cases (Fig. 2), much as is observed in hulled oat. In hulled oat, kernels from double-kernel spikelets were in such abundance that the primary and second-
ary kernels from these form distinct subpopulations that can be identified graphically and statistically, by bimodal analysis. The increased frequency of single-kernel spikelets and the presence of four-, five-, and six-kernel spikelets may diminish any bimodal tendency derived from preponderance of the double-kernel spikelets, as evidenced by the decreased bimodal coefficient in the naked genotypes (Table 3).

Most naked genotypes had smaller kernels size than the hulled genotypes (Table 1), but this can be attributed largely to the absence of the hull. Peltonen-Sainio (1994) also reported that hulled oat kernels were significantly more massive than naked oat kernels. A comparison of groats from the hulled lines with the groats of the naked genotypes indicated that the hulled oat groats were smaller than some of the naked oat groats, indicating that some naked oat had relatively large groats compared to the hulled genotypes. This is notable in light of our previous observations that AC Assiniboia had larger kernels (including hull) than most oat hulled cultivars studied (Doehlert et al., 2004).

A remarkable difference between size distributions of naked oat and hulled oat is the difference in total variation in size. The variance of image area in the naked genotypes was frequently only 20% that of the hulled genotypes (including the hull). Thus, it can be concluded that naked oat are much more uniform in size than hulled oat. The source of the variation can be traced to the hulls. The variance of image size of hulled oat groats was about the same as that of naked oat groats.

Whereas dehulling decreased groat image size to about 60% of the whole oat size, the variance was reduced to 15 to 30% of that of the whole oat. Thus, it appears the presence of hulls contributes greatly to the size and the amount of variation in size of oat kernels.

Naked oat kernel size distributions were less bimodal in their distributions than hulled oat, although all samples were still significantly bimodal (Table 3). This indicated that their distributions were better described by a bimodal model than by a normal distribution model. Although some naked oat size distributions did not significantly differ from normal distributions, several failed the Shapiro-Wilk Normality Test. The basis of the bimodal distribution in hulled oat has been shown to be related to the preponderance of double-kernel spikelets that results in two distinct sub-populations (Doehlert et al., 2005). Our results (Table 2) indicate that double-kernel spikelets were much less abundant in naked oat than in hulled oat, and in many genotypes, double-kernel spikelets were not the predominant spikelet type. It seems likely that the increased frequency of single-kernel spikelets and the presence of three-, four-, five-, and six-kernel spikelets, diminished the bimodality caused by the preponderance of two-kernel spikelets, characteristic to hulled oat. Consequently, kernel size distributions appeared more normal among hull-less oat, although these distributions still frequently failed the test for normality. This hypothesis for the naked oat kernel size distributions is consistent with our previous observations that increased frequencies of triple-kernel spikelets in hulled oat led to a decrease in the extent of bimodality of size distributions (Doehlert et al., 2005).

There were few differences in panicle architecture that were discernable between the naked genotypes and the hulled oat cultivars. Some naked genotypes had fewer spikelets per panicle than hulled genotypes, but these naked genotypes tended to have more kernels per spikelet, so there were fewer differences in kernels per panicle. Grain mass per panicle was greater in the hulled cultivars than in most naked genotypes, but this difference can be attributed entirely to the mass of the hull adding to the grain mass in the hulled kernels. Peltonen-Sainio (1994) reported that hulled oat carried more grain mass per panicle than naked oat, had more spikelets per panicle, but did not differ in number of kernels per panicle.

To conclude, naked oat panicles differed little from hulled oat panicles, except in the structure of their spikelets. Naked oat kernels contained much less size variation than hulled oat, when the hulled oat retained their hulls. Much of the differences in size variation could be attributed to the presence of the hull. Naked oat groats had about the same amount of size variation as did the hulled oat groats. Most of the variation in naked oat kernel size can be attributed to size differences of kernels within the multiflorous spikelet.

REFERENCES


