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RESEARCH ARTICLE

Variable expressivity and heritability of multiflorous spikelets in oat panicles

C. M. Zimmer*, I. P. Ubert, M. T. Pacheco and L. C. Federizzi

Department of Crop Science, Faculty of Agronomy, Federal University of Rio Grande do Sul, 7712 Bento Gonçalves Avenue, Porto Alegre, Rio Grande do Sul State, Brazil
*Corresponding author. Email: cmz.zimmer@gmail.com

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Abstract

Multiflorous spikelets are found in oat *Avena sativa* L. subsp. *nudisativa*, which is characterised by elongated rachilla and variable number of florets per spikelet. One of the main factors limiting the exploration of multiflorous spikelets in oats, aiming to produce naked grains, is its variable expressivity. This work aimed to detect the environmental influence on the variable expressivity of multiflorous spikelet formation in oats and to estimate the heritability of this trait by analysing its expression in lower, middle and upper third of the panicle in 94 inbred lines of two crosses each. Two populations of recombinant inbred lines were screened for the spikelet formation in 2 years and sowing dates under field experiments. The results demonstrate that the variable expressivity of the multiflorous spikelet formation was highly influenced by the environmental conditions. The variable expressivity varied according to the genetic background, as well as the panicle third where spikelets were produced. The upper third of the panicle showed greater stability for the multiflorous spikelet formation, which is confirmed by the highest heritability coefficients observed in this third, regardless of the assessed population. Our results provide substantial evidences of the contribution exerted by environmental conditions in multiflorous spikelet formation in oats.

Keywords: Naked oats; Indeterminate spikelets; Heritability

Introduction

Oat grains are highly recommended for human nutrition due to a superior nutritional composition. Among oat benefits, lowering blood cholesterol levels, low glycemic index and enhancing the immune system have been reported (Davis *et al.*, 2004; Liu, 2007). In order to provide oat varieties highly appreciated by farmers and industry, oat breeding programmes are looking into different grain-related traits. One of these traits is the incorporation of naked grains into elite oat germplasm. Naked grains are present in *Avena sativa* L. subsp. *nudisativa* (Husnot.) Rod. et Sold., generally in indeterminate spikelets termed as multiflorous. Multiflorous spikelets are characterised by elongated rachilla and variable number of florets per spikelet, ranging from four to eight (Peltonen-Sainio *et al.*, 2004). Although these spikelets are able to produce up to eight grains, high floret infertility is verified mainly at terminal florets (Valentine, 1995). The indeterminate spikelet growth pattern observed in multiflorous spikelets is not verified in common cultivated hexaploid oats [*A. sativa* subsp. *sativa*; *A. sativa* subsp. *byzantina* (C. Koch) Romero Zarco], which have hulled spikelets with two or three fertile florets per spikelet (Valentine, 1995). To date, indeterminate spikelets/inflorescences have been studied in several grasses, such as barley (Brown and Bregitzer, 2011; Poursarebani *et al.*, 2015), wheat

(Poursarebani *et al.*, 2015), rice (Lee *et al.*, 2007), maize (Chuck *et al.*, 1998, 2007a, 2008) and oat (Zimmer *et al.*, 2017).

One of the main factors limiting the understanding of multiflorous spikelet formation in oats is the variable expressivity. The phenotypic manifestation is not uniform among individuals of the same genotype, varying among plants, panicles and within the panicle (Pellizzaro *et al.*, 2016). This variable expressivity is not completely understood to date, and epigenetic factors could be involved in its modulation. The *microRNA172* (*miR172*) of maize, which is transcribed from the *TASSELSEED4* (*TS4*) gene, regulates the APETALA2 (AP2) gene family members *INDETERMINATE SPIKELET1* (*IDS1*) and *SISTER OF INDETERMINATE SPIKELET1* (*SID1*). Mutant *ts4* plants showed spikelets with indeterminate meristem (Chuck *et al.*, 2007b). Rice plants overexpressing *miR172* showed reduced mRNA levels of *OsIDS1* and *SUPERNUMERARY BRACT* (*SNB*), which are also AP2 family genes (Lee and An, 2012). The *miR172* overexpression delayed the transition from spikelet meristem to floral meristem in rice, causing defects in inflorescences and seed development, as well as changes in the number and identity of floral organs (Zhu *et al.*, 2009). Recently, oat nucleotide sequences showing similarity to an AP2 gene family member were isolated from the 'URS Taura' and 'UFRGS 017004-2' lines, which are contrasting for the multiflorous spikelet formation. Important genetic polymorphisms and a putative *miR172* target site were identified in these sequences, indicating its putative involvement in multiflorous spikelet formation in oats (Zimmer *et al.*, 2017). The *miR172* expression is regulated by environmental stimuli, including temperature and day length (Jung *et al.*, 2007; Lee *et al.*, 2010). Under short-day length conditions, *miR172* gene expression was reduced, while under long-day conditions elevated levels of gene expression were verified (Jung *et al.*, 2007).

Although the genetic mechanisms controlling multiflorous spikelet formation in oats remain unclear, an expressive environmental influence on its expression can be assumed. Environmental signals such as temperature and photoperiod may explain the variable expressivity observed in oat panicles derived from naked by hulled crosses. A detailed panicle screening in different years and sowing dates could clarify the environmental influence on the variable expressivity of multiflorous spikelets in oats. This work aimed to detect the environmental influence on the variable expressivity of multiflorous spikelet formation in oats and to estimate the heritability of this trait on line-mean basis in each third of the panicle.

Materials and Methods

Plant material

Two oat populations of 94 recombinant inbred lines (RILs) at the $F_{5:6}$ and $F_{5:7}$ generations and their parental lines were assessed for the multiflorous spikelet trait. These populations were derived from the single cross between 'UFRGS 01B7114-1-3' and 'UFRGS 006013-1' and between 'URS Taura' and 'UFRGS 017004-2'. The names of these populations are abbreviated hereby as 'U01B/U006' and 'UTau/U017', respectively. The parents 'UFRGS 01B7114-1-3' and 'URS Taura' are hulled lines presenting panicles with 100% normal spikelet phenotypes (Supplementary Material Figure S1). On the other hand, 'UFRGS 006013-1' and 'UFRGS 017004-2' are naked lines and show different levels of variable expressivity for spikelet formation. 'UFRGS 017004-2' presents panicles with 100% multiflorous spikelets, while 'UFRGS 006013-1' shows variable expressivity (Supplementary Material Figure S1). Panicles with variable expressivity contain different spikelet types: (i) multiflorous spikelets, which contain at least four florets per spikelet, elongated rachilla, and usually produce naked grains and/or (ii) mosaic spikelets, which contain variable number of florets per spikelet, with naked and hulled grains in the same spikelet. The naked lines were derived from the same cross, 'Coker 492/Starter-1//UFRGS 8'. The hulled parent 'UFRGS 01B7114-1-3' has the pedigree 'Pc68/5*Starter (F_4)//UFRGS 10', while 'URS Taura' is descent from the cross 'UFRGS 970216-2 ($F_{3:4}$)/UFRGS 970461 ($F_{7:8}$)'. Both populations were

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developed by the modified single-seed descent method. All parental lines and RIL populations were developed by the Federal University of Rio Grande do Sul (UFRGS) Oat Breeding Program.

Field experiments

RILs and their respective parental lines were assessed in different years and sowing dates in Eldorado do Sul (30°07′S, 51°40′W, at 70 m altitude), RS, Southern Brazil. For both populations, RILs in the $F_{5:6}$ generation were assessed in the growing season of 2013 and RILs in the $F_{5:7}$ generation were assessed in the growing season of 2014. In 2013, seeds from a panicle of each genotype were sown mechanically on 18 June. Each experimental unit was composed by double rows that were 2 m in length with 0.20 m width between rows and 0.40 m apart, in a single replication. In 2014, the same genotypes were sown in two sowing dates, 12 June and 4 July, in a randomized complete block with two replications. Each experimental unit was composed by a hill, with 0.30 m width between hills in the row and 0.40 m apart rows. Sowing was carried out manually at a seed density of 15 and 20 seeds per hill for hulled and naked RILs, respectively. Two sowing densities were used due to the lower germination of naked seeds compared to hulled ones. Fertilization was performed using 300 kg ha^{-1} of a 5–30–15 N–P–K formula. Topdressing nitrogen, in the form of dry urea, was applied twice at a rate of 33 kg ha^{-1} of N per application, when plants showed three and six fully expanded leaves. Pests and fungal diseases were chemically controlled when needed and weeds were manually controlled.

Phenotypic assessment

Six panicles from each plot were screened for multiflorous spikelet trait in 2013. In 2014, four panicles from each experimental unit were screened for the same trait. Initially, each panicle was divided into three thirds: (i) basal third; (ii) middle third; and (iii) upper third (Supplementary Material Figure S2). This division was performed in order to characterise the environmental influence on spikelet formation in each third of the panicle. The number of normal, multiflorous and mosaic spikelets was quantified. Normal spikelets were classed as determinate, showing two to three fertile florets and grains adhered to well-lignified lemma and palea. On the other hand, multiflorous and mosaic spikelets were classed as indeterminate, with variable expressivity according to the environmental conditions. Multiflorous spikelets show four or more florets, elongated rachilla, and grains coated with little lignified lemma and palea, while mosaic spikelets have a variable number of florets with naked and hulled grains in the same spikelet.

RILs of each population were classed in seven panicle categories: (i) type A, composed by panicles that produce 100% normal spikelets (Figure 1a); (ii) type B, with panicles that produce normal and mosaic spikelets (Figure 1b); (iii) type C, with panicles that produce 100% mosaic spikelets (Figure 1c); (iv) type D, with panicles that produce normal, mosaic and multiflorous spikelets (Figure 1d); (v) type E, with panicles that produce mosaic and multiflorous spikelets (Figure 1e); (vi) type F, with panicles that produce multiflorous and normal spikelets (Figure 1f); and (vii) type G, with panicles that produce 100% multiflorous spikelets (Figure 1g).

Statistical analyses

The observed number of normal, multiflorous and mosaic spikelets was converted in percentage in order to allow the comparison among RILs, once there is a wide phenotypic variation for the total number of spikelets produced per panicle. Analyses of variance were carried out separately for each population: (i) to identify the influence of years and sowing dates on multiflorous spikelet formation among panicle phenotypes and (ii) to estimate the heritability of the trait in each third of the panicle. For the first analysis, 'panicle-type categories' were included in factorial designs with year or sowing date. For the second one, RILs (without panicle-type categories) were included

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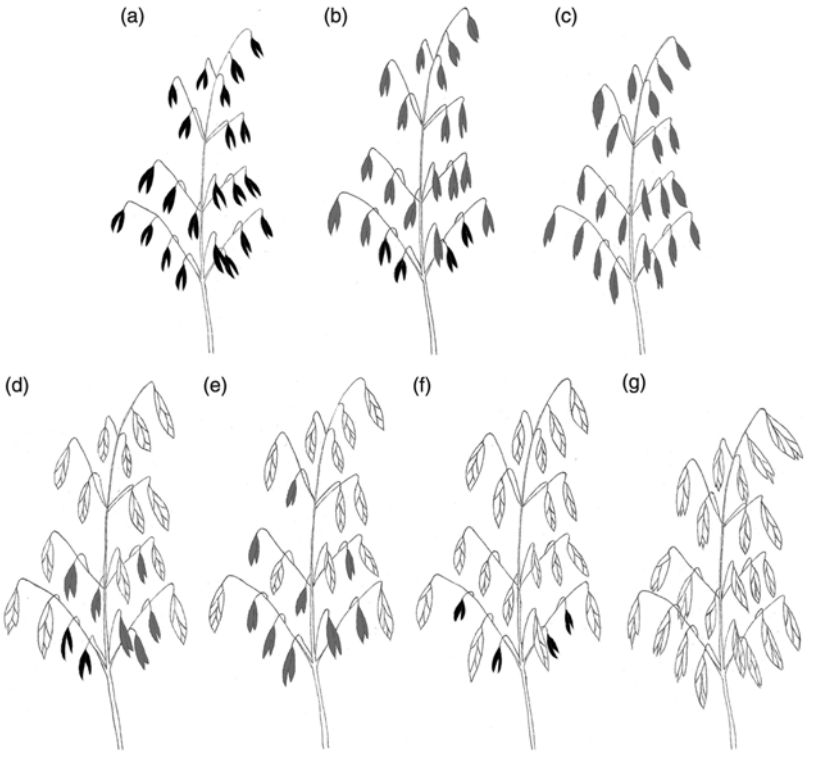

Figure 1. Panicle phenotypes observed among oat RILs. Black spikelet = normal spikelet; grey spikelet = mosaic spikelet; and white spikelet = multiflorous spikelet; (a) type A panicle phenotype showing 100% normal spikelets; (b) type B panicle phenotype showing normal and mosaic spikelets; (c) type C panicle phenotype showing 100% mosaic spikelets; (d) type D panicle phenotype showing normal, mosaic and multiflorous spikelets; (e) type E panicle phenotype showing mosaic and multiflorous spikelets; (f) type F panicle phenotype showing multiflorous and normal spikelets; and (g) type G panicle phenotype showing 100% multiflorous spikelets.

in factorial designs with year. Both analyses considered 'panicle-type categories' or 'RILs' as aleatory effects, as well as their interaction with year/sowing date. The option 'test' was also included in the random statement in all analyses. The comparison between years or sowing dates was performed by the *t*-test. All statistical analyses were carried out using the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Estimate of heritability on line-mean basis

The heritability of the multiflorous spikelet trait was estimated for both oat populations using the analysis of variance, partitioning the total phenotypic variance (σ_p^2) in genetic (σ_g^2) and environmental (σ_e^2) variances. The partition was performed using the expected mean squares $E(MS)$. The heritability on line-mean basis was estimated for each third of the panicle using the average of each RIL assessed in different years, as described by Vencovsky and Barriga (1992):

$$h^2 = \frac{(\sigma_g^2)}{(\sigma_p^2)}, \tag{1}$$

$$\sigma_p^2 = \sigma_g^2 + \frac{\sigma_{ge}^2}{y} + \frac{\sigma_e^2}{ry}, \tag{2}$$

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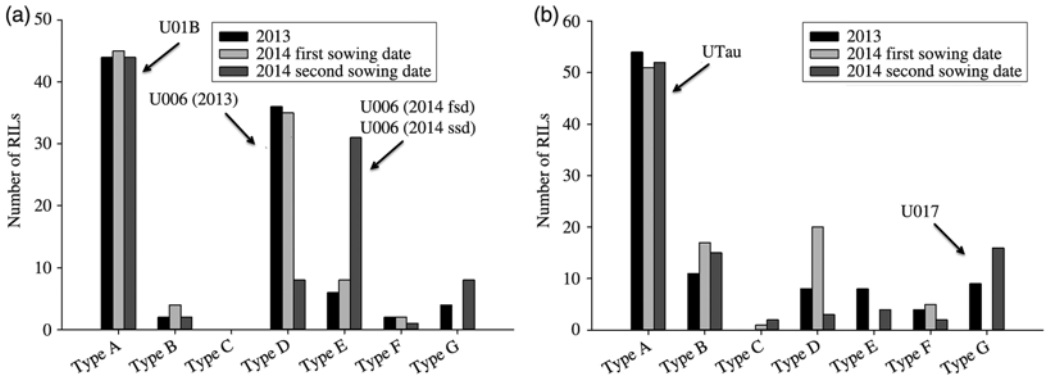

Figure 2. Frequency of panicle phenotypes of two oat populations assessed in different years and sowing dates. (a) 'U01B/U006' population and (b) 'UTau/U017' population. Parental lines are presented by arrows. U01B = UFRGS 01B7114-1-3; U006 = UFRGS 006013-1; UTau = URS Taura; and U017 = UFRGS 017004-2.

$$\sigma_g^2 = \frac{MS_g - MS_{gy}}{ry}, \tag{3}$$

$$\sigma_e^2 = MS_e, \tag{4}$$

$$\sigma_{ge}^2 = \frac{MS_{ge} - MS_e}{r}, \tag{5}$$

where h^2 is heritability coefficient, σ_p^2 is phenotypic variance, σ_g^2 is genotypic variance, σ_e^2 is environmental variance, σ_{ge}^2 is variance of the genotype by environment interaction, MS_g is mean squared genotype, MS_e is mean squared error, MS_{ge} is mean square of the genotype by environment interaction, r is number of replications and y is number of years.

Results

Identification of different panicle mosaic phenotypes

Different panicle phenotypes showing variable expressivity were identified among RILs from both populations (Figure 1). For the 'U01B/U006' population, nearly 50% of RILs showed type A panicle phenotype (determinate panicle growth habit), with 100% normal spikelets, regardless of year and sowing date (Figure 2). On the other hand, indeterminate phenotypes, which present variable expressivity, showed a broad variation due to year and/or sowing date influence (Figure 2). RILs exhibiting type C (Figure 1c) were not identified in this population, while RILs presenting type G panicle phenotype (Figure 1g) were identified only in 2013 and in the second sowing date of 2014 (Figure 2).

For the 'UTau/U017' population, the number of RILs classed as type A panicle phenotype was higher than for the 'U01B/U006' population (Figure 2). Similarly, the number of RILs showing type B, type C and type G panicle phenotypes was also higher for the 'UTau/U017' population (Figure 2). Otherwise, the number of RILs showing the type D panicle phenotype was lower in the 'UTau/U017' population compared to the 'U01B/U006' population (Figure 2). RILs classed as type C and type E were not identified in 2013 and in the first sowing date of 2014, respectively. Similar to the 'U01B/U006' population, RILs classed as type G panicle phenotype were not identified in the first sowing date of 2014 (Figure 2).

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Influence of year and sowing date on multiflorous spikelet formation

Spikelet formation in all thirds of the panicle varied due to year (Table 1) or sowing date (Table 2). For the 'U01B/U006' population, spikelet formation changed in different thirds of the panicle due to year conditions (Table 1). Similarly, spikelet formation in the 'UTau/U017' population also changed widely in all thirds of the panicle, especially in the middle and upper ones. Except for normal and multiflorous spikelets in the basal third, all spikelet types varied among the thirds of the panicles according to crop year in the 'UTau/U017' population (Table 1). A significant influence of the year as the cause of variation was observed. Although this occurred in both populations, the reduction was more accentuated for the 'U01B/U006' population than for the 'UTau/U017' population.

Differences in spikelet formation were also identified in both populations when assessed in two sowing dates. For the 'U01B/U006' population, spikelet formation was highly influenced by sowing date among the thirds of the panicle, except for normal spikelets in the basal and middle thirds (Table 2). In this population, the second sowing date provided an increase of nearly 20% of multiflorous spikelets in all thirds of the panicle, accompanied by a decrease of mosaic spikelets (Table 2). The 'UTau/U017' population was less affected by sowing date than the 'U01B/U006' population. For the 'UTau/U017' population, only the formation of multiflorous spikelets in the basal and middle thirds was affected. In this population, the second sowing date provided an increase of 11 and 8.7% in multiflorous spikelets in the basal and middle thirds, respectively (Table 2).

Variable expressivity of spikelet formation between years and sowing dates

The 'U01B/U006' and 'UTau/U017' populations showed differences in spikelet formation in response to environmental stimuli exercised by each crop year. Panicle categories type A, type C and type G were not discussed here because these categories showed only one type of spikelet in their panicles (Figure 1a,c,g). For the 'U01B/U006' population, RILs classed as type B panicle phenotype (Figure 1b) showed a reduction in the percentage of normal spikelets and an increase in the percentage of mosaic spikelets in 2014, for all thirds of the panicle (Table 3). For RILs classed as type D (Figure 1d), only the basal and middle thirds were influenced by year effects. In the basal third, a reduction in multiflorous spikelets and an increase in mosaic spikelets were observed in 2014 (Table 3). In the middle third, multiflorous spikelet formation also showed a reduction in 2014, while no differences were observed for normal and mosaic spikelets (Table 3). For RILs classed as type E (Figure 1e), the effect of year was uniform during panicle formation. The percentage of multiflorous spikelets was reduced in 2014, while the percentage of mosaic spikelets increased dramatically (Table 3). RILs classed as type F (Figure 1f) did not show any alteration in spikelet formation in the basal and middle thirds in response to crop year. On the other hand, reduction in the percentage of normal spikelets and increase of multiflorous spikelets in the upper third of the panicle were observed in 2014 (Table 3).

For the 'UTau/U017' population and when considering RILs classed as type B (Figure 1b), only the middle third of the panicle showed reduction in normal spikelets and increase in mosaic spikelet formation (Table 3). The variation observed for RILs classed as type D (Figure 1d) was uniform in the panicle. The increase of multiflorous spikelets was observed in all thirds of the panicle, being more pronounced in the upper one. At the same time, a reduction in mosaic spikelet formation was also observed (Table 3). Type E panicle phenotype (Figure 1e) was not compared between years because RILs showing this phenotype were not identified in the first sowing date of 2014 (Figure 2 and Table 3). For RILs classed as type F (Figure 1f), reduction in normal spikelets and increase in multiflorous spikelet formation were observed in the middle and upper thirds of the panicle (Table 3).

Differences in spikelet formation were also observed in response to sowing date in both populations. For the 'U01B/U006' population, RILs classed as type B panicle phenotype showed a

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Table 1. Summary of variance analyses for multiflorous spikelet trait in two oat populations assessed in two crop years

		Basal third			Middle third			Upper third		
Cause of variation	DF	Normal	Multiflorous	Mosaic	Normal	Multiflorous	Mosaic	Normal	Multiflorous	Mosaic
U01B/U006 population										
Rep(Y)	1	35.77	640.47	146.57	172.34	933.84	303.84	111.84	1668.08	916.09
Y	1	4879.66**	2550.01**	11276.12**	2113.52*	3310.10**	10739.69**	5561.75**	195.18ns	7865.78**
PT	5	58530.35**	10765.04**	25371.49**	65212.29**	13563.51**	25310.93**	81694.44**	25029.34**	19768.40**
PT*Y	4	1548.47**	5171.84**	5432.18**	1088.74*	5725.64**	6412.84**	1428.45**	5263.20**	4959.19**
Experimental error	270	465.19	184.71	509.02	403.32	223.62	480.67	286.89	376.04	446.05
$\bar{X}$ RILs 2013 (%)	–	66.80	16.79	16.41	62.92	19.64	17.45	59.40	23.69	16.92
$\bar{X}$ RILs 2014 (%)	–	66.54	4.36	29.10	64.34	7.70	27.97	57.63	18.60	23.78
Response to year†	–	−0.26	−12.43	12.69	1.42	−11.94	10.52	−1.77	−5.09	6.86
UTau/U017 population										
Rep(Y)	1	99.84	70.35	0.08	4.17	3.60	14.38	98.38	24.60	223.54
Y	1	558.19ns	568.32ns	9.35ns	3713.85**	4617.06**	47.38ns	6423.90**	10755.41**	572.72ns
PT	6	42624.76**	28215.85**	8420.59**	47061.37**	31620.34**	8111.56**	50645.20**	34135.71**	8527.49ns
PT*Y	3	378.29ns	300.78ns	779.23*	1688.07**	1699.94**	1290.45**	2196.16**	4067.67**	1537.08**
Experimental error	270	361.50	221.32	311.68	358.37	230.11	315.72	317.48	233.54	279.33
$\bar{X}$ RILs 2013 (%)	–	72.15	20.47	7.38	72.52	20.34	7.14	72.62	19.30	8.08
$\bar{X}$ RILs 2014 (%)	–	77.60	10.92	11.48	75.49	13.61	10.91	73.33	17.02	9.65
Response to year†	–	5.45	−9.55	4.10	2.97	−6.73	3.77	0.71	−2.28	1.57

DF = degrees of freedom; PT = panicle type; RILs = recombinant inbred lines; Y = year.
*Significant at 5% probability of error by the *F*-test ($p \leq 0.05$).
**Significant at 1% probability of error by the *F*-test ($p \leq 0.01$).
nsNot significant.
†RILs average of 2014 minus the RILs average of 2013.

Table 2. Summary of variance analyses for multiflorous spikelet trait in two oat populations assessed in two sowing dates of 2014

		Basal third			Middle third			Upper third		
Cause of variation	DF	Normal	Multiflorous	Mosaic	Normal	Multiflorous	Mosaic	Normal	Multiflorous	Mosaic
U01B/U006 population										
Rep(SD)	2	246.20	584.13	1224.79	338.47	947.30	1573.09	300.09	949.11	1155.24
SD	1	549.36^{ns}	1929.46*	3992.01*	1017.40^{ns}	987.95^{ns}	3992.19^{ns}	4647.27**	21.06^{ns}	4049.44**
PT	5	102932.25**	23797.39**	53190.06**	105508.88**	27157.26**	48420.10**	115643.02**	44538.08**	28063.12**
SD*PT	3	687.53^{ns}	4797.67**	4630.94**	402.98^{ns}	6382.04**	6214.02**	1424.66**	9340.28**	7521.45**
Experimental error	364	361.38	363.08	640.30	336.75	354.17	601.24	237.66	357.59	434.56
$\overline{X}$ RILs first SD	–	66.54	4.36	29.10	64.34	7.70	27.97	57.63	18.60	23.78
$\overline{X}$ RILs second SD	–	52.67	25.15	22.18	52.37	28.18	19.45	51.79	36.78	11.43
Response to late SD†	–	−13.87	20.79	−6.92	−11.97	20.48	−8.52	−5.84	18.18	−12.35
UTau/U017 population										
Rep(SD)	2	53.56	40.32	0.90	15.87	2.66	14.95	54.82	14.69	112.54
SD	1	558.85^{ns}	527.69^{ns}	5.85^{ns}	124.69^{ns}	132.33^{ns}	0.17^{ns}	8.46^{ns}	15.37**	1.01^{ns}
PT	6	64950.63**	52318.49**	19767.29**	70165.77**	58889.69**	19162.23**	75256.61**	65955.37**	17126.23**
SD*PT	4	564.14^{ns}	477.64*	6.97^{ns}	345.69^{ns}	463.47*	37.63^{ns}	402.86^{ns}	333.25^{ns}	272.39^{ns}
Experimental error	362	362.06	205.38	366.32	377.56	201.73	380.83	346.29	195.08	328.15
$\overline{X}$ RILs first SD	–	77.59	10.92	11.49	75.49	13.61	10.91	73.33	17.02	9.65
$\overline{X}$ RILs second SD	–	67.55	22.03	10.42	67.42	22.33	10.25	67.33	22.86	9.81
Response to late SD†	–	−10.04	11.11	−1.07	−8.07	8.72	−0.66	−6.00	5.84	0.16

DF = degrees of freedom; PT = panicle type; RILs = recombinant inbred lines; SD = sowing date.
*Significant at 5% probability of error by the *F*-test ($p \leq 0.05$).
**Significant at 1% probability of error by the *F*-test ($p \leq 0.01$).
^{ns}Not significant.
†Average of the second sowing date minus the average of the first one.

Table 3. Percentage of normal, multiflorous and mosaic spikelets in different panicle phenotypes, from two oat populations, assessed in two years and sowing dates

	Basal third						Middle third						Upper third					
	Normal		Multiflorous		Mosaic		Normal		Multiflorous		Mosaic		Normal		Multiflorous		Mosaic	
Panicle type within year	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
U01B/U006 population																		
Type B (N + MS)	94^{a}	36^{b}	0^{a}	0^{a}	6^{b}	64^{a}	90^{a}	38^{b}	0^{a}	0^{a}	10^{b}	62^{a}	86^{a}	37^{b}	0^{a}	0^{a}	14^{b}	63^{a}
Type D (N + MS + MF)	43^{a}	43^{a}	20^{a}	9^{b}	37^{b}	48^{a}	34^{a}	36^{a}	25^{a}	18^{b}	41^{a}	46^{a}	25^{a}	19^{a}	35^{a}	43^{a}	40^{a}	38^{a}
Type E (MS + MF)	0^{a}	0^{a}	73^{a}	3^{b}	27^{b}	97^{a}	0^{a}	0^{a}	80^{a}	4^{b}	20^{b}	96^{a}	0^{a}	0^{a}	84^{a}	20^{b}	16^{b}	80^{a}
Type F (MF + N)	100^{a}	83^{a}	0^{a}	17^{a}	0^{a}	0^{a}	84^{a}	75^{a}	16^{a}	25^{a}	0^{a}	0^{a}	92^{a}	49^{b}	8^{b}	51^{a}	0^{a}	0^{a}
UTau/U017 population																		
Type B (N + MS)	81^{a}	70^{a}	0^{a}	0^{a}	19^{a}	30^{a}	85^{a}	72^{b}	0^{a}	0^{a}	15^{b}	28^{a}	83^{a}	76^{a}	0^{a}	0^{a}	17^{a}	24^{a}
Type D (N + MS + MF)	40^{a}	42^{a}	23^{b}	35^{a}	37^{a}	23^{b}	31^{a}	34^{a}	28^{b}	44^{a}	41^{a}	22^{b}	31^{a}	23^{a}	23^{b}	57^{a}	46^{a}	20^{b}
Type E (MS + MF)	0	–	76	–	24	–	0	–	78	–	22	–	0	–	75	–	25	–
Type F (MF + N)	42^{a}	33^{a}	58^{a}	67^{a}	0^{a}	0^{a}	58^{a}	20^{b}	42^{b}	80^{a}	0^{a}	0^{a}	58^{a}	9^{b}	42^{b}	91^{a}	0^{a}	0^{a}

	Basal third						Middle third						Upper third					
	Normal		Multiflorous		Mosaic		Normal		Multiflorous		Mosaic		Normal		Multiflorous		Mosaic	
Panicle type within sowing date	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
U01B/U006 population																		
Type B (N + MS)	43^{b}	75^{a}	0^{a}	0^{a}	57^{a}	25^{b}	51^{b}	74^{a}	0^{a}	0^{a}	49^{a}	26^{a}	48^{a}	61^{a}	0^{a}	0^{a}	52^{a}	39^{a}
Type D (N + MS + MF)	41^{a}	41^{a}	10^{a}	17^{a}	49^{a}	42^{a}	36^{a}	39^{a}	18^{a}	21^{a}	46^{a}	40^{a}	19^{b}	35^{a}	43^{a}	38^{a}	38^{a}	27^{a}
Type E (MS + MF)	0^{a}	0^{a}	3^{b}	45^{a}	97^{a}	55^{b}	0^{a}	0^{a}	4^{b}	53^{a}	96^{a}	47^{b}	0^{a}	0^{a}	20^{b}	75^{a}	80^{a}	25^{b}
Type F (MF + N)	66^{a}	63^{a}	34^{a}	37^{a}	0^{a}	0^{a}	50^{a}	62^{a}	50^{a}	38^{a}	0^{a}	0^{a}	7^{b}	62^{a}	93^{a}	38^{b}	0^{a}	0^{a}
UTau/U017 population																		
Type B (N + MS)	70^{a}	70^{a}	0^{a}	0^{a}	30^{a}	30^{a}	72^{a}	69^{a}	0^{a}	0^{a}	28^{a}	31^{a}	76^{a}	69^{a}	0^{a}	0^{a}	24^{a}	31^{a}
Type D (N + MS + MF)	42^{a}	20^{b}	35^{a}	54^{a}	23^{a}	26^{a}	34^{a}	18^{a}	44^{a}	63^{a}	22^{a}	19^{a}	23^{a}	18^{a}	57^{a}	68^{a}	20^{a}	14^{a}
Type E (MS + MF)	–	0	–	40	–	60	–	0	–	40	–	60	–	0	–	48	–	52
Type F (MF + N)	33^{a}	27^{a}	67^{a}	73^{a}	0^{a}	0^{a}	20^{a}	25^{a}	80^{a}	75^{a}	0^{a}	0^{a}	9^{a}	25^{a}	91^{a}	75^{a}	0^{a}	0^{a}

Different superscript letters between years or sowing dates are statistically significant.
N = normal spikelets; MS = mosaic spikelets; MF = multiflorous spikelets.

Table 4. Analyses of variance partitioning the total phenotypic variance of each third of the panicle in genetic and environmental components and estimation of heritability on line-mean basis for the multiflorous spikelet formation

Source of variation	DF	Basal third	Middle third	Upper third	Expected(MS)
U01B/U006 population					
Replication(Year)	1	640.47	933.84	1668.08	
Year	1	10495.55**	8904.40**	1613.68**	
RIL	93	944.66**	1274.60**	2282.50**	$\sigma_e^2 + r\sigma_{ge}^2 + ry\sigma_g^2$
RIL*Year	93	446.41**	359.56**	199.36*	$\sigma_e^2 + r\sigma_{ge}^2$
Experimental error	93	84.13	86.13	133.90	σ_e^2
σ_{ge}^2	–	271.72	205.08	49.10	
σ_g^2	–	186.85	343.15	781.20	
σ_p^2	–	354.26	477.98	855.96	
σ_e^2	–	84.13	86.13	133.90	
h^2	–	0.53	0.72	0.91	
UTau/U017 population					
Replication(Year)	1	70.35	3.60	24.60	
Year	1	5738.30**	2761.53**	327.84ns	
RIL	93	2320.45**	2640.15**	2993.93**	$\sigma_e^2 + r\sigma_{ge}^2 + ry\sigma_g^2$
RIL*Year	93	243.62**	218.73**	146.72ns	$\sigma_e^2 + r\sigma_{ge}^2$
Experimental error	93	140.45	122.04	123.75	σ_e^2
σ_{ge}^2	–	77.38	72.52	17.23	
σ_g^2	–	778.83	908.05	1067.73	
σ_p^2	–	870.19	990.08	1122.75	
σ_e^2	–	140.45	122.04	123.75	
h^2	–	0.90	0.92	0.95	

DF = degrees of freedom; MS = mean square; RILs = recombinant inbred lines; h^2 = heritability coefficient on line-mean basis; σ_p^2 = phenotypic variance; σ_g^2 = genotypic variance; σ_e^2 = environmental variance; σ_{ge}^2 = variance of the genotype by environment interaction; r = number of replications; and y = number of years.
*Significant at 5% probability of error by the *F*-test ($p \leq 0.05$).
**Significant at 1% probability of error by the *F*-test ($p \leq 0.01$).
nsNot significant ($p > 0.05$).

reduction of mosaic spikelets in the basal third and an increase of normal spikelets in the middle one in response to sowing date. RILs classed as type D (Figure 1d) showed changes only in the percentage of normal spikelets in the upper third of the panicle (Table 3). For RILs classed as type E (Figure 1e), increase in multiflorous spikelets and decrease in mosaic spikelet formation were noticed in the second sowing date in all thirds of the panicle (Table 3). RILs classed as type F (Figure 1f) showed a reduction in the percentage of multiflorous spikelets and an increase of normal spikelets in the upper third of the panicle in response to the second sowing date (Table 3).

The 'UTau/U017' population indicated a stable response to sowing date when compared to the 'U01B/U006' population. Among all panicle phenotypes with variable expressivity, only RILs classed as type D (Figure 1d) varied the spikelet phenotype in response to sowing date (Table 3). This panicle category showed a reduction in normal spikelet formation in the basal third when sown in the second sowing date (Table 3). All other panicle phenotypes did not change in response to sowing date exchange.

Heritability on line-mean basis

Based on the joint analysis of data from 2013 and 2014, the heritability coefficients for the multiflorous spikelet trait were estimated for both oat populations in all thirds of the panicle. For the 'U1B7/U006' population, the phenotypic variances observed among the thirds of the panicle were 354.26 for the basal third, 477.98 for the middle third and 855.96 for the upper third. In these thirds the genotypic variances were 186.85, 343.15 and 781.20, respectively (Table 4). The lowest heritability was observed in the basal third, with a coefficient of 0.53. The middle third also showed low heritability coefficient (0.72), while the highest heritability was verified in the upper

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one, with a coefficient of 0.91 (Table 4). For the 'UTau/U017' population, the phenotypic and genotypic variances observed among thirds of the panicle were higher than for the 'U01B/U006' population. The phenotypic variance ranged from 870.19 in the basal third to 1122.75 in the upper one, while the genotypic variance ranged from 778.83 to 1067.73 in these thirds, respectively (Table 4). High heritability coefficients for the multiflorous spikelet formation were verified in all thirds of the panicle. The heritability coefficient was 0.90 for the basal third, 0.92 for the middle third and 0.95 for the upper third (Table 4). These results indicate that multiflorous spikelet formation in the 'UTau/U017' population is less influenced by environment when compared to the 'U1B7/U006' population. Genetic variances and heritability coefficients showed that multiflorous spikelet formation varies among thirds of the panicle, being the basal and middle panicle segments less stable.

Discussion

The dynamics of variable expressivity in oat lines derived from naked by hulled crosses is poorly understood. Panicle phenotypes observed in the 'U01B/U006' population suffered a large influence of the year in all thirds of the panicle, while RILs from the 'UTau/U017' population altered the spikelet pattern only in the middle and upper thirds (Table 1). Considering the effects induced by varying sowing date, mosaic and multiflorous spikelet formation were highly affected in the 'U01B/U006' population in all thirds of the panicle, while only multiflorous spikelets in the basal and middle thirds were altered in the 'UTau/U017' population (Table 2). The significant influence of and interactions between year and sowing date indicate a strong environmental influence on spikelet formation in oats. In this context, the variable expressivity in both populations may be associated with temperature effects. When a high percentage of multiflorous spikelets was verified in 2013, a temperature peak was detected during flowering time, approximately 80–85 days after sowing (Supplementary Material Figure S3). Similarly, the average temperature in 2014 was higher for the second sowing date (Supplementary Material Figure S3). These environmental conditions reduced the multiflorous spikelets instability, as indicated by the higher number of RILs expressing exclusively multiflorous spikelets in their panicles in 2013 and in the second sowing date of 2014 (Figure 2).

One of the first studies involving the variable expressivity of multiflorous spikelet formation in oats was reported by Love and McRostie (1919). These authors identified plants in the F_4 generation exhibiting multiflorous and normal spikelets within the same panicle, with normal spikelets rates ranging from 10 to 87.9% of the spikelets. The variable expressivity in different levels, among individuals with the same genotype and among/within panicles, was also identified in rice. The rice mutant *fickle spikelet1* (*fsp1*), which shows spikelets with a high number of floral organs, is directly influenced by environmental signals, mainly temperature (Suzuki *et al.*, 2015). Although studies associating temperature and multiflorous spikelet formation are scarce in the literature, temperature effects on naked grains formation have been observed in oats (Lawes and Boland, 1974; Ubert *et al.*, 2017). Assessing eight oat genotypes under varying temperature, Lawes and Boland (1974) identified a high formation of naked grains under high temperature. Ubert *et al.* (2017) also reported the increase of naked grains due to temperature variations. Similar results involving the interaction of genotype and sowing date, as well as the effect of temperature, were also identified in wheat with supernumerary spikelets (Pennell and Halloran, 1984).

The 'U01B/U006' population showed higher variable expressivity for the multiflorous spikelet formation than the 'UTau/U017' population. This can be seen by the higher response to year and sowing date of the 'U01B/U006' population (Tables 1 and 2). The differences of variable expressivity between populations may be explained by contrasting genetic mechanisms controlling spikelet formation. These genetic factors must come from the hulled parental lines, once both naked ones share the same cross 'Coker 492/Starter-1//UFRGS 8'. Recently, genetic analyses

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demonstrated the action of one major gene and three modifying genes acting in multiflorous spikelet formation in the 'U01B/U006' population, while one major gene and only two modifying genes best fit in the 'UTau/U017' population (Zimmer *et al.*, 2017). The major gene was associated with the growth pattern definition (determinate or indeterminate), while the modifying genes would interact with the major gene according to the environmental stimuli. In these same populations, a genetic analysis indicated the action of a major gene termed as *N1* and modifying genes controlling the formation of naked grains (Ubert *et al.*, 2017). A quantitative trait loci (QTL) mapping for multiflorous spikelet formation in the 'U01B/U006' population showed that the SNP marker GMI_ES17_c5923_221 was strongly associated with the trait (Pellizzaro *et al.*, 2016). This same SNP marker was also associated to a QTL controlling naked grain formation in the 'U01B/U006' population in different years, being located 6.7 cM from the QTL peak (Ubert *et al.*, 2017). Together, these results indicate that multiflorous spikelets and naked grain traits could share a specific gene (Ougham *et al.*, 1996; Simons *et al.*, 1978) and this may explain the phenotypic association between multiflorous spikelet and naked grain formation.

The multiflorous spikelet formation was also differently influenced among the thirds of the panicle, mainly in the 'U01B/U006' population. The upper third was more stable for multiflorous spikelet formation than either the middle or basal ones. This stability is evidenced by the higher heritability coefficient estimated in this third of the panicle (Table 4). Considering that the stability of multiflorous spikelet formation is different through the panicle, the selection of stable naked oat lines could be based on the most challenging panicle segment, where the trait is less stable. In this sense, a panicle screening focusing on the basal third of the panicle could facilitate the selection process.

The contrasting heritability coefficients observed among thirds of the panicle in the 'U01B/U006' population (Table 4) indicate that variable expressivity may be a consequence of distinct vascularisation pattern through meristem development. In this context, the transport of mRNA from genes acting in multiflorous spikelet formation could be regulated. Small RNAs, including miRNAs, can be transported long distances by the phloem (Yoo *et al.*, 2004). Recently, *miR172* was cloned from phloem exudates in *Brassica napus* (Buhtz *et al.*, 2008). In this way, *miR172* could be a strong candidate modifying gene, once the expression of this gene is regulated by environmental conditions (Jung *et al.*, 2007; Lee *et al.*, 2010) and its target genes, which are AP2 gene family members, are highly associated with indeterminate spikelets in grasses (Chuck *et al.*, 2008; Lee *et al.*, 2007; Poursarebani *et al.*, 2015). Nucleotide sequences showing similarity to an AP2 gene family member were isolated from 'URS Taura' and 'UFRGS 017004-2' parents, where different genetic polymorphisms and a putative *miR172* target site were identified (Zimmer *et al.*, 2017). However, these genetic polymorphisms detected in *AP2* candidate sequences were not validated in RILs derived from the cross 'URS Taura' by 'UFRGS 017004-2'.

Conclusions

The elucidation of the underlying factors associated with multiflorous spikelets in oats requires considerable research. Herein, a detailed screening of spikelet formation was performed among thirds of the panicle in two oat populations, enabling the identification of different panicle phenotypes. The multiflorous spikelet formation was highly influenced by year and sowing date, depending on the third of the panicle and the genetic background. The upper third of the panicle was more stable for multiflorous spikelet formation than the others, showing the highest heritability coefficients, regardless of the assessed oat population. Our results provide substantial evidence of the contribution exerted by environmental conditions in multiflorous spikelet formation in oats.

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S0014479718000418.

Author ORCIDs. C. M. Zimmer 0000-0002-2108-8529

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