

# Regulation of Subsoiling Tillage on the Grain Filling Characteristics of Maize Varieties From Different Eras

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## Research Article

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# 1 Regulation of subsoiling tillage on the grain filling 2 characteristics of maize varieties from different eras

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11

12 Abstract: Grain filling is the key stage for achieving high grain yield. Subsoiling tillage has been  
13 widely used as a conservation tillage method in the maize planting region of China. This study  
14 was conducted to explore the effects of subsoiling on the grain filling characteristics of maize  
15 varieties of different eras. Five typical maize varieties from different eras (1970s, 1980s, 1990s,  
16 2000s and 2010s) were used as trial materials with two tillage modalities: rotation tillage and  
17 subsoiling tillage. The characteristic parameters and rate parameters of grain filling were  
18 compared and analyzed using the selected tillage modalities. The results showed that the grain  
19 filling parameters of the 2010s variety were better than those of the other varieties, and these  
20 differences mainly manifested in the filling rate parameters of the rapidly increasing and  
21 slowly increasing periods. In comparison with rotation tillage, subsoiling improved the  
22 maximum grain filling rate and the grain growth during the period of the maximum grain  
23 filling rate to different degrees. In addition, subsoiling delayed the appearance time of the  
24 maximum grain filling rate, extended the grain filling duration, and improved the mean filling  
25 rate. These differences are major reasons for the significant increase in 100-kernel dry weight  
26 at harvest for subsoiling in comparison with rotation tillage. Moreover, subsoiling enhanced  
27 the filling rate parameters during the rapidly increasing and slowly increasing periods. The  
28 filling stage filling duration and filling rate of maize varieties of different eras showed different  
29 responses to subsoiling. For example, the grain filling rate parameters of the 2010s variety  
30 during the rapidly increasing period were more sensitive to subsoiling in comparison with  
31 those of the other varieties.

32 **Key words:** spring maize; subsoiling tillage; grain filling

## 33 1.Introduction

34 Analysis of reports about maize high yield records in China and elsewhere show that  
35 increasing maize yield depends on sufficient water and fertilizer conditions, varieties with high  
36 yield and density tolerance, high planting density and reasonable cultivation measures<sup>[1-2]</sup>.  
37 Therefore, in the context of the rigid demand for maize grain yield, the reduction of cultivated  
38 land area and aggravation of water resource shortages in China, soil fertility improvement,  
39 maize variety improvement and innovative cultivation techniques have become effective ways

40 to optimize maize yield per unit area.

41 It is inevitable that increasing plant density leads to decreased grain yield. In addition, the  
42 advantages and disadvantages of topsoil structure further affect the population capacity<sup>[3]</sup>. A  
43 tillage layer with good structure can alleviate the adverse effects of increased density, and  
44 practices that optimize this structure are key measures for optimizing maize yield in China. As  
45 a conservation tillage measure, subsoiling can effectively improve soil physical and chemical  
46 properties, enhance plant self-regulation ability, alleviate the cluster effect of densification,  
47 effectively stabilize population yield and realize densification and yield improvement<sup>[4-5]</sup>.

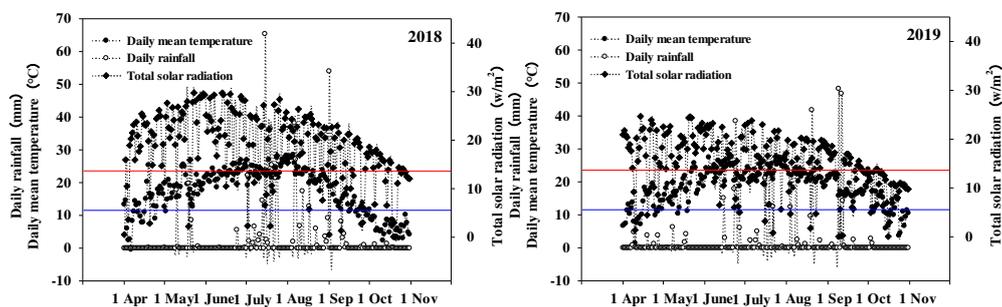
48 Previous work has shown that grain filling is an important physiological process that  
49 determines the yield and quality of maize grain<sup>[6-7]</sup>. Therefore, by matching appropriate tillage  
50 practices<sup>[8-9]</sup>, varieties<sup>[10-11]</sup>, planting density<sup>[12-13]</sup> and management measures<sup>[14]</sup>, as well as  
51 constructing a reasonable group structure to realize full utilization of light, heat, water and  
52 fertilizer resources, good filling conditions can be established to achieve coordination of ear  
53 number, ear grain number and grain weight, which finally increase the maize yield per unit  
54 area.

55 Studies have shown that subsoiling promoted maize root penetration, increased the  
56 photosynthetic rate and delayed leaf senescence, which increased planting density and maize  
57 yield<sup>[15-17]</sup>. However, few studies have assessed the effects of subsoiling on maize grain filling  
58 characteristics. Therefore, using maize varieties from different eras as experimental materials,  
59 the aim of this study was to analyze the effects of subsoiling on grain filling characteristics to  
60 provide a basis for further exploration of the mechanisms underlying increases in planting  
61 density and maize yield.

## 62 2. Materials and methods

### 63 2.1 Trials and measurements

64 Field experiment were carried out at the Tumoteyou Qi Experimental Station of the Inner  
65 Mongolia Agricultural University (40°33' N, 110°31' E) during 2018 and 2019. The soil type  
66 was sandy loam, and the 0-30 cm soil layer contained 22.27 g·kg<sup>-1</sup> of organic matter, 103.75  
67 mg·kg<sup>-1</sup> available nitrogen, 15.76mg·kg<sup>-1</sup> available phosphorus, and 219.60 mg·kg<sup>-1</sup> available  
68 potassium (pH 8.23). The main meteorological factors during the maize growth period are  
69 given in Fig. 1.



70

71 Fig. 1 Main meteorological factors during the growth period in the experimental area

72 The trial used a two-factor split-plot design (tillage treatment and variety). Tillage  
73 treatment was the main plot, and the two tillage practices were subsoiling (SS) with a depth of  
74 35 cm and rotary tillage (RT) with a depth of 15 cm. The main plot contained subplots with  
75 each variety: ZD2 (1970s), DY13 (1980s), YD13 (1990s), XY 335 (2000s), and DH618 (2010s).  
76 These varieties are sold in the Chinese market and allowed to be purchased as test materials.

77 The planting density was 75000 plants•hm<sup>2</sup>. Interline planting was used with a row spacing of  
 78 0.6 m. The plot length and width were each 6 m, and the trial area was 6 × 6 m. The dosages of  
 79 N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were 465 kg•hm<sup>2</sup>, 210 kg•hm<sup>2</sup>, and 202.5 kg•hm<sup>2</sup>, respectively. P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O  
 80 were applied as basal fertilizer at seeding. At V6, V12, and R2, N was applied as fertilizer at the  
 81 ratio of 3:6:1. Subsoiling was achieved with a five-shovel subsoiling plough, and the traction  
 82 power source was a John Deere 1654 tractor. The plots were irrigated four times during the  
 83 growth period (seeding stage, V12, R1 and R2) at a rate of 750 m<sup>3</sup>/hm<sup>2</sup>. The main soil physical  
 84 characteristic indexes in the trial area are given in Table 1.

85

86

Table 1 Main soil physical properties in the test area

Years	Tillage method	VE		R3	
		Soil compaction (Kpa)	Soil moisture content	Soil compaction (Kpa)	Soil moisture content
2018	RT	2194.19	0.17	2675.5	0.15
	SS	1775.23	0.19	2443.14	0.16
2019	RT	2352.46	0.17	1333.41	0.19
	SS	1850.77	0.14	1143.86	0.16

87

## 88 2.2 Measurement

### 89 2.2.1 Grain filling characteristics

90 From 15 days after pollination, samples were collected at 3-day intervals until the end of  
 91 filling. At each sampling point, three ears were collected per plot, and 100 kernels were  
 92 collected from the middle of each ear. The kernels were weighed and placed into an oven for  
 93 30 min at 105 °C, after which they were dried at 80 °C to a constant weight, and their weight  
 94 was measured<sup>[10]</sup>. The collection of experimental material, which complied with relevant  
 95 institutional, national, and international guidelines and legislation.

96 A logistic equation<sup>[18]</sup> was used to fit the grain filling process, calculate grain filling  
 97 characteristic parameters, and analyze grain filling growth. The logistic equation was  
 98 calculated as follows:

99

$$W=A/(1+Be^{-Ct})$$

100 In the equation above,  $t$  is the number of days after flowering (blooming day  $t_0=0$ ),  $w$  is  
 101 the 100-kernel weight after flowering (grain weight on flowering day= $w_0$ ),  $A$  is the theoretical  
 102 maximum 100-kernel weight, and  $B$  and  $C$  are shape parameters. The filling parameters were  
 103 derived from the first and second derivatives of the equation.

104  $t_1$  (the start date of the filling peak period) $=(\ln B-1.317)/C$ , corresponding to the grain  
 105 weight ( $w_1$ ) at this time:  $w_1=A/(1+Be^{-Ct_1})$ ;

106  $t_2$  (the end date of the filling peak period) $=(\ln B+1.317)/C$ , corresponding to the grain weight  
 107 ( $w_2$ ) at this time:  $w_2=A/(1+Be^{-Ct_2})$ ;

108  $t_3$  (the grain weight reaches 99% after flowering, the effective filling  
 109 period) $=(\ln B+4.59512)/C$ , corresponding to the grain weight ( $w_3$ ) at this time.

110 The filling duration of the gradually increasing period was calculated as  $T_1=t_1-t_0$ . The  
 111 increase in grain weight during the rapidly increasing period was calculated as  $w_1=W_1-W_0$ . The  
 112 mean filling rate of the gradually increasing period was calculated as  $v_1=w_1/T_1$ .

113 The filling duration of the rapidly increasing period was calculated as  $T_2=t_2-t_1$ . The increase  
 114 in grain weight during the rapidly increasing period was calculated as  $w_2=W_2-W_1$ . The mean  
 115 filling rate of the rapidly increasing stage was calculated as  $v_2=w_2/T_2$ .

116 The filling duration of the slowly increasing period was calculated as  $T_3=t_3-t_2$ . The increase  
 117 in grain weight of the slowly increasing period was calculated as  $w_3=W_3-W_2$ . The mean filling  
 118 rate of the slowly increasing stage was calculated as  $v_3=w_3/T_3$ ;

119 The final grain growth was  $A$ .  $T_{max}$  (maximum filling rate time)= $\ln B/C$ ,  $W_{max}$  (growth at  
 120 the maximum filling rate)= $A/2$ ,  $R_{max}$  (the maximum filling rate)= $(CW_{max}) \cdot (1 - W_{max}/A)$ ,  $P$   
 121 (time to complete approximately 90% of total accumulation)= $6/C$ , and  $v_{mean}$  (the mean filling  
 122 rate)= $W_3/t_3$ .

### 123 2.2.2 Determination of grain weight

124 At physiological maturity, ten ears were randomly selected from each plot and air-dried,  
 125 after which 100 kernels were collected from the middle of each ear and weighed, and this  
 126 weight was converted to the hundred grain weight with 14% moisture content<sup>[19]</sup>. The  
 127 determination of grain weight, which complied with GB/T 5519-2008 national standard.

### 128 2.3 Statistical analysis

129 Statistical analysis was performed using Microsoft Excel 2016 and SAS 9.4 statistical  
 130 software. The factor analysis was carried out using SPSS 25.0. The filling dynamic fitting was  
 131 carried out using Curve Expert 1.4 software, and Sigmaplot 12.5 was used to create figures.

## 132 3. Results

### 133 3.1 Effect of subsoiling on the 100-kernel weight of maize varieties from different eras

134 Analysis of variance showed that the effects of the different tillage methods, varieties and  
 135 years on the 100-grain weight were significant at  $p < 0.01$ . The interactions of tillage  
 136 method\*varieties and years\*years were also significant, but the effect of tillage method\*variety  
 137 was not significant (Table 2).

138

139 Table 2 Variance analysis of the effect of tillage method and variety on the 100-grain weight of maize

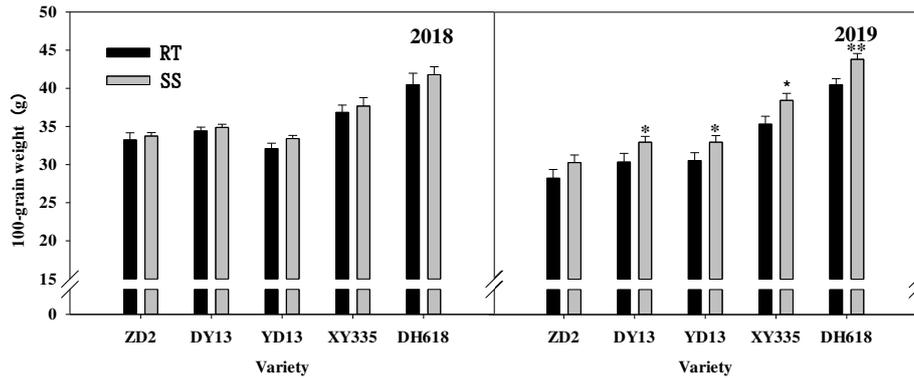
Source	DF	Mean Square
Tillage method (M)	1	38.53**
Main area error	2	1.27
Variety (V)	4	181.46**
V*M	4	0.40
Secondary area error	16	0.54
Years	1	7.95**

140 Note: "\*" means significant difference, and "\*\*" means extremely significant difference.

141

142 Under the rotation tillage (RT) condition, the mean grain weight of each of the 1970s-1990s  
 143 varieties was lower than that of the 2010s variety, but the 100-kernel weight of DH618 (2010s)  
 144 was significantly increased ( $P < 0.05$ ). Compared with ZD2, the 100-kernel weight of DY13, YD13,  
 145 XY335, and DH618 increased by 1.17 g, -1.14 g, 3.62 g and 7.24 g in 2018, respectively, and  
 146 increased by 1.81 g, 1.99 g, 6.07 g and 10.50 g in 2019, respectively (Fig. 2).

147 Subsoiling tillage significantly increased the 100-kernel weight of each variety ( $P < 0.05$ ).  
 148 However, there were significant differences in the responses of the varieties to subsoiling.  
 149 Compared with RT, the mean 100-kernel weight of ZD2, DY3, YD13, XY335 and DH618 for  
 150 2018-2019 increased by 1.14 g, 1.35 g, 1.69 g, 1.75 g and 2.09 g, respectively.



151

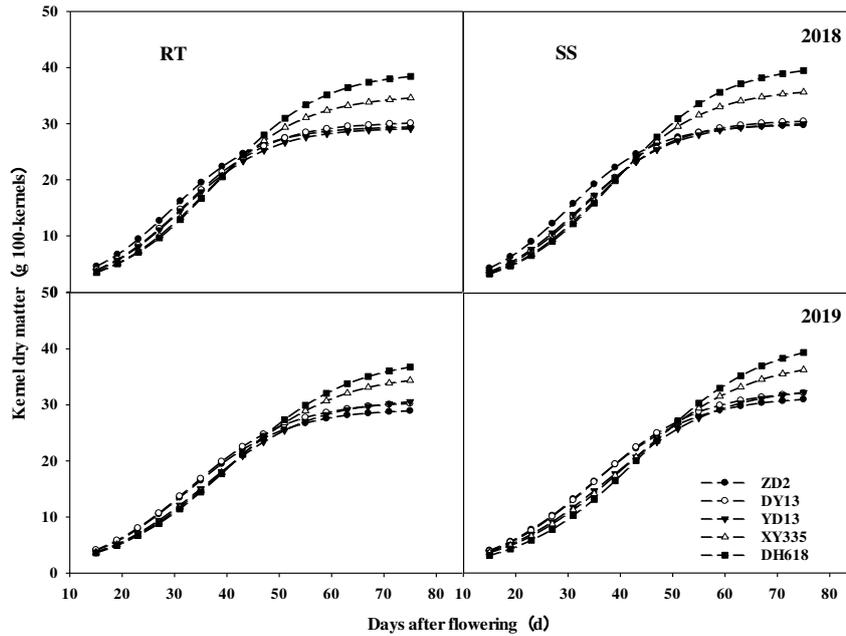
152 Fig. 2 Effects of deep loosening tillage on the 100-grain weight of different ages maize varieties

153 3.2 Effect of subsoiling on the kernel dry matter accumulation of maize varieties from different  
154 eras

155 As shown in Fig. 3, from 15 days after silking to physiological maturity, the kernel dry  
156 weight increased gradually, and maximum dry weight was achieved at physiological maturity.  
157 The kernel dry weight of the tested varieties showed no significant difference from 0-45 d after  
158 flowering; however, as the filling process continued, the difference increased progressively.  
159 The kernel weight of ZD2, DY13, YD13, XY335, and DH618 increased by 24.66 g, 23.91 g, 23.34  
160 g, 24.07 g and 24.49 g, respectively, from 0-43 d after flowering in 2018, and it increased by 4.74  
161 g, 6.19 g, 5.76 g, 10.52 g and 13.95 g, respectively, from 43-75 d after flowering. In 2019, the  
162 kernel weight of ZD2, DY13, YD13, XY335, and DH618 increased by 22.03 g, 22.54 g, 20.91 g,  
163 21.18 g and 21.16 g, respectively, from 0-43 d after flowering, and it increased by 6.89 g, 7.69 g,  
164 9.67 g, 13.19 g and 15.60 g, respectively, from 43-75 d after flowering. These results show that  
165 the period from 45-75 d after flowering was the main stage during which significant differences  
166 between the kernel dry weight of the old and new varieties manifested.

167 Compared with RT, subsoiling tillage improved the kernel dry weight of the tested  
168 varieties, but the extent of the improvement of kernel dry weight by subsoiling was inconsistent  
169 for different filling processes. From 0-43 d after flowering in 2018, the kernel weight of ZD2,  
170 DY13, YD13, XY335, and DH618 changed by -0.03 g, -0.64 g, -0.10 g, -0.33 g and -0.58 g,  
171 respectively. However, from 43-75 d after flowering, the kernel weight increased by 0.43 g, 1.00  
172 g, 1.01 g, 1.38 g and 1.65 g, respectively. In 2019, the kernel weight of ZD2, DY13, YD13, XY335,  
173 and DH618 changed by 0.18 g, -0.10 g, -0.23 g, -0.53 g and -1.13 g, respectively, from 0-43 d after  
174 flowering, and the kernel weight increased by 1.84 g, 1.94 g, 1.85 g, 2.38 g and 3.69 g,  
175 respectively, from 43-75 d after flowering. These results show that subsoiling mainly increased  
176 the kernel dry weight from 43-75 d after maize flowering, and the effect became more obvious  
177 as the originating era of the variety became more recent.

178



179

180 Fig. 3 Effects of deep loosening tillage on the dry weight of 100 grains of maize varieties in different ages

181

### 182 3.3 Effect of subsoiling on the filling characteristic of maize varieties from different eras

#### 183 3.3.1 Fitting equation for the filling dynamic curves of maize varieties from different eras

184 The days after flowering ( $t$ ) and weight after flowering ( $W$ ) were used as the independent  
 185 variable and dependent variable, respectively, in the logistic equation to simulate the grain  
 186 filling process. According to the functional relationship between dry weight and days after  
 187 flowering, the grain filling process was divided into three stages: gradually increasing stage,  
 188 rapidly increasing stage and slowly increasing stage. The parameters of the fitting equation  
 189 allowed the characteristic parameters of each filling stage to be obtained. The fitting equation  
 190 determination coefficient ( $R^2$ ) of the filling process of the tested maize varieties was greater  
 191 than 0.99, so the logistic equation was determined to fit the grain filling process of maize  
 192 varieties from different eras (Table 3).

193

194

Table 3. Fitting equation of the grain growth curve of each treatment

Year	Tillage method	Variety	Fitting equation	$R^2$
2018	RT	ZD2	$y=29.48/(1+33.92*\exp(-0.12x))$	0.997
		DY13	$y=30.26/(1+39.44*\exp(-0.12x))$	0.998
		YD13	$y=29.24/(1+39.76*\exp(-0.12x))$	0.998
		XY335	$y=35.04/(1+45.05*\exp(-0.11x))$	0.998
		DH618	$y=39.33/(1+48.03*\exp(-0.10x))$	0.998
	SS	ZD2	$y=29.89/(1+37.33*\exp(-0.12x))$	0.997
		DY13	$y=31.40/(1+32.01*\exp(-0.10x))$	0.997
		YD13	$y=30.68/(1+46.74*\exp(-0.12x))$	0.998
		XY335	$y=32.60/(1+34.28*\exp(-0.10x))$	0.997
		DH618	$y=30.20/(1+41.96*\exp(-0.12x))$	0.998

2019	RT	ZD2	$y=29.21/(1+32.02*\exp(-0.11x))$	0.997
		DY13	$y=30.57/(1+31.34*\exp(-0.10x))$	0.997
		YD13	$y=31.13/(1+33.04*\exp(-0.10x))$	0.998
		XY335	$y=35.60/(1+34.97*\exp(-0.09x))$	0.997
		DH618	$y=38.48/(1+37.24*\exp(-0.09x))$	0.998
	SS	ZD2	$y=33.11/(1+32.95*\exp(-0.09x))$	0.997
		DY13	$y=36.23/(1+50.59*\exp(-0.11x))$	0.999
		YD13	$y=38.02/(1+36.39*\exp(-0.09x))$	0.997
		XY335	$y=40.56/(1+53.28*\exp(-0.10x))$	0.999
		DH618	$y=41.89/(1+46.78*\exp(-0.09x))$	0.998

195

196 3.3.2 Effect of subsoiling on the filling characteristic parameters of maize varieties from  
197 different eras

198 As shown in Table 4, the trends of the two-year trial data were basically consistent for  
199 filling characteristic parameters under the RT and subsoiling conditions. The filling parameters  
200 tended to increase, decrease, and then increase again during variety replacement over the last  
201 several decades; YD13 had the lowest values, and DH618 had the highest values. Therefore, the  
202 data for 2018 and 2019 were averaged for the following analysis. Under the RT condition, the  
203 *A* values of ZD2, DY13, YD13, XY335 and DH618 were 29.35 g, 30.42 g, 30.19 g, 35.32 g and  
204 38.91 g, respectively; the *T*<sub>max</sub> values were 30.91 d, 32.25 d, 33.44 d, 37.23 d and 39.42 d,  
205 respectively; the *W*<sub>max</sub> values were 14.67 g 100-kernel<sup>-1</sup>, 15.21 g 100-kernel<sup>-1</sup>, 15.10 g 100-kernel<sup>-1</sup>,  
206 17.66 g 100-kernel<sup>-1</sup> and 19.45 g 100-kernel<sup>-1</sup>, respectively; the *R*<sub>max</sub> values were 0.83 g 100-  
207 kernel<sup>-1</sup>d<sup>-1</sup>, 0.84 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.81 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.88 g 100-kernel<sup>-1</sup>d<sup>-1</sup> and 0.93 g 100-  
208 kernel<sup>-1</sup>d<sup>-1</sup>, respectively; the *P* values were 53.09 d, 54.45 d, 56.02 d, 60.84 d and 63.31 d,  
209 respectively; the *G*<sub>mean</sub> values were 0.41 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.41 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.39 g 100-  
210 kernel<sup>-1</sup>d<sup>-1</sup>, 0.42 g 100-kernel<sup>-1</sup>d<sup>-1</sup> and 0.44 g 100-kernel<sup>-1</sup>d<sup>-1</sup>. During period when the kernel  
211 weight was increasing, all of the filling parameters of the modern varieties improved to  
212 different degrees.

213 For the subsoiling condition in comparison with RT, the *A* values of ZD2, DY13, YD13,  
214 XY335 and DH618 increased by 4.43%, 4.03%, 5.02%, 5.11%, 5.96%, respectively; the *W*<sub>max</sub>  
215 values increased by 4.46%, 4.01%, 4.84%, 5.12%, and 5.96%, respectively, the *R*<sub>max</sub> values  
216 increased by 1.81%, 1.79%, 1.23%, 2.29%, and 4.86%, respectively, the *P* values increased by  
217 2.79%, 2.12%, 4.03%, 2.67%, and 1.19%, respectively, the *G*<sub>mean</sub> values increased by 1.13%,  
218 0.29%, 0.55%, 1.56%, and 2.59%, respectively. These results indicate that subsoiling tillage can  
219 effectively control the filling characteristic parameters of different ages maize varieties, and the  
220 effect was more obvious with varieties that were generated more recently.

221 Table 4 Response of the grain filling characteristic parameters of maize varieties from different eras to  
222 tillage methods

Year	Tillage method	Variety	<i>A</i>	<i>T</i> <sub>max</sub>	<i>W</i> <sub>max</sub>	<i>R</i> <sub>max</sub>	<i>P</i>	<i>G</i> <sub>mean</sub>
			(g)	(d)	(g 100- kernel <sup>-1</sup> )	(g 100-kernel <sup>-1</sup> d <sup>-1</sup> )	(d)	(g 100-kernel <sup>-1</sup> d <sup>-1</sup> )
2018	RT	ZD2	29.48	29.36	14.74	0.88	49.99	0.432
		DY13	30.26	31.47	15.13	0.88	51.39	0.423

		YD13	29.24	31.22	14.62	0.86	50.86	0.413
		XY335	35.04	35.64	17.52	0.94	56.16	0.441
		DH618	39.33	38.07	19.66	1.00	58.99	0.468
		ZD2	29.89	30.08	14.95	0.90	49.86	0.433
		DY13	30.68	33.07	15.34	0.89	51.61	0.418
	SS	YD13	30.20	32.47	15.10	0.87	52.14	0.413
		XY335	36.23	36.96	18.12	0.96	56.52	0.447
		DH618	40.56	39.40	20.28	1.02	59.46	0.473
		ZD2	29.21	32.46	14.60	0.78	56.18	0.383
		DY13	30.57	33.02	15.29	0.80	57.51	0.393
	RT	YD13	31.13	35.66	15.57	0.76	61.17	0.374
		XY335	35.60	38.82	17.80	0.81	65.52	0.396
		DH618	38.48	40.77	19.24	0.85	67.62	0.412
2019		ZD2	31.40	34.24	15.70	0.79	59.27	0.390
		DY13	32.60	35.11	16.30	0.82	59.60	0.400
	SS	YD13	33.20	37.51	16.55	0.77	64.40	0.377
		XY335	38.02	40.98	19.01	0.83	68.41	0.403
		DH618	41.89	44.01	20.94	0.92	68.66	0.429

223 Note: Tmax is the time when the maximum grouting rate was reached; Wmax is the grain growth at the maximum  
 224 filling rate; Rmax is the maximum grouting rate; P is the active grouting stage; Gmean is the average grouting rate; A  
 225 is the ultimate growth rate of the grain.

226

### 227 2.3.3 Path analysis of grain filling characteristic parameters and 100-kernel weight

228 In order to clarify the direct and indirect relationships between grain filling parameters  
 229 and maize grain kernel weight, path-coefficient analysis was performed for five filling  
 230 characteristic parameters and 100-kernel weight (Table 5). Highly significant positive  
 231 correlations were identified between Tmax, Wmax, P and 100-kernel weight ( $P < 0.01$ ), and  
 232 significant positive correlations were identified between Rmax, Gmean and 100-kernel weight  
 233 ( $P < 0.05$ ). The correlation coefficients were ranked as follows:  $W_{max} > T_{max} > P > R_{max} > G_{mean}$ .  
 234 Wmax and 100-kernel weight had a positive direct correlation, and their correlation coefficient  
 235 was 0.939. Tmax, Rmax, P and Gmean showed positive indirect correlations mainly through  
 236 Wmax and 100-kernel weight, and their correlation coefficients were 0.866, 0.495, 0.667, and  
 237 0.484, respectively. Comprehensive analysis suggests the use of rational cultivation to postpone  
 238 the appearance of the maximum filling rate during the kernel filling process, while increasing  
 239 the maximum filling rate to increase kernel growth and achieve the goal of increasing 100-  
 240 kernel weight.

241

Table 5 Path analysis of grain filling characteristic parameters

Index	Correlation coefficient	Direct path coefficient	Coupling diameter factor				
			X1	X2	X3	X4	X5
X1	0.893**	0.209		0.866	-0.021	-0.174	0.013
X2	0.976**	0.939	0.193		-0.059	-0.138	0.041
X3	0.540*	-0.113	0.039	0.495		0.040	0.079
X4	0.683**	-0.191	0.191	0.677	0.023		-0.017

X5	0.527*	0.080	0.033	0.484	-0.111	0.041
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Note: X1 stands for Tmax, X2 stands for Wmax, X3 stands for Rmax, X4 stands for P, X5 stands for Gmean, and Y stands for 100-grain weight. Tmax is the time when the maximum grouting rate was reached; Wmax is the grain growth at the maximum filling rate; Rmax is the maximum grouting rate; P is the active grouting stage; Gmean is the average grouting rate; A is the ultimate growth rate of the grain.

#### 2.3.4 Effect of subsoiling on the filling rate parameters of maize varieties from different eras

The durations of each stage of maize grain filling were ranked as follows: slowly increasing stage>rapidly increasing stage>gradually increasing stage. The mean filling rates of each stage were ranked as follows: rapidly increasing stage>gradually increasing stage>slowly increasing stage (Table 6). With the exception of the grain filling rate of the gradually increasing stage, the other grain filling rate parameters increased to different degrees during maize variety replacement over the last several decades. Under RT, the two-year mean T1 durations of ZD2, DY13, YD13, XY335, and DH618 were 19.26 d, 20.30 d, 21.14 d, 23.87 d and 25.52 d, respectively, whereas those of T2 were 23.31 d, 23.91 d, 24.59 d, 26.71 d and 27.79 d, respectively; and those of T3 were 29.01 d, 29.75 d, 30.61 d, 33.24 d and 34.59 d. For ZD2, DY13, YD13, XY335, and DH618, the V1 values were 0.33 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.32 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.31 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.32 g 100-kernel<sup>-1</sup>d<sup>-1</sup> and 0.32 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, whereas their V2 values were 0.73 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.74 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.72 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.77 g 100-kernel<sup>-1</sup>d<sup>-1</sup> and 0.82 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, respectively, and their V3 values were 0.21 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.21 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.20 g 100-kernel<sup>-1</sup>d<sup>-1</sup>, 0.22 g 100-kernel<sup>-1</sup>d<sup>-1</sup> and 0.23 g 100-kernel<sup>-1</sup>d<sup>-1</sup>.

Under the subsoiling condition, the grain filling duration was prolonged during different stages of grain filling in comparison with RT, and the increase in filling duration was greatest during the gradually increasing stage. The averaged two-year data revealed that the filling durations of ZD2, DY13, YD13, XY335, and DH618 were prolonged by 4.80%, 7.83%, 5.01%, 5.80% and 8.33%, respectively, during the gradually increasing stage. In addition, subsoiling increased the filling rate of ZD2, DY13, YD13, XY335, and DH618 during the rapidly increasing stage by 2.05%, 2.04%, 0.70%, 2.61%, and 4.29%, respectively, but it had little effect on the filling rate during other stages. These results indicate that subsoiling mainly extended the filling duration during the gradually increasing stage and improved the filling rate during the rapidly increasing stage. Moreover, maize varieties from more recent eras were found to be more sensitive to the effect of subsoiling in comparison with relatively older varieties.

Table 6 Response of grain filling rate parameters of different ages maize varieties to tillage methods

Year	Tillage method	Variety	T1	V1	T2	V2	T3	V3
			(d)	(g 100-kernel <sup>-1</sup> d <sup>-1</sup> )	(d)	(g 100-kernel <sup>-1</sup> d <sup>-1</sup> )	(d)	(g 100-kernel <sup>-1</sup> d <sup>-1</sup> )
2018	RT	ZD2	18.39	0.34	21.95	0.78	27.31	0.22
		DY13	20.19	0.32	22.56	0.77	28.08	0.22
		YD13	20.05	0.31	22.33	0.76	27.79	0.21
		XY335	23.31	0.32	24.65	0.82	30.68	0.23
		DH618	25.12	0.33	25.90	0.88	32.23	0.25
	SS	ZD2	19.14	0.33	21.89	0.79	27.24	0.22

		DY13	21.74	0.30	22.66	0.78	28.20	0.22
		YD13	21.03	0.30	22.89	0.76	28.48	0.21
		XY335	24.55	0.31	24.81	0.84	30.88	0.24
		DH618	26.35	0.33	26.10	0.90	32.49	0.25
		ZD2	20.13	0.31	24.66	0.68	30.70	0.19
		DY13	20.40	0.32	25.25	0.70	31.42	0.20
	RT	YD13	22.23	0.30	26.85	0.67	33.42	0.19
		XY335	24.43	0.31	28.77	0.71	35.80	0.20
		DH618	25.92	0.31	29.68	0.75	36.94	0.21
2019		ZD2	21.23	0.31	26.02	0.70	32.38	0.20
		DY13	22.03	0.31	26.17	0.72	32.57	0.20
	SS	YD13	23.37	0.30	28.27	0.68	35.18	0.19
		XY335	25.96	0.31	30.03	0.73	37.37	0.20
		DH618	28.94	0.31	30.14	0.80	37.51	0.22

275 Note: T1 represents the grain filling duration of the gradually increasing stage, V1 represents the average grain filling  
276 rate of the gradually increasing stage, T2 represents the grain filling duration of the rapidly increasing stage, V2  
277 represents the average grain filling rate of the rapidly increasing stage, T3 represents the grain filling duration of the  
278 slowly increasing stage, and V3 represents the average grain filling rate of the slowly increasing stage.

279

### 280 3.3.5 Analysis of the grouting rate parameter factor

281 In order to clarify the internal dependence of the grain filling rate and duration at each  
282 stage, factor analysis was performed using the two-year trial data (Table 7). Under RT, the factor  
283 load difference of the filling duration was small at each stage (T1=0.96, T2=0.96, T3=0.96), which  
284 indicated that the proportion of the filling duration of each stage was nearly identical. Under  
285 subsoiling, the factor load difference of the grain filling duration between T1 and T2/T3 was  
286 large (T1=0.91, T2=0.97, T3=0.97), which indicated that the effect of subsoiling on the grain  
287 filling duration was stronger during the rapidly increasing and slowly increasing stages. In  
288 addition, the factor load of the filling rate during the rapidly and slowly increasing stages was  
289 higher than that of the gradually increasing stage, which showed that the filling rate during the  
290 rapidly and slowly increasing stages contributed more to the mean filling rate in comparison  
291 with that of the gradually increasing stage. These results demonstrate that appropriate tillage  
292 measures can improve the filling rate during the rapidly increasing and slowly increasing  
293 stages, and thus improve the mean filling rate.

294

Table 7 Parameter factor analysis of grain filling rate

Farming methods	SS		SS	
	Y1	Y2	Y1	Y2
T1	0.961	0.249	0.914	0.340
V1	-0.414	0.766	-0.055	0.721
T2	0.958	-0.233	0.970	-0.211
V2	0.021	0.985	-0.001	0.983
T3	0.958	-0.234	0.971	-0.210
V3	0.022	0.992	-0.069	0.971

295 Note: the extraction method was principal component analysis, Y1 represents the grouting duration, and Y2 represents

296 the average grouting rate. T1 represents the grain filling duration of the gradually increasing stage, V1 represents the  
297 average grain filling rate of the gradually increasing stage, T2 represents the grain filling duration of the rapidly  
298 increasing stage, V2 represents the average grain filling rate of the rapidly increasing stage, T3 represents the grain  
299 filling duration of the slowly increasing stage, and V3 represents the average grain filling rate of the slowly increasing  
300 stage.

#### 301 302 4. Discussion

303 Previous studies have shown that the grain weight of maize is mainly determined by the  
304 filling rate and filling duration. Therefore, improving the grain filling rate and ensuring that  
305 grain filling is maintained for an appropriate duration could increase grain weight and yield<sup>[20-  
306 23]</sup>. Fang et al.<sup>[24]</sup> showed that the grain filling rate determined dry matter accumulation and  
307 affected yield, and they showed that the implementation of a reasonable planting method could  
308 improve the grain filling rate. Gasura et al.<sup>[25]</sup> reported that extending the active grain filling  
309 period and increasing the mean filling rate increased maize yield. Daynard et al.<sup>[26]</sup> found that  
310 prolonging the filling duration increased the 100-kernel dry weight of maize. On the basis of  
311 previous studies, the present study further explored the characteristics of maize grain filling  
312 and revealed that improving the mean filling rate and delaying the occurrence of the maximum  
313 filling rate led to increased grain growth during the period of the maximum filling rate. In  
314 addition, we found that the filling rates of the rapidly increasing and slowly increasing stages  
315 primarily determined the mean filling rate, whereas that of the gradually increasing stage had  
316 a weaker influence on the mean filling rate. Therefore, during the period from 20 days after  
317 silking to physiological maturity, which encompasses the rapidly increasing and slowly  
318 increasing stages of grain filling, the level of plant material accumulation was strongly  
319 correlated with the mean filling rate, and implementing practices that maximize plant dry  
320 matter accumulation will be beneficial to the further improvement of grain weight.

321 Conservation agriculture, such as the use of no tillage, less tillage, and straw mulch, has  
322 been an important strategy for the sustainable development of worldwide agriculture in the  
323 past few decades, because conservation agriculture can improve soil properties while  
324 increasing income and crop yields<sup>[27]</sup>. Farming methods are a key factor affecting soil systems  
325 in China, where soil management and seeding are mainly performed using small tractors, and  
326 a significant portion of farmland is subjected to methods involving less tillage or no tillage,  
327 which increase the surface soil bulk density and osmotic resistance, which have deleterious  
328 effects on crop growth<sup>[28-30]</sup>. Previous studies demonstrated that the use of optimized farming  
329 methods increased crop yield<sup>[31-32]</sup>. For example, subsoiling increased yield by improving the  
330 100-kernel weight of maize<sup>[33]</sup>. Zhai et al.<sup>[34]</sup> showed that subsoiling increased the maximum and  
331 mean kernel filling rates, which significantly increased the maximum kernel weight at harvest.  
332 Cai et al.<sup>[35]</sup> et al. showed that subsoiling increased kernel weight, improved plant resistance to  
333 environmental stresses, and increased yield. In this study, we further analyzed the filling  
334 characteristics of maize varieties from different eras using subsoiling, and we showed that,  
335 compared with conventional rotation, subsoiling significantly improved kernel weight by  
336 increasing the maximum filling rate and prolonging the duration of the maximum grain filling  
337 rate. The beneficial effects of subsoiling on filling characteristics were likely observed because  
338 subsoiling tillage the bottom of the ploughed stratum, increased the topsoil depth, improved  
339 water storage and moisture conservation, promoted root growth and development<sup>[36-37]</sup>,

340 maintained a high leaf area index and photosynthetic rate after anthesis, and slowed the  
341 subsequent decrease in photosynthetic rate. Therefore, the implementation of subsoiling tillage  
342 was beneficial for maize because it led to the production of more photosynthetic products<sup>[38]</sup>,  
343 thus increasing the grain filling rate and kernel weight.

344

#### 345 4 Conclusion

346 During variety replacement over the last several decades, kernel weight was significantly  
347 increased, while kernel growth during the period of the maximum kernel filling rate was  
348 improved, the durations of the active filling period and maximum filling rate were prolonged,  
349 and the maximum filling rate and mean filling rate were increased. Compared with the other  
350 trial varieties, the 2010s variety had a longer gradually increasing period, and its filling rates  
351 during the rapidly increasing and slowly increasing period were improved to different degrees.  
352 Subsoiling tillage increased the maximum grain filling rate, postponed the occurrence of the  
353 maximum filling rate, prolonged the duration of the gradually increasing period, improved the  
354 filling rates of the rapidly increasing period and slowly increasing period, and increased grain  
355 weight. The filling rate of the rapidly increasing period of the 2010s variety was more sensitive  
356 to subsoiling tillage in comparison with the other trial varieties.

357

358 **Author Contributions:** Performed the experiments: L-q.W., J-l.G. and S-p.H. Analyzed the data:  
359 L-q.W., D-l.M. and X-f.Y.. Revised the manuscript critically for important intellectual content:  
360 X-f.Y., J-l.G. and D-l.M. ,Wrote the paper: L-q.W, L.L. and X-f.Y. .

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367 **Conflicts of Interest:** Page: 12

368 The authors declare no conflicts of interest.

369

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# Figures

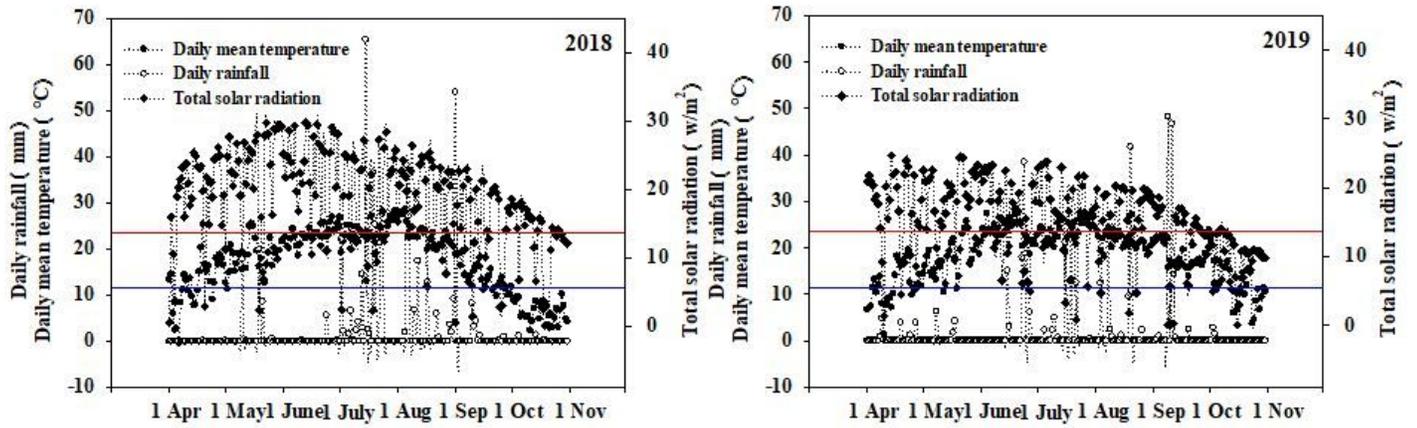


Figure 1

Main meteorological factors during the growth period in the experimental area

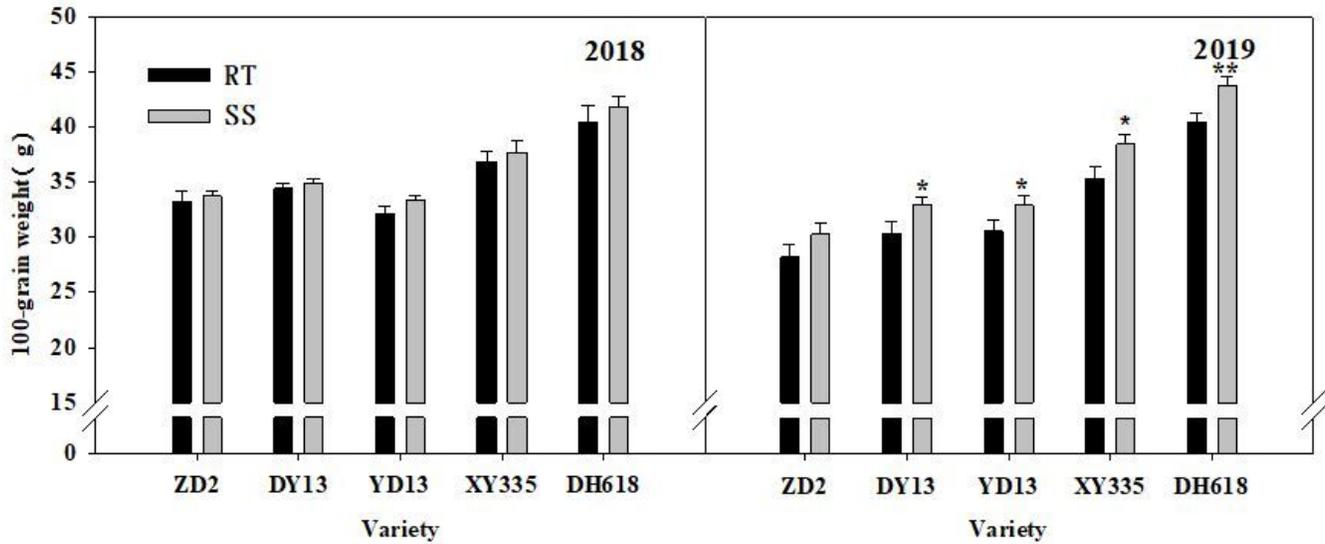


Figure 2

Effects of deep loosening tillage on the 100-grain weight of different ages maize varieties 3.2 Effect of subsoiling on the kernel dry matter accumulation of maize varieties from different eras

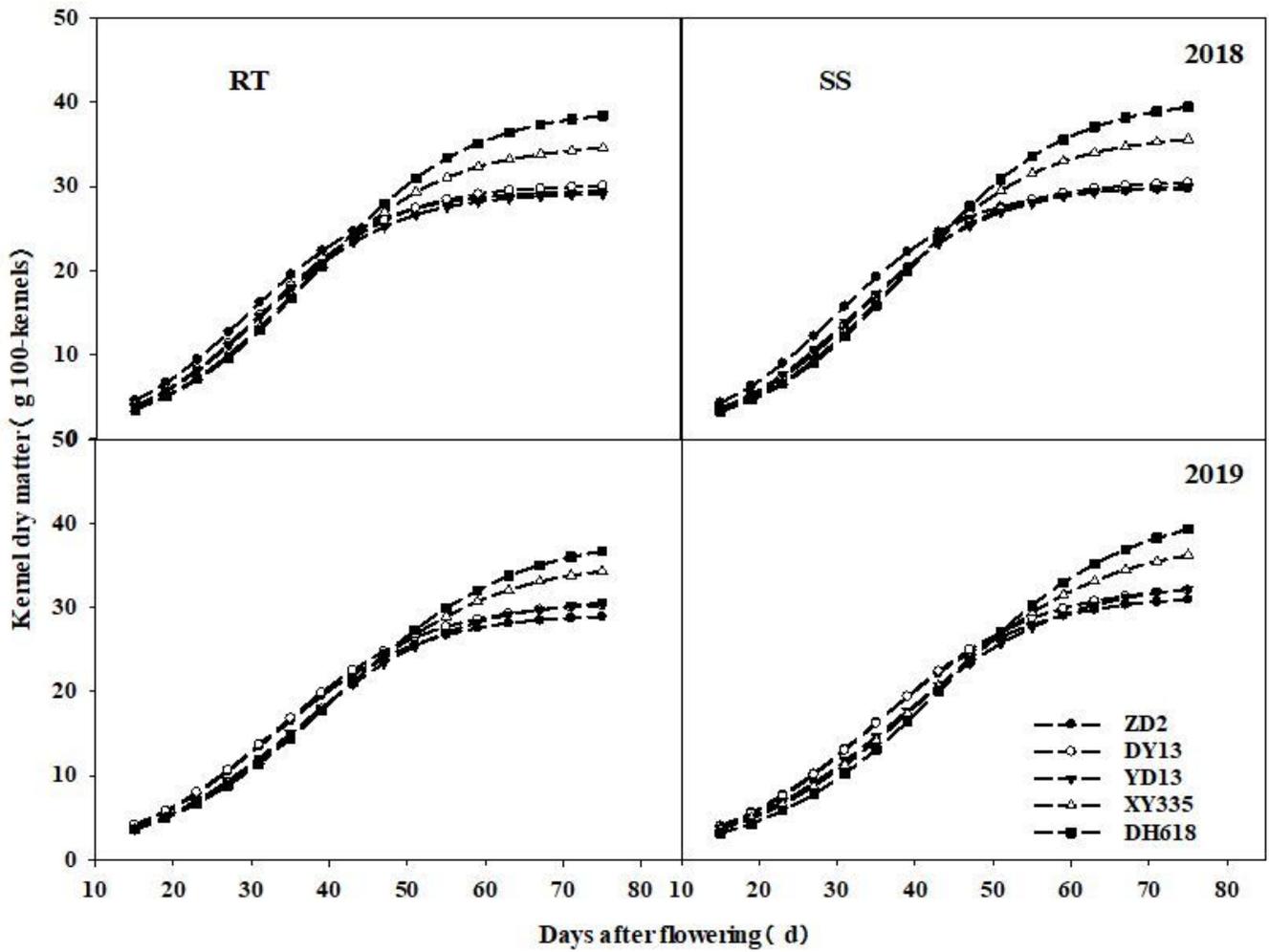


Figure 3

Effects of deep loosening tillage on the dry weight of 100 grains of maize varieties in different ages