



## Effect of planting densities on grain filling and kernel dehydration of maize (*Zea mays*)

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### ABSTRACT

Maize (*Zea mays* L.) yield is greatly affected by the environmental and cultivate pattern. Grain filling and dehydration play important roles in maize grain dry mass accumulation and are important biological processes in maize growth and development. Four maize cultivars were planted at Hebei province with planting densities of 60000 and 75000 plants/ha during 2017-18. The kernel fresh weight and dry weight of four cultivars were measured at 15, 22, 29, 36, 43, 50 and 57 days after pollination of two planting densities. Logistic model was used to estimate the grain filling process in different environments. The results showed that the maximum filling rates of all cultivars under two planting densities were attained at about 25 days after pollination. The grain filling period was longer under the planting density of 60000 plants/ha. Meanwhile, the grain dehydration was slower. With the increased of planting density, 100-grain weight of per plant decreased while the yield increased. The higher yield was contributed by the larger numbers of plants. It indicated that suitable higher planting density will enhance the yield of maize. Bract, cob and grain water content were significantly correlated. This study indicated a negative effect of dense planting on grain filling.

**Key words:** Grain filling, Kernel dehydration, Planting density

Maize (*Zea mays* L.) is an important food, feed, and energy crop in the world, and its consumption increases with population (Shiferaw *et al.* 2011). Maize yield is greatly affected by environmental, geographical factors and cultivation patterns (Trachsel *et al.* 2016). Optimization of the row spacing with improving the field structure could increase the yield of maize. Narrow rows maize planting with a more uniform root and leaf distribution contribute to soil water and light utilization, and the field microenvironment could be improved by reducing soil temperature and evaporation (Sharratt 2005). In the USA, planting densities increased from 3 plants/m<sup>2</sup> (1930) to 9 plants/m<sup>2</sup> (2005) and yield increased by 8 t/ha (Duvick 2005). Genotype differences also affect the yield of maize under high planting density conditions (Lee 2007). To optimize the potential of maize yield per unit area in current production systems, modern breeding efforts must consider high plant density conditions (Li *et al.* 2015).

The number and weight of maize kernels per unit area determines the grain yield of maize, and factors such as

kernel filling rate and duration are important (Eichenberger *et al.* 2015). The filling rate is closely related to environmental conditions and genotypic differences. Borrás *et al.* characterized a large set of maize inbred lines for kernel growth traits, found high variation in kernel growth rate and grain-filling duration across diverse maize inbred lines (Borrás *et al.* 2009). The grain filling rate and drying rate of all stages showed broad phenotypic variations (Zhang *et al.* 2016). Previous studies have shown that maize grain filling is significantly related to moisture of maize kernels (Gambin *et al.* 2007). The kernel drying rate is an important trait that determines harvest time and yield of maize, and main factors affecting it are cultivars, temperature and humidity. Therefore, it is necessary to study the effect of planting density on the drying rate of different cultivars maize ear. To provide basic data and theoretical support on breeding of high-yield and densely planted maize, an experiment was conducted to reveal the effect of planting densities on grain filling and dehydration of different maize cultivars.

### MATERIALS AND METHODS

*Plant material and site description:* Four maize hybrid cultivars were investigated in our study, Zhengdan 958 (ZD958), Hengyu147 (HY147), Hengyu1587 (HY1587) and Hengyu6105 (HY6105). Zhengdan 958 is an elite

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commercial hybrid, which has been the most widely planted maize hybrid since 2005 in China. Hengyu147, Hengyu1587 and Hengyu6105, which have a high yield, were bred by Dry Land Farming Research Institute, Hebei Academy of Agriculture and Forestry Sciences. All four hybrid cultivars were planted in five-row plot (five meters length and 0.6 meters row spacing) in an incompletely randomized block design following three replicates on 14 June 2017 and 15 June 2018 at the farmland of the Hebei Academy of Agriculture and Forestry Sciences (Shijiazhuang, Hebei, China, 38°07'N, 114°22'E). The soil type was clay loam composed of 1.96% organic matter, 86.10 mg/kg available nitrogen, 32.75 mg/kg available phosphorus and 185.40 mg/kg available potassium at pH 7.74. Each material was planted in two planting densities of 60000 and 75000 plants/ha.

**Sampling and measurement of grain filling rate (GFR):** Sixty to sixty-five ears in each block were self-pollinated and the pollination dates were determined. Three ears were hand-collected at each plot at 15, 22, 29, 36, 43, 50 and 57 days after pollination (DAP) in 2017-18. The sampling dates were chosen starting at 15 DAP because previous studies suggested that starch synthesis in the kernel begins from 12 to 15 DAP (Zhang *et al.* 2013). These harvested ears were split into three parts: cob, kernels and bract. From each ear, 100 kernels were obtained from the middle part of the ear. After measured fresh weight, all parts of the ear were dried by oven then measured the dry weight. Water content and dehydration rate were calculated followed Liu's methods which described in his study (Liu *et al.* 2016).

**The model of kernel filling of maize:** In this study, the Logistic model was used to fit the kernel filling of maize. Its mathematical expression followed Yin's methods which described in his study (Yin *et al.* 2018).

**Data analysis:** All data were processed by the software of Microsoft Excel 2007, analyzed by SPSS19.0 and plotted by OriginPro 9.1. Basic statistical analysis were performed with the method of one-way ANOVA and followed by multiple comparisons (Duncan's Multiple Range Test), correlations were measured using a Pearson correlation coefficient.

## RESULTS AND DISCUSSION

The grain dry weight of most cultivars reduced with the increasing of planting density at 15 DAP, and HY6105 reduced significantly ( $P < 0.05$ ). However, the grain dry weight of HY147 increased at higher planting densities in 2017. In the middle stage of grain development (36 DAP), the grain dry weight of HY1587 at 60000 plants/ha was the heaviest in 2017-18, significantly higher ( $P < 0.05$ ) than HY147, effect of planting density on grain dry weight is not significant. After 57 days of pollination, the grain dry weight of HY1587 at 60000 plants/ha was still the heaviest of all cultivars, and HY147 (30.4 g) was the lightest at 60000 plants/ha, higher planting density significantly reduced ( $P < 0.05$ ) the grain dry weight of HY1587 and HY6015, but ZD958 and HY147 were less affected (Table 1). The grain filling is an important biological process and plays an

important role in grain dry weight accumulation (Gambin *et al.* 2007, Eichenberger *et al.* 2015). Depend on the grain filling rate of 4 cultivars, ZD958 showed a positive correlation of grain filling traits with the planting density while the other 3 cultivars showed a negative correlation. Different maize cultivars have their own suitable planting density. The best planting density will significantly enhance the yield. The result may help farmer select suitable planting density for different cultivars.

Grain dry matter accumulation is related to grain filling rate. The grain filling rate of the four cultivars showed a single-peak type under the two planting densities in the whole grain filling period, which was similar to previous studies (Wang *et al.* 2012, Zhang *et al.* 2015, Yin *et al.* 2018). Logistic model were fitted to study grain filling process and deeply revealed the interaction relationship for key factors which affecting grain filling (Table 1). The maximum filling rates of all cultivars under two planting densities were attained at about 25 DAP. HY1587 (1.41 g/d) had higher  $G_{max}$  under conventional planting density (60000 plants/ha) than under higher planting density. ZD958 (1.39 g/d) had highest  $G_{max}$  under 75000 plants/ha. With the planting density increased,  $G_{max}$  of HY147 was decreased in 2017-18. The maximum filling rate of HY6105 increased at density of 75000 plants/ha in both two years, while HY1587 and HY147 decreased. HY6105 reached the maximum value of grain filling rate for the shortest time (24.2 DAP) in 2018, while HY1587 needed 28.68 days. Compared with 60000 plants/ha,  $T_{max}$  of HY147 were delayed at density of 75000 plants/ha in both two years. HY1587 had the shortest duration of active growth period at 60000 plants/ha in 2017 and was the longest in 2018. Compared  $G_{max}$ ,  $T_{max}$  and  $T_{active}$ ,  $G_{max}$  was the most stable parameter and the other two were effect by the environment. Among four cultivars, grain filling parameters showed that 75000 plants/ha was the suitable planting density for HY147.

With the increased of planting density, grain water content of HY147, HY1587 and HY6105 were increased after 57 days of pollination in 2017 and had slightly difference in 2018 (Figure 1). The dehydrating rate was slightly different in each part of the ear. The grain dehydrating rate was faster in the early stages of development and showed a fast-to-slow trend. Under density of 75000 plants/ha, most of the grain dehydration rates were increased. The grain dehydration rate was mainly affected by ear traits, grain types and environment characters. The less ear rows and thinner ear diameter, the faster grain dehydration (Zhang *et al.* 2016).

Generally, the water content of grain decreased with ear development. The grain water contents and dehydration rate of all cultivars under two planting densities showed a fast-slow trend. Compare with 75000 plants/ha, the grain filling period and grain dehydration rate of four cultivars were longer and lower under the 60000 plants/ha. It is consistent with the previous studies (Yue *et al.* 2018).

With the increase of planting densities, the yield increased (Table 2). Interestingly, the 100 grain weight of four cultivars showed a decrease trend by the increase

Table 1 Grain dry weight development and fitting parameters of different maize cultivars at two planting densities in 2017 and 2018

| Year | Cultivars | Planting density (plants/ha) | Days after pollination |         |         |          |          |         |          |       | Fitting parameters |       |               |                  |                  |                   |                     |
|------|-----------|------------------------------|------------------------|---------|---------|----------|----------|---------|----------|-------|--------------------|-------|---------------|------------------|------------------|-------------------|---------------------|
|      |           |                              | 15d                    | 22d     | 29d     | 36d      | 43d      | 50d     | 57d      | a     | b                  | k     | Adj. R-square | G <sub>max</sub> | T <sub>max</sub> | G <sub>mean</sub> | T <sub>active</sub> |
| 2017 | HY1587    | 60000                        | 5.2abcd                | 13.1abc | 21.0bc  | 30.8e    | 33.6de   | 36.4bc  | 36.3cde  | 36.62 | 58.16              | 0.154 | 0.991         | 1.41             | 26.38            | 0.94              | 38.96               |
|      |           | 75000                        | 4.5ab                  | 12.0abc | 19.7ab  | 27.2bcde | 31.9bcde | 33.7abc | 34.4bcd  | 34.87 | 50.97              | 0.145 | 0.9957        | 1.26             | 27.11            | 0.84              | 41.38               |
|      | HY6105    | 60000                        | 6.1def                 | 13.0abc | 21.0bc  | 27.5bcde | 33.5de   | 36.4bc  | 37.7defg | 38.8  | 29.76              | 0.121 | 0.9979        | 1.17             | 28.04            | 0.78              | 49.59               |
|      |           | 75000                        | 5.5bcde                | 11.8abc | 20.8abc | 26.8bcd  | 33.0cde  | 37.4c   | 40.3def  | 41.77 | 29.24              | 0.112 | 0.9945        | 1.17             | 30.14            | 0.78              | 53.57               |
|      | HY147     | 60000                        | 4.3a                   | 11.3ab  | 18.2a   | 25.2abc  | 30.9bcde | 31.8ab  | 33.3abc  | 33.74 | 46.43              | 0.138 | 0.9958        | 1.16             | 27.81            | 0.78              | 43.48               |
|      |           | 75000                        | 4.6ab                  | 10.8a   | 18.1a   | 24.8abc  | 30.7bcd  | 33.2abc | 34.5bcd  | 35.43 | 40.15              | 0.128 | 0.9964        | 1.13             | 28.85            | 0.76              | 46.88               |
| 2018 | ZD958     | 60000                        | 4.8abc                 | 13.9abc | 19.3ab  | 26.6bcd  | 28.5abc  | 30.6a   | 31.2ab   | 31.57 | 50.85              | 0.147 | 0.9956        | 1.16             | 26.73            | 0.77              | 40.82               |
|      |           | 75000                        | 4.8abc                 | 11.5ab  | 18.1a   | 24.3ab   | 30.8bcd  | 33.2abc | 34.7bcd  | 35.92 | 32.84              | 0.12  | 0.9912        | 1.08             | 29.10            | 0.72              | 50.00               |
|      | HY1587    | 60000                        | 6.8ef                  | 14.6bcd | 21.1bc  | 30de     | 32.6cde  | 37.5c   | 40.9f    | 41.88 | 21.02              | 0.106 | 0.9872        | 1.11             | 28.68            | 0.74              | 56.51               |
|      |           | 75000                        | 5.6bvde                | 14.6bc  | 20.6bc  | 26.7bcd  | 30.3bcd  | 34.2abc | 35.2def  | 35.73 | 24.02              | 0.12  | 0.9865        | 1.07             | 26.56            | 0.71              | 50.14               |
|      | HY6105    | 60000                        | 6.5ef                  | 14.3bcd | 22.5cd  | 28.6cde  | 30.7bcd  | 35.8bc  | 36.9defg | 37.11 | 24.3               | 0.122 | 0.986         | 1.13             | 26.26            | 0.75              | 49.37               |
|      |           | 75000                        | 5.2abcd                | 15.3c   | 20.9bc  | 26.4bcd  | 30.4bcd  | 33.1abc | 30.7ab   | 32.11 | 34.78              | 0.147 | 0.973         | 1.18             | 24.2             | 0.79              | 40.91               |
| 2019 | HY147     | 60000                        | 5.3abcd                | 13abc   | 20ab    | 23.9ab   | 27.8ab   | 29.6a   | 30.4ab   | 30.33 | 29.63              | 0.136 | 0.9908        | 1.03             | 24.83            | 0.69              | 43.96               |
|      |           | 75000                        | 5.1abc                 | 12.1abc | 18.2a   | 22.3a    | 25.6a    | 29.8a   | 29a      | 30.02 | 23.95              | 0.121 | 0.9832        | 0.91             | 26.18            | 0.61              | 49.45               |
|      | ZD958     | 60000                        | 5.7cde                 | 13.9abc | 23.6d   | 28.8cde  | 33.1cde  | 38c     | 40.4ef   | 40.59 | 26.71              | 0.118 | 0.9834        | 1.2              | 27.78            | 0.8               | 50.75               |
|      |           | 75000                        | 5abc                   | 13.8abc | 22.7cd  | 29.3de   | 35.5e    | 37c     | 37.1defg | 37.87 | 48.9               | 0.147 | 0.9944        | 1.39             | 26.43            | 0.93              | 40.77               |

Note: Different letters in the same column indicate significant differences ( $P < 0.05$ ). At fitting parameters part, a, b and k are parameters of Logistic model which fit the kernel filling. A, the final growth amount; k, the relative growth rate; G<sub>max</sub>, the maximum value of grain filling rate; T<sub>max</sub>, the time for maximum value of grain filling rate; G<sub>mean</sub>, The mean value of grain filling rate and T<sub>active</sub>, active growth period.

Table 2 The ear and yield characteristics of different cultivars

| Year | Cultivars | Density<br>(plants/ha) | Ear length<br>(cm) | Ear diameter<br>(cm) | Ear rows       | Kernels per<br>row | Cob diameter<br>(cm) | Grain width<br>(mm) | Yield<br>(kg/ha)  | Kernels<br>percentage (%) | 100-grain<br>weight (g) |
|------|-----------|------------------------|--------------------|----------------------|----------------|--------------------|----------------------|---------------------|-------------------|---------------------------|-------------------------|
| 2017 | HY1587    | 60000                  | 14.7±0.92abcde     | 4.26±0.17a           | 13.2±0.80a     | 33.6±4.24ab        | 2.34±0.07abc         | 4.43±0.36cdefg      | 9446.7±309.76abc  | 88.68±1.16bc              | 31.65±0.75abc           |
|      | HY1587    | 75000                  | 15.1±1.59abcde     | 4.54±0.09cde         | 14.0±0.80abcd  | 29.3±2.73ab        | 2.39±0.15abc         | 4.45±0.24cdefg      | 11760±426.25e     | 89.26±0.52c               | 32.03±1.79abc           |
|      | HY6105    | 60000                  | 16.8±0.42e         | 4.49±0.14bcde        | 14.7±0.23cde   | 31.4±1.63ab        | 2.42±0.04abcd        | 4.27±0.12bcde       | 10237.5±302.25bcd | 89.16±0.43c               | 31.99±1.08abc           |
|      | HY6105    | 75000                  | 14.9±0.08abcde     | 4.41±0.14abcd        | 15.1±0.23de    | 33.9±1.68ab        | 2.49±0.14bcde        | 4.20±0.10bcd        | 10756.7±225.02cde | 84.46±0.72ab              | 32.11±3.13bc            |
|      | HY147     | 60000                  | 14.2±1.60abcd      | 4.31±0.05ab          | 15.1±0.83de    | 28.9±2.31ab        | 2.24±0.09a           | 3.99±0.16ab         | 8775±234a         | 83.97±3.02a               | 27.92±0.84a             |
|      | HY147     | 75000                  | 14.0±1.40abc       | 4.55±0.13cde         | 16.3±0.61fg    | 31.9±2.66ab        | 2.31±0.06ab          | 3.98±0.09ab         | 10546.7±340.13cde | 88.92±0.90c               | 29.10±2.01ab            |
|      | ZD958     | 60000                  | 16.0±0.56cde       | 4.67±0.12e           | 13.2±0.01a     | 36.0±2.28b         | 2.61±0.17de          | 4.85±0.15h          | 9403.3±129.84abc  | 90.09±0.15c               | 31.01±0.44abc           |
|      | ZD958     | 75000                  | 15.8±1.10bcde      | 4.63±0.13de          | 13.9±0.61abc   | 34.4±3.17b         | 2.51±0.18bcde        | 4.52±0.05defgh      | 11846.3±447.29e   | 91.06±0.97c               | 31.45±2.01abc           |
|      | HY1587    | 60000                  | 14.3±2.04abcd      | 4.31±0.06ab          | 13.2±0.80a     | 31.2±1.13ab        | 2.33±0.13ab          | 4.61±0.33efgh       | 8997.7±109.30ab   | 88.16±1.70bc              | 39.22±3.93e             |
|      | HY1587    | 75000                  | 15.6±0.13bcde      | 4.37±0.07abc         | 13.2±0.40a     | 27.0±2.76a         | 2.37±0.11ab          | 4.72±0.16gh         | 10027±157.08abcd  | 88.66±0.64bc              | 36.89±3.32de            |
| 2018 | HY6105    | 60000                  | 15.2±1.34abcde     | 4.31±0.05ab          | 14.0±0.80abcd  | 33.0±3.67ab        | 2.39±0.06abc         | 4.34±0.15cdef       | 9807.3±102.76abcd | 88.85±0.53c               | 36.45±1.34de            |
|      | HY6105    | 75000                  | 16.3±0.34de        | 4.55±0.13cde         | 14.4±0.69bcde  | 31.6±3.33ab        | 2.38±0.18abc         | 4.33±0.11cde        | 10898.6±176.40de  | 89.59±0.18c               | 34.76±1.76cd            |
|      | HY147     | 60000                  | 13.6±1.17ab        | 4.35±0.14abc         | 16.5±0.46g     | 30.8±2.99ab        | 2.28±0.08ab          | 3.74±0.13a          | 8654.9±248.44a    | 89.70±0.91c               | 32.33±0.13bc            |
|      | HY147     | 75000                  | 13.2±1.48a         | 4.53±0.03bcde        | 15.3±0.83ef    | 31.3±3.18ab        | 2.37±0.10abc         | 4.15±0.12bc         | 9380.9±89.15abc   | 88.28±2.45bc              | 28.99±2.92ab            |
|      | ZD958     | 60000                  | 15.3±1.06abcde     | 4.71±0.18e           | 14.3±0.23abcde | 33.7±1.75ab        | 2.65±0.09e           | 4.61±0.01efgh       | 8753.9±302.68a    | 88.66±1.00bc              | 31.36±2.71abc           |
|      | ZD958     | 75000                  | 14.4±1.57abcd      | 4.65±0.08e           | 13.3±0.46ab    | 33.5±3.50ab        | 2.56±0.04cde         | 4.68±0.22fgh        | 11194.1±177.94de  | 88.38±0.23bc              | 37.22±1.87de            |

Note: Different letters in the same column indicate significant differences ( $P < 0.05$ ).

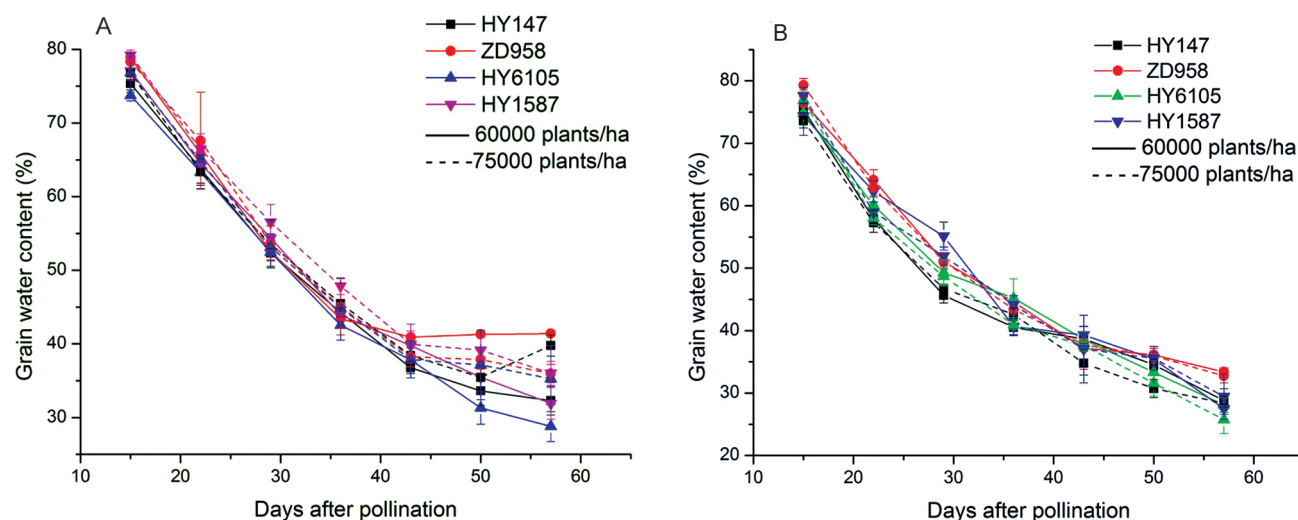


Fig 1 Grain water content of different maize cultivars under two planting densities in 2017 and 2018. (A) Grain water content at density of 60000 plants/ha and 75000 plants/ha in 2017, (B) Grain water content at density of 60000 plants/ha and 75000 plants/ha in 2018.

planting density. The kernels percentage changed slightly under different planting densities. At 60000 plants/ha, ears of four cultivars were 14.2 to 16.0 cm long and 4.26 to 4.67 cm diameter in 2017, were 13.6 to 15.3 cm long and 4.31 to 4.71 cm diameter in 2018, while ZD958 was the longest and had largest diameter. Under the condition of 75000 plants/ha, the ear length of HY1587 increased by 0.4 cm (2017) and 0.81cm (2018), and the higher density could increase the ear diameter of HY1587 and reduce the ear diameter of ZD958. HY147 had the largest number of ear rows among four cultivars. The planting density of 75000 plants/ha has a greater impact on kernels per row of HY147. Higher planting density increased the cob diameter of most cultivars, and had negative effect on ZD958. The grain width of HY1587 and HY147 were larger at 75000 plants/ha, and higher planting density reduced the grain width of HY6105 and ZD958.

Grain quantity was significantly affected by different cultivars and closely related to the grain filling period (Paponov *et al.* 2005, Chen *et al.* 2016). Suitable planting density can enhance maize yield potential. With the increase of plant density, the dry matter weight per plant decreased. The higher yield of high planting density may contribute by the larger number of plants. Different planting densities affect the grain filling period and ear dehydration progress. Under the 60000 plants/ha, the grain filling period became longer and the grain dehydration was slower. With the increasing of planting density, 100 grain weight per plant decreased while the yield increased. The higher production was enhanced by the larger number of plants. Suitable higher planting density will enhance the yield of maize. There is a significant correlation between bract, cob and grain water content.

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