

Article

# Husk Leaf Senescence Characteristics of Spring Maize (*Zea mays* L.) Cultivated in Two Row Directions and Three Plant Spacings in Northeast China

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**Abstract:** Row direction and plant spacing influence crop senescence. An experiment was conducted to analyze the effect of row direction and plant spacing on the husk leaf senescence. Physiological indicators related to husk leaf senescence at days after silking (DAS) 12, 22, and 40 were investigated under two row directions (east to west and south to north, abbreviated as EW and SN, respectively) and three plant patterns (single rows spaced at 65 cm, 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows, and 40 cm twin rows spaced at 160 cm between the paired rows of narrow–wide rows, abbreviated as SR, WN1, and WN2, respectively). Row direction affects the chlorophyll content and dehydration rate according to our results. Superoxide dismutase activity at DAS 22, catalase (CAT) activity at DS, and abscisic acid (ABA) concentrations at DAS 12, 22, and 40 were significantly affected by plant spacing. The CAT activities of WN1 and WN2 were significantly higher than those of SR, and WN2 had a lower ABA concentration than WN1 and SR. Our results suggest that row direction from SN and plant spacing from WN1 and WN2 were the suitable conditions for delaying the senescence of husk leaves of maize in the experimental site.

**Keywords:** *Zea mays* L.; dehydrating rate; row spacing; row orientation

## 1. Introduction

Maize (*Zea mays* L.) is an important food crop worldwide, particularly in China. Maize is one of the largest crops planted in China because of its high efficiency and the competitiveness of its crop planting system per area unit. Nevertheless, growers hope to harvest an increased grain yield of maize [1]. Increasing planting density is currently one of the most effective ways to increase the yield per unit area of maize in China [2].

The increase of planting density can increase maize yield. However, maize is sensitive to planting density [3]. An increase in planting density could change the growing conditions of maize, competition of light in canopy increases, the quantity of the solar radiation intercepted of the lower leaves was reduced, as well as red/far-red (R/FR) light ratio [4]. Therefore, the senescence of lower maize leaves is accelerated in this changed microenvironment of canopy [5–7]. Senescence is a highly complex regulatory process in crops [8–10], and thus is closely related to the yield of fruit and/or seed [11]. Therefore, senescence of crop has been widely studied. In maize, researchers have focused on the senescence of maize leaf. Leaf senescence is initiated before the flowering period and is accelerated during the filling period in maize [5]. In the process of leaf senescence, the chlorophyll content, antioxidant system, abscisic acid (ABA) concentration, and dehydrating rate of leaf could

be changed [12–14]. Premature leaf senescence reduces the area of green leaves and shortens the photosynthetic time of leaves, thus reducing maize yield [4,15].

The husk leaf is a special leaf, similar to leaves, containing chlorophyll for photosynthesis, and the photosynthetic products of husk leaves provide grains and contribute to the dry weights of grains [16,17]. The contribution rate of husk leaves to yield is approximately 15% in maize, including the contribution of photosynthesis in husk leaves [16,17]. Therefore, the senescence of husk leaves is closely related to maize yield.

Water content in husk leaves is correlated with the dehydration rate of grain in maize [18], and the low water contents of husk leaves are favorable to grain dehydration. Therefore, the early senescence of husk leaves facilitates grain dehydration because it lowers the water contents of husk leaves. Dehydration is an important part of aging, and a suitable row direction and plant spacing can result in a good canopy structure, improve canopy microenvironment, and increase the yield for maize [19]. Numerous studies reported that crop yield is higher and crop quality is better in the north–south row direction than those in the east–west row direction [20,21]. Many studies, particularly those about the influence of plant spacing, reported that a suitable plant spacing can increase yield [22]. Balkcom et al. reported that a narrow row can increase light retention in canopies [15] and promote canopy growth and closure [23]. However, ventilation in this setup is poor, and thus the plants forming the canopy are prone to disease and lodging. Plant spacing consisting of wide–narrow rows (maize planted on two narrow rows) can improve field ventilation [24]. Our previous study showed that maize yield obtained using wide–narrow row patterns was high in north China [25,26], and the senescence of the lower leaves of maize planted using these patterns was delayed because the chlorophyll content and the activities of superoxide dismutase (SOD), polyphenoloxidase (POD), and catalase (CAT) in the leaves changed. However, how row direction and plant spacing affect the senescence of maize husk leaves is unclear. On the basis of the important role of husk leaves in maize yield and dehydration, the effects of row direction and planting mode on the physiological characteristics of the senescence of husk leaves were explored. A field experiment with two row direction (east to west and south to north) and three plant spacing (single rows spaced at 65 cm, 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows, and 40 cm twin rows spaced at 160 cm between the paired rows of narrow–wide rows) was designed to examine the influence of row direction and plant spacing on the husk leaf senescence of spring maize during post-flowering in Northeast China.

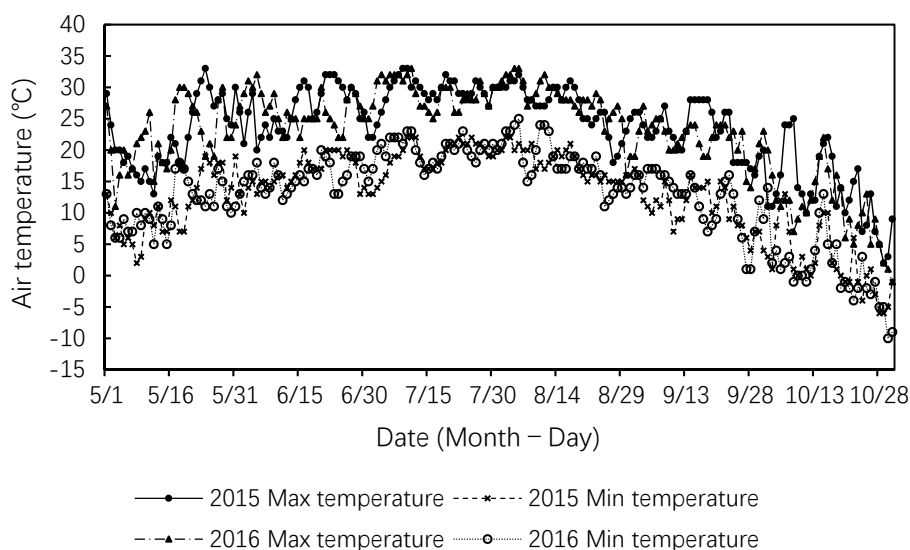
## 2. Materials and Methods

The experiment was performed in the agricultural experimental field of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun City, Jilin Province, China (44.00° N, 125.40° E). The soil is a black soil layer of 0–20 cm containing 1.3 g·kg<sup>-1</sup> total nitrogen, 18.8 mg·kg<sup>-1</sup> Olsen phosphorus, and 16.4 g·kg<sup>-1</sup> total potassium.

The following treatments were applied in a split plot design with three replications: two row directions and three plant spacings (Table 1). The length and width of each plot are both 10.4 m with three repeats. The plots were sown on 1 May 2015 and 2016. The highest and lowest temperatures (Figure 1) were from 1 May to 30 October 2015 and 2016. The variety of maize was LY 99, and the planting density was 6.5 plants per m<sup>2</sup>. The distance between plants was the 23.67 cm for SR (single rows spaced at 65 cm) and WN1 (40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows), and 15.38 cm for WN2 (40 cm twin rows spaced at 160 cm between the paired rows of narrow–wide rows) (the density does not change, but increases the distance between wide rows [26]). For each plot, 200 kg of N, 100 kg of P<sub>2</sub>O<sub>5</sub>, and 100 kg of K<sub>2</sub>O per ha were applied. Weed growth was controlled with herbicide, and no irrigation was performed.

**Table 1.** The experiment design.

Row Direction	Plant Spacing
	SR (single rows spaced at 65 cm)
EW (east to west)	WN1 (40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows)
SN (south to north)	WN2 (40 cm twin rows spaced at 160 cm between the paired rows of narrow–wide rows)

**Figure 1.** Max and min air temperatures from 1 May to 30 October in 2015 and 2016.

At the silking stage, the uniform plants in the two middle rows of each plot were marked (30 July 2016). For later samplings, we selected three marked plants in each plot for analysis. Three biological replicates were included for each treatment.

The total area of husk leaves per plant was measured by Li-3000C (LI-COR, USA) at DAS 22 in 2015 and 2016.

At days after silking (DAS) 12, 22, and 40, fresh husk leaf samples were taken in 2016. After rapid grounding with liquid nitrogen, 500 mg fresh weight of tissues was mixed with 10 mL of phosphate buffer solution that was pre-stored at 4 °C (100 mM phosphate buffer, pH = 7.0). After oscillating at 4 °C for 30 min, the mixture was centrifuged at 14,000× g for 10 min at 4 °C, and the supernatant was used to determine the activities of the following: SOD by measuring the inhibition of nitroblue tetrazolium reduction according to the protocol of Fridovich [27], POD by calculating the oxidation pyrogallol according to the protocol of Kumar and Khan [28], and CAT using ultraviolet absorption at 240 nm according to the protocol of Aebi [29].

Fresh husk leaves were obtained at DAS 12, 22, and 40 in 2016. After rapid grounding with liquid nitrogen, 40 mg fresh weight of tissues was added with 1 mL of ultrapure water, placed in a shaker to be shaken at 4 °C overnight for ABA extraction, and then centrifuged at 14,000× g for 10 min at 4 °C. The supernatant was used to determine the ABA concentration by ELISA [30].

The chlorophyll content of second husk leaf was measured by a portable SPAD-502 plus chlorophyll meter at DAS 12 and 40 in 2016.

All husk leaves per plant were collected; weighed for fresh weight (FW); placed in a 70 °C oven to dry to constant weight; and then weighed again for dry weight (DW) at DAS 12, 22, and 40 in 2016. The water content of husk at DAS 12, 22, and 40 was recorded as  $W_1$ ,  $W_2$ , and  $W_3$ , respectively. The days

from DAS 12 to DAS 22 and from DAS 22 to DAS 40 were recorded  $d_1$  and  $d_2$ . The dehydrating rate of husk leaves is calculated according to the following equations:

$$\text{Dehydrating rate from BS to MS} = (W_1 - W_2)/d_1 \times 100\%$$

$$\text{Dehydrating rate from MS to DS} = (W_2 - W_3)/d_2 \times 100\%$$

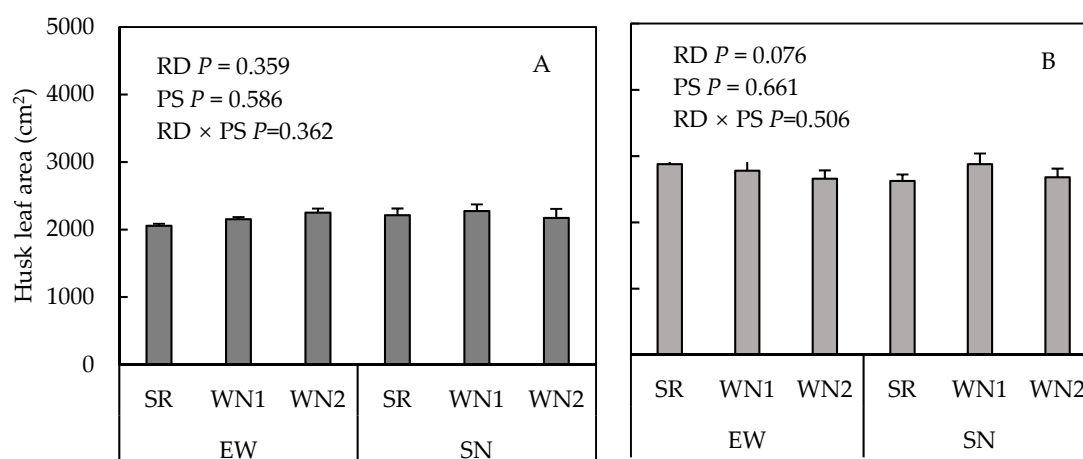
At the maturity of maize, four middle rows of maize per plot were harvested to measure grain yield of maize per plot. Grain yield of maize was standardized to 14% moisture in 2015 and 2016.

Experimental results are expressed as the mean  $\pm$  standard error. Data were analyzed by one-way analysis of variance (ANOVA) (SPSS 19.0 software). The different stages were analyzed separately, and significant differences among the treatments were determined using Duncan test ( $P$  values = 0.05).

### 3. Results

#### 3.1. Area of Husk Leaves

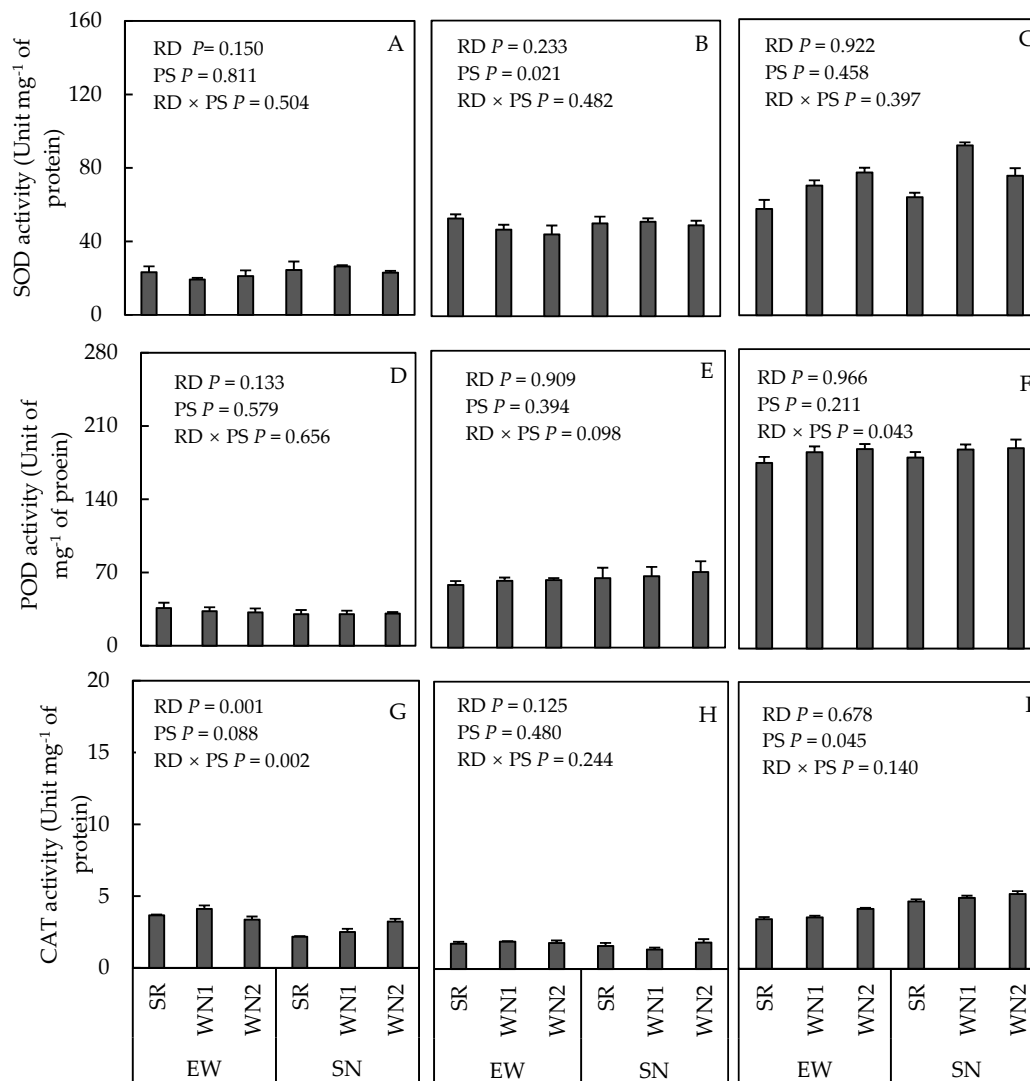
The area of husk leaf was 2056–2274 cm<sup>2</sup> (2015) and 2625.46–2879.70 cm<sup>2</sup> (2016) per plant, and it had no significant difference ( $P > 0.05$ ) under two row directions and three plant spacings (Figure 2).



**Figure 2.** Area of husk maize leaf under two row directions (RD) and three plant spacings (PS) in 2015 (A) and 2016 (B) (EW and SN indicate the row direction of east to west and south to north, respectively. SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow–wide rows.). Bars denote the SE of the mean ( $n = 3$ ).

#### 3.2. SOD, POD, and CAT Activities

SOD activity was not significantly affected by plant spacing, row direction, and not by their interaction either (Figure 3A–C), but was significantly affected by plant spacing at DAS 22 (Figure 3B). POD activity was not significantly affected by plant spacing, row direction, or their interaction (Figure 3D–F). CAT activities were significantly affected by row direction at DAS 12 (Figure 3G) and by plant spacing at DAS 40 (Figure 3I). SOD, POD, and CAT activities was significantly affected by stages (at DAS 12, 22, and 40) (Table 2).



**Figure 3.** The superoxide dismutase (SOD), polyphenoloxidase (POD), and catalase (CAT) activities of husk leaf at days after silking (DAS) 12 (A,D,G), DAS 22 (B,E,H), and DAS 40 (C,F,I) of maize under two row directions (RD) and three plant spacings (PS) (EW and SN indicate row direction of east to west and south to north, respectively. SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow–wide rows.). Bars denote the SE of the mean ( $n = 3$ ).

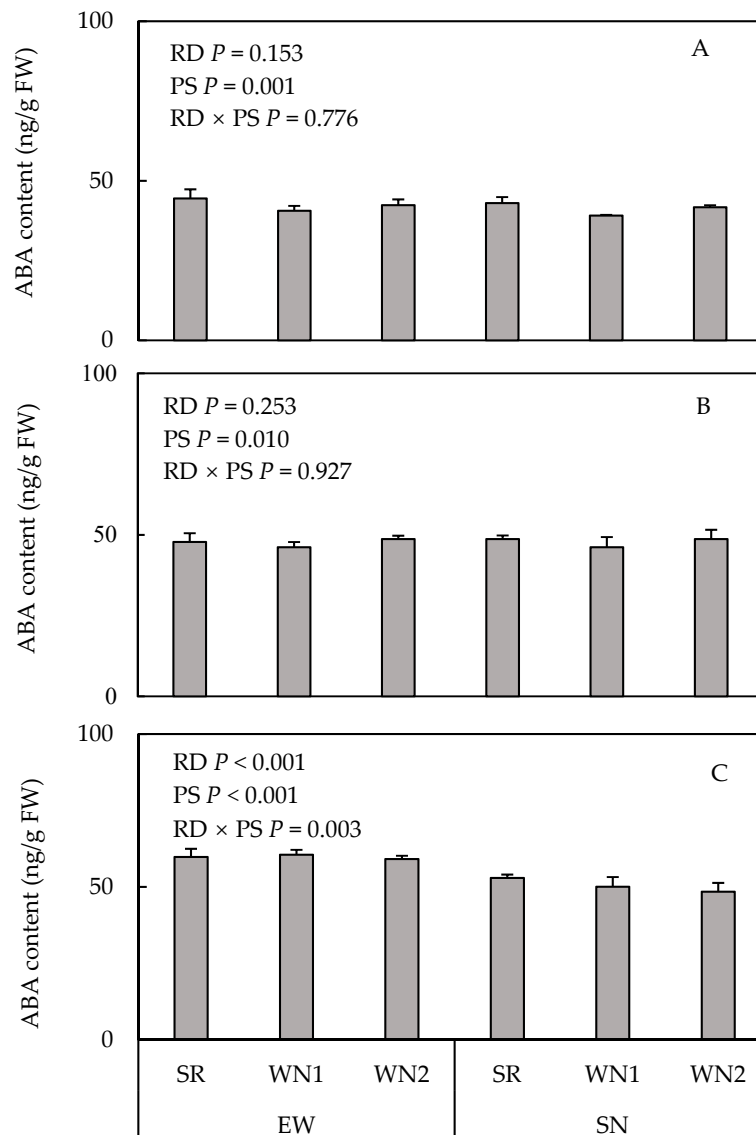
**Table 2.** Out-put of one-way analysis of variance (ANOVA) for stages on superoxide dismutase (SOD), polyphenoloxidase (POD), and catalase (CAT) activities; abscisic acid (ABA) concentration; and SPAD value at days after silking (DAS) 12, 22, and 40, as well as the dehydrating rate from DAS 12 to 22 and DAS 22 to 40.

Physiological Index	Significance Level at $P \leq 0.05$
SOD activities	*
POD activities	*
CAT activities	*
ABA concentration	*
SPAD value	*
Dehydrating rate	*

\* indicate significance level at  $P \leq 0.05$ .

### 3.3. ABA Concentration

The ABA concentration of husk leaf gradually increased from DAS 12 to DAS 40 (Figure 4). At DAS 12 and DAS 22, the ABA concentration of husk leaves was significantly affected by plant spacing, but not by row direction and the interaction of these two factors. At DAS 40, the ABA concentration of husk leaves was significantly influenced by row direction, plant spacing, and their interaction. The ABA concentration was significantly affected by stages (at DAS 12, 22, and 40) (Table 2).

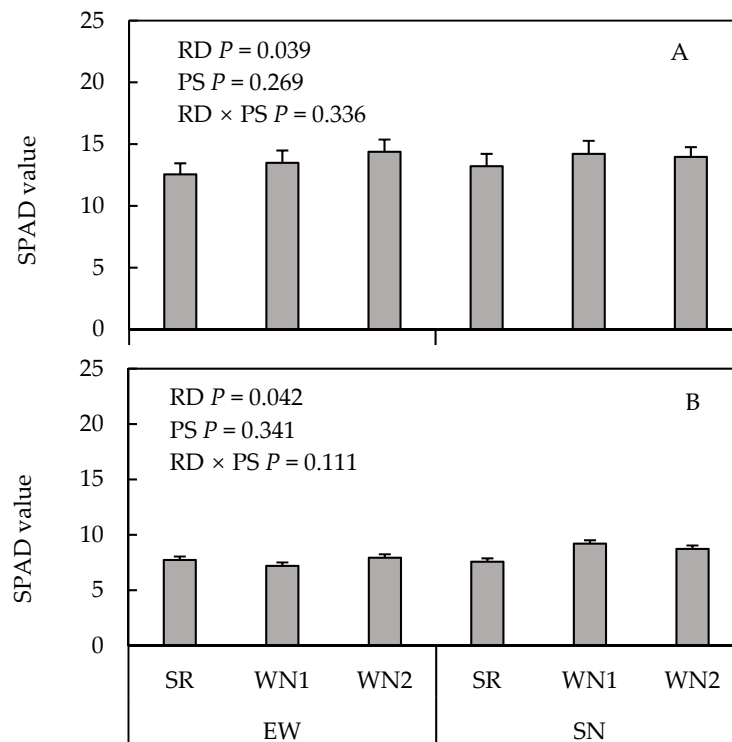


**Figure 4.** The abscisic acid (ABA) concentration of husk leaf at days after silking (DAS) 12 (A), DAS 22 (B), and DAS 40 (C) of maize under two row directions (RD) and three plant spacings (PS) (EW and SN indicate row direction of east to west and south to north, respectively. SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow-wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow-wide rows.). Bars denote the SE of the mean ( $n = 3$ ).

### 3.4. Chlorophyll Content of Husk Leaves

The chlorophyll content of husk leaves at DAS 12 was higher and that at DAS 40 was lower. The chlorophyll content of husk leaves decreased gradually from DAS 12 to DAS 40 (Figure 5).

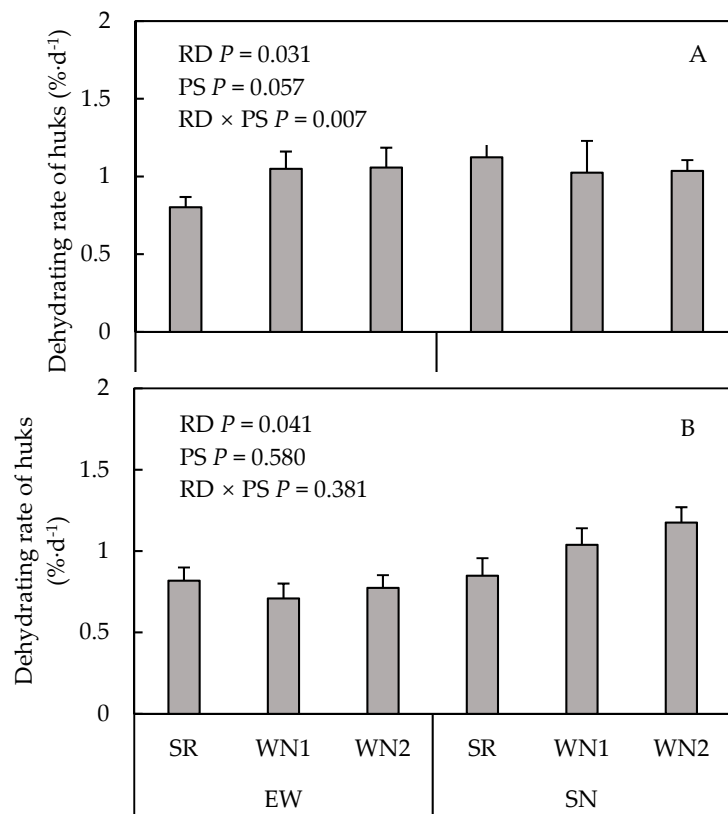
The chlorophyll content of husk leaves was significantly affected by row direction at DAS 12 and DAS 40. The chlorophyll content was significantly affected by stages (at DAS 12 and DAS 40) (Table 2).



**Figure 5.** SPAD value of husk leaf at days after silking (DAS) 12 (A) and DAS 40 (B) of maize under two row directions (RD) and three plant spacings (PS) (EW and SN indicate row direction of east to west and south to north, respectively. SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow–wide rows.). Bars denote the SE of the mean ( $n = 3$ ).

### 3.5. Dehydrating Rate of Husk Leaves

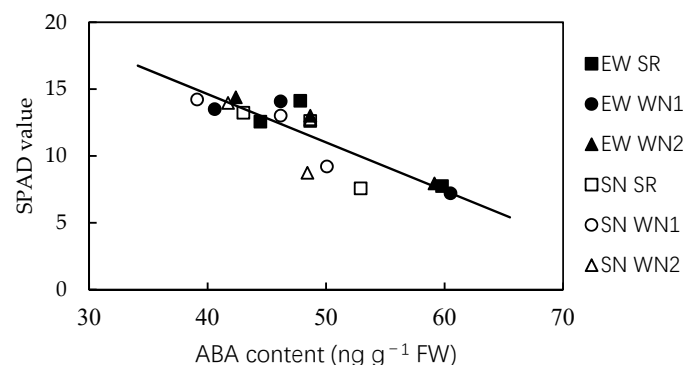
The dehydrating rate of husk leaves from DAS 12 to DAS 22 (Figure 6A) and from DAS 22 to DAS 40 (Figure 6B) was significantly affected by row direction, but not by plant spacing. The dehydrating rate from DAS 22 to DAS 40 of SN was higher than that of EW. The interaction between these factors significantly influenced the dehydrating rate of husk leaves from DAS 12 to DAS 22, but not from DAS 22 to DAS 40 (Figure 6). The dehydrating rate was significantly affected by stages (DAS 12 to DAS 22 and DAS 22 to DAS 40) (Table 2).



**Figure 6.** Dehydrating rate of husk leaf during from days after silking (DAS) 12 to DAS 22 (A) and during from DAS 22 to DAS 40 (B) of maize under two row directions (RD) and three plant spacings (PS) (EW and SN indicate row direction of east to west and south to north, respectively. SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow–wide rows.). Bars denote the SE of the mean ( $n = 3$ ).

### 3.6. Correlation Analysis

The correlation analysis showed that ABA concentration of was significantly negative correlated with chlorophyll content of husk leaves at DAS 12, DAS 22, and DAS 40 ( $r^2 = 0.74$ ,  $P < 0.05$ ) (Figure 7).



**Figure 7.** Relationship between abscisic acid (ABA) concentration and chlorophyll content (SPAD value) at days after silking (DAS) 12, 22, and 40 of maize under two row directions and three plant spacings (EW and SN indicate row direction of east to west and south to north, respectively. SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow–wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow–wide rows).



### 3.7. Grain Yield

Significant differences in grain yield of maize were found among row directions, as well as an interaction of factors of row direction and plant spacing in 2015 and 2016. Grain yield of WN1 and WN2 of SN was higher than that of SR of SN, as well as WN1 and WN2 of EW, in 2015 and 2016.

## 4. Discussion

Husk leaves growing on the shortening ear-stalk of maize are special leaves. The development of husk leaves generally stopped [17]. Therefore, the area of husk leaves in this study was measured at DAS 22, because the area did not increase at this time. Our results show that row direction and plant spacing had significant effects on total area per plant (Figure 2). This result is similar to that obtained from the areas of maize leaves [25].

Crop senescence is influenced by genetic and external environmental factors [8,9]. In the study of the senescence physiology of leaves, tissue senescence leads to the degradation of organelles [31,32], and chloroplasts are among the organelles that are degraded first [33]. Chlorophyll content decreases as chloroplast structure changes. Husk leaves contain chlorophyll for photosynthesis. Row direction significantly affects the chlorophyll contents of husk leaves (Figure 5). The chlorophyll contents of husk leaves in the north–south row direction was significantly higher than those in the east–west row direction. Therefore, the high chlorophyll contents of husk leaves in the north–south row direction is beneficial to the synthesis of photosynthetic products because of the photosynthetic rates of chlorophyll [34]. Under different planting densities, the decrease of distance between plants in the same row may cause the change of chlorophyll content owing to the poor light. In the present study, the planting density of three planting spacing was the same. Compared with SR and WN1, the distance between plants in the same row of WN2 decreased and the distance between the wide rows increased. The increase in the distance of wide rows of WN2 improves the light transmission in the canopy. Therefore, in the present study, plant spacing had no significant effect on the chlorophyll contents of the husk leaves (Figure 5). Moreira et al. showed that chlorophyll content in leaves is affected by row spacing [35,36].

The free radical hypothesis has received wide attention from studies about plant senescence. Reactive oxygen scavenging systems can effectively protect cells from photooxidative damage [37], inhibit the accumulation of reactive oxygen species, and reduce damage to cell structures [12]. Therefore, senescence is accompanied by changes in SOD, POD, and CAT activities [38]. After flowering, maize leaves gradually lose their ability to scavenge oxygen free radicals, and the activities of membrane protective enzymes decrease [39]. In the present study, SOD activities of husk leaves at DAS 22 (Figure 3B) and the CAT activities of husk leaves at DAS 40 were significantly affected by plant spacing (Figure 3I). This indicates that WN1 and WN2 delayed the senescence by improving SOD activities of SN at DAS 22 and CAT activities at DAS 40 [40,41]. Our results were different from those of a previous study of maize leaves, which showed high SOD, POD, and CAT activities in the plants in a “160 + 40” wide and narrow row pattern [26]. This difference in results may be related to the difference between leaves and husk leaves.

ABA is involved in crop senescence and is the main hormone promoting senescence. In this study, the ABA concentrations in husk leaves increased from DAS 12 to DAS 40 (Figure 4). This result suggests that the senescence of husk leaves was intensified at this stage. Previous studies have shown that plant senescence intensifies when the ABA level increases [42]. At DAS 40, ABA concentrations in the husk leaves of SN were significantly lower than those of the husk leaves of EW. This result suggests that the senescence of the husk leaves of EW was intensified at this stage. Plant spacing affected the ABA concentrations in the husk leaves at DAS 40. The husk leaves of WN1 and WN2 had the lowest ABA concentrations, suggesting that the senescence of the husk leaves of WN1 and WN2 maize was weaker than that of the husk leaves of SR at this stage.

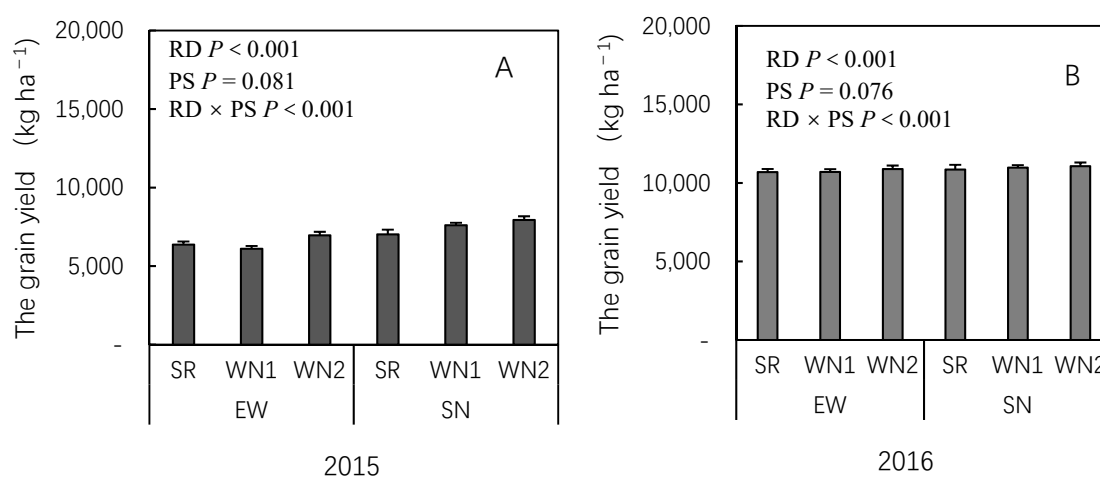
The water content of maize grain directly affects the breakage rate and drying cost of grain in mechanical harvest [43], and thus farmers expect the grain to have a lower moisture content at harvest.

The water contents of the husk leaves are related to maize grains, and the rapid dehydration rates of husk leaves are beneficial to grain dehydration [36]. Plant dehydration reflects the senescence process. In this study, the dehydration rate of the husk leaves of SN was significantly higher than that of EW from DAS 22 to DAS 40 (Figure 6), indicating that the water contents of the husk leaves of SN decreased rapidly. Meanwhile, the water contents of the husk leaves were related to those of maize grains, and the rapid dehydration rates of the husk leaves were beneficial to grain dehydration [36].

In this study, row direction significantly affected the dehydration rates of husk leaves, and the dehydration rates of the husk leaves in the north–south row direction were significantly higher than those in the east–west row direction from DAS 22 to DAS 40 (Figure 6). This result indicates that the water contents of the husk leaves in the north–south row direction decreased rapidly during this stage, and the water contents of the husk leaves in the later stage decreased, and thus the seeds were dehydrated [18]. Yoon and Johnson reported that the temperature at the bottom of the canopy in the north–south row direction was higher and relative humidity was lower than those in the east–west row orientation. This result indicated that the canopy microenvironment in the north–south row direction is favorable to husk leaf dehydration.

ABA concentrations in the husk leaves were negatively correlated with chlorophyll contents (Figure 7). Zhang and He [44] reported similar results in cotton leaves. Wan et al. [45] focused on the relationship between ABA accumulation and dehydration rate and found that ABA concentration in maize grains is negatively correlated with dehydration rate. Our study found similar results. This relationship may have occurred because ABA accumulation in the husk leaves may regulate and promote dehydration. In addition, the dehydration of husk leaves is related to grain dehydration [46]; therefore, ABA accumulation in husk leaves is beneficial to grain dehydration in maize [45].

In the present study, the grain yield in 2015 was lower than in 2016 (Figure 8). This may be owing to the drought in July 2015 at the experimental site. The rainfall in July 2015 was only 30% of the annual average rainfall of July (Data not shown).



**Figure 8.** Grain yield of maize under two row directions (RD) and three plant spacings (PS) in 2015 (A) and 2016 (B) (EW and SN indicate row direction of east to west and south to north, respectively). SR indicates single rows spaced at 65 cm; WN1 indicates 40 cm twin rows spaced at 90 cm between the paired rows of narrow-wide rows; and WN2 indicates 40 cm twin rows, spaced at 160 cm between the paired rows of narrow-wide rows). Bars denote the SE of the mean ( $n = 3$ ).

The planting spacing had a significant effect on the activities of SOD at DAS 22; CAT at DAS 40 (Figure 3); and ABA concentration at DAS 12, 22, and 40 (Figure 4) in the present study. The difference among three planting spacing could be either owing to the changed spacing within the rows or owing to the changed distance between the rows. Although the density of the three planting spacings is the same, the canopy structure of three planting spacings is different.

Agricultural practices, such as row direction and row spacing, are beneficial to the production of maize [15]. Suitable row direction and plant configuration can create good canopy structure for crop production and obtain high yield [19]. Some studies reported that the yield in the north–south row direction is higher than that in the east–west row direction, but other studies reported that the yield in the east–west row direction is better than that in the south–north row direction. Other studies reported that row direction has no significant effect on yield [20,25,47]. These different results may be owing to latitude differences in study sites [48]. This experiment was carried out in the main maize producing areas in north China, and the results showed that the chlorophyll contents and dehydration rates of husk leaves in the north–south and east–west directions were significantly different.

## 5. Conclusions

In this paper, that husk leaves of maize growing in WN1 and WN2 with row direction of south–north could help to delay chlorophyll degradation, improve the activity of protective enzymes, and accelerate dehydration at later stages. Therefore, these results suggest that row direction from south to north and plant spacing from WN1 and WN2 were suitable conditions for maize in the experimental site.

**Author Contributions:** Experimental design, S.L.; data collecting, X.L. and S.L.; writing—original draft preparation, Y.W.; writing—review and editing, X.W.; project administration, Y.G.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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