Effects of Excessive Ear Removal on Senescence Order of Wheat Functional Leaves

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Abstract: We studied the effects of different ear removal treatments on the senescence order of functional leaves and the effects of removal of 3/4 ear on the chlorophyll content, net photosynthetic rate, transpiration rate, stomata conductance, malonaldehyde content and catalase activity in the functional leaves in wheat. The results showed that different ear removal resulted in different frequency of plants with leaf-color inversion (i.e., the green color of both the 2nd and 3rd leaf from the top or only the 2nd leaf was deeper than that of the flag leaf). Removal of 3/4 ear or whole ear, obviously increased the frequency of plants with leaf-color inversion. The chlorophyll content, photosynthetic rate, transpiration rate, stomata conductance and catalase activity in the flag leaf of plants with leaf-color inversion were mostly lower than those in the 2nd and 3rd leaf from the top, but the malonaldehyde content of flag leaf was obviously higher than that of the 2nd and 3rd leaf from the top. The experiments demonstrated that removal of 3/4 ear accelerated the senescence of flag leaf, delayed the senescence of the 2nd and the 3rd leaf from the top and altered the senescence order of wheat functional leaves.

Key words: Chlorophyll content, Ear removal, Leaf senescence, Photosynthetic rate, Source/sink, Wheat.

Source capacity is determined by the photosynthetic activity of the crop and by the availability of carbohydrate reserves (Uhart and Andrade, 1991; Rajcan and Tollenaar, 1999). Sink capacity is the product of sink number and sink activity (Yang et al., 2004). In general, kernel number is the main determinant of sink capacity. Manipulation of sink size has been used widely to study source-sink relationships of wheat. Partial decrease of kernel number per spike reduces total sink size and increases the source-sink ratio (Fischer and HilleRisLambers, 1978; Martinez-Carrasco and Thorne, 1979; Radley and Thorne, 1981; Simmons et al., 1982).

During grain filling, source capacity can be defined as the ability of the crop to supply assimilates to the developing grains, whereas sink capacity represents the potential of the grains to accumulate assimilates. The impact of the source-sink ratio during grain filling on crop senescence has been the subject of several studies. Leaf area decline was delayed after sink removal, it was inferred that nutrient drainage by sinks was a driving force of crop senescence (Biswas and Mandal, 1993). Other authors have reported, however, that removal of sinks accelerated or had no effect on leaf senescence in wheat (Guitman et al., 1991; Feller, 1979). Tollenaar and Daynard (1982) described two types of premature leaf senescence that can occur during grain filling due to an imbalance of assimilate supply and demand: senescence due to low source-sink ratio (i.e. assimilate starvation); and senescence due to high source-sink ratio (i.e. excessive assimilate accumulation). Leaf senescence is accelerated when supply and demand of assimilates are not equal during grain filling.

The objective of this study was to examine the effects of high source-sink ratio on the senescence of the functional leaves during grain filling, and to explore the mechanism of leaf-color inversion and flag leaf senescence after excessive ear removal in wheat.

Materials and Methods

Experiments were conducted in the 2004/05 and 2005/06 growing seasons at the experimental station of the Northwest A&F University, which is located in the uppermost terrace area of Weihe River valley in Shaanxi province. It is a part of the most important wheat production area, the winter wheat production area of Huanghuai Plain in China, which belongs to the warm temperate semi-humid climate.

Wheat varieties Xiaoyan6, 9430 and TU2 were sown on 8 October, 2004 and 6 October, 2005. Plots are free of stubs before planting. Xiaoyan6 and TU2 have longer functional periods of leaves than 9430, and TU2 has larger grains than Xiaoyan6. The experiment was a split-plot design with three replications, with the wheat variety assigned to main plots and the ear-removal treatments to subplots. The size of the main plots was ten rows 0.25 m apart and 2.67 m long and the size of subplots was two rows. Nutrients and water were not limiting to growth. Before planting, 150 kg ha⁻¹ urea, 225 kg ha⁻¹ ammonium dihydrogen phosphate and 30...
kg ha\(^{-1}\) potassium dihydrogen phosphate were applied. In early spring when wheat was irrigated, 75 kg ha\(^{-1}\) urea was applied. Weeds and insects were adequately controlled during the wheat growing period.

On the anthesis date, which was around April 24, 26, and 27 each year, in wheat 9430, TU2 and Xiaoyan 6, respectively, 1/4, 2/4, 3/4 and the whole ear which accounted for 0.25, 0.5, 0.75 and all of the spikelets, respectively, were removed.

The color changes in the functional leaves were observed by eye and recorded every three days after ear-removal in the field. After evident leaf-color inversion phenomenon (i.e., the green color of 2nd and 3rd leaf or only the 2nd leaf from the top was deeper than that of the flag leaf) appeared during the late grain-filling period, the frequency of leaf-color inversion plants in the control (without ear removal) and four ear-removal treatments were counted. About 60 plants in each treatment in a plot were randomly selected, 2 leaf-color inversion plants, 3 leaf-color inversion plants and no leaf-color inversion plants were counted, respectively. The frequency of leaf-color inversion plants was calculated as follows:

\[
\text{Frequency of leaf-color inversion plants} = \frac{\text{number of leaf-color inversion plants}}{\text{total number of counted plants} \times 100}
\]

Three plants with leaf-color inversion were randomly selected from the control and 3/4 ear-removal treatment groups, and separated into flag leaves, and the 2nd and 3rd leaves from the top to determine chlorophyll content, malonaldehyde (MDA) content, and the activity of catalase (CAT) using the acetone extraction method, thiobarbituric acid colorimetric method, and ultraviolet spectrophotometry, respectively (Gao, 2000). In the field, from 9:00 to 11:00 on May 29 (a clear, cloudless day), three plants with leaf-color inversion randomly selected from the control and 3/4 ear-removal treatment groups were collected to determine net photosynthetic rate, transpiration rate and stomata conductance in the flag leaf, and the 2nd and 3rd leaves from the top with a portable LI-6400 photosynthesis system (LI-COR, USA).

Analysis of variance was performed by using the procedures of SPSS (15.0) for all data to calculate the significance of the differences among the values in the ear-removal treatment and control groups and among the values at different leaf positions.

### Results

1. **Effects of different ear-removal treatments on the frequency of plants with leaf-color inversion**

Normally, the senescence of functional leaves in wheat starts from the 3rd leaf from the top followed by the 2nd leaf and the flag leaf in this order. After excessive ear removal (removal of 3/4 or the whole ear), however, the senescence order obviously changed. The flag leaf showed senescence first followed by the 2nd and 3rd leaves. If the flag leaf showed senescence earlier than the 2nd but not the 3rd leaf, this is called 2-leaf color inversion. If the flag leaf senescence is earlier than the 2nd and 3rd leaves, this is called 3-leaf color inversion.

| Year | Treatments         | Xiaoyan6 | TU2 | 9430 |
|------|--------------------|----------|-----|------|
|      | 2 leaf-color       | 3 leaf-color | 2 leaf-color | 3 leaf-color | 2 leaf-color | 3 leaf-color |
|      | inversion          | inversion | inversion | inversion   | inversion   | inversion   |
| 2004/05 | No ear-removal    | 20.5 ±0.6 | 0     | 0     | 12.4 ±1.8 | 0     |
|       | 1/4 ear removed    | 89.7 ±4.3 | 0     | 2.1±0.8 | 76.8±3.5  | 0     |
|       | 3/4 ear removed    | 92.6 ±3.5 | 4.2±0.4 | 3.4±1.2 | 95.2±3.4  | 3.9±0.4 |
|       | whole ear removed  | 21.8 ±0.9 | 79.8±2.1 | 17.3±0.8 | 20.9±1.7  | 13.9±2.1 | 86.1±2.3 |
|       |                    | 13.8 ±0.4 | 87.6±1.8 | 6.9±0.8  | 36.4±2.3  | 3.4±0.5  | 95.8±2.4 |
| One-way ANOVA | ** | ** | ** | ** | ** | ** |
| 2005/06 | No ear-removal    | 24.5±1.8  | 0     | 0     | 8.4±0.9   | 0     |
|       | 1/4 ear removed    | 82.3±2.3  | 0     | 1.9±0.5 | 84.8±2.3  | 0     |
|       | 3/4 ear removed    | 94.8±3.8  | 3.8±0.6 | 2.6±0.9 | 96.4±1.9  | 4.5±0.8 |
|       | whole ear removed  | 17.4±1.2  | 81.0±1.6 | 12.7±1.2 | 24.1±0.7  | 14.7±0.6 | 85.3±2.1 |
|       |                    | 16.2±1.6  | 82.4±2.0 | 8.5±1.6  | 30.2±0.6  | 3.0±0.3  | 94.6±1.5 |
| One-way ANOVA | ** | ** | ** | ** | ** | ** |

Leaf-color inversion frequency data are mean ± S.E. (n=3). ** represents significant difference at 1% among no ear-removal, 1/4 ear removed, 1/2 ear removed, 3/4 ear removed and whole ear removed.

| Year | Treatments         | Xiaoyan6 | TU2 | 9430 |
|------|--------------------|----------|-----|------|
|      | 2 leaf-color       | 3 leaf-color | 2 leaf-color | 3 leaf-color | 2 leaf-color | 3 leaf-color |
|      | inversion          | inversion | inversion | inversion   | inversion   | inversion   |
| 2004/05 | No ear-removal    | 20.5 ±0.6 | 0     | 0     | 12.4 ±1.8 | 0     |
|       | 1/4 ear removed    | 89.7 ±4.3 | 0     | 2.1±0.8 | 76.8±3.5  | 0     |
| One-way ANOVA | ** | ** | ** | ** | ** | ** |

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**Table 1.** The frequency of plants with leaf-color inversion in different wheat varieties with different ear removal treatments in 2004/05 and 2005/06 (%).
Compared with Xiaoyan6 and 9430, the frequency of plants with 2-leaf and 3-leaf color inversion in TU2 was relatively lower in the whole-ear removal treatment. The frequency of plants with leaf-color inversion showed an extremely significant difference with the treatment (Table 1).

Ear removal experiments indicated that artificial increase in the source-sink ratio significantly increased the frequency of plants with leaf-color inversion; excessive ear removal obviously lengthened the life period of the 2nd and 3rd leaves from the top, and the response to ear removal treatment differed with the wheat variety.

2. Physiological characteristics of functional leaves in the plants with leaf-color inversion

Table 1 showed that removal of 3/4 ear obviously increased the occurring frequency of plants with 3-leaf color inversion. To determine the cause, we examined some physiological characteristics of the three functional leaves in the plants with leaf-color inversion after removal of 3/4 ear.

### 2.1 Chlorophyll content of functional leaves

One of the marked characteristics of leaf senescence is the decrease in chlorophyll content. The lower the chlorophyll content, the faster the leaf senescence. The chlorophyll content of the functional leaves in the control was the highest in the flag leaf followed by the 2nd leaf, and the 3rd leaf from the top. A represents significant difference at 1%, a represents significant difference at 5%, and NS indicates the absence of a significant difference between control and 3/4 ear-removal treatment at the same leaf position. The bars show S.E. (n=3).

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**Table 1.** Effects of removal of 3/4 ear on chlorophyll content of functional leaves in wheat at late grain filling. The 3rd leaf from the top of 9430 in the control withered at late grain filling. ** represents significant difference at 5% and 1%, respectively, and NS indicates the absence of a significant difference among flag leaf, the 2nd leaf and the 3rd leaf from the top. A represents significant difference at 1%, a represents significant difference at 5%, and NS indicates the absence of a significant difference between control and 3/4 ear-removal treatment at the same leaf position. The bars show S.E. (n=3).
and delayed the senescence of the 2nd and 3rd leaves, resulting in functional leaf-color inversion.

2.2 MDA content of functional leaves

The malonaldehyde (MDA) content was an important indicator of lipid peroxidation. In the plants suffering from serious injury, the MDA content significantly increased, which indicated rapid senescence. At late grain filling, the MDA content of functional leaves in the control was highest in the 3rd leaf, followed by the 2nd leaf, and flag leaf in this order (Fig. 2). Removal of 3/4 ear significantly altered the MDA content order of functional leaves. The MDA content of Xiaoyan6 functional leaves was highest in the flag leaf, followed by the 2nd and 3rd leaves. In TU2 and 9430, the MDA content was highest in the flag leaf, followed by the 3rd and 2nd leaves. Fig. 2 demonstrated that removal of 3/4 ear obviously increased MDA accumulation in the flag leaf and reduced it in the 2nd and 3rd leaves. These results also indicated that removal of 3/4 ear accelerated senescence in the flag leaf and delayed it in the 2nd and 3rd leaves, resulting in functional leaf-color inversion.

2.3 CAT activity in functional leaves

The H$_2$O$_2$ produced by plant metabolism would lead to cell-destructive oxidation. Catalase (CAT) was one of key enzymes in the biological defense system, which would separate H$_2$O$_2$ into oxygen and water to eliminate H$_2$O$_2$ poison. At late grain filling, the CAT activity in the 3rd leaf of 9430 in the control withered at late grain filling. The CAT activity in the flag leaf was significantly higher than that in the 2nd leaf. In 9430, the CAT activity in the flag leaf was obviously lower than that in the 2nd leaf, which was significantly lower than that in the 3rd leaf. The CAT activity in the functional leaves in Xiaoyan6 and TU2 was not significantly affected by the removal of 3/4 ear.
However, compared with the control, removal of 3/4 ear obviously increased the CAT activity in the 2nd and 3rd leaves, and delayed the senescence of these leaves, resulting in functional leaf-color inversion.

2.4 Net photosynthetic rate in functional leaves

Removal of 3/4 ear obviously affected the net photosynthetic rate in the functional leaves at late grain filling in wheat (Fig. 4). In the control, the net photosynthetic rate in the flag leaf was obviously higher than that in the 2nd leaf which was obviously higher than that in the 3rd leaf. After removal of 3/4 ear, the net photosynthetic rate in the 2nd and 3rd leaves was significantly higher than that in the flag leaf in TU2 and 9430, but not in Xiaoyan6. Fig. 4 showed that removal of 3/4 ear obviously increased the net photosynthetic rate of the 2nd and 3rd leaves at late grain filling, and lengthened the functional period of these leaves.

2.5 Transpiration rate in functional leaves

In the control, the transpiration rate in the functional leaves was the highest in the flag leaf followed by the 2nd and 3rd leaves in all varieties (Fig. 5). After removal of 3/4 ear, the transpiration rate in the 2nd and 3rd leaves was obviously higher than that in the flag leaf in TU2 and 9430, but not in Xiaoyan6, in which the transpiration rate was highest in the 2nd leaf followed by the flag leaf and the 3rd leaf in this order. Fig. 5 showed that removal of 3/4 ear obviously increased the transpiration rate in the 2nd and 3rd leaves and reduced the transpiration rate in flag leaf at late grain filling.

2.6 Stomata conductance in the functional leaves

The stomata conductance in the functional leaves shown in Fig. 6 was closely correlated with the transpiration rate shown in Fig. 5. This shows that removal of 3/4 ear affected the transpiration rate by altering the stomata conductance in all varieties.

Discussion

The photosynthetic rate, transpiration rate, stomata conductance, catalase activity and the chlorophyll
content in the flag leaf were reduced, but those in the 2nd and 3rd leaves from the top were increased by removal of 3/4 ear at anthesis. On the other hand, the malonaldehyde content of the flag leaf was increased and that of the 2nd and 3rd leaves was reduced by the same treatment. Thus, removal of 3/4 ear obviously accelerated the senescence of flag leaf and delayed the senescence of the 2nd and 3rd leaves. Because the chlorophyll content of the 2nd and 3rd leaf from the top were higher than that of flag leaf, the green color of the 2nd and 3rd leaves was deeper than that of the flag leaf, which was called leaf-color inversion phenomena. Rajcan and Tollenaar (1999) reported that the difference in leaf senescence between an old hybrid and new hybrid of maize could be attributed, in part, to lower source-sink ratio in the old hybrid. Leaf senescence was delayed by either the high or the low source-sink ratio. The source-sink relationship and nitrogen status in the plant regulate leaf senescence at the whole-plant level (Christensen et al., 1981; Tollenaar and Daynard, 1982; Crafts-Brandner et al., 1984; Feller and Fischer, 1994). A low source-sink ratio and high source-sink ratio accelerated leaf senescence because of the imbalance of supply and demand of assimilates during grain filling (Tollenaar and Daynard, 1982). Nitrogen status also affects leaf senescence. Grain N is supplied from vegetative tissue as well as from N uptake by roots (Pan et al., 1986). N uptake is dependent upon the availability of soluble carbohydrates for the roots (Tolley-Henry et al., 1988). Reduction in N uptake by roots during reproductive growth when carbohydrates are partitioned to ear will enhance N mobilization from leaves and stems. N mobilization from leaves lowers photosynthetic activity and, eventually, leaf senescence (Wada et al., 1993). In the present study, excessive assimilate accumulation could be the capital reason for earlier flag leaf senescence. Wheat grain dry matter primarily supplied by flag leaf photosynthesis, but a higher source-sink ratio could result in excessive assimilate accumulation in the flag leaf which accelerated flag leaf senescence. Removal of 3/4 ear excessively raised the source-sink ratio, greatly reduced partitioning of carbohydrates to the ear and increased that to the roots, which could promote N uptake by roots and reduced N mobilization from leaves, resulting in prolonged leaf life. Nitrogen uptake capability of roots could maintain deeper green color of the 2nd and 3rd leaves.

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