Article

Foliar Brassinolide Sprays Ameliorate Post-Silking Heat Stress on the Accumulation and Remobilization of Biomass and Nitrogen in Fresh Waxy Maize

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Abstract: Heat stress (HS) during grain filling is an extreme environmental factor and affects plant growth and development. Foliar application of exogenous brassinolide (BR) is an effective practice to relieve HS injuries, but the influence on the accumulation and remobilization of biomass and nitrogen is still unclear. In the present study, the effect of foliar BR application at the silking stage on the accumulation and remobilization of biomass and nitrogen in fresh waxy maize under ambient (28/20 °C) and high (35/27 °C) temperatures during grain filling were studied in a phytotron using heat-sensitive Yunuo7 (YN7) and heat-tolerant Jingkenuo2000 (JKN2000) as materials. HS reduced the fresh ear yield by 21.8% and 19.8% in YN7 and JKN2000, respectively, but fresh grain yield was only reduced in the heat-sensitive hybrid (6.9%) and unaffected in the heat-tolerant hybrid. BR application improved the yields of fresh ears (11.3% and 10.9% in YN7 and JKN2000, respectively) and grains (19.9% and 13.2% in YN7 and JKN2000, respectively) under HS, and the increases were higher in YN7. HS decreased the post-silking biomass accumulation by 67.3% and 51.8%, and nitrogen deposition by 61.9% and 50.5%, in YN7 and JKN2000, respectively. The remobilization of pre- and post-silking biomass and nitrogen were increased and decreased by HS in YN7, respectively, but both were unaffected in JKN2000. Under HS, BR application increased the remobilization of post-silking biomass and nitrogen in both hybrids. The grain nitrogen concentration was increased by HS but decreased by BR application in both hybrids. The harvest index of biomass and nitrogen was increased by HS, and it was improved in YN7 and unaffected in JKN2000 by BR application under HS. In conclusion, BR application at the silking stage can relieve HS injuries on fresh waxy maize yields by improving the remobilization of biomass and nitrogen to grain and increasing the harvest index, especially in the heat-sensitive hybrid. Therefore, foliar BR application is a simple, feasible, efficient practice in fresh waxy maize production and is worth popularizing, especially under warmer climates.

Keywords: fresh waxy maize; high temperature; nitrogen remobilization; plant growth regulator

1. Introduction

Global climate changes, such as higher temperatures, are predicted to deteriorate plant growth and development, causing a catastrophic loss in crop productivity. Heat stress (HS) is defined as the increased temperature level that is sufficient to cause irreversible damage to plant growth and is a serious threat to global crop production [1]. The Intergovernmental Panel for Climate Change predicts a 3.7 ± 1.1 °C increase in global mean surface temperature by the end of this century if the current global warming rate continues without any mitigation strategies [2]. For every 1 °C increase, the yields of
maize, wheat, rice, and soybean are expected to decrease by 7.4%, 6.0%, 3.2%, and 3.1%, respectively [3]. At different stages, HS negatively affects plant growth the most during the anthesis, silking, and grain-filling phases [4,5]. Low grain yield under post-silking HS is mainly caused by the reduced remobilization of assimilates to the developing grains or the weakened enzymatic activities that accumulate assimilates in grains [6,7]. Under post-silking HS conditions, the plant growth rate is accelerated and ceases early, leaf senescence is advanced, photosynthetic capacity is decreased, carbon–nitrogen metabolism is imbalanced, the grain-filling duration is shortened, and the accumulation and remobilization of biomass and nutrient are reduced, ultimately resulting in a yield penalty [8,9].

The source of grain filling mainly relies on leaf direct photosynthesis [10]. The reduced leaf photosynthetic capacity under HS cannot meet the grain sink demand, and the water-soluble carbohydrate reserved from the vegetative organs begins to be mobilized to the developing grains [11–12]. In wheat, HS restrains the current photosynthesis, promotes the remobilization of biomass, and reduces grain yield, indicating that the increased remobilization cannot compensate for the loss of photosynthesis [13]. HS is sink limited, relating to a shorter grain-filling duration and nitrogen accumulation rate [14]. Under the booting-stage HS condition, the stem carbohydrate concentration increases, whereas the panicles’ carbohydrate concentration, stem carbohydrate remobilization efficiency, and contribution of the stem carbohydrate to rice grain yield decrease [15]. However, previous studies have mainly focused on the biomass and nitrogen accumulation and remobilization in cereals that are harvested at maturity, and limited information is available about the crops that are harvested at the milk stage.

Brassinolides (BRs) are a class of naturally occurring steroidal phytohormones, and are best known for their role in stress resistance by regulating physiological and molecular processes, such as cell division, nucleic acid and protein biosynthesis, gene expression, and photosynthesis. BRs are widely used to alleviate HS damage by preventing denaturation and facilitating the refolding of damaged proteins, enhancing activities of enzymes involved in the antioxidant system, and protecting leaf photosynthesis [16–18]. Exogenous BR application is a feasible practice for alleviating heat injuries by increasing the accumulation of osmotic adjustment substances and endogenous hormone content [19]. Exogenous BR application promotes sucrose transport to young rice panicles and improves sucrose utilization under ambient and high temperatures [20]. BR application remarkably alleviates the effect of post-silking HS on the starch quality of waxy maize [21]. However, there is a knowledge gap regarding BR application on the accumulation and remobilization of biomass and nitrogen in fresh food crops, such as waxy maize, sweet maize, soybean, and horse beans, under HS during grain filling. Fresh waxy maize is a special crop whose starch is composed of nearly pure amylopectin, which endows it with high viscosity and low retrograde properties. Fresh waxy maize in China is mainly harvested at the milk stage for direct eating or production of frozen ears and grains [22]. Considering that the fresh waxy maize plants are harvested at the mid-grain-filling (milk) stage, the accumulation and remobilization of biomass and nitrogen may be different to those in cereal crops that are harvested at maturity. Therefore, we hypothesize that the spraying of BR at the silking stage can alleviate the negative effects of post-silking HS on fresh waxy maize. To test this hypothesis, two waxy maize hybrids with different thermotolerance levels were cultivated in an ambient environment before silking. Then, plants were foliar sprayed with BR at the silking stage, and subjected to HS during grain filling. The accumulation and remobilization of biomass and nitrogen under the interaction of BR application and post-silking HS were investigated. The results can provide a new perspective about the effects of BR on fresh waxy maize under warm conditions.
2. Materials and Methods

2.1. Experimental Design

Two fresh waxy maize hybrids that are widely planted in southern China, namely, Jingkenuo2000 (JKN2000, a heat-tolerant hybrid having the largest planted area in China) and Yunuo7 (YN7, a heat-sensitive hybrid used in the national fresh waxy maize regional test as the control hybrid), were employed in the study. The pot experiment was conducted at Yangzhou University experimental farm in 2020. Plants (two at the seedling stage, and one left at the jointing stage) were sown on March 15 and provided with 16 g control-released compound fertilizer (N/P₂O₅/K₂O = 27%/9%/9%) per pot at sowing time. Plants were grown in a natural environment before the silking stage. Plants with similar appearance were sampled and manually pollinated on the same morning. Using Tween 20 (0.05%) as the surfactant, exogenous BR (0.25 mg/L, 100 mL/plant; this concentration is recommended by the manufacturer and achieved the highest yield in our trial test from 0.1 to 1 mg/L, unpublished data) was sprayed on the plants in the afternoon. Pots were moved to a phytotron the next day for temperature treatments. The temperatures (day/night) in the phytotron were set at 28/20 °C (AT) and 35/27 °C (HS). The stress duration was the whole grain-filling stage. The water transpiration was supplied using weighing methods, and the soil moisture was about 75% during plant growth.

2.2. Yield Determination

At 23 days after pollination (DAP), 10 ears in each treatment were harvested. After stripping off the bracts, the fresh ear weight (g/plant) was determined immediately. After measuring the fresh ear weight, the fresh grains (g/plant) were scraped from the ear and weighed.

2.3. Accumulation and Remobilization of Biomass and Nitrogen

Maize plants were sampled and separated into leaves and stems (including sheaths and tassels) at the silking stage (silks protrude from bracts by 1–2 cm), and into leaves, stems (including sheaths and tassels), cobs (including bracts), and grains at the fresh stage (23 DAP). All samples were oven dried to a constant weight at 80 °C after de-enzyming at 105 °C for 30 min.

The nitrogen content in different organs was determined using a modified Kjeldahl digestion method. The nitrogen accumulations of each fraction were calculated as the product of the concentration and biomass. The various parameters were calculated as previously described [12].

1. Post-silking biomass (nitrogen) accumulation = biomass (nitrogen) accumulation at the fresh stage — biomass (nitrogen) accumulation at the silking stage;
2. Biomass (nitrogen) remobilization amount from pre-silking leaf (stem) to grain (REP) = biomass (nitrogen) accumulation amount in leaf (stem) at the silking stage — biomass (nitrogen) accumulation amount in leaf (stem) at the fresh stage;
3. 

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<th>Biomass (nitrogen) remobilization (%)</th>
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4. Remobilization amount of biomass (nitrogen) of post-silking vegetative organ photosynthate = grain dry weight at the fresh stage — biomass (nitrogen) remobilization from pre-silking vegetative organs to grain;
5. Harvest index (HI, %) = 100 × grain dry weight
2.4. Statistical Analysis

The data reported in all figures and tables represent the average of three replicates. Data were subjected to ANOVA using a data processing system (DPS 7.05). The LSD test was used to compare the means at the 0.05 level.

3. Results and Discussion

3.1. Ear and Grain Yield

The fresh ear yield ranged from 122.4 to 135.5 g/plant and from 95.8 to 116.5 g/plant at AT and HS, respectively. Post-silking HS decreased the ear yield by 21.8% and 19.8% without BR application, and by 19.9% and 14.0% with BR application, in YN7 and JKN2000, respectively (Figure 1). This result is consistent with that from crops harvested at maturity [3,8]. Smaller ear size under HS has also been reported in normal maize [23].

The fresh grain yield was reduced by 6.9% in YN7 and unaffected in JKN2000 after plants suffered from HS without BR application. With BR application, the fresh grain yield was unaffected in YN7 and increased by 4.4% in JKN2000 under HS condition. The result was consistent with previous findings on fresh waxy maize [22], but contrary to plants harvested at maturity under HS condition [24]. The discrepancy can be attributed to the accelerated grain-filling rate under HS, as grain weight gradually increased with the development [25].

![Figure 1.](image)

Foliar BR application at the silking stage increased the yields of fresh ear and grain under both temperature regimes. For YN7 and JKN2000, the fresh ear yield was increased by 8.8% and 3.4% under AT and by 11.3% and 10.9% under HS, and the fresh grain yield was increased by 10.8% and 6.3% under AT and by 19.9% and 13.2% under HS. The improved yield may be due to the enhanced source–sink capacity [26] and carbon–nitrogen metabolism [16,17]. The increased yield with BR under HS in both hybrids indicates that BR ameliorated the negative effects of HS. Therefore, BR application can be considered as an efficient practice to alleviate HS in fresh waxy maize production because it can increase cereal tolerance and acclimation [27,28].
3.2. Post-Silking Biomass and Nitrogen Accumulation

High biomass and nitrogen accumulation are the target traits in crops to improve grain yield [29]. The post-silking biomass and nitrogen accumulation were 35.4–58.7 g/plant and 307.9–449.2 mg/plant under AT, and 13.6–30.7 g/plant and 128.0–222.5 mg/plant under HS. These findings indicate that the biomass and nitrogen accumulation were reduced by HS regardless of whether the plants were sprayed with BR or not (Figure 2). For YN7 and JKN2000, respectively, the biomass was reduced by 67.3% and 51.8% without BR application, and by 66.7% and 30.1% with BR application; the nitrogen accumulation was reduced by 61.9% and 50.5% without BR application, and by 60.4% and 39.9% with BR application, when plants suffered from HS. HS reduced biomass and nitrogen were also reported on waxy maize [12] and wheat [30] harvested at maturity, and this phenomenon may be caused by the decreased photosynthetic rate, accelerated leaf senescence, and weakened carbon–nitrogen metabolism [12,31,32]. A study on wheat showed that stem water-soluble carbohydrate and nitrogen accumulation in response to HS depends on the cultivar [33]. Another study on rice showed that a 2 °C increase after transplanting improves the biomass and nitrogen accumulation for the whole plant but decreases it for the grain [34]. This discrepancy is caused by differences in heat duration, stage, and degree, and genotypic differences in thermotolerance.

For YN7, BR application increased the biomass and nitrogen accumulation by 41.1% and 33.7% at AT and 43.5% and 39.0% at HS, respectively. For JKN2000, BR application increased the biomass and nitrogen accumulation by 24.0% and 20.3% at AT and 79.9% and 46.1% at HS, respectively. This observation is consistent with findings from normal maize, as BR application improves biomass accumulation by enhancing the activity of carbon–nitrogen metabolism, increasing the photosynthetic pigment content, delaying senescence, and stimulating the flow of assimilate from the source to the sink [35,36]. BR application can increase the biomass and nitrogen accumulation under waterlogged [37] and acid [38] conditions, mainly due to the enhanced antioxidant system and alleviated membrane lipid peroxidation [16].

3.3. Remobilization of Post-Silking Biomass and Nitrogen

In the present study, the remobilization of post-silking biomass and nitrogen were reduced by HS in heat-sensitive YN7 (without BR, 38.0% and 33.4%; with BR, 47.0% and 36.5%, respectively) and unaffected in heat-tolerant JKN2000, regardless of whether the plants were sprayed by BR (Figure 3). In rice, HS during grain filling decreases carbon and nitrogen allocation to the panicles [39], and reduces nitrogen accumulation in grains [40]. A study on pea showed that the plant nitrogen uptake under HS was not allocated to seeds, and a substantial quantity of nitrogen remains available in vegetative organs at maturity [14]. In addition, the decreased grain number and grain size/mass resulted in
reduced storage capacity/demand for carbon and nitrogen in the grains, thus reducing the remobilization [14,15].

BR application increased the remobilization of post-silking biomass by 55.2% and 38.7% at AT, and by 32.7% and 45.6% at HS; and increased the remobilization of post-silking nitrogen by 40.1% and 28.3% at AT, and by 33.5% and 28.8% at HS in YN7 and JKN2000, respectively. The increased remobilization was because BR application can enhance the leaf activity, delay senescence, and stimulate the transportation of carbohydrate in maize leaf by increasing the cell inclusions in the phloem conducting tissue, which increases the activity of sucrose synthase and sucrose phosphatase [26,35,36]. Improved photosynthesis and reduced membrane injury were also observed in BR-treated heat-acclimated plants compared with untreated plants [27].

**Figure 3.** Effects of foliar BR application at the silking stage on post-silking remobilization of biomass and nitrogen of fresh waxy maize under post-silking ambient and high temperatures. AT, ambient temperature; HS, heat stress; BR0, application of water; BR1, application of 0.25 mg/L BR. The different letters on the column mean the difference was significant at the $p < 0.05$ level.

### 3.4. Remobilization of Pre-Silking Biomass and Nitrogen

The crop grain yield depends on the direct deposition of post-silking photo-assimilate and the remobilization of the pre-silking stored photo-assimilate in vegetative organs [31]. Remobilized biomass and nitrogen reserves play a vital role during the grain-filling period when photosynthesis is suppressed by HS [41]. In the present study, the remobilization of pre-silking biomass and nitrogen in the leaf were unaffected by BR and HS, and was similar between the two hybrids (Table 1). The unaffected leaf remobilization may be due to the initialization of the leaf nitrogen remobilization at approximately 30 DAP under optimal conditions; remobilization had not started at the milk stage even this time was advanced under stress conditions [42]. A study on normal maize also observed that leaf senescence before 20 DAP was unaffected by BR application at the tasseling stage [26]. In wheat, nitrogen remobilization may be initiated immediately after anthesis, as indicated by the decrease in the nitrogen contents of the flag leaves [43].

**Table 1.** Effects of BR application at the silking stage on the nutrient translocation in the stem under post-silking ambient and high temperatures.
The remobilization amount and rate of pre-silking biomass and nitrogen in the stem were increased by HS in YN7 and unaffected in JKN2000 without BR application. With BR application, the remobilization amount and rate of pre-silking biomass and nitrogen in the stem were increased by HS in both hybrids. In rice, nitrogen remobilization efficiency in the stem and leaf is reduced by HS [44]. In wheat, the nitrogen remobilization is inhibited, whereas biomass remobilization is increased by post-anthesis HS [45]. The finding in the present study that remobilization increased in the stem and was unaffected in the leaf can be attributed to the earlier initiation of the remobilization from the stem than from the leaf in maize [46]. The discrepancy may be because the harvest stage of fresh waxy maize is at approximately 23 DAP, when the leaf maintains a high photosynthetic rate and the remobilization is not initiated [42]. The increased remobilization in the stem indicates that the stored carbohydrate in the stem compensates for the reduced direct photo-assimilate, and the rapid and full exhaustion of the temporary carbohydrate store is a promising strategy under HS [47]. The remobilization of pre-silking biomass and nitrogen in the stem in both hybrids was reduced and unaffected by BR application under AT and HS conditions, respectively, mainly because BR application increased the remobilization of post-silking biomass and nitrogen under AT and HS conditions (Figure 3). BR application protects plant photosynthesis by improving the photosynthetic pigment contents, carboxylation rate of Rubisco, photochemical activity of PSI, and energy charge [17].

### 3.5. Nitrogen Concentration in Stem, Leaf, and Grain

The nitrogen concentration (NC) in the stem, leaf, and grain among different treatments was 5.7–6.0, 12.8–13.7, and 18.3–19.9 mg/g, respectively (Figure 4). The stem NC was increased by HS in YN7 (1.8%) and unaffected in JKN2000 without BR application. With BR application, stem NC was decreased in both hybrids (Figure 4). This result was consistent with the change trend in the remobilization of pre-silking nitrogen in the stem, but the pre-silking stem biomass was increased by HS without BR application. The higher remobilization of biomass than of nitrogen resulted in high stem NC. The different response between the two hybrids may be because the remobilization of nitrogen was unaffected in JKN2000, and pre-silking nitrogen was increased in YN7. The leaf NC was unaffected by HS in both hybrids without BR application. With BR application, the leaf NC was unaffected by HS in YN7 and reduced in JKN2000. The grain NC was increased by HS in both hybrids without BR application. This result was consistent with studies on mature cereal crops [48,49]. The grain NC may have increased because the effect of HS on carbon/starch accumulation was more severe than that of nitrogen [49,50]. With BR application, the grain NC was reduced by HS in JKN2000 and unaffected in YN7. A study on rice observed that ear NC was unaffected by HS, whereas the NC in the stem, leaf, and root was increased [39]. However, another study reported that rice leaf NC was unaffected by HS, but stem and grain NC were reduced at maturity [44]. An increase of 2 °C improves the grain and shoot NC in rice [34]. In normal maize, HS does not affect the NC in all plant parts [51]. In wheat, the leaf NC is reduced, but stem and spike NC depend on the cultivar after the plant suffers from HS [33]. The different changes in NC in different organs indicate that the nitrogen remobilization is dependent on the HS stage, duration, and degree.

Under AT conditions, the stem NC was increased by BR application in both hybrids. Under HS conditions, the value was unaffected by BR application in YN7, and reduced in JKN2000. The leaf NC in both hybrids was unaffected and reduced by BR at AT and HS, respectively. The grain NC was decreased by BR in both hybrids under HS conditions, and the value at AT decreased in YN7 and increased in JKN2000. This is mainly because
BR application improved the grain biomass accumulation, resulting in a dilutive effect. In rice, silicon application does not affect the NC in the stem and panicles but reduces the leaf NC at maturity under AT, whereas silicon application under HS reduces leaf NC and increases stem and panicle NC [44].

Figure 4. Effects of foliar BR application at the silking stage on nitrogen contents in leaf, stem, and grain of fresh waxy maize under post-silking ambient and high temperatures. AT, ambient temperature; HS, heat stress; BR0, application of water; BR1, application of 0.25 mg/L BR. The different letters on the column mean the difference was significant at the \( p < 0.05 \) level.

3.6. Harvest Index of Biomass and Nitrogen

The intensified sensitivity in HI mainly results from the high sensitivity of yield to post-silking HS, which explains more than half of the yield reduction [52]. In the present study, HI was 19.7–22.0% at AT and 24.7–28.0% at HS, and NHI was 24.7–28.0% at AT and 29.3–32.1% at HS; these findings indicate that both HI and NHI in both hybrids were increased by HS regardless of whether the plants were sprayed by BR (Figure 5). HI and NHI remarkably decrease in rice [39,44] and wheat [33]. The discrepancy can be attributed to the decreased post-silking biomass, and nitrogen accumulation and remobilization, which induced high remobilization of pre-silking biomass and nitrogen from stems. Considering that the fresh waxy maize was harvested at the milk stage, the high grain weight caused by the high grain-filling rate under HS resulted in the high HI.

Figure 5. Effects of foliar BR application at the silking stage on harvest index of biomass and nitrogen of fresh waxy maize under post-silking ambient and high temperatures. AT, ambient temperature; HS, heat stress; BR0, application of water; BR1, application of 0.25 mg/L BR. The different letters on the column mean the difference was significant at the \( p < 0.05 \) level.

The HI and NHI at AT were unaffected by BR application in both hybrids. Under HS, both were increased by BR application in YN7 and unaffected in JKN2000. In rice, BR application promotes sucrose transport to young panicles [20]. Foliar application of silicon fertilizer can increase the HI under both AT and HS conditions in rice [43]. The increased HI and NHI in heat-sensitive YN7 may be caused by the increased remobilization of the pre- and post-silking biomass and nitrogen.
4. Conclusions

HS during grain filling decreased the post-silking biomass and nitrogen accumulation and remobilization, but did not affect the remobilization of pre-silking leaf biomass, and increased the HI, NHL, and grain NC in both fresh waxy maize hybrids. Application of BR at the silking stage improved the fresh ear and grain yields under both temperature regimes by improving the remobilization of post-silking biomass and nitrogen accumulation. BR application promoted the remobilization of post-silking direct assimilates of carbohydrate and nitrogen, and the influence under HS was less for nitrogen, resulting in low grain nitrogen content. Our results indicate that foliar BR application at the silking stage can improve the yields of ear and grain of fresh waxy maize, especially under HS condition. Thus, foliar BR application should be popularized in fresh waxy maize production in warmer environments.

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Reference


