



Article Effects of Exogenous Brassinolide Application at the Silking Stage on Nutrient Accumulation, Translocation and Remobilization of Waxy Maize under Post-Silking Heat Stress

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Abstract: Exogenous brassinolide (BR) application is a feasible measure to alleviate abiotic stresses on crop productivity. The effects of BR application at the silking-stage on the accumulation, translocation, and remobilization of dry matter (DM) and nutrients (nitrogen, phosphorus, and potassium) of waxy maize exposed to post-silking high temperature (HT) were studied using Jingkenuo2000 (JKN2000, heat-tolerant) and Yunuo7 (YN7, heat-sensitive) as materials. BR application mitigated the penalty of HT on grain yield. HT reduced the post-silking accumulation and increased the translocation of pre-silking DM and nutrients in YN7. In JKN2000, accumulation and remobilization of DM were unaffected by HT. The contribution rate of DM and nutrients translocation to grain yield were unaffected by HT in JKN2000 and increased in YN7. Under HT, the accumulation, translocation, and remobilization of DM were unaffected by BR application, whereas the nitrogen, phosphorus, and potassium response were dependent on hybrids. The harvest index of DM and nutrients in response to HT and BR were different between the two hybrids. In conclusion, BR application relieved the negative effects of HT mainly caused by the increased post-silking accumulation and remobilization of DM and nutrients by the increased post-silking accumulation and remobilization of DM and nutrients by the increased post-silking accumulation and remobilization of DM and nutrients between the two hybrids. In conclusion, BR application relieved the negative effects of HT mainly caused by the increased post-silking accumulation and remobilization of DM and nitrogen, and the alleviation was more obvious in the heat-tolerant hybrid.

Keywords: brassinolide; dry matter; high temperature; nutrient; waxy maize

1. Introduction

High temperature (HT) is a prime abiotic constraint severely reducing crop productivity. The slump in food productivity and quality, primarily due to extreme temperatures, impresses a serious destruction to agriculture production [1]. Minimizing the yield penalty has become a major challenge and has received a global concern in the changing climate [2]. Maize is the multipurpose cereal crop with the greatest production and is severely affected by global warming [3]. It is deduced that about half of the maize production area in the world will face a mean of five-day HT (temperature higher than 35 °C) during the reproductive stage annually by 2050 [4]. The optimum temperature range for maize growth is around 25–33 °C, day/17–23 °C, night [5]. In comparison with the optimum temperature (mean 25 °C), 1 °C increase of mean temperature in the growing season induced a 0.83 t/ha (5.8%) yield loss in maize [6]. HT suppressed the plant growth, decreased photosynthesis, reduced mineral nutrients accumulation, and ultimately induced grain yield loss, and those were widely reviewed at morphological, physiological, and biochemical scales [1,7]. Although HT obstructs productivity at all growth stages, yield penalty extent at the grain-filling stage is critical because this phase is vital to grain formation and grain composition deposition [8]. In cereal crops, the grain filling relies on two carbohydrate sources, mobilization



Citation: Xu, Y.; Zhang, X.; Yang, H.; Lu, D. Effects of Exogenous Brassinolide Application at the Silking Stage on Nutrient Accumulation, Translocation and Remobilization of Waxy Maize under Post-Silking Heat Stress. *Agriculture* 2022, *12*, 572. https://doi.org/ 10.3390/agriculture12050572

Academic Editors: Pirjo Mäkelä and Daniel Tan

Received: 13 March 2022 Accepted: 18 April 2022 Published: 19 April 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of the current assimilates directly to grains and the translocation of vegetative organs [9]. Post-silking HT reduces the photosynthesis capacity and accelerates the leaf senescent rate, and the increased translocation amount from vegetative organs cannot fully compensate the direct reduction of photoassimilate deposition during grain filling, ultimately resulting in yield penalty [10,11]. Under HT, the increased grain dry matter (DM) accumulation rate cannot compensate for the concomitant shortened grain-filling duration completely, and the grain yield reductions are likely because the requirements of the accelerated grain DM accumulation rate are not fully meted by an assimilate supply [12,13]. HT induces a decrease in carbon and nitrogen (N) and an increase of sucrose transport from the vegetative organs to the rice panicles [14]. In wheat, N is allocated more in grains, while the phosphorus (P) content is reduced by HT [15]. Two weeks of HT after tasseling reduced the plant grain yield but did not affect the nutrient concentration in different organs, but the harvest index (HI) was unaffected in heat-tolerant, and decreased in heat-sensitive, maize hybrid [16]. The effects of HT on the grain yield and quality have been essentially clarified in maize [5]. However, information of post-silking HT affecting the accumulation, translocation, and remobilization of N, P, and potassium (K) in maize remains scarce. Conducting related study is helpful for improving nutrition utilization under HT.

Exogenous plant growth regulators (PGRs) application is one of the most effective practices to relieving HT-stress-induced harmful effects in plants [1,7]. Brassinolides (BRs) are a class of PGRs that play crucial roles in plant growth and development [17]. BR improves grain yield by increasing DM production and harmonizing the source-to-sink relationship under HT. BR also increases carbon assimilation rate, enhances solute accumulation, and reduces oxidative damage [18]. The higher grain production by BR and other PGRs is caused by the enhanced photosynthetic rate, spikelet fertility, and grain filling rate, which counter the adverse impact of HT [19]. HT obstructs the sucrose transport from leaves to young panicles and suppresses the enzymatic activities involved in sucrose metabolism, and those adverse effects can be mitigated by exogenous BR application [20]. The biosynthesis of photosynthetic pigments, antioxidant enzymes, and osmolytes system in Leymus chinensis under ambient temperature (AT) and HT were enhanced by BR application and improve the plant growth [21]. In maize production, exogenous BR application can enhance the source and sink capacity [22] and enhance the resistance to abiotic adversities such as water deficit [23], chilling [24], waterlogging [25], and acid [26]. Though PGRs are widely reported to enhance the maize plant's response to HT [7,27,28], there is a lag of exogenous BR application on maize under HT. Based on the performance of exogenous BR on the other cereal crops, it is speculated that exogenous BR application at the silking stage can enhance the thermotolerance of maize during grain filling. To test this hypothesis, the plants were sprayed with exogenous BR at the silking stage, and the grain yield, accumulation, translocation, and remobilization of DM and nutrients (N, P, and K) were studied using two waxy maize hybrids as materials. The results may improve knowledge on the BR mitigative effects under HT during the grain-filling period in waxy maize production.

2. Materials and Methods

2.1. Experimental Design

A pot trial was conducted at the Yangzhou University Experimental Farm $(32^{\circ}39' \text{ N}, 119^{\circ}42' \text{ E})$ in 2020 using Jingkenuo2000 (JKN2000, heat-tolerant) and Yunuo7 (YN7, heat-sensitive) as materials. The pots (h = 38 cm, d = 43 cm) were loaded with 30 kg of field-sieved sandy loam soil. The plants (two at seedling and one left at jointing stage) were provided with 16 g control-released compound fertilizer (N/P₂O₅/K₂O = 27%/9%/9%) per pot at sown time (March 15). Plants were grown in the natural environment before the silking stage. The plants with a similar appearance were sampled and manually pollinated on the same day. With Tween 20 (0.05%) as the surfactant, exogenous BR (0.25 mg/L) was sprayed on the plants at a volume of 100 mL/plant in the afternoon (16:00–18:00), with plants sprayed with 100 mL water per plant (also with Tween 20) as the control (CK) [22].

After manual pollination on the same day, pots were moved to the greenhouse on the next day for temperature treatments. The temperatures (12 h day/12 h night) in the smart greenhouse were set at 28/20 °C (AT) and 35/27 °C (HT), respectively. The water transpiration was supplied by the weighing method and the soil moisture was about 75% during plant growth. The stress duration is the whole grain-filling stage (about 40 days). Each treatment included 50 pots.

2.2. Yield Determination

Ten ears were harvested upon maturity, grains were manually stripped from the cobs and air-dried, and the grain yields (g/plant) were weighed.

2.3. Accumulation, Translocation, and Remobilization of Dry Matter (DM) and Nutrients

Three maize plants were sampled and separated into leaves and stems (including sheaths and tassels) upon silking stage (silks protrude from bracts 1–2 cm); and into leaves, stems (including sheaths and tassels), cobs (including bracts), and grains upon maturity. In the study, the accumulation of DM and nutrients is limited to aboveground because the root system is not included. All samples were oven-dried to a constant weight at 80 °C. To estimate DM and nutrient translocation, all the loss from vegetative parts was assumed to be translocated to the grain because DM and nutrient loss due to respiration was not determined.

The N content in different organs was determined by a modified Kjeldahl digestion method. P and K concentrations were measured with an inductively coupled plasma emission spectrometer (IRIS Intrepid II XSP, Thermal Electron Corporation, Waltham, Massachusetts, USA) after digesting the sample with 5 mL of concentrated HNO₃ at 180 °C for 10 min using a microwave-accelerated reaction system (CEM Corporation, Matthews, North Carolina, USA). The N, P, and K accumulations of each fraction were calculated as the product of the N, P, and K concentrations and DM. The various parameters referring to DM, N, P, and K were calculated as previously described [10].

- 1. Post-silking DM (N, P, and K) accumulation (g/plant) = DM (N, P, and K) accumulation at maturity-DM (N, P, and K) accumulation at silking.
- DM (N, P, and K) translocation from pre-silking vegetative organs to grain (g/plant) (TAP) = DM (N, P, and K) accumulation in vegetative organs at silking-DM (N, P, and K) accumulation in vegetative organs at maturity.
- 3. DM (N, P, and K) translocation efficiency (%) (TRP) = $100 \times DM$ (N, P, and K) translocation from pre-silking vegetative organs to grain/DM (N, P, and K) accumulation in vegetative organs at silking.
- Contribution rate of DM (N, P, and K) translocation from pre-silking vegetative organs to grain (%) (CTP) = 100 × DM (N, P, and K) translocation from pre-silking vegetative organs to grain/grain dry weight.
- 5. Remobilization amount of DM (N, P, and K) of post-silking vegetative organ photosynthate (g/plant) = Grain dry weight at maturity—DM (N, P, and K) translocation from pre-silking vegetative organs to grain.
- 6. Harvest index (HI, %) = $100 \times$ Grain dry weight/DM accumulation at maturity;
- 7. N (P, K) HI (%) = $100 \times$ N (P, K) accumulation in grain at maturity/N (P, K) accumulation in total plants at maturity.

2.4. Statistical Analysis

The data reported in all figures and table were subjected to ANOVA using a data processing system (DPS 7.05). The Duncan test was used to compare the means at the 0.05 level, and correlation was calculated by Microsoft Excel 2010.

3. Results

3.1. Grain Yield

Application of exogenous BR increased the grain yield under both temperature regimes, and the increase was significant for YN7 under AT condition (13.1%) and for JKN2000 under HT condition (11.2%) (Figure 1). The grain yield was decreased in YN7 (without BR, 8.5%; with BR, 15.6%) and unaffected in JKN2000 by post-silking HT regardless of the exogenous BR application.



Grain yield

Figure 1. Effects of exogenous BR application at the silking stage on the grain yield of waxy maize under post-silking ambient and high temperatures. YN7, Yunuo7; JKN2000, Jingkenuo2000; AT, ambient temperature; HT, high temperature; CK, control, plants sprayed with 100 mL water; BR, brassinolide, plants sprayed with 100 mL 0.25 mg/L BR. Within columns, those followed by the same letters are not significantly different (p < 0.05).

3.2. Accumulation of DM and Nutrients at Maturity

Without BR application, post-silking HT decreased the plant DM and nutrient (N, P, and K) accumulation in YN7, whereas HT did not affect the DM, N, and P, and increased K accumulation in JKN2000 (Figure 2). With exogenous BR application at the silking stage, post-silking HT decreased the DM and nutrient accumulation in both hybrids. Under AT condition, exogenous BR application increased the accumulation of DM and nutrient at maturity in both hybrids. Under HT condition, exogenous BR application did not affect the DM, N, and K accumulation but increased the P accumulation in YN7; and it did not affect the DM and K accumulation, and increased P accumulation but decreased N accumulation in JKN2000.



Figure 2. Effects of exogenous BR application at the silking stage on the DM and nutrients accumulation at maturity of waxy maize under post-silking ambient and high temperatures. YN7, Yunuo7; JKN2000, Jingkenuo2000; AT, ambient temperature; HT, high temperature; CK, control, plants sprayed with 100 mL water; BR, brassinolide, plants sprayed with 100 mL 0.25 mg/L BR. Within columns, those followed by the same letters are not significantly different (p < 0.05).

3.3. Post-Silking Accumulation of DM and Nutrients

Post-silking accumulation of DM and nutrient were affected by HT, BR, and hybrid (Figure 3). Under AT condition, exogenous BR application improved the post-silking DM and nutrient accumulations in both hybrids. Under HT condition, BR application did not affect the post-silking DM, N, and K accumulations, but improved P accumulation in YN7. Post-silking DM accumulation was unaffected by BR application, whereas N and K were decreased and P was improved by BR application in JKN2000. Without BR application, post-silking HT reduced the DM, N and K accumulation but did not affect P accumulation in YN7. In JKN2000, HT did not affect the DM, N, and P accumulation, and increased K accumulation. With BR application, the post-silking DM and nutrient accumulation was decreased by HT in both hybrids.



Figure 3. Effects of exogenous BR application at the silking stage on the post-silking DM and nutrients accumulation of waxy maize under post-silking ambient and high temperatures. YN7, Yunuo7; JKN2000, Jingkenuo2000; AT, ambient temperature; HT, high temperature; CK, control, plants sprayed with 100 mL water; BR, brassinolide, plants sprayed with 100 mL 0.25 mg/L BR. Within columns, those followed by the same letters are not significantly different (p < 0.05).

3.4. Translocation of Pre-Silking DM and Nutrient in Vegetative Organs

The translocation amount and percentage of pre-silking DM and nutrients and its contribution percentage to grain yield were reduced by exogenous BR application in both hybrids under AT condition (Table 1). Under HT condition, the translocation amount and percentage of pre-silking DM, P, and K and its contribution percentage to grain yield were unaffected by exogenous BR application in both hybrids, whereas the translocation amount and percentage of pre-silking N and its contribution percentage to grain yield were unaffected in YN7 and increased in JKN2000. With exogenous BR application at silking stage, the translocation amount and percentage to grain yield were increased by HT in both hybrids. Without exogenous BR application at silking stage, the translocation amount and percentage to grain yield were increased by HT in both hybrids. Without exogenous BR application at silking stage, the translocation amount and percentage of pre-silking DM and nutrient and its contribution percentage to grain yield were increased by HT in both hybrids. Without exogenous BR application at silking stage, the translocation amount and percentage of pre-silking DM, N, and K and its contribution percentage to grain yield were increased and those values for P were unaffected by HT in YN7. In JKN2000, the translocation amount and percentage of pre-silking DM and P were unaffected. N was increased but K was decreased by HT, and the contribution percentage of DM and nutrient to grain yield were unaffected by HT.

			DM			Ν			Р			K		
Hybrid	Temperature	Hormone	TAP	TRP	CTP	TAP	TRP	CTP	TAP	TRP	CTP	TAP	TRP	CTP
-			(g/Plant)	(%)	(%)	(mg/Plant)	(%)	(%)	(mg/Plant)	(%)	(%)	(mg/Plant)	(%)	(%)
YN7	AT	СК	$20.9\pm4.1b$	$19.1\pm3.7~\mathrm{c}$	$35.4\pm6.3b$	194.3 ± 25.8 de	$15.1\pm2.0~\mathrm{c}$	$22.4\pm2.3~\mathrm{e}$	$23.8\pm2.8~\mathrm{a}$	$17.6\pm2.1~\mathrm{a}$	$28.5\pm2.6~\mathrm{a}$	$121.4\pm12.8~\mathrm{c}$	$22.7\pm2.4~\mathrm{c}$	$146.0\pm17.7\mathrm{b}$
		BR	$3.8\pm1.5~c$	$3.4\pm1.4~d$	$5.6\pm2.1~\mathrm{c}$	$47.9\pm18.1~\mathrm{f}$	$3.7\pm1.4~\mathrm{e}$	$6.0\pm2.2~\mathrm{f}$	$-9.2\pm4.4~\mathrm{c}$	$-6.8\pm5.4~\mathrm{c}$	$-8.5\pm3.8~\mathrm{c}$	$21.3\pm5.9~\mathrm{e}$	$4.0\pm3.0~\mathrm{e}$	$19.3\pm14.4d$
	HT	СК	$38.6\pm3.8~\text{a}$	$35.2\pm3.5~\text{a}$	$72.0\pm9.5~a$	393.8 ± 42.0 bc	$30.5\pm3.3~\text{a}$	$53.8\pm7.1~bc$	$30.8\pm1.0~\text{a}$	$22.7\pm0.7~a$	$36.9\pm1.0~\mathrm{a}$	$255.7\pm8.3~a$	$47.9\pm1.6~\mathrm{a}$	$307.4\pm37.2~\mathrm{a}$
		BR	$35.6\pm1.3~\mathrm{a}$	$32.4\pm1.2~\text{ab}$	$63.3\pm3.2~\mathrm{a}$	453.1 ± 43.8 ab	$35.1\pm3.4~\mathrm{a}$	$57.5\pm5.9~\mathrm{ab}$	$29.2\pm4.0~\text{a}$	$21.5\pm2.9~\mathrm{a}$	$30.6\pm5.1~\mathrm{a}$	$237.8\pm14.9~\text{a}$	$44.5\pm2.8~\mathrm{a}$	$327.2\pm15.9~\mathrm{a}$
JKN2000	AT	CK	34.6 ± 5.0 a	$26.8\pm3.9b$	$67.0\pm9.7~\mathrm{a}$	$226.4\pm32.6~\mathrm{d}$	$15.1\pm2.2~\mathrm{c}$	$37.4\pm7.5~\mathrm{d}$	-5.8 ± 5.6 bc	$-4.0\pm3.9\mathrm{bc}$	-7.7 ± 7.3 bc	$182.3\pm10.5\mathrm{b}$	$29.0\pm1.7b$	$176.7\pm17.1\mathrm{b}$
		BR	$17.0\pm4.2\mathrm{b}$	$13.2\pm3.3~\mathrm{c}$	$30.7\pm6.8\mathrm{b}$	$136.5 \pm 26.9 \text{ e}$	$9.1\pm1.8~{ m d}$	$19.8\pm3.4~\mathrm{e}$	-45.8 ± 6.0 d	-31.8 ± 4.2 d	-50.4 ± 6.3 d	$77.0 \pm 25.3 \text{ d}$	$12.3 \pm 4.0 \text{ d}$	$88.7\pm26.1~\mathrm{c}$
	HT	CK	$37.7 \pm 2.1 a$	$29.2\pm1.6~\mathrm{ab}$	74.6 ± 2.2 a	$329.2 \pm 12.9 \text{ c}$	22.0 ± 0.9 b	$44.7\pm1.7~{ m cd}$	$2.8\pm5.5\mathrm{b}$	$1.9\pm5.9\mathrm{b}$	$3.0\pm9.3b$	$111.1 \pm 35.6 \text{ c}$	$17.7\pm5.7~\mathrm{cd}$	$146.2\pm37.1~\mathrm{b}$
		BR	$40.2\pm2.8~\mathrm{a}$	31.1 ± 2.2 ab	71.6 ± 5.0 a	467.8 ± 14.7 a	31.3 ± 1.0 a	$64.2 \pm 2.1 \text{ a}$	-6.3 ± 4.7 bc	$-4.4\pm3.3\mathrm{bc}$	$-7.0\pm5.7~\mathrm{bc}$	$120.7\pm13.5~\mathrm{c}$	$19.2\pm2.2~\mathrm{c}$	$181.1\pm17.1~\mathrm{b}$
F-value														
Hybrid			20.5 **	3.2	27.9 **	1.4	2.4	7.4 *	181.2 **	184.7 **	200.5 **	21.8 **	64.3 **	21.3 **
Temperature			125.3 **	134.4 **	124.2 **	294.0 **	282.0 **	192.8 **	93.8 **	94.6 **	92.7 **	108.4 **	144.1 **	139.8 **
Hormone			27.2 **	28.4 **	37.0 **	0.4	0.6	1.2	75.3 **	75.3 **	82.7 **	47.4 **	53.4 **	12.7 **
Hybrid \times Temperature			11.7 **	19.2 **	12.7 **	7.9 *	15.5 **	10.3 **	0.1	0.0	0.4	148.5 **	188.3 **	82.2 **
$Hybrid \times Hormone$			0.6	1.4	0.0	5.0 *	5.0 *	2.3	2.2	1.5	0.8	0.5	1.8	1.4
Temperature × Hormone			25.4 **	25.2 **	18.0 **	51.4 **	47.8 **	34.9 **	41.7 **	42.1 **	36.2 **	40.3 **	43.5 **	35.9 **
Hybrid \times Temperature $ imes$ Hormone		0.8	0.2	0.9	0.1	0.0	3.1	0.0	0.1	0.0	1.1	0.3	0.3	

Table 1. Effects of exogenous BR application at the silking stage on the translocations of pre-silking DM and nutrient of waxy maize under post-silking ambient and high temperatures.

YN7, Yunuo7; JKN2000, Jingkenuo2000; AT, ambient temperature; HT, high temperature; CK, control, plants sprayed with 100 mL water; BR, brassinolide, plants sprayed with 100 mL 0.25 mg/L BR; TAP, DM (N, P, and K) translocation from pre-silking vegetative organs to grain; TRP, DM (N, P, and K) translocation efficiency; CTP, contribution efficiency of DM (N, P, and K) translocation from pre-silking vegetative organs to grain. Within columns, those followed by the same letters are not significantly different (*p* < 0.05). *, *p* < 0.05; **, *p* < 0.01.

3.5. Remobilization of Post-Silking DM and Nutrient

Application of BR at the silking stage increased the post-silking DM and nutrient remobilization in both hybrids at AT condition (Figure 4). Under HT condition, it did not affect the DM and K remobilization and increased P remobilization in both hybrids, the N remobilization was unaffected and decreased in YN7 and JKN2000. Without BR application, post-silking DM and nutrient remobilization were reduced by post-silking HT in YN7, whereas DM, N, and P remobilization were unaffected and K was improved in JKN2000. With BR application, the post-silking DM and nutrient remobilization were reduced by HT in both hybrids.



Figure 4. Effects of exogenous BR application at the silking stage on the remobilization of post-silking DM and nutrients of waxy maize under post-silking ambient and high temperatures. YN7, Yunuo7; JKN2000, Jingkenuo2000; AT, ambient temperature; HT, high temperature; CK, control, plants sprayed with 100 mL water; BR, brassinolide, plants sprayed with 100 mL 0.25 mg/L BR. Within columns, those followed by the same letters are not significantly different (p < 0.05).

3.6. Distribution of DM, N, P, and K at Maturity

The distribution of DM and nutrient in grains can be used to estimate the HI. Without exogenous BR application at the silking stage, post-silking HT increased the HI and KHI but unaffected the NHI and PHI in YN7, whereas the HI was unaffected, KHI was decreased,

and NHI and PHI were increased in JKN2000 (Figure 5). With BR application at the silking stage, post-silking HT did not affect the KHI but increased the HI, NHI, and PHI in both hybrids. Under AT condition, the HI, PHI, and KHI were unaffected but NHI was reduced by exogenous BR application in YN7, and the NHI and PHI were unaffected but HI and KHI were reduced in JKN2000. Under HT condition, the HI was unaffected and KHI was decreased, but NHI and PHI were increased by BR application in YN7, whereas BR application did not affect the PHI and KHI but increased HI and NHI in JKN2000.



Figure 5. Effects of exogenous BR application at the silking stage on the harvest index of DM and nutrients of waxy maize under post-silking ambient and high temperatures. YN7, Yunuo7; JKN2000, Jingkenuo2000; AT, ambient temperature; HT, high temperature; CK, control, plants sprayed with 100 mL water; BR, brassinolide, plants sprayed with 100 mL 0.25 mg/L BR. Within columns, those followed by the same letters are not significantly different (p < 0.05).

3.7. Correlation Analysis

Based on the results in both hybrids, grain weight (yield) was positively correlated to post-silking DM and N accumulation and negatively correlated to translocation amount of pre-silking DM (Figure 6).



Figure 6. Correlation of grain weight and post-silking accumulation and pre-silking translocation amount biomass and nutrient. **, p < 0.01.

4. Discussion

4.1. Effects of Post-Silking HT on Accumulation and Remobilization of DM and Nutrient

The plant DM and grain yield are mainly dependent on two carbon resources: (1) postsilking directly formed by photoassimilates, and (2) translocation of the non-structural carbohydrate reserves from the vegetative organs [9]. Under the optimal condition, the main carbon resource is from the leaf directly photosynthesized during the grain filling, but HT decreased the photosynthesis rate by weakening the enzymatic activities involved in nitrogen and carbon metabolism [11] and accelerated the leaf senescence [10]. In the present study, the grain yield was reduced in heat-sensitive YN7 and slightly affected in heat-tolerant JKN2000, which identify with the change of post-silking DM and nutrient accumulation and the remobilization amount of DM of post-silking vegetative organ photosynthate. The grain yield loss may be due to the reduced duration of assimilate translocation during grain filling [29]. A study on rice also observed that the DM reduction was more severely susceptible than the tolerant cultivar [30]. This may be due to the fact that HT inhibits carbohydrate transportation and retards sink organ growth [31]. Though the TAP, TRP, and CTP were increased in the heat-sensitive YN7, this increase cannot fully compensate the direct reduction of post-silking photoassimilate, ultimately leading to grain yield penalty. The HI was unaffected by HT in JKN2000 and increased in YN7. The increased HI in the heat-sensitive hybrid may be due to the increased TAP. A study on rice also reported that the DM in stem and sheath was decreased by the HT [20].

In heat-sensitive YN7, the whole and post-silking nutrient accumulation and remobilization amount of post-silking DM were reduced; the translocation amounts of pre-silking N and K were increased but P was unaffected; NHI and PHI was unaffected and KHI was increased by HT. In heat-tolerant JKN2000, the whole and post-silking accumulation and remobilization of post-silking N and P was unaffected and K was increased; remobilization and HI of N and P was increased, but K was decreased by HT. A study on rice reported that HT decreased the translocation efficiencies of N and P in vegetative organs (including leaves, stems, and sheaths), and reduced the allocation rates of N, P, and K in panicles at maturity, ultimately causing yield loss [32]. A study on pea reported that seed weight and N accumulation were reduced, and N remobilization from vegetative organs to seed was also reduced by HT during grain filling [33]. A previous study on rice observed that HT at vegetative stage promoted the DM and N accumulation [34]. The discrepancy may be due to the HT at vegetative stage accelerating the plant growth, whereas at late stage it shortened the grain-filling duration [29,33].

In YN7, K still accumulated after pollination under AT condition, whereas under HT condition, it mainly relied on the translocation from the pre-silking stored K in vegetative organs. This change was contrary to JKN2000. The remobilization from the post-silking K under different treatments without BR were negative, indicating there was no remobilization of K, but it was reduced in YN7 and increased in JKN2000 by the HT. The results underline the asynchronous accumulation of N, P, and K over the various growth stages in maize, and plants at grain-filling stage still accumulated about 16–43% N and 16–55% P of the total accumulation, whereas K mainly accumulated before silking [35]. A study on rice also reported that K translocation efficiency in stems and sheaths was increased by HT [32]. This observation may be because K+ is a key osmotic adjustment substance relieving the adverse effect under stressed conditions [36]. These results may indicate that the endogenous hormones were stimulated in heat-tolerant JKN2000 to adjust lateral root development and to maintain the physiological function, thereby mitigating HT [37].

4.2. Effects of Exogenous BR Application on Accumulation and Remobilization of DM and Nutrient under Post-Silking HT Condition

Many reports suggest that BRs may act as immunomodulators to defend plants from HT injuries [17]. At the maize silking stage, exogenous BR application can increase the grain yield of both hybrids in HT conditions by increasing the accumulation of DM and nutrients and promoting the remobilization of post-silking DM and nutrients but reducing the translocation of pre-silking vegetative organs. The high DM accumulation may be due to exogenous BR application postponing leaf senescence and promoting leaf photosynthesis [22]. The increased rice yield and yield components and DM (whole plant, leaf, stem, and sheath and grain) at AT and HT conditions were also improved by exogenous BR application [20]. The maize grain yield, DM, and nutrient accumulation were also improved by application of exogenous salicylic acid under AT conditions [38]. Under HT conditions, the grain yield was only significantly increased in heat-tolerant JKN2000 and slightly increased in heat-sensitive YN7. Under HT conditions, the postsilking DM accumulation was unaffected in both hybrids, the N and K accumulation were unaffected and decreased by BR application in YN7 and JKN2000, whereas P accumulation was increased by exogenous BR application both hybrids. The increased P accumulation was also reported in wheat following the application of PGRs, as those PGRs promoted the nutrient uptake [39]. Those results indicated that the exogenous BR application can alleviate the negative influence of post-silking HT, but the application effects are less on AT. BR application reduced the contents of Na, Cl, malondialdehyde, and H₂O₂, increased

the contents of N, P, K, and Ca, and enhanced the activities of key antioxidant enzymes, thereby mitigating the salinity stress on maize plants [40]. A previous study also reported that the plant growth, photosynthetic pigments, and accumulation of N, P, and K under Pb stress was minimized by exogenous PGRs such as jasmonic acid, salicylic acid, and proline [41]. The rice grain yield and allocation rates of N and K in panicles at maturity under HT were increased by silicon fertilizer application [32]. In the present study, the remobilization of post-silking DM and K was unaffected and P was increased in both hybrids, but those were unaffected by exogenous BR application in YN7 and decreased in JKN2000. The HT at seedling stages increased the leaf N and P concentration and content of a heat-stable rapeseed cultivar because it increased the plant growth rate, leading to an increased biomass accumulation [42]. The discrepancy may be due to the fact that HT at vegetative stage can promote microbial mineralization of N and P, providing more nutrients for plants uptake [42], whereas the vegetative organs had ceased growth and gradually became senescent with the grain development. The HT may induce the early senescence of the root, which results in low nutrient uptake. Grain yield was positively correlated to post-silking DM and N accumulation and negatively correlated to translocation amount of pre-silking DM. A study on rice also reported that the grain yield was negatively related to the N and P allocation rates in vegetative organs at maturity [32]. The increased post-silking DM and nutrient accumulation is vital to provide the photoassimilation for grain formation and development. A study on wheat reported that application of exogenous cytokinin can enhance the sucrose and nitrogen accumulation in tiller bud and improve the grain yield under low nitrogen levels [43]. Nevertheless, knowledge of the underlying physiological mechanisms for single and reciprocal effect of HT and exogenous BR application on the accumulation and remobilization of N, P, and K in waxy maize remain scanty, and worthy of digging deeper.

5. Conclusions

Accumulation, translocation, and distribution of biomass and nutrient were affected by exogenous BR application and HT during grain filling. The grain yield was decreased by HT in YN7 and unaffected in JKN2000 regardless of the BR application. However, BR application improved the grain yield in JKN2000 but not in YN7. HT decreased the plant DM and nutrient accumulation in YN7, whereas it did not affect the DM, N, and P, and it increased K accumulation in JKN2000. Under HT conditions, BR application did not affect the post-silking DM, and improved P accumulation in both hybrids, whereas N and K accumulations were unaffected in YN7 and decreased in JKN2000. The translocation amount and percentage of pre-silking DM and nutrients and its contribution percentage to grain yield were increased by HT in both hybrids. Under HT condition, the translocation amount and percentage of pre-silking DM, P, and K and its contribution percentage to grain yield were unaffected in both hybrids, whereas the translocation amount and percentage of pre-silking N and its contribution percentage to grain yield were unaffected by BR application in YN7 and increased in JKN2000. Under HT condition, HI was unaffected, KHI was decreased, but NHI and PHI were increased by BR application in YN7, whereas the PHI and KHI were unaffected but HI and NHI were increased in JKN2000. Grain yield was positively correlated to post-silking DM and N accumulation and negatively correlated to translocation amount of pre-silking DM. In waxy maize production, BR application at the silking can improve the grain yield and accumulation of DM and nutrients at optimal conditions, but it also can alleviate the deterioration of HT during grain filling, especially for heat-tolerant hybrids.

Author Contributions: Conceptualization, D.L. and H.Y.; methodology, D.L. software, Y.X.; validation, D.L.; formal analysis, Y.X.; investigation, Y.X. and X.Z.; resources, D.L.; data curation, Y.X. and D.L.; writing—original draft preparation, Y.X.; writing—review and editing, Y.X. and D.L.; visualization, Y.X. and X.Z.; supervision, D.L.; project administration, D.L.; funding acquisition, D.L. and Y.X. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China (32071958, 31771709), Jiangsu Agriculture Science and Technology Innovation Fund (CX[20]3147), Key Research & Development Program of Jiangsu Province (BE2021317), Coordinated Promotion of Major Agricultural Technologies (2021-ZYXT-05-1), Jiangsu Key Laboratory of Crop Genetics and Physiology (YCSL202101), Priority Academic Program Development of Jiangsu Higher Education Institutions, and High-end Talent Support Program of Yangzhou University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data and codes used in this study can be requested by email to the corresponding author Dalei Lu at dllu@yzu.edu.cn.

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

References

- 1. Ali, S.; Rizwan, M.; Arif, M.S.; Ahmad, R.; Hasanuzzaman, M.; Ali, B.; Hussain, A. Approaches in enhancing thermotolerance in plants: An updated review. J. Plant Growth Regul. 2020, 39, 456–480. [CrossRef]
- Prasad, P.V.V.; Bheemanahalli, R.; Jagadish, S.V.K. Field crops and the fear of heat stress—Opportunities, challenges and future directions. *Field Crop. Res.* 2017, 200, 114–121. [CrossRef]
- 3. Lizaso, J.I.; Ruiz-Rarnos, M.; Rodriguez, L.; Gabaldon-Leal, C.; Oliveira, J.A.; Lorite, I.J.; Sanchez, D.; Garcia, E.; Rodriguez, A. Impact of high temperatures in maize: Phenology and yield components. *Field Crops Res.* **2018**, *216*, 129–140. [CrossRef]
- 4. Gourdji, S.M.; Sibley, A.M.; Lobell, D.B. Global crop exposure to critical high temperatures in the reproductive period: Historical trends and future projections. *Environ. Res. Lett.* **2013**, *8*, 024041. [CrossRef]
- 5. Li, Z.X.; Howell, S.H. Heat stress responses and thermotolerance in maize. Int. J. Mol. Sci. 2021, 22, 948. [CrossRef] [PubMed]
- Hou, P.; Liu, Y.E.; Liu, W.M.; Yang, H.S.; Xie, R.Z.; Wang, K.R.; Ming, B.; Liu, G.Z.; Xue, J.; Wang, Y.H.; et al. Quantifying maize grain yield losses caused by climate change based on extensive field data across China. *Resour. Conserv. Recycl.* 2021, 174, 105811. [CrossRef]
- Waqas, M.A.; Wang, X.; Zafar, S.A.; Noor, M.A.; Hussain, H.A.; Azher Nawaz, M.; Farooq, M. Thermal stresses in maize: Effects and management strategies. *Plants* 2021, 10, 293. [CrossRef]
- Sehgal, A.; Sita, K.; Siddique, K.H.M.; Kumar, R.; Bhogireddy, S.; Varshney, R.K.; HanumanthaRao, B.; Nair, R.M.; Prasad, P.V.V.; Nayyar, H. Drought or/and heat-stress effects on seed filling in food crops: Impacts on functional biochemistry, seed yields, and nutritional quality. *Front. Plant Sci.* 2018, *9*, 1705. [CrossRef]
- 9. Yang, J.C.; Zhang, J.H. Grain filling of cereals under soil drying. New Phytol. 2006, 169, 223–236. [CrossRef]
- 10. Yang, H.; Huang, T.Q.; Ding, M.Q.; Lu, D.L.; Lu, W.P. High temperature during grain filling impacts on leaf senescence in waxy maize. *Agron. J.* 2017, 109, 906–916. [CrossRef]
- 11. Yang, H.; Gu, X.T.; Ding, M.Q.; Lu, W.P.; Lu, D.L. Weakened carbon and nitrogen metabolisms under post-silking heat stress reduce the yield and dry matter accumulation in waxy maize. *J. Integr. Agric.* **2020**, *19*, 78–88. [CrossRef]
- 12. Kobata, T.; Uemuki, N. High temperatures during the grain-filling period do not reduce the potential grain dry matter increase of rice. *Agron. J.* **2004**, *96*, 406–414. [CrossRef]
- 13. Impa, S.M.; Sunoj, V.S.J.; Krassovskaya, I.; Bheemanahalli, R.; Obata, T.; Jagadish, S.V.K. Carbon balance and source-sink metabolic changes in winter wheat exposed to high night-time temperature. *Plant. Cell Environ.* **2019**, *42*, 1233–1246. [CrossRef] [PubMed]
- Ito, S.; Hara, T.; Kawanami, Y.; Watanabe, T.; Thiraporn, K.; Ohtake, N.; Sueyoshi, K.; Mitsui, T.; Fukuyama, T.; Takahashi, Y.; et al. Carbon and nitrogen transport during grain filling in rice under high-temperature conditions. *J. Agron. Crop Sci.* 2009, 195, 368–376. [CrossRef]
- 15. Cabral, C.; Ravnskov, S.; Tringovska, I.; Wollenweber, B. Arbuscular mycorrhizal fungi modify nutrient allocation and composition in wheat (*Triticum aestivum* L.) subjected to heat-stress. *Plant Soil* **2016**, *408*, 385–399. [CrossRef]
- Hussain, H.A.; Men, S.; Hussain, S.; Chen, Y.; Ali, S.; Zhang, S.; Zhang, K.; Li, Y.; Xu, Q.; Liao, C.; et al. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Sci. Rep.* 2019, *9*, 3890. [CrossRef]
- 17. Yang, J.C.; Miao, W.Q.; Chen, J. Roles of jasmonates and brassinosteroids in rice responses to high temperature stress—A review. *Crop J.* **2021**, *9*, 977–985. [CrossRef]
- 18. Tanveer, M. Role of 24-epibrassinolide in inducing thermo-tolerance in plants. J. Plant Growth Regul. 2019, 38, 945–955. [CrossRef]
- Fahad, S.; Hussain, S.; Saud, S.; Hassan, S.; Ihsan, Z.; Shah, A.N.; Wu, C.; Yousaf, M.; Nasim, W.; Alharby, H.; et al. Exogenously applied plant growth regulators enhance the morpho-physiological growth and yield of rice under high temperature. *Front. Plant Sci.* 2016, 7, 1250. [CrossRef]
- Chen, Y.H.; Chen, H.Z.; Xiang, J.; Zhang, Y.K.; Wang, Z.G.; Zhu, D.F.; Wang, J.K.; Zhang, Y.P.; Wang, Y.L. Rice spikelet formation inhibition caused by decreased sugar utilization under high temperature is associated with brassinolide decomposition. *Environ. Exp. Bot.* 2021, 190, 104585. [CrossRef]

- Niu, J.H.; Anjum, S.A.; Wang, R.; Li, J.H.; Liu, M.R.; Song, J.X.; Zohaib, A.; Lv, J.; Wang, S.G.; Zong, X.F. Exogenous application of brassinolide can alter morphological and physiological traits of Leymus chinensis (Trin.) Tzvelev under room and high temperatures. *Chil. J. Agric. Res.* 2016, *76*, 27–33. [CrossRef]
- Gao, Z.; Liang, X.G.; Zhang, L.; Lin, S.; Zhao, X.; Zhou, L.L.; Shen, S.; Zhou, S.L. Spraying exogenous 6-benzyladenine and brassinolide at maize yield by enhancing source and sink capacity tasseling increases. *Field Crops Res.* 2017, 211, 1–9. [CrossRef]
- 23. Anjum, S.A.; Wang, L.C.; Farooq, M.; Hussain, M.; Xue, L.L.; Zou, C.M. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J. Agron. Crop Sci. 2011, 197, 177–185. [CrossRef]
- 24. Sun, Y.J.; He, Y.H.; Irfan, A.R.; Liu, X.M.; Yu, Q.Q.; Zhang, Q.; Yang, D.G. Exogenous brassinolide enhances the growth and cold resistance of maize (*Zea mays* L.) seedlings under chilling stress. *Agronomy* **2020**, *10*, 488. [CrossRef]
- 25. Otie, V.; Ping, A.; Udo, I.; Eneji, E. Brassinolide effects on maize (*Zea mays* L.) growth and yield under waterlogged conditions. *J. Plant Nutr.* **2019**, *42*, 954–969. [CrossRef]
- Otie, V.; Ping, A.; Eneji, E. Interactive effect of brassinolide and lime on growth and yield of maize (*Zea mays* L.) on acid soils of South-East Nigeria. *Commun. Soil Sci. Plant Anal.* 2018, 49, 2918–2931. [CrossRef]
- 27. Tao, Z.Q.; Chen, Y.Q.; Li, C.; Zou, J.X.; Yan, P.; Yuan, S.F.; Wu, X.; Sui, P. The causes and impacts for heat stress in spring maize during grain filling in the North China Plain—A review. *J. Integr. Agric.* 2016, *15*, 2677–2687. [CrossRef]
- Cairns, J.E.; Sonder, K.; Zaidi, P.H.; Verhulst, N.; Mahuku, G.; Babu, R.; Nair, S.K.; Das, B.; Govaerts, B.; Vinayan, M.T.; et al. Maize production in a changing climate: Impacts, adaptation, and mitigation strategies. *Adv. Agron.* 2012, 114, 1–58.
- 29. Arshad, M.S.; Farooq, M.; Asch, F.; Krishna, J.S.V.; Prasad, P.V.V.; Siddique, K.H.M. Thermal stress impacts reproductive development and grain yield in rice. *Plant Physiol. Biochem.* **2017**, *115*, 57–72. [CrossRef]
- 30. Sharma, N.; Yadav, A.; Khetarpal, S.; Anand, A.; Sathee, L.; Kumar, R.R.; Singh, B.; Soora, N.K.; Pushkar, S. High day-night transition temperature alters nocturnal starch metabolism in rice (*Oryza sativa* L.). *Acta Physiol. Plant.* **2017**, *39*, 74. [CrossRef]
- Zhang, C.X.; Feng, B.H.; Chen, T.T.; Fu, W.M.; Li, H.B.; Li, G.Y.; Jin, Q.Y.; Tao, L.X.; Fu, G.F. Heat stress-reduced kernel weight in rice at anthesis is associated with impaired source-sink relationship and sugars allocation. *Environ. Exp. Bot.* 2018, 155, 718–733. [CrossRef]
- 32. Liu, Q.H.; Ma, H.; Sun, Z.W.; Lin, X.Q.; Zhou, X.B. Translocation efficiencies and allocation of nitrogen, phosphorous and potassium in rice as affected by silicon fertilizer under high daytime temperature. *J. Agron. Crop Sci.* **2019**, 205, 188–201. [CrossRef]
- 33. Larmure, A.; Munier-Jolain, N.G. High Temperatures during the seed-filling period decrease seed nitrogen amount in pea (*Pisum sativum* L.): Evidence for a sink limitation. *Front. Plant Sci.* **2019**, *10*, 1608. [CrossRef] [PubMed]
- Chen, S.; Zhang, X.G.; Zhao, X.; Wang, D.Y.; Xu, C.M.; Ji, C.L.; Zhang, X.F. Response of rice nitrogen physiology to high nighttime temperature during vegetative stage. *Sci. World J.* 2013, 2013, 649326. [CrossRef]
- 35. Ning, P.; Li, S.; Yu, P.; Zhang, Y.; Li, C.J. Post-silking accumulation and partitioning of dry matter, nitrogen, phosphorus and potassium in maize varieties differing in leaf longevity. *Field Crops Res.* **2013**, *144*, 19–27. [CrossRef]
- Sardans, J.; Penuelas, J. Potassium Control of Plant Functions: Ecological and Agricultural Implications. *Plants* 2021, 10, 419. [CrossRef]
- 37. Zhao, X.H.; Yu, H.Q.; Wen, J.; Wang, X.G.; Du, Q.; Wang, J.; Wang, Q. Response of root morphology, physiology and endogenous hormones in maize (*Zea mays* L.) to potassium deficiency. *J. Integr. Agric.* **2016**, *15*, 785–794. [CrossRef]
- Tucuch-Haas, C.; Alcantar-Gonzalez, G.; Trejo-Tellez, L.I.; Volke-Haller, H.; Salinas-Moreno, Y.; Larque-Saavedra, A. Effect of salicylic acid on growth, nutritional status, and performance of maize (*Zea mays*). Agrociencia 2017, 51, 771–781.
- 39. Jadwiga, W.; Teresa, B.; Stanislaw, S. Nitrogen and phosphorus management of spring wheat plants following the application of growth regulators and increasing potassium fertilization rates. *Pol. J. Nat. Sci.* 2006, 20, 91–100.
- 40. Kaya, C.; Aydemir, S.; Akram, N.A.; Ashraf, M. Epibrassinolide Application regulates some key physio-biochemical attributes as well as oxidative defense system in maize plants grown under saline stress. J. Plant Growth Regul. 2018, 37, 1244–1257. [CrossRef]
- 41. Sofy, M.R.; Seleiman, M.F.; Alhammad, B.A.; Alharbi, B.M.; Mohamed, H.I. Minimizing adverse effects of Pb on maize plants by combined treatment with jasmonic, salicylic acids and proline. *Agronomy* **2020**, *10*, 699. [CrossRef]
- Biswas, D.K.; Ma, B.L.; Morrison, M.J. Changes in leaf nitrogen and phosphorus content, photosynthesis, respiration, growth, and resource use efficiency of a rapeseed cultivar as affected by drought and high temperatures. *Can. J. Plant Sci.* 2019, *99*, 488–498. [CrossRef]
- Yang, D.Q.; Luo, Y.L.; Kong, X.; Huang, C.; Wang, Z.L. Interactions between exogenous cytokinin and nitrogen application regulate tiller bud growth via sucrose and nitrogen allocation in winter wheat. J. Plant Growth Regul. 2021, 40, 329–341. [CrossRef]