Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Comparative Analysis of the Regulation of Grain Filling and Physiological Metabolism by Foliar-Applied Pro-Ca and GA_3 in Rice under Salt Stress

Qicheng Zhang
Guangdong Ocean University
Dianfeng Zheng
Guangdong Ocean University
Naijie Feng
fengnj@gdou.edu.cn
Guangdong Ocean University
Ziming Chen
Guangdong Ocean University
Wenji Zhong
Guangdong Ocean University
Yin Lin
Guangdong Ocean University
Xiao Wang
Guangdong Ocean University
Juezhen Zheng
Guangdong Ocean University
Siyu Liu
Guangdong Ocean University
Yushawu Zakaria Mohammed
Guangdong Ocean University

Research Article

Keywords: rice, salt stress, prohexadione-calcium, gibberellin, grain filling, carbon metabolism

Posted Date: June 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9905922/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

This study systematically compared the regulatory effects of prohexadione-calcium (Pro-Ca) and gibberellin (GA_3) on the grain-filling process of rice under salt stress. Using the rice varieties Guanghong 3 (GH3) and FL478 as experimental materials, foliar applications of Pro-Ca and GA_3 were conducted at the three-leaf-one-heart stage, rupture stage, and a combination of both stages under NaCl stress conditions.

Results

The results demonstrated marked differences in the regulation of yield between Pro-Ca and GA under salt stress. Pro-Ca treatment increased the effective panicle number, grains per panicle, seed-setting rate, and 1000-grain weight. In contrast, the GA application reduced the effective panicle number and seed-setting rate despite increasing grains per panicle and 1000-grain weight. Regarding grain-filling regulation, Pro-Ca enhanced the grain-filling rate while maintaining a stable grain-filling duration. Conversely, GA_3 significantly accelerated grain-filling rate but shortened the effective grain-filling period, resulting in a “fast but unfilled” phenotype. Further analysis revealed that Pro-Ca promoted efficient conversion of sucrose to starch by enhancing the activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS), while reducing the activities of acid invertase (AI) and neutral invertase (NI), and maintaining a prolonged period of high activity for key starch synthesis enzymes (AGPase, SSS, GBSS, SBE). In contrast, GA_3 treatment resulted in the retention of reducing sugars.

Conclusions

In conclusion, Pro-Ca optimized grain carbon metabolic flux and maintained a balance between grain-filling rate and duration, thereby achieving stable, high yields in rice under salt stress. The combined application at the three-leaf-one-heart and rupture stages proved to be the most effective treatment. These findings provide a theoretical basis and an optimization strategy for high, stable rice production in saline-alkaline soils.

1. Background

Soil salinization poses a severe challenge to global food security, with approximately 20% of the world’s arable land currently affected by salt stress, resulting in substantial crop yield losses [1]. Rice (Oryza sativa L.) is not only one of the most important staple food crops but also a salt-sensitive species[2]. Salt stress typically inflicts multiple damages on rice, including ion toxicity, osmotic stress, and oxidative injury, which not only inhibits normal growth and development but also alters grain composition and

starch properties[3], severely constraining rice growth and yield[4]. Therefore, investigating agronomic practices that alleviate salt damage is crucial to enhancing crop productivity.

The reproductive growth stage of rice encompasses panicle initiation, flowering, and grain filling, which represent both the critical period determining rice yield and one of the most salt-sensitive phases [5]. Salt stress occurring during this period significantly reduces rice yield, and the magnitude of the reduction increases with stress severity [6]. Several studies have suggested that salt stress treatment at the booting stage exerts the greatest impact on traits such as plant height, filled grain number, effective panicle number, seed-setting rate, 1000-grain weight, and overall yield[7], followed by the jointing and heading stages[8]. Research has demonstrated that salt stress during the booting and heading stages reduces spikelets per panicle, decreases seed-setting rate, and lowers 1000-grain weight, resulting in yield reduction or total crop failure [9]. For instance, experimental findings have revealed that yield reduction in rice under salt stress is primarily associated with a marked decrease in grains per panicle, with the greatest reductions observed in the number of secondary branches, total grain number on secondary branches, and total grain weight on secondary branches[10]. Regarding seed-setting rate and 1000-grain weight, Wei et al.[6] reported that under moderate and high salt stress, both salt-sensitive and salt-tolerant rice varieties exhibited significantly lower seed-setting rates and grain weights than the control, with the greatest reduction in grain weight occurring on secondary branches.

The application of exogenous plant growth regulators (PGRs) has become an effective strategy for mitigating salt stress damage in crops. For instance, gibberellin (GA3) application has been demonstrated to be an effective approach for enhancing crop salt tolerance. Relevant studies have shown that GA supplementation can rescue seed germination and seedling growth under salt stress by upregulating α-amylase gene expression and modulating endogenous GA metabolism[11]. As an inhibitor of GA biosynthesis, prohexadione-calcium (Pro-Ca) enhances salt tolerance in rice during growth stages, such as the tillering stage, by improving photosynthetic capacity, antioxidant defense systems, and physical lodging resistance [12]. Studies have indicated that Pro-Ca pretreatment can help rice establish a more robust photosynthetic apparatus to cope with salt stress [13].

Based on this, the present study used two salt-tolerant varieties, Guanghong 3 (GH3) and FL478, as experimental materials, with foliar application treatments applied at different stages (the three-leaf-one-heart stage, the rupture stage, and a combination of both). By measuring yield components, grain-filling dynamics, activities of key starch synthesis enzymes, grain non-structural carbohydrate content, and activities of key sucrose metabolism enzymes, this study aimed to systematically compare the regulatory effects and mechanistic differences between Pro-Ca and GA3 on the grain-filling and yield formation processes of rice under salt stress. These findings are expected to provide a theoretical basis and optimization strategies for high-yield, stable rice cultivation in saline-alkaline soils.

2. Materials and Methods

2.1 Plant Materials

The experiment was conducted from June 2024 to August 2025 in an outdoor cultivation field at the Coastal Agricultural College of Guangdong Ocean University. The tested rice varieties were Guanghong 3 (GH3) and FL478, both moderately salt-tolerant, and the regulators used were Pro-Ca and GA_3. The Coastal Agricultural College of Guangdong Ocean University provided both the plant varieties and regulators.

2.2 Experimental Design

Plump, intact rice seeds were selected and sterilized with 3% hydrogen peroxide for 15 min, then rinsed thoroughly with distilled water. The seeds were then soaked in distilled water for 24 hours and germinated in the dark at 30°C for another 24 hours. Subsequently, the seeds were sown in seedling trays with holes at the bottom (54 cm long × 28 cm wide, 21 holes per tray). When the seedlings reached the three-leaf-one-heart stage, they were foliar-sprayed with 100 mg/L Pro-Ca or 1 mg/L GA3. Twenty-four hours after treatment, uniformly grown seedlings were selected and transplanted into impermeable plastic pots (upper diameter: 27.5 cm, bottom diameter: 18.5 cm, height: 23 cm). Each pot was pre-filled with 8 kg of lateritic soil and 3 L of 80 mM NaCl solution to simulate salt stress.

Three plants were planted per pot, with two seedlings per hill, spaced 10 cm apart and planted at a depth of 1.5 cm. After transplanting, the water level was marked and regularly replenished. The salt concentration was maintained by real-time monitoring using a soil salinity meter (STEPS, Nuremberg, Germany, PNT Combi 5000).

A second treatment was applied at the breakthrough stage. One set of plants from the Pro-Ca-sprayed treatments of both varieties was selected and foliar-sprayed with 100 mg/L Pro-Ca. Similarly, one set from the GA_3-sprayed treatments was selected and foliar-sprayed with 1 mg/L GA_3. Additionally, one set from each control group of both varieties was selected and foliar-sprayed with either 100 mg/L Pro-Ca or 1 mg/L GA_3. Plants sprayed with distilled water served as the control. Each variety comprised 7 treatments, yielding a total of 14. The experimental materials and treatment conditions are presented in Table 1. The experiment was conducted using a completely randomized block design with three biological replicates per treatment.

Table 1
Plant materials, regulators, and treatment methods

Varieties	Treatments	Plant regulators	Stages
GH3	HS	Fresh Water	
	HSCa1	Pro-Ca	three-leaf-one-heart
	HSCa2		breakthrough
	HSCa3		three-leaf-one-heart+ breakthrough
	HSGA1	GA_3	three-leaf-one-heart
	HSGA2		breakthrough
	HSGA3		three-leaf-one-heart+ breakthrough
FL478	FS	Fresh Water	
	FSCa1	Pro-Ca	three-leaf-one-heart
	FSCa2		breakthrough
	FSCa3		three-leaf-one-heart+ breakthrough
	FSGA1	GA_3	three-leaf-one-heart
	FSGA2		breakthrough
	FSGA3		three-leaf-one-heart+ breakthrough

2.2 Determination of Yield Components

The experiment was conducted from June 2024 to August 2025 in an outdoor cultivation field at the Coastal Agricultural College of Guangdong Ocean University. The tested rice varieties were Guanghong 3 (GH3) and FL478, both moderately salt-tolerant, and the regulators used were Pro-Ca and GA_3. Both the plant varieties and regulators were provided by the Coastal Agricultural College of Guangdong Ocean University.

2.3 Determination of Grain-Filling Dynamics

At the heading stage, 40 panicles that initiated flowering on the same day and exhibited essentially uniform growth were selected from each treatment. From anthesis to maturity, five panicles with similar growth were sampled every five days. The grains were oven-dried at 80°C for 72 h to a constant weight, hulled, and weighed to determine the dynamics of grain weight increase.

Following the method of Zhu et al.[14] The grain-filling process was fitted to the Richards equation, and the corresponding grain-filling characteristic parameters were calculated to analyze grain-filling traits. Using grain weight W (after hull removal) as the dependent variable and days after anthesis (with the day

of anthesis designated as 0) as the independent variable, the Richards equation was fitted by the nonlinear least squares method:

$W = A\ (1 + Be^{-kt})^{-1/N}$

where W is the grain weight at each stage (mg), A is the final growth amount (mg), t is the number of days after anthesis (d), and B, N, and K are equation parameters.

The specific calculation formulas for the grain-filling characteristic parameters were as follows:

Maximum grain-filling rate (mg grain^{-1} d^{-1}) = $AK/(1 + N)^{(N+1)/N}$

Mean grain-filling rate (mg grain^{-1} d^{-1}) = $AK/2(2 + N)$

The time to achieve the maximum grain-filling rate (d) = (lnB-lnN)/K

Effective grain-filling days (d) = $-(\ln((100/99)^N-1)/B)/K$

The calculation formulas for grain-filling parameters for the grain-filling early stage (0-t_1)、middle stage (t_2-t_1), and late stage (t_3-t_2):

$t_1=-(\ln((N^2 + 3N + N(N^2 + 6N + 5)^{1/2})/2B)/K$

$t_2=-(\ln((N^2 + 3N-N(N^2 + 6N + 5)^{1/2})/2B)/K$

$t_3=-(\ln((100/99)^N-1)/g)/K$

The mean grain-filling rate of early stage (mg grain^{-1} d^{-1}) = $(A(1 + Be^{-Kt1})^{-1/N})/t_1$

The mean grain-filling rate of middle stage (mg grain^{-1} d^{-1}) = $(A(1 + Be^{-Kt2})^{-1/N}-A(1 + Be^{-Kt1})^{-1/N})/(t_2-t_1)$

The mean grain-filling rate of early stage (mg grain^{-1} d^{-1}) = $(A(1 + Be^{-Kt3})^{-1/N}-A(1 + Be^{-Kt2})^{-1/N})/(t_3-t_2)$

2.4 Determination of Key Starch Synthesis Enzyme Activities in Grains

From five days after heading until full maturity, five panicles were sampled from each treatment every five days. The samples were immediately frozen in liquid nitrogen and stored at − 80°C. The activities of ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), and granule-bound starch synthase (GBSS) in grains were determined according to the method of Cheng et al.[15], while starch branching enzyme (SBE) activity was measured following the method of Li et al.[16].

2.5 Determination of Non-Structural Carbohydrate Contents in Rice Grains

The fructose content in rice grains was determined according to the method of Du et al.[17]. Soluble sugar content was measured according to the method described by Du et al.[18], and sucrose content was determined according to the method of Du et al.[19]. Starch content in grains was measured using the method of Kuai et al.[20].

2.6 Determination of Key Sucrose Metabolism Enzyme Activities

The activities of AI/NI (EC 3.2.1.26) were measured according to the method of Mirajkar et al.[21]. The activities of SPS (EC 2.4.1.14) and SS (EC 2.4.1.13) were determined based on the methods of Wongmetha et al.[22] and Baxter et al.[23].

All data were processed using Microsoft Excel 2019 software. Results are expressed as the mean ± standard error of the mean (SEM). The presence of different lowercase letters indicates statistically significant differences ($p < 0.05$). Statistical analysis was performed using IBM SPSS Statistics 26.0 software (SPSS Inc., Chicago, IL, USA) and one-way analysis of variance (ANOVA). Graphs and charts were generated using Origin 2021 (OriginLab, Northampton, MA, USA) and Microsoft PowerPoint 2016 software.

3. Result

3.1 Effects of Pro-Ca and GA_3 Application on Yield Components in Rice under Salt Stress

Both Pro-Ca and GA_3 treatments alleviated the adverse effects of salt stress on panicle morphology in GH3 and FL478. Compared with the S treatment, the SCa1, SCa2, and SCa3 treatments increased panicle length in GH3 by 2.35%, 5.65%, and 9.94%, respectively (Fig. 1A), and increased panicle weight by 0.95%, 14.04%, and 28.07%, respectively (Fig. 1C). Similarly, the SGA1, SGA2, and SGA3 treatments increased panicle length in GH3 by 1.7%, 12.23%, and 14.11%, respectively (Fig. 1A), and increased panicle weight by 3%, 9.46%, and 10.53%, respectively (Fig. 1C). Compared with the S treatment, the SCa1, SCa2, and SCa3 treatments increased panicle length in FL478 by 3.56%, 6.84%, and 9.78%, respectively (**Figure. 1B**), and increased panicle weight by 7.43%, 25.45%, and 37.45%, respectively (**Figure. 1D**). Similarly, the SGA1, SGA2, and SGA3 treatments increased panicle length in FL478 by 5.37%, 11.24%, and 15.15%, respectively (Fig. 1B), and increased panicle weight by 3.64%, 8.52%, and 13.51%, respectively (**Figure. 1D**).

Likewise, compared with the S treatment, both SCa and SGA treatments significantly increased the number of primary branches (Fig. 2B, E) and secondary branches (Fig. 2C, F) in the panicles of GH3 and FL478. Notably, in contrast, compared with the S treatment, SCa treatment significantly increased the effective panicle number in both GH3 and FL478, whereas SGA treatment significantly decreased the

effective panicle number in both varieties. In GH3, compared with the S treatment, the SCa1, SCa2, and SCa3 treatments increased the effective panicle number by 12.38%, 19.05%, and 25.71%, respectively (**Figure. 2A**), whereas the SGA1, SGA2, and SGA3 treatments decreased it by 6.67%, 12.38%, and 10.48%, respectively (**Figure. 2A**). In FL478, compared with the S treatment, the SCa1, SCa2, and SCa3 treatments increased the effective panicle number by 14.29%, 28.57%, and 33.93%, respectively (**Figure. 2D**), whereas the SGA1, SGA2, and SGA3 treatments decreased it by 6.25%, 12.5%, and 17.86%, respectively (**Figure. 2D**).

Similarly, differences were observed in the regulation of rice yield between Pro-Ca and GA3. Both Pro-Ca and GA_3 treatments increased grains per panicle and 1000-grain weight in GH3 and FL478 under salt stress; however, their regulatory effects on seed-setting rate were opposite. Compared with the S treatment, the SCa1, SCa2, and SCa3 treatments increased grains per panicle in GH3 by 6.3%, 13.05%, and 19.1%, respectively (Fig. 3A), increased seed-setting rate by 3.35%, 7.7%, and 11.18%, respectively (**Figure. 3B**), increased 1000-grain weight by 2.55%, 5.63%, and 9.04%, respectively (Fig. 3C), and increased yield by 15.5%, 35.13%, and 54.72%, respectively (Fig. 3D). In contrast, under the SGA1, SGA2, and SGA3 treatments, grains per panicle in GH3 increased by 3.86%, 14.79%, and 17.43%, respectively (Fig. 3A), seed-setting rate decreased by 2.11%, 6.46%, and 9.81%, respectively (Fig. 3B), 1000-grain weight increased by 0.03%, 4.02%, and 4.43%, respectively (Fig. 3C), and yield increased by 1.38%, 4.09%, and 2.66%, respectively (Fig. 3D).

Compared with the S treatment, the SCa1, SCa2, and SCa3 treatments increased the number of grains per panicle in FL478 by 8.45%, 18.38%, and 28.72%, respectively (Fig. 3E), and increased the seed-setting rate by 4.42%, 8.59%, and 12.64%, respectively (Fig. 3F), and increased the 1000-grain weight by 3.68%, 7.36%, and 11.82%, respectively (Fig. 3G), and increased the yield by 23.87%, 61.6%, and 92.68%, respectively (Fig. 3H). Under the SGA1, SGA2, and SGA3 treatments, the grains per panicle in FL478 increased by 5.27%, 20.81%, and 24.73%, respectively (Fig. 3E), the seed-setting rate decreased by 4.42%, 13.5%, and 17.55%, respectively (Fig. 3F), and the 1000-grain weight increased by 1.23%, 4.5%, and 4.91%, respectively (Fig. 3G), and the yield decreased by 6.59%, 10.16%, and 15.75%, respectively (Fig. 3H).

3.2 Effects of Pro-Ca and GA_3 Application on Grain-Filling Dynamics in Rice under Salt Stress

Using days after heading as the independent variable and the corresponding grain weight as the dependent variable, the grain-filling processes of GH3 and FL478 rice panicles under salt stress and Pro-Ca and GA_3 treatments were fitted and analyzed. The optimal fitting curves for each treatment are presented in Table 2. As shown in Table 2, the grain-filling processes of GH3 and FL478 under salt stress and Pro-Ca and GA_3 treatments were all appropriately fitted by the Richards equation, with coefficients of determination (R^2) generally around 0.950 (Table 2).

Table 2
Fitting Equations for Grain Filling of Rice under Salt Stress after Foliar Application of Pro-Ca and GA_3

Treatments	Parameter				Simulated equation	
	A	B	K	N		
HS	25.38	12.86	0.245	1.032	$Y = 25.38(1 + 12.86e^{-0.245X})^{-1/1.032}$	R^2=0.976
HSCa1	26.88	12.06	0.243	0.978	$Y = 26.88(1 + 12.06e^{-0.243X})^{-1/0.978}$	R^2=0.982
HSCa2	27.97	11.24	0.244	0.926	$Y = 27.97(1 + 11.24e^{-0.244X})^{-1/0.926}$	R^2=0.982
HSCa3	28.65	9.84	0.244	0.857	$Y = 28.65(1 + 9.84e^{-0.244X})^{-1/0.857}$	R^2=0.982
HSGA1	26.21	6.89	0.251	0.739	$Y = 26.21(1 + 6.89e^{-0.251X})^{-1/0.739}$	R^2=0.951
HSGA2	26.79	14.03	0.291	0.997	$Y = 26.79(1 + 14.03e^{-0.291X})^{-1/0.997}$	R^2=0.931
HSGA3	27.06	18.81	0.310	1.129	$Y = 27.06(1 + 18.81e^{-0.310X})^{-1/1.129}$	R^2=0.913
FS	24.14	13.38	0.246	1.062	$Y = 24.14(1 + 13.38e^{-0.246X})^{-1/1.062}$	R^2=0.976
FSCa1	27.04	8.54	0.224	0.845	$Y = 27.04(1 + 8.54e^{-0.224X})^{-1/0.845}$	R^2=0.985
FSCa2	28.80	8.16	0.232	0.783	$Y = 28.80(1 + 8.16e^{-0.232X})^{-1/0.783}$	R^2=0.982
FSCa3	30.32	6.37	0.228	0.670	$Y = 30.32(1 + 6.37e^{-0.228X})^{-1/0.670}$	R^2=0.983
FSGA1	25.47	16.60	0.305	1.085	$Y = 25.47(1 + 16.60e^{-0.305X})^{-1/1.085}$	R^2=0.938
FSGA2	26.49	35.62	0.355	1.390	$Y = 26.49(1 + 35.62e^{-0.355X})^{-1/1.390}$	R^2=0.942
FSGA3	27.00	54.98	0.389	1.583	$Y = 27.00(1 + 54.98e^{-0.389X})^{-1/1.583}$	R^2=0.910

Regarding the grain-filling characteristic parameters, both Pro-Ca and GA3 treatments increased the maximum and mean grain-filling rates in GH3 and FL478. For instance, the maximum grain-filling rates (mg grain-1 d-1) of the HSCa3 and HSGA3 treatments were 1.828 and 2.017, representing increases of 18.78% and 31.06%, respectively, compared with the HS treatment (Table 3); the mean grain-filling rates (mg grain-1 d-1) were 1.223 and 1.340, representing increases of 19.32% and 30.73%, respectively (Table 3). The maximum grain-filling rates of the FSCa3 and FSGA3 treatments were 1.925 and 2.233, representing increases of 32.12% and 53.26%, respectively, compared with the FS treatment (Table 3); the mean grain-filling rates were 1.294 and 1.466, representing increases of 33.4% and 51.13%, respectively (Table 3).

Compared with salt stress alone, Pro-Ca treatment did not significantly affect the time to reach the maximum grain-filling rate in GH3 and FL478, nor did it significantly alter the effective grain-filling period. However, GA3 treatment significantly advanced the time to reach the maximum grain-filling rate and

significantly shortened the effective grain-filling period in both varieties. For example, under HSGA3 treatment, the time to reach the maximum grain-filling rate was 9.1 d and the effective grain-filling period was 20.19 d, representing reductions of 11.65% and 18.42%, respectively, compared with the HS treatment (Table 3). Under FSGA3 treatment, the time to reach the maximum grain-filling rate was 9.1 d and the effective grain-filling period was 18.42 d, representing reductions of 11.65% and 25.99%, respectively, compared with the FS treatment (Table 3).

Table 3
Effects of Foliar Application of Pro-Ca and GA_3 on Grain-Filling Characteristic Parameters of Rice under Salt Stress

Treaments	**Grain-filling parameters**			
	Maximum grain-filling rate (mg grain^{-1} d^{-1})	**Mean grain-filling rate (mg grain^{-1} d^{-1})**	**The time to achieve the maximum grain-filling rate(d)**	**Effective grain-filling days(d)**
HS	1.539	1.025	10.3	24.75
HSCa1	1.644	1.097	10.3	24.51
HSCa2	1.746	1.166	10.2	23.98
HSCa3	1.828	1.223	10.0	23.42
HSGA1	1.789	1.201	8.9	21.82
HSGA2	1.951	1.301	9.1	20.60
HSGA3	2.017	1.340	9.1	20.19
FS	1.457	0.970	10.3	24.89
FSCa1	1.590	1.064	10.3	25.40
FSCa2	1.790	1.200	10.1	23.99
FSCa3	1.925	1.294	9.9	23.42
FSGA1	1.893	1.259	8.9	20.23
FSGA2	2.102	1.387	9.1	19.10
FSGA3	2.233	1.466	9.1	18.42

In terms of the early, middle, and late stages of the grain-filling process, the regulatory effects of Pro-Ca and GA3 treatments on GH3 and FL478 showed that both treatments enhanced the mean grain-filling rate and amount across all three stages in both varieties. However, from the middle stage onward, the duration of grain filling under the GA3 treatment decreased markedly compared with the S treatment. For

example, under the HSGA3 treatment, the durations of the middle and late grain-filling stages were 9.8 d and 10.5 d, representing reductions of 18.3% and 21.05%, respectively, compared with the HS treatment (Table 4). Under the FSGA3 treatment, the durations of the middle and late grain-filling stages were 8.6 d and 8 d, representing reductions of 28.93% and 39.85%, respectively, compared with the FS treatment (Table 4).

Table 4
Effects of Foliar Application of Pro-Ca and GA_3 on the Early, Middle, and Late Stages of Grain Filling in Rice under Salt Stress

Treaments	Early stage			Middle stage			Last stage		
	Days(d)	MGR (mg $grain^{-1}$ d^{-1})	GFA (mg)	Days(d)	MGR (mg $grain^{-1}$ d^{-1})	GFA (mg)	Days	MGR (mg $grain^{-1}$ d^{-1})	GFA (mg)
HS	3.7	1.175	4.303	12.0	1.309	15.769	13.3	0.379	5.055
HSCa1	3.8	1.177	4.427	12.0	1.398	16.733	13.5	0.403	5.453
HSCa2	3.8	1.181	4.473	11.8	1.484	17.440	13.5	0.427	5.777
HSCa3	3.7	1.186	4.398	11.5	1.554	17.901	13.6	0.445	6.064
HSGA1	3.0	1.233	3.728	10.8	1.520	16.424	13.4	0.433	5.792
HSGA2	3.6	1.252	4.458	10.0	1.658	16.665	11.3	0.479	5.399
HSGA3	3.7	1.310	4.819	9.8	1.716	16.750	10.5	0.499	5.218
FS	3.6	1.145	4.157	12.1	1.239	14.981	13.3	0.359	4.759
FSCa1	3.5	1.178	4.120	12.5	1.351	16.899	14.8	0.387	5.747
FSCa2	3.6	1.157	4.218	11.8	1.521	18.028	14.4	0.434	6.261
FSCa3	3.6	1.148	4.109	11.6	1.636	19.026	14.8	0.464	6.879
FSGA1	3.5	1.258	4.438	9.8	1.610	15.794	10.7	0.467	4.983
FSGA2	4.1	1.298	5.301	9.1	1.790	16.216	8.9	0.527	4.707
FSGA3	4.3	1.356	5.817	8.6	1.902	16.376	8.0	0.565	4.534

MGR: Mean grain-filling rate; GFA: Grain filling amount

3.3 Effects of Pro-Ca and GA_3 Application on Non-Structural Carbohydrate Contents in Rice Grains under Salt Stress

During the grain-filling process of GH3 and FL478 under salt stress, fructose content continuously decreased, while sucrose and soluble sugar contents initially increased before declining, and starch

content steadily increased. The regulatory effects of Pro-Ca and GA3 treatments on non-structural carbohydrate contents in grains differed significantly. Compared with salt stress alone, Pro-Ca treatment significantly reduced fructose content and increased sucrose, soluble sugar, and starch contents in rice grains under salt stress. In contrast, GA_3 treatment significantly increased fructose content but significantly decreased sucrose content. Furthermore, although GA_3 treatment also significantly increased soluble sugar and starch content in rice under salt stress, the magnitude of the increase was notably smaller than that observed under Pro-Ca treatment.

Under salt stress, fructose content in grains of GH3 and FL478 gradually decreased during the grain-filling process. SCa treatment significantly reduced fructose content in rice grains under salt stress. At 30 days after heading, compared with the HS treatment, the HSCa1, HSCa2, and HSCa3 treatments decreased fructose content by 22.6%, 25.97%, and 32%, respectively (Fig. 4A); compared with the FS treatment, the FSCa1, FSCa2, and FSCa3 treatments decreased fructose content by 26.5%, 51.6%, and 62.12%, respectively (Fig. 5A). In contrast, SGA treatment significantly increased fructose content in rice grains under salt stress. At 30 days after heading, compared with the HS treatment, the HSGA1, HSGA2, and HSGA3 treatments increased fructose content by 20.82%, 32%, and 39.99%, respectively (Fig. 4A); compared with the FS treatment, the FSGA1, FSGA2, and FSGA3 treatments increased fructose content by 27.17%, 38.7%, and 40.47%, respectively (Fig. 5A).

The sucrose and soluble sugar contents in GH3 and FL478 under salt stress showed an initial increase followed by a decrease, both peaking at 15 days after heading. SCa treatment significantly increased the sucrose content in rice grains under salt stress. At 15 days after heading, compared with the HS treatment, the HSCa1, HSCa2, and HSCa3 treatments increased sucrose content by 26.89%, 47.6%, and 64.56%, respectively (Fig. 4B); compared with the FS treatment, the FSCa1, FSCa2, and FSCa3 treatments increased sucrose content by 29.08%, 61.07%, and 83.61%, respectively (Fig. 5B). In contrast, SGA treatment significantly decreased sucrose content in rice grains under salt stress. At 15 days after heading, compared with the HS treatment, the HSGA1, HSGA2, and HSGA3 treatments decreased sucrose content by 8.4%, 19.49%, and 28.77%, respectively (Fig. 4B); compared with the FS treatment, the FSGA1, FSGA2, and FSGA3 treatments decreased sucrose content by 25.95%, 42.07%, and 53.87%, respectively (Fig. 5B). Regarding soluble sugars, both SCa and SGA treatments increased their content under salt stress. At 15 days after heading, compared with the HS treatment, the HSCa1, HSCa2, and HSCa3 treatments increased soluble sugar content by 10.03%, 19.37%, and 30.83%, respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments increased it by 3.36%, 7.36%, and 8.7%, respectively (Fig. 4C). Compared with the FS treatment, the FSCa1, FSCa2, and FSCa3 treatments increased soluble sugar content by 14.64%, 31.52%, and 42.42%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments increased it by 10.91%, 14.55%, and 18.18%, respectively (Fig. 5C).

In contrast to the changes in fructose, the starch content in grains of GH3 and FL478 under salt stress gradually increased during the grain-filling process. Both SCa and SGA treatments significantly increased starch content in rice grains under salt stress. At 30 days after heading, compared with the HS treatment, the HSCa1, HSCa2, and HSCa3 treatments increased starch content by 8.27%, 13.71%, and 18.51%,

respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments increased it by 0.26%, 3.54%, and 2.82%, respectively (Fig. 4D). Compared with the FS treatment, the FSCa1, FSCa2, and FSCa3 treatments increased starch content by 12.9%, 24.56%, and 31.84%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments increased it by 1.25%, 2.7%, and 4.16%, respectively (Fig. 5D).

3.4 Effects of Pro-Ca and GA $_3$ Application on the Activities of Key Sucrose Metabolism Enzymes in Rice Grains under Salt Stress

During the grain-filling process of GH3 and FL478 under salt stress, the activities of the key sucrose metabolism enzymes AI, NI, SS, and SPS all exhibited an initial increase, followed by a decline, with AI and NI activities peaking 10 days after heading and SS and SPS activities peaking 15 days after heading. However, compared with salt stress alone, Pro-Ca and GA_3 treatments exhibited distinctly different regulatory patterns in rice. Compared with the S treatment, SCa treatment significantly reduced the activities of AI and NI and significantly increased the activities of SS and SPS in the grains of GH3 and FL478 (Figs. 6 and 7). In contrast, SGA treatment significantly increased the activities of AI and NI and significantly decreased the activities of SS and SPS (Figs. 6, 7).

Similar to the regulatory patterns observed for the key enzymes of sucrose metabolism, the regulation of AMS and SP activities by Pro-Ca and GA3 treatments differed markedly. Under salt stress, the activities of AMS and SP in rice grains initially increased, then decreased, peaking at 15 days after heading. Compared with the S treatment, the SCa treatment significantly decreased AMS activity and increased SP activity, whereas the SGA treatment had the opposite effect, significantly increasing AMS activity while decreasing SP activity.

Compared with the HS treatment, at 10 days after heading, the HSCa1, HSCa2, and HSCa3 treatments significantly decreased AI activity by 11.63%, 22.16%, and 32.24%, respectively. In contrast, the HSGA1, HSGA2, and HSGA3 treatments significantly increased AI activity by 27.7%, 44.31%, and 55.4%, respectively (Fig. 6A). Similarly, at 10 days after heading, the HSCa1, HSCa2, and HSCa3 treatments significantly decreased NI activity by 12.26%, 22.99%, and 34.48%, respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments significantly increased NI activity by 26.05%, 41.38%, and 52.93%, respectively (Fig. 6B).

Compared with HS treatment, at 15 days after heading, HSCa1, HSCa2, and HSCa3 treatments significantly increased SS activity by 19.45%, 39.9%, and 59.35%, respectively, whereas HSGA1, HSGA2, and HSGA3 treatments significantly decreased SS activity by 4.99%, 10.47%, and 12.97%, respectively (Fig. 6C). Similarly, at 15 days after heading, the HSCa1, HSCa2, and HSCa3 treatments significantly increased SPS activity by 16.37%, 35.09%, and 51.46%, respectively. In contrast, the HSGA1, HSGA2, and HSGA3 treatments significantly decreased SPS activity by 5.85%, 12.7%, and 17.54%, respectively (Fig. 6D).

Compared with HS treatment, at 15 days after heading, HSCa1, HSCa2, and HSCa3 treatments significantly decreased AMS activity by 16.97, 29.48, and 45.37%, respectively, whereas HSGA1, HSGA2,

and HSGA3 treatments significantly increased AMS activity by 29.04, 49.97, and 57.92%, respectively (Fig. 6E). The HSCa1, HSCa2, and HSCa3 treatments significantly increased SP activity by 10.03%, 20.07%, and 30.1%, respectively. In contrast, the HSGA1, HSGA2, and HSGA3 treatments significantly decreased SP activity by 6.02%, 13.38%, and 19.4%, respectively (Fig. 6F).

Similarly, in FL478, compared with the FS treatment, at 10 days after heading, the FSCa1, FSCa2, and FSCa3 treatments significantly decreased AI activity by 10.03%, 21.11%, and 31.66%, respectively. In contrast, the FSGA1, FSGA2, and FSGA3 treatments significantly increased AI activity by 47.49%, 69.13%, and 84.96%, respectively (Fig. 7A). At 10 days after heading, the FSCa1, FSCa2, and FSCa3 treatments significantly decreased NI activity by 13.62%, 24.37%, and 35.84%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments significantly increased NI activity by 43.44%, 64.52%, and 79.57%, respectively (Fig. 7B).

Compared with FS treatment, at 15 days after heading, the FSCa1, FSCa2, and FSCa3 treatments significantly increased SS activity by 19.95%, 40.92%, and 60.87%, respectively. In contrast, the FSGA1, FSGA2, and FSGA3 treatments significantly decreased SS activity by 10.74%, 17.9%, and 23.02%, respectively (Fig. 7C). Similarly, at 15 days after heading, the FSCa1, FSCa2, and FSCa3 treatments significantly increased SPS activity by 20.72%, 40.49%, and 58.9%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments significantly decreased SPS activity by 11.04%, 23.31%, and 33.13%, respectively (Fig. 7D).

Compared with the FS treatment, at 15 days after heading, the FSCa1, FSCa2, and FSCa3 treatments significantly decreased AMS activity by 23.93%, 39.32%, and 56.41%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments significantly increased AMS activity by 52.99%, 80.34%, and 97.44%, respectively (Fig. 7E). The FSCa1, FSCa2, and FSCa3 treatments significantly increased SP activity by 13.75%, 26.8%, and 41.24%, respectively. In contrast, the FSGA1, FSGA2, and FSGA3 treatments significantly decreased SP activity by 13.75%, 24.05%, and 35.05%, respectively (Fig. 7F).

3.5 Effects of Pro-Ca and GA_3 Application on the Activities of Key Starch Synthesis Enzymes in Rice Grains under Salt Stress

The results showed that, compared with salt stress alone, both Pro-Ca and GA3 enhanced the activities of key starch synthesis enzymes during grain filling in GH3 and FL478. Still, the two treatments differed markedly in their regulatory effects. The results demonstrated that among the key starch synthesis enzymes during rice grain filling, the activities of AGPase, SSS, GBSS, and SBE all peaked at 15–20 days after heading and then gradually declined. Compared with salt stress alone, enzyme activities under both Pro-Ca and GA_3 treatments were markedly enhanced during grain filling. However, compared with Pro-Ca treatment, enzyme activities under GA_3 treatment declined more rapidly after reaching peak levels.

Compared with the S treatment, the AGPase activity in GH3 under both SCa and SGA treatments peaked at 15 days after heading. The HSCa1, HSCa2, and HSCa3 treatments increased AGPase activity by 15.41%, 30.83%, and 46.63%, respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments

increased it by 23.51%, 38.54%, and 46.24%, respectively. Compared with the peak values, the AGPase activity of the HSCa1, HSCa2, and HSCa3 treatments at 30 days after heading decreased by 60.1%, 50.07%, and 47.43%, respectively, whereas that of the HSGA1, HSGA2, and HSGA3 treatments decreased by 78%, 75.1%, and 73.52%, respectively (Fig. 8A). Similarly, the AGPase activity in FL478 under both SCa and SGA treatments peaked at 15 days after heading. The FSCa1, FSCa2, and FSCa3 treatments increased AGPase activity by 24.45%, 48.1%, and 68.14%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments increased it by 40.08%, 60.12%, and 68.54%, respectively. Compared with the peak values, the AGPase activity of the FSCa1, FSCa2, and FSCa3 treatments at 30 days after heading decreased by 55.07%, 40.56%, and 33.13%, respectively, whereas that of the FSGA1, FSGA2, and FSGA3 treatments decreased by 82.98%, 80.1%, and 79.9%, respectively (Fig. 8E).

Similarly, the activities of SSS and SBE in both GH3 and FL478 peaked at 15 days after heading, whereas GBSS activity peaked at 20 days after heading. Compared with the S treatment, at 15 days after heading, the HSCa1, HSCa2, and HSCa3 treatments increased SSS activity by 14.98%, 29.89%, and 45.98%, respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments increased it by 22.22%, 38.31%, and 45.61%, respectively. Compared with the peak values, the SSS activities of the HSCa1, HSCa2, and HSCa3 treatments at 30 days after heading decreased by 60.35, 49.79, and 47.24%, respectively, whereas those of the HSGA1, HSGA2, and HSGA3 treatments decreased by 77.74, 74.79, and 73.93%, respectively (Fig. 8C). Compared with the peak values, the SSS activity of FSCa1, FSCa2, and FSCa3 treatments at 30 days after heading decreased by 56.43, 38.87, and 28.95%, respectively, whereas that of FSGA1, FSGA2, and FSGA3 treatments decreased by 84.79, 82.48, and 80.68%, respectively (Fig. 8G). Compared with the peak values, the SSS activities of the FSCa1, FSCa2, and FSCa3 treatments at 30 days after heading decreased by 56.43%, 38.87%, and 28.95%, respectively, whereas those of the FSGA1, FSGA2, and FSGA3 treatments decreased by 84.79%, 82.48%, and 80.68%, respectively (Fig. 8G).

Compared with the S treatment, at 15 days after heading, the HSCa1, HSCa2, and HSCa3 treatments increased SBE activity by 16.4%, 32.35%, and 46.01%, respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments increased it by 30.07%, 43.28%, and 47.84%, respectively. Compared with the peak values, the SBE activities of the HSCa1, HSCa2, and HSCa3 treatments at 30 days after heading decreased by 64.94%, 55.3%, and 51.54%, respectively, whereas those of the HSGA1, HSGA2, and HSGA3 treatments decreased by 80.91%, 77.9%, and 76.73%, respectively (Fig. 8D). At 15 days after heading, compared with the FS treatment, the FSCa1, FSCa2, and FSCa3 treatments increased SBE activity by 24.34%, 47.73%, and 69.69%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments increased it by 52.98%, 73.99%, and 79.24%, respectively. Compared with the peak values, the SBE activities of the FSCa1, FSCa2, and FSCa3 treatments at 30 days after heading decreased by 61.8%, 48.47%, and 41.05%, respectively, whereas those of the FSGA1, FSGA2, and FSGA3 treatments decreased by 84.24%, 83.68%, and 82.8%, respectively (Fig. 8H).

Compared with the S treatment, GBSS activity in GH3 peaked at 20 days after heading. The HSCa1, HSCa2, and HSCa3 treatments increased GBSS activity by 19.16%, 37.55%, and 52.87%, respectively, whereas the HSGA1, HSGA2, and HSGA3 treatments increased it by 10.73%, 26.05%, and 30.65%,

respectively. Compared with the peak values, the GBSS activity of the HSCa1, HSCa2, and HSCa3 treatments at 30 days after heading decreased by 35.37%, 24.51%, and 19.62%, respectively, whereas that of the HSGA1, HSGA2, and HSGA3 treatments decreased by 51.21%, 48.02%, and 47.51%, respectively (Fig. 8B). Similarly, the GBSS activity in FL478 peaked at 20 days after heading. The FSCa1, FSCa2, and FSCa3 treatments increased GBSS activity by 32.13%, 61.04%, and 84.96%, respectively, whereas the FSGA1, FSGA2, and FSGA3 treatments increased it by 28.92%, 44.18%, and 49%, respectively. Compared with the peak values, the GBSS activity of the FSCa1, FSCa2, and FSCa3 treatments at 30 days after heading decreased by 32.83%, 20.27%, and 15.1%, respectively, whereas that of the FSGA1, FSGA2, and FSGA3 treatments decreased by 62.31%, 58.17%, and 56.6%, respectively (Fig. 8F).

4 Discussion

4.1 Effects of Pro-Ca and GA $_3$ Application on Yield Components and Grain-Filling Dynamics in Rice under Salt Stress

Although both Pro-Ca and GA_3 alleviated the adverse effects of salt stress on yield formation in rice to some extent, marked differences were observed in their regulatory mechanisms and final yield outcomes. Pro-Ca treatment significantly increased yield by synergistically increasing effective panicle number, grains per panicle, seed-setting rate, and 1000-grain weight, whereas GA3 treatment reduced effective panicle number and seed-setting rate, despite increasing grains per panicle and 1000-grain weight, leading to yield reduction in FL478. This difference was closely associated with the regulatory patterns governing the grain-filling process. The core of grain filling lies in the balance between grain-filling rate and duration: Pro-Ca treatment enhanced the grain-filling rate while maintaining a stable effective grain-filling period, ensuring adequate grain filling. In contrast, GA_3 treatment significantly accelerated the grain-filling rate but shortened the effective grain-filling period by 18.42%–25.99%, exhibiting a “fast but unfilled” phenotype. In terms of stage-specific grain-filling characteristics, GA_3 treatment markedly reduced the duration of the middle and late grain-filling stages, with FGA3 shortening them by 28.93% and 39.85%, respectively, which may directly cause the decline in seed-setting rate. Previous studies have demonstrated that salt stress affects rice grain filling primarily by inhibiting the grain-filling rate and prolonging the time to reach the maximum grain-filling rate[24], and the regulation of grain-filling duration by exogenous regulators is a key determinant of grain weight. Pro-Ca ensured sustained grain filling by optimizing the efficiency of the carbon metabolic pathway and maintaining the stability of enzyme activities related to sucrose–starch metabolism[25].

In contrast, although GA_3 upregulated α-amylase gene expression to promote grain-filling initiation, it may have shortened the starch accumulation period by accelerating endosperm cell development or by promoting ethylene signal transduction [26, 27]. Furthermore, the promoting effect of GA_3 on shoot elongation may have intensified resource competition between vegetative and reproductive growth, resulting in a large “sink” but an insufficient “source,” and ultimately a decline in seed-setting rate [25, 28].

Taken together, Pro-Ca maintained a balance between grain-filling rate and duration, thereby ensuring grain plumpness and a stable seed-setting rate, and synergistically improving effective panicle number, grains per panicle, and 1000-grain weight to achieve stable, high yield under salt stress. In contrast, although GA_3 transiently enhanced grain-filling rate and single-grain weight potential, it disrupted coordination among yield components, resulting in a shortened grain-filling duration and reduced seed-setting rate, ultimately leading to a limited yield increase or even a yield reduction.

4.2 Effects of Pro-Ca and GA_3 Application on Non-Structural Carbohydrate Contents in Rice Grains under Salt Stress

The regulatory differences between Pro-Ca and GA_3 in the accumulation patterns of non-structural carbohydrates were primarily manifested in the divergent directions of carbon metabolic flux. The results revealed that Pro-Ca treatment exhibited a highly efficient conversion-type accumulation pattern characterized by “low fructose, high sucrose, and high starch,” whereas GA_3 treatment displayed a reducing sugar accumulation pattern featuring “high fructose, low sucrose, and a smaller increase in starch.” This difference was highly consistent with the direction of regulation exerted by the two regulators on key enzymes of sucrose metabolism. Liu et al.[25] confirmed that Pro-Ca treatment was significantly superior to GA_3 treatment in improving the efficiency of the sucrose metabolic pathway in rice seedlings, as evidenced by higher activities of sucrose synthase and starch phosphorylase, thereby promoting the conversion of sucrose to starch. Deng et al.[29] further demonstrated that Pro-Ca promoted sugar translocation from source organs (leaves) to sink organs (grains), increased grain sugar content, and enhanced grain starch synthesis by elevating starch phosphorylase activity. In contrast, under GA_3 treatment, sucrose degradation was enhanced while starch synthesis capacity was relatively insufficient, leading to the accumulation of reducing sugars such as fructose. Previous studies have indicated that although reducing sugar accumulation contributes to osmotic adjustment and energy supply under salt stress, it is not conducive to dry matter accumulation in sink organs[30]. Boriboonkaset et al.[31] reported that the salt-tolerant variety Pokkali maintained carbon energy reserves by enhancing soluble sugar and starch accumulation under salt stress, thereby ensuring yield formation. Huo et al.[32] also confirmed that reprogramming of carbon metabolic pathways is a key mechanism regulating salt tolerance. Furthermore, Yuan et al.[33] demonstrated that the efficiency of sucrose-to-starch conversion under salt stress directly affects plant growth adaptability and yield performance. Therefore, the regulatory mode of Pro-Ca in promoting efficient sucrose-to-starch conversion renders it superior to GA_3 treatment in sustaining grain filling and yield formation.

4.3 Effects of Pro-Ca and GA_3 Application on the Activities of Key Carbon Metabolism Enzymes in Rice Grains under Salt Stress

Sucrose metabolism and starch synthesis are two tightly linked processes during grain filling, and the changes in the activities of their key enzymes are closely associated with the direction of carbon flux partitioning and the efficiency of starch accumulation. This study revealed marked differences in the

regulatory patterns of Pro-Ca and GA_3 on the activities of sucrose–starch metabolic enzymes. Pro-Ca treatment significantly increased the activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS), while simultaneously reducing the activities of acid invertase (AI) and neutral invertase (NI), indicating that it promoted metabolic flux toward sucrose synthesis and starch production. In contrast, GA_3 treatment increased AI/NI activities and decreased SS/SPS activities, thereby shifting metabolic flux toward sucrose degradation and reducing sugar accumulation. These findings are consistent with those of Deng et al.[29], who reported that Pro-Ca promotes sugar translocation from source organs to grains by enhancing SS activity, thereby alleviating photosynthetic feedback inhibition. Molecular mechanism studies have revealed that GA signaling can regulate the nucleocytoplasmic distribution of sucrose synthase OsSUS1 by competing with SLR1 for binding to OsBURP3, thereby affecting sucrose content [34]. This provides a mechanistic explanation for the changes in sucrose metabolic enzyme activities observed under GA_3 treatment.

During starch synthesis, although both Pro-Ca and GA_3 enhanced the peak activities of key starch synthesis enzymes, such as AGPase, SSS, GBSS, and SBE, the two treatments differed significantly in their ability to sustain enzyme activity. Under Pro-Ca treatment, the activities of all these enzymes declined slowly after reaching their peaks, whereas under GA_3 treatment, enzyme activities decayed rapidly after peaking, with the extent of decline at 30 days after heading being 20%–40% greater than that observed under Pro-Ca treatment. As the rate-limiting enzyme in starch synthesis, the duration of the high-activity period of AGPase directly determines the sustained intensity of starch accumulation[35]. Pro-Ca ensured the continuous progression of starch synthesis by maintaining prolonged enzyme activity, consistent with the findings of Deng et al.[29] that Pro-Ca enhances starch phosphorylase activity and promotes grain filling. In contrast, although GA_3 transiently stimulated enzyme activities, the shortened grain-filling period was insufficient for starch accumulation, ultimately resulting in reduced grain plumpness. In summary, Pro-Ca constructed an efficient and sustained metabolic pathway from sucrose supply to starch accumulation by upregulating the activities of enzymes in the sucrose synthesis direction, inhibiting those in the decomposition direction, and prolonging the period of high activity of starch synthesis enzymes. Conversely, GA_3 led to low carbon accumulation efficiency due to a reversal of metabolic flux direction and insufficient capacity to maintain enzyme activity. These enzymatic differences provide direct evidence for the differential regulation of the two types of regulators during grain filling, characterized as “stable and full” versus “fast but unfilled,” respectively.

5. Conclusion

This study systematically compared the regulatory effects of foliar-applied prohexadione-calcium (Pro-Ca) and gibberellin (GA_3) on carbon metabolism and the grain-filling process in rice under salt stress. The results demonstrated that Pro-Ca and GA_3 influenced yield formation through distinctly different mechanisms. Pro-Ca achieved significant yield enhancement by synergistically increasing effective panicle number, grains per panicle, seed-setting rate, and 1000-grain weight. In contrast, GA_3 increased

grains per panicle and 1000-grain weight at the expense of reduced effective panicle number and seed-setting rate, resulting in either a limited yield increase or even a yield reduction. Regarding grain-filling regulation, Pro-Ca moderately enhanced the grain-filling rate while maintaining a stable grain-filling duration, thereby ensuring adequate grain filling. GA_3 significantly accelerated the grain-filling rate but shortened the effective grain-filling period, exhibiting a “fast but unfilled” phenotype. Carbon metabolism analysis revealed that Pro-Ca promoted efficient conversion of sucrose to starch by increasing the activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) while reducing the activities of acid invertase (AI) and neutral invertase (NI), and maintained a prolonged period of high activity for key starch synthesis enzymes (AGPase, SSS, GBSS, SBE).

In contrast, GA_3 treatment resulted in the retention of sugars as reducing sugars and a rapid decline in starch synthesis enzyme activities after peaking. In conclusion, Pro-Ca achieved stable, high yields in rice under salt stress by optimizing carbon metabolic flux and maintaining a balance between grain-filling rate and duration. These findings suggest that Pro-Ca has considerable potential for application in rice production on saline-alkaline soils, with combined applications at the three-leaf-one-heart and rupture stages proving the most effective.

Abbreviations

GH3
Guanghong 3
GA_3
gibberellin
Pro-Ca
prohexadione-calcium
MGR
Mean grain-filling rate
GFA
Grain filling amount
AI
acid invertase
NI
neutral Invertase
ADPase
ADP-glucose pyrophosphorylase
GBSS
granule-bound starch synthase
SSS
soluble starch synthase
SBE

starch branching enzyme

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Clinical trial

Not applicable.

Funding

This research is supported by Guangdong Provincial Department of Agriculture and Rural Affairs (2024KJ31), Guangdong Provincial Department of Education General Higher Education Key Area Special Project (2021ZDZX4027), Guangdong Provincial General Universities Innovation Team Project (2021KCXTD011).

Author Contribution

Conceptualization, N.F. and D.Z.; formal analysis and investigation, Q.Z.; data curation, Q.Z.; writing—original draft preparation, Q.Z.; writing—review and editing, Q.Z., N.F. and D.Z.; visualization, Q.Z. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Riaz M, Arif MS, Ashraf MA, et al. A Comprehensive Review on Rice Responses and Tolerance to Salt Stress[M]//Advances in Rice Research for Abiotic Stress Tolerance. Elsevier; 2019. pp. 133–58.
2. Pires IS, Negrão S, Oliveira MM, et al. Comprehensive phenotypic analysis of rice (*Oryza sativa* L.) response to salinity stress[J]. Physiol Plant. 2015;155(1):43–54.
3. Thitisaksakul M, Tananuwong K, Shoemaker CF, et al. Effects of Timing and Severity of Salinity Stress on Rice (*Oryza sativa* L.) Yield, Grain Composition, and Starch Functionality[J]. J Agric Food Chem. 2015;63(8):2296–304.
4. Li Q, Zhu P, Yu X, et al. Physiological and Molecular Mechanisms of Rice Tolerance to Salt and Drought Stress: Advances and Future Directions[J]. Int J Mol Sci. 2024;25(17):9404.
5. Quan S. Advances in Physiological and Genetic Research on Saline-Alkali Tolerance in Rice[J]. South Agricultural Mach. 2020;51(8):39.
6. Huanhe W, Xubin Z, Jialin G, et al. Effects of salinity stress on spikelets formation and grains filling in rice (*Oryza sativa* L.) [J]. Acta Agron Sinica. 2021;47(12):2471–80.
7. Jialiu Z, Zhongqi Y. Screening of Saline-Alkali Tolerant Rice Varieties in the Saline-Alkali Land of Zhoushan[J]. J Zhejiang Agricultural Sci. 2021;62(10):1913–5.
8. Jialiu Z, Guocan F. Effects of Salt Stress at Different Growth Stages on Yield Traits of Rice[J]. J Zhejiang Agricultural Sci. 2021;62(7):1299–300.
9. Xiangran Z, Jian W, Lin J, et al. Expression Profiling of Rice RNase T2 Proteins in Leaves and Seedlings under Salt-stressed Conditions[J]. J Nuclear Agricultural Sci. 2013;27(6):743–9.
10. Fu Y, Zhengwei L, Zhichun W. Effect of Soda Saline-sodic Stress on the Panicle Traits and Yield Components of Rice Variety Changbai 9[J]. Acta Agriculturae Boreali-Sinica. 2010;25(S2):59–61.
11. Farooq M, Khan MA, Zhao DD, et al. Extrinsic role of gibberellin mitigating salinity effect in different rice genotypes[J]. Front Plant Sci. 2022;13:1041181.
12. Zhang R, Zheng D, Feng N, et al. Prohexadione calcium enhances rice growth and tillering under NaCl stress[J]. PeerJ. 2023;11:e14804.
13. Zuo G, Huo J, Yang X, et al. Photosynthetic mechanisms underlying NaCl-induced salinity tolerance in rice (*Oryza sativa* L.) [J]. BMC Plant Biol. 2024;24(1):41.
14. Qisen Z, Xianzu C, Yiqi L. Growth analysis on the process of grain filling in rice[J]. Acta Agron Sinica. 1988;14(3):182–93.
15. Cheng F, Jiang D, Wu P, et al. The Dynamic Change of Starch Synthesis Enzymes during the Grain Filling Stage and Effects of Temperature upon It[J]. Acta Agron Sinica. 2001;27(2):201–6.
16. li T, Shen B, Chen N, et al. Effect of Q-Enzyme on the Chalkiness Formation of Rice Grain[J]. Acta Agron Sinica. 1997;23(3):338–44.

17. Du J, Shu S, An Y, et al. Influence of exogenous spermidine on carbon–nitrogen metabolism under $Ca(NO_3)_2$ stress in cucumber root[J]. Plant Growth Regul. 2017;81(1):103–15.
18. Xu W, Cui K, Xu A et al. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings[J]. Acta Physiol Plant, 2015, 37(2).
19. Du Y, Zhao Q, Chen L, et al. Effect of drought stress on sugar metabolism in leaves and roots of soybean seedlings[J]. Plant Physiol Biochem. 2020;146:1–12.
20. Kuai J, Liu Z, Wang Y, et al. Waterlogging during flowering and boll forming stages affects sucrose metabolism in the leaves subtending the cotton boll and its relationship with boll weight[J]. Plant Sci. 2014;223:79–98.
21. Mirajkar SJ, Suprasanna P, Vaidya ER. Spatial distribution and dynamics of sucrose metabolising enzymes in radiation induced mutants of sugarcane[J]. Plant Physiol Biochem. 2016;100:85–93.
22. Wongmetha O, Ke LS, Liang YS. The changes in physical, bio-chemical, physiological characteristics and enzyme activities of mango cv. Jinhwang during fruit growth and development[J]. NJAS: Wageningen J Life Sci, 2015, 72–3(1): 7–12.
23. Baxter CJ. Elevated sucrose-phosphate synthase activity in transgenic tobacco sustains photosynthesis in older leaves and alters development[J]. J Exp Bot. 2003;54(389):1813–20.
24. Wei H, Zuo B, Zhu J, et al. Grain-Filling Characteristics and Yield Formation of Rice at Saline Field[J]. Agronomy. 2024;14(11):2687.
25. Liu M, Feng N, Zheng D, et al. Comparative study of the mechanisms underlying the effects of prohexadione-calcium and gibberellin on the morphogenesis and carbon metabolism of rice seedlings under NaCl stress[J]. Plants. 2025;14(8):1240.
26. Guo L, Zhang C, Yu H, et al. The Role of Phytohormones in Alleviating Salt Stress in Rice[J]. Phyton. 2024;93(12):3131–49.
27. Liu S, Zhang R, Hussain S, et al. research progress in alleviating effects of exogenous substances on salt stress in rice[J]. Chin J Rice Sci. 2023;37(1):1–15.
28. Ikram M, Batool M, Ullah M, et al. Molecular alchemy: converting stress into resilience via secondary metabolites and calcium signaling in rice[J]. Rice. 2025;18(1):32–58.
29. Deng R, Zheng D, Feng N, et al. Prohexadione Calcium Improves Rice Yield Under Salt Stress by Regulating Source–Sink Relationships During the Filling Period[J]. Plants. 2025;14(2):211.
30. Zhu Y, Guo J, Feng R, et al. The regulatory role of silicon on carbohydrate metabolism in *Cucumis sativus* L. under salt stress[J]. Plant Soil. 2016;406(1–2):231–49.
31. Boriboonkaset T, Theerawitaya C, Yamada N, et al. Regulation of some carbohydrate metabolism-related genes, starch and soluble sugar contents, photosynthetic activities and yield attributes of two contrasting rice genotypes subjected to salt stress[J]. Protoplasma. 2013;250(5):1157–67.
32. Huo J, Yu M, Feng N, et al. Integrated transcriptome and metabolome analysis of salinity tolerance in response to foliar application of choline chloride in rice (*Oryza sativa* L.) [J]. Front Plant Sci. 2024;15:1440663.

33. Yuan Y, Zhong M, Shu S, et al. Effects of exogenous putrescine on leaf anatomy and carbohydrate metabolism in cucumber (*Cucumis sativus* L.) under salt stress[J]. J Plant Growth Regul. 2015;34(3):451–64.
34. Huang J, Xie B, Xian F, et al. Gibberellin signalling mediates nucleocytoplasmic trafficking of Sucrose Synthase 1 to regulate the drought tolerance in rice[J]. Plant Biotechnol J. 2025;23(6):1909–26.
35. Jiang H, Xu X, Sun A, et al. Silicon nutrition improves the quality and yield of rice under dry cultivation[J]. J Sci Food Agric. 2024;104(4):1897–908.

Figures

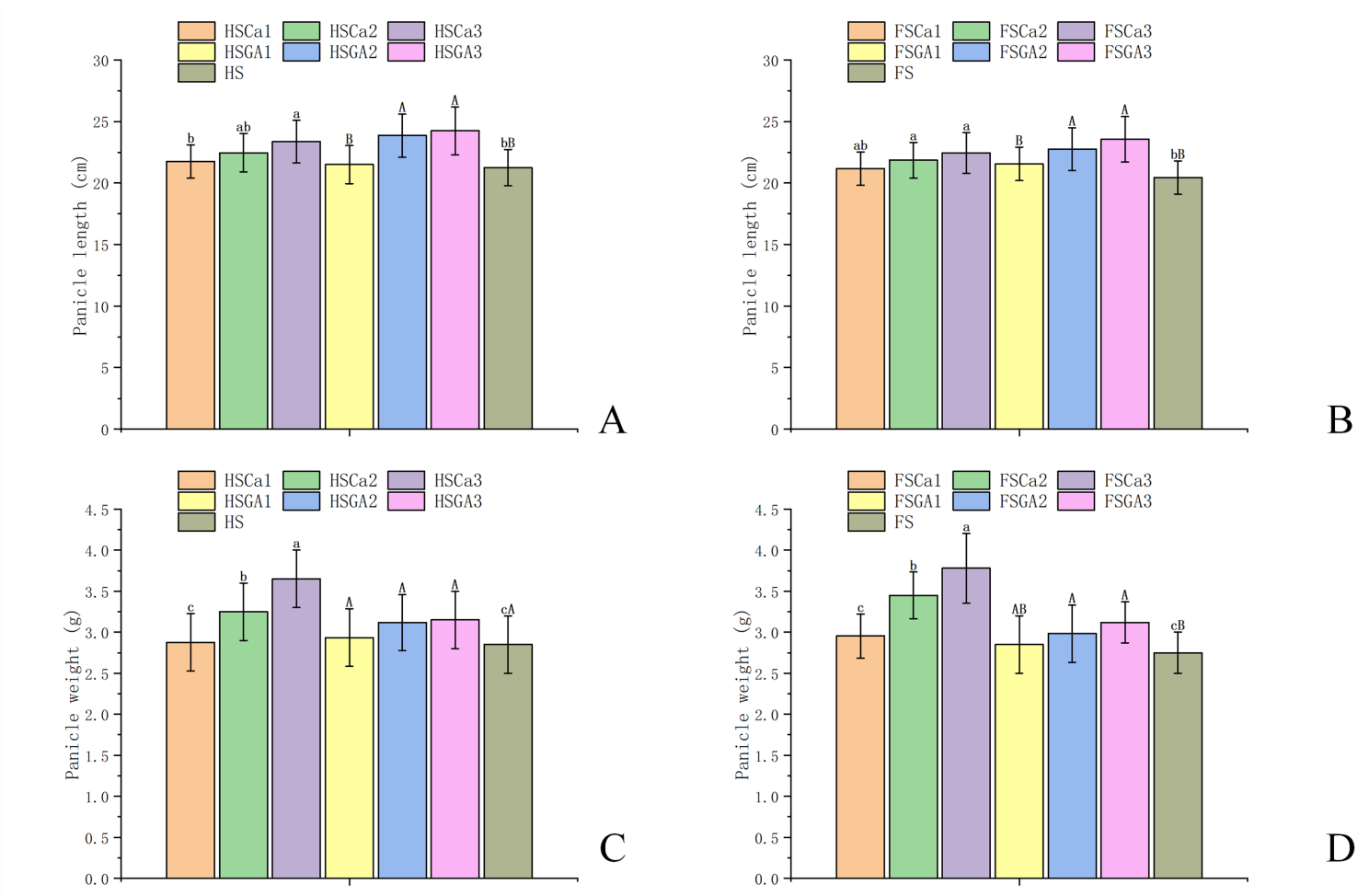


Figure 1

Effects of foliar application of Pro-Ca and GA3 on panicle length and panicle weight in rice under salt stress. Different lowercase letters indicate significant differences among Pro-Ca treatments at different stages, and different uppercase letters indicate significant differences among GA_3 treatments at different stages ($P < 0.05$). Same below.

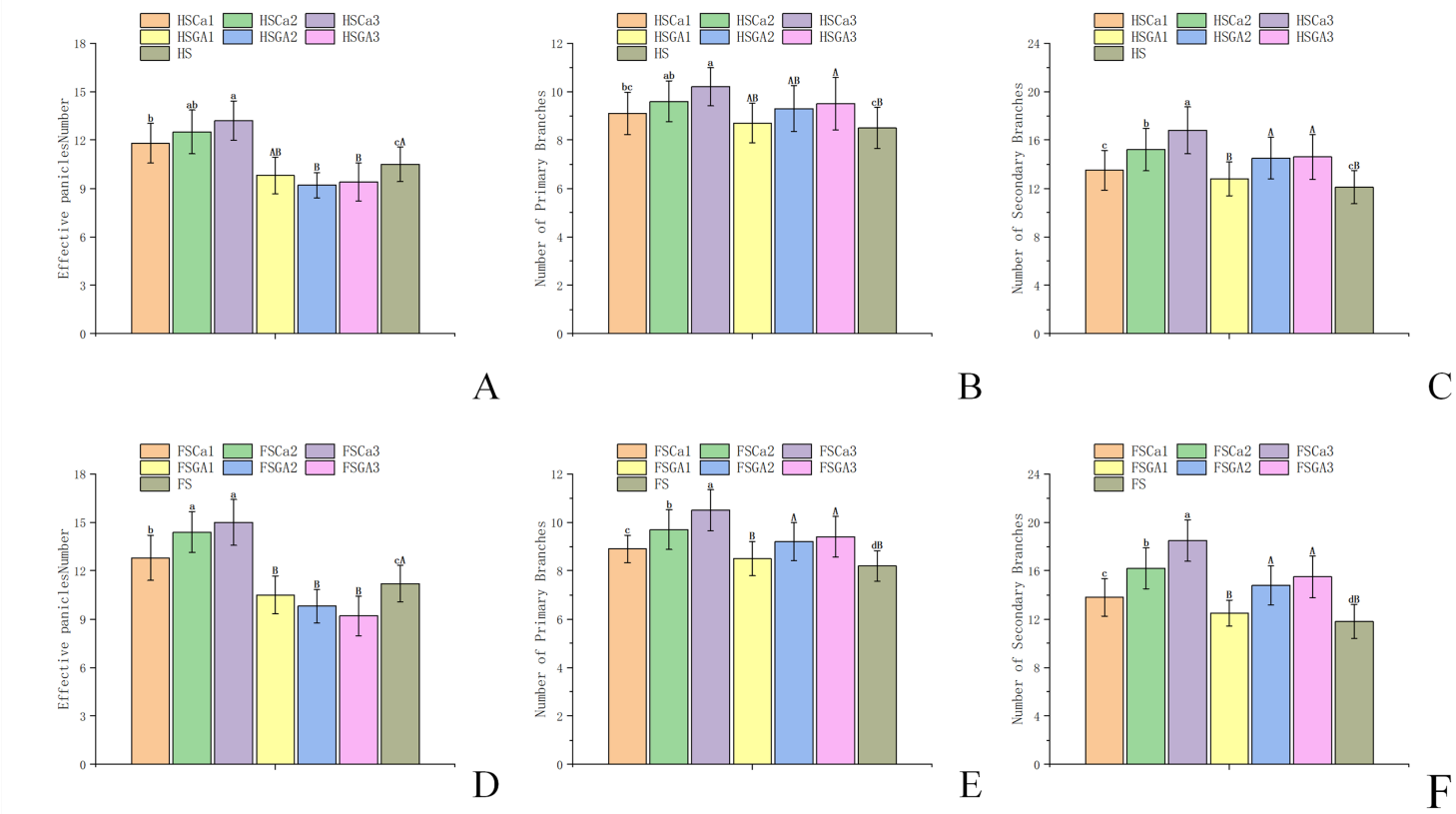


Figure 2

Effects of Foliar Application of Pro-Ca and GA3 on Effective Panicle Number, Primary Branch Number, and Secondary Branch Number in Rice under Salt Stress

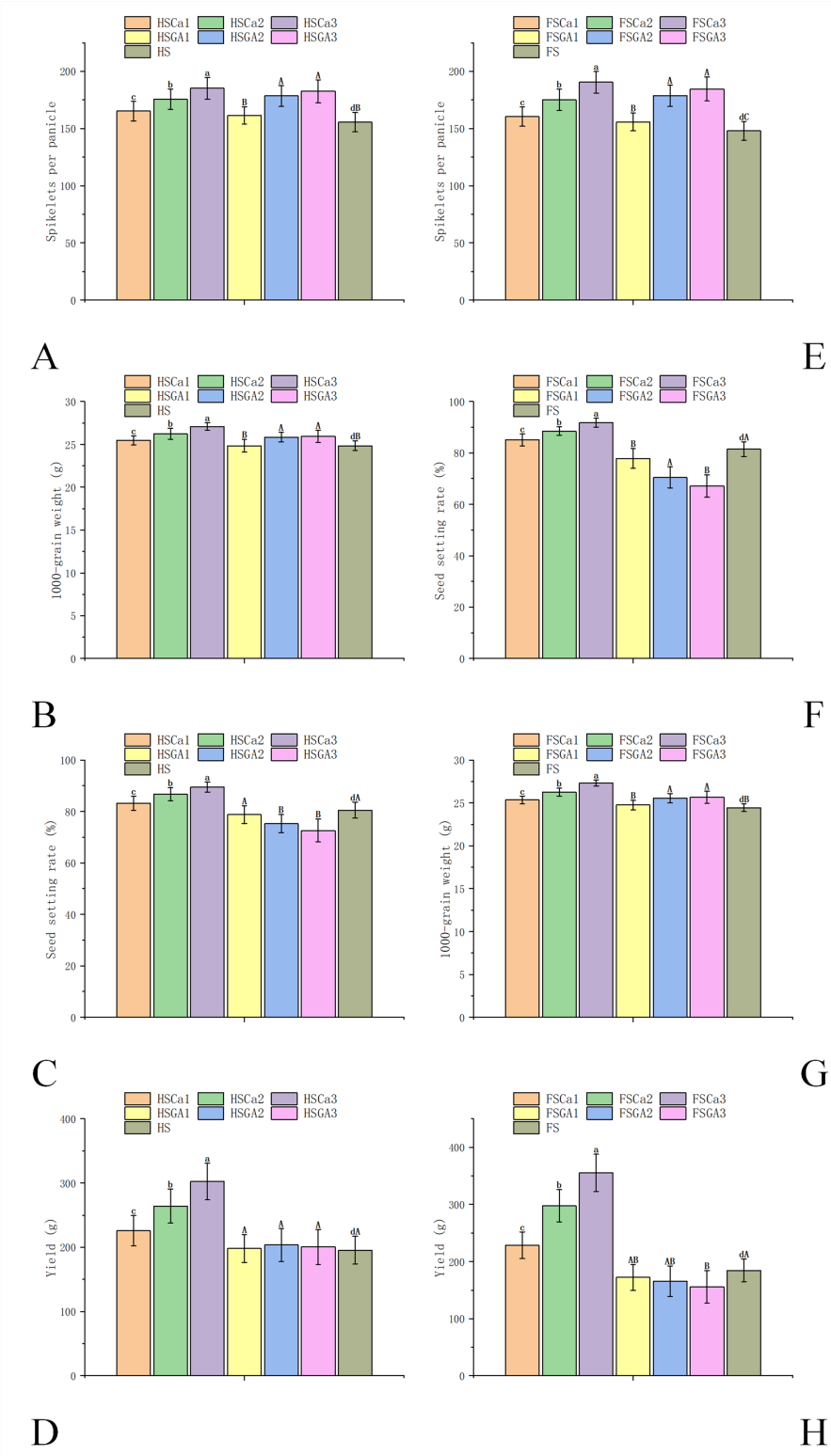


Figure 3

Effects of Foliar Application of Pro-Ca and GA3 on Grains per Panicle, Seed-Setting Rate, 1000-Grain Weight, and Yield in Rice under Salt Stress

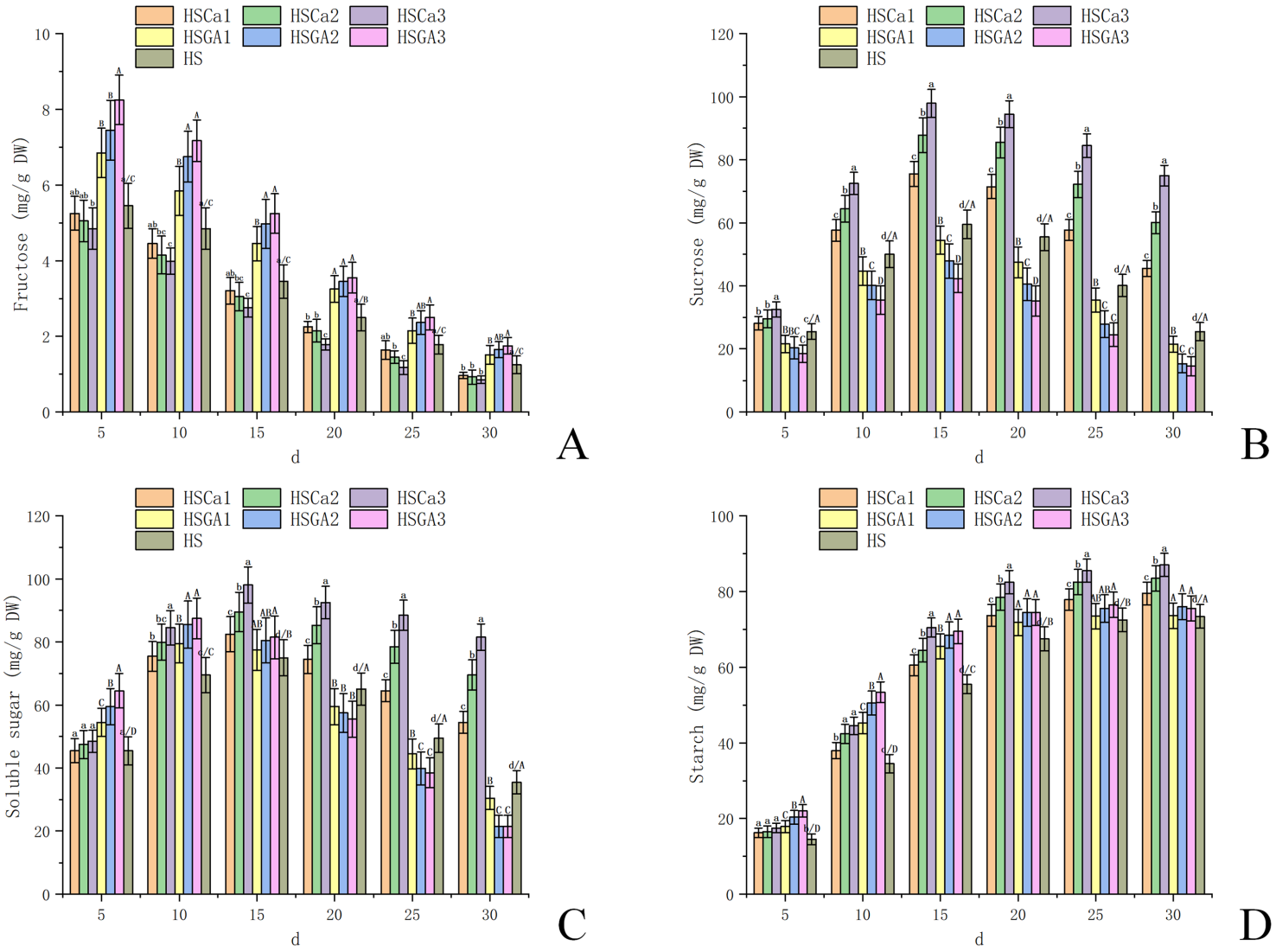


Figure 4

Effects of Foliar Application of Pro-Ca and GA_3 on the Contents of Fructose, Sucrose, Soluble Sugar, and Starch in Grains of GH3 under Salt Stress

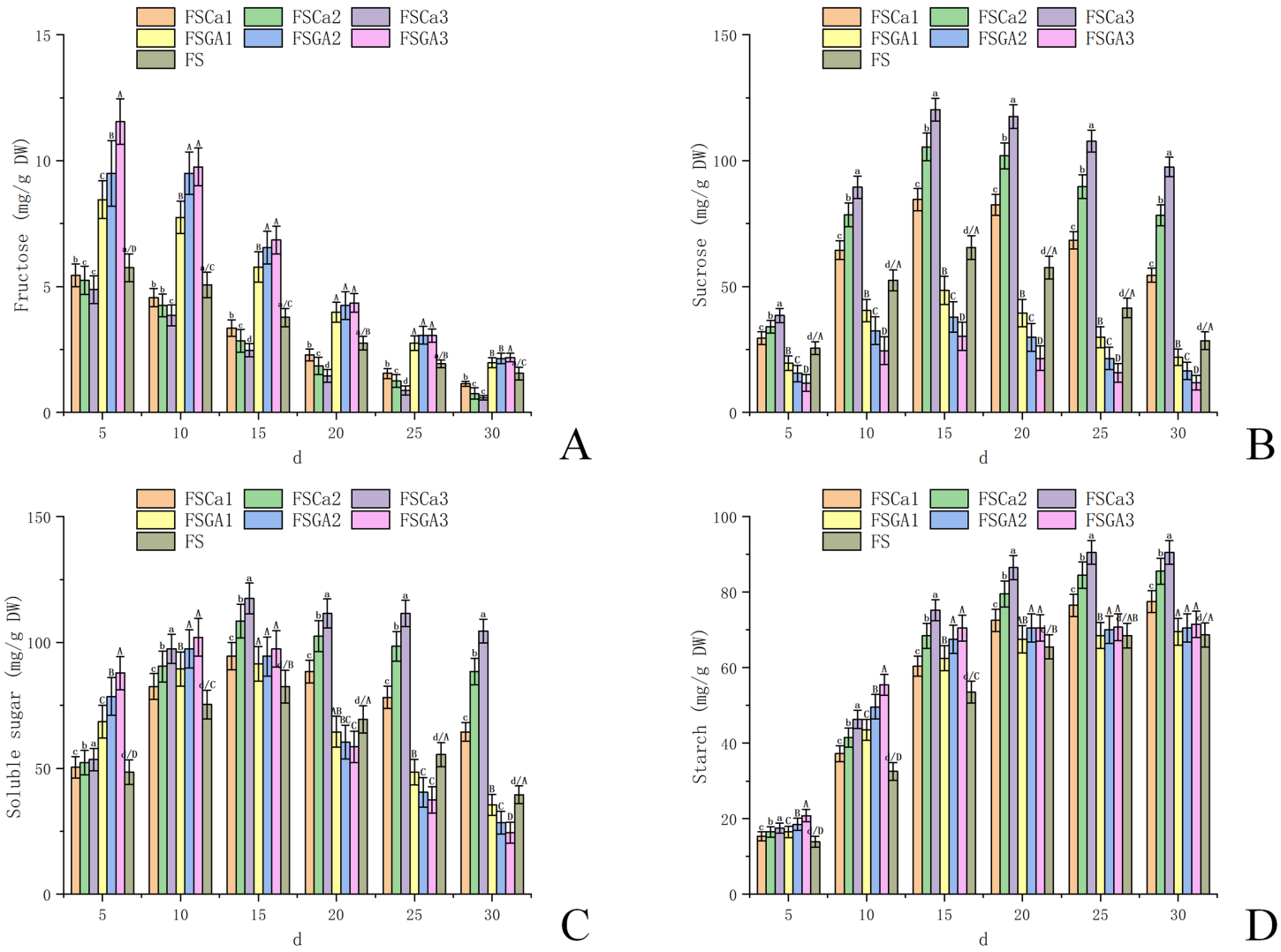


Figure 5

Effects of Foliar Application of Pro-Ca and GA_3 on the Contents of Fructose, Sucrose, Soluble Sugar, and Starch in Grains of FL478 under Salt Stress

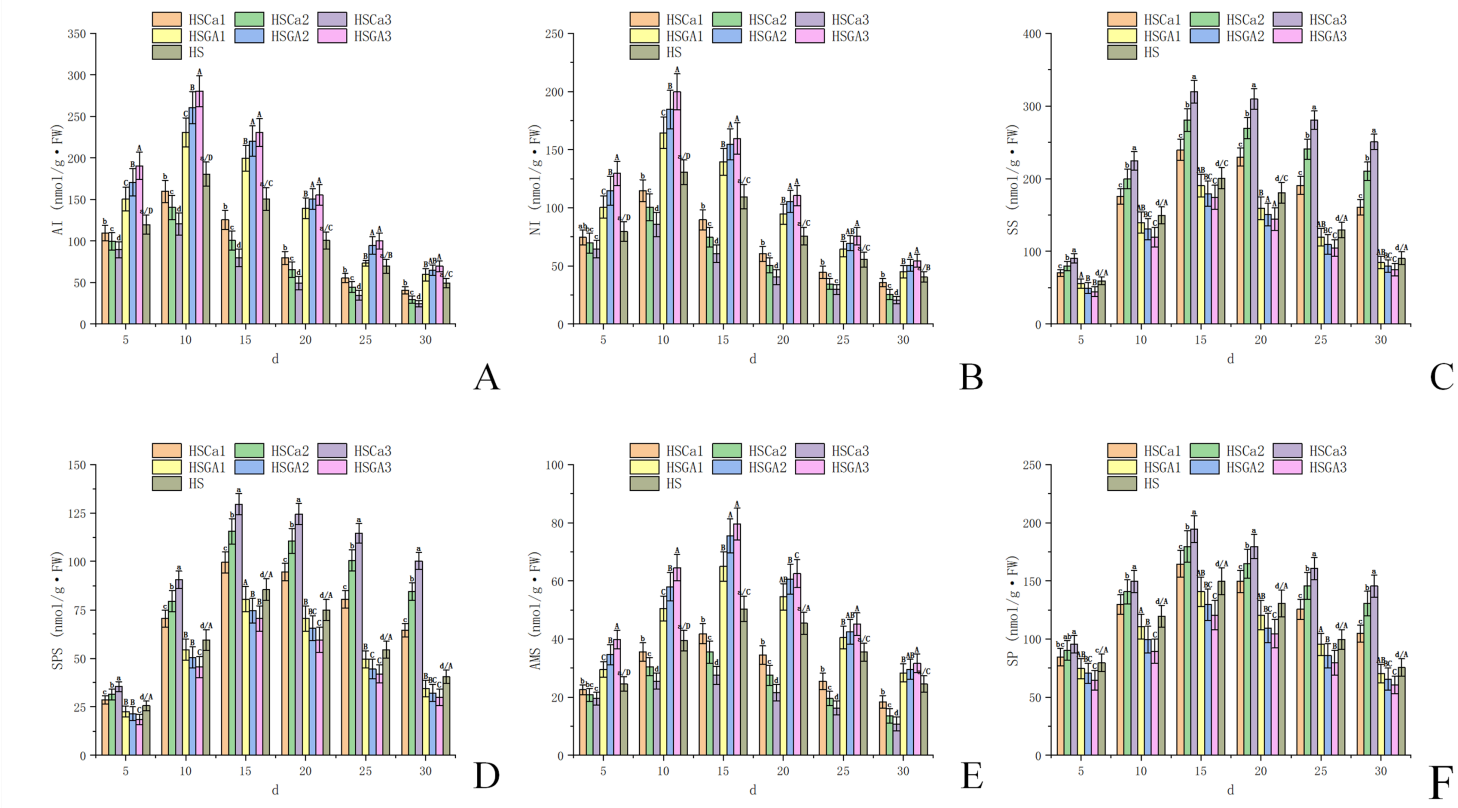


Figure 6

Effects of Foliar Application of Pro-Ca and GA_3 on the Activities of AI, NI, SS, SPS, AMS, and SP in Grains of GH3 under Salt Stress

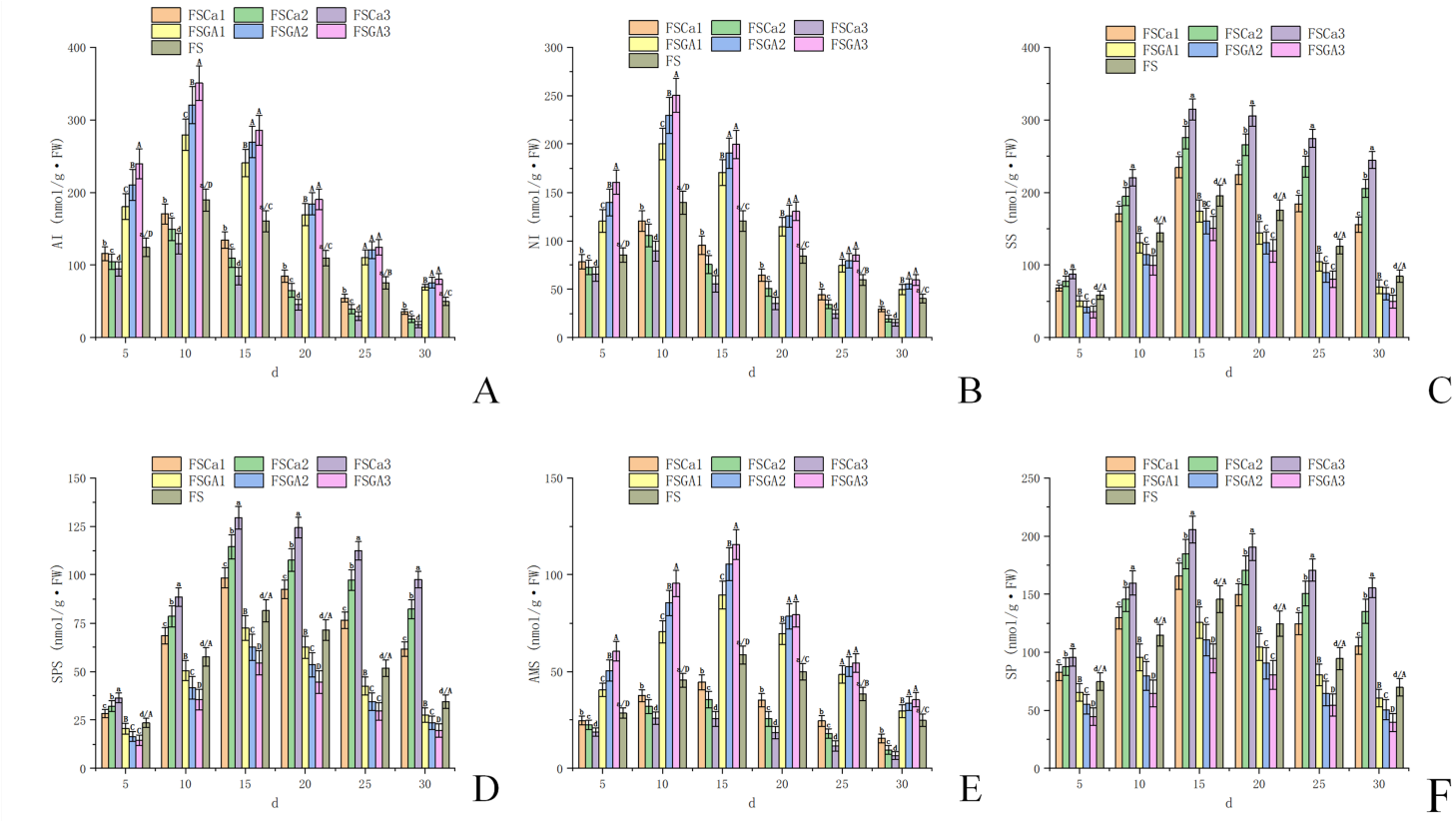


Figure 7

Effects of Foliar Application of Pro-Ca and GA_3 on the Activities of AI, NI, SS, SPS, AMS, and SP in Grains of FL478 under Salt Stress

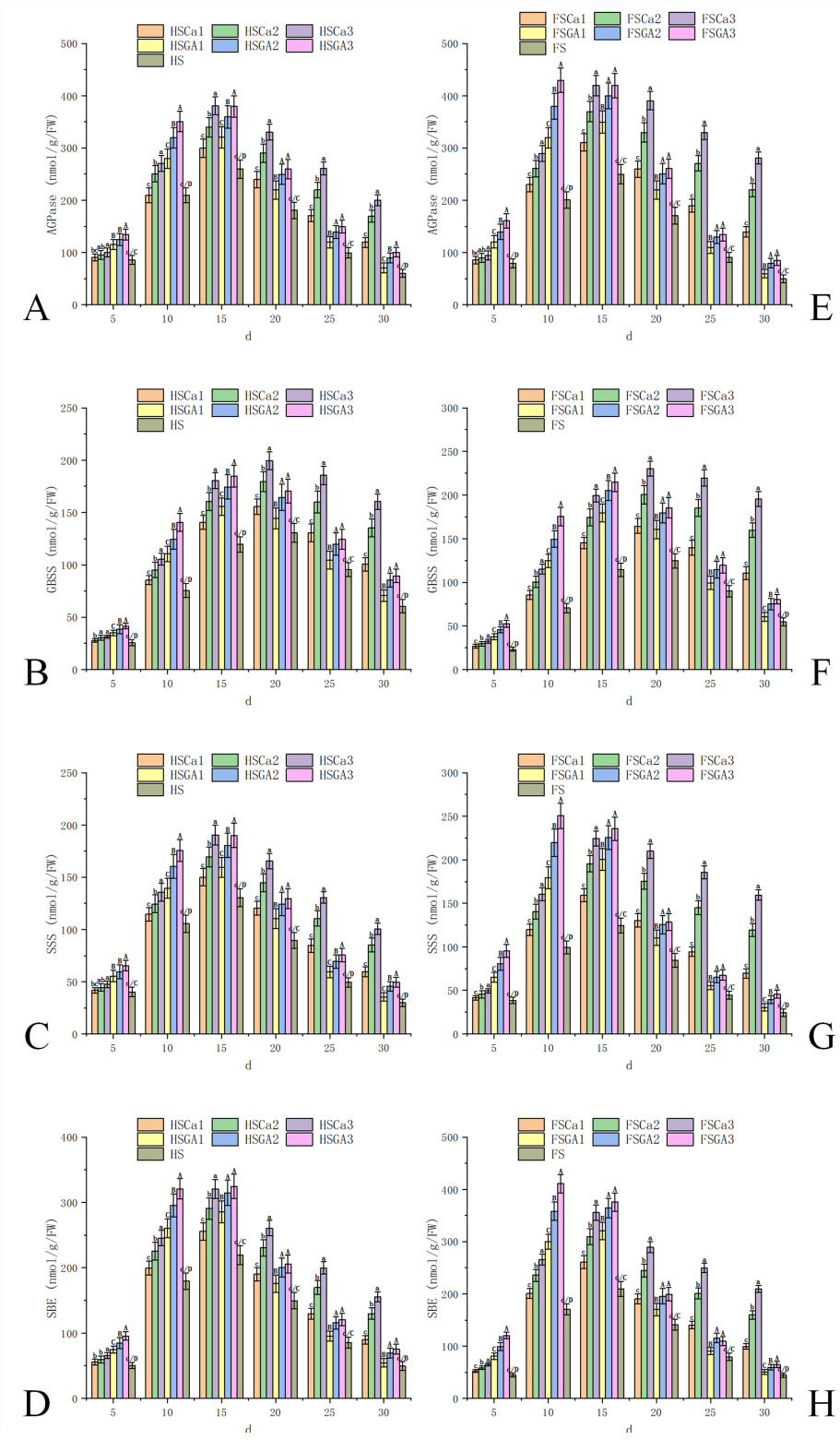


Figure 8

Effects of Foliar Application of Pro-Ca and GA_3 on the Activities of ADPase, GBSS, SSS, and SBE in Rice Grains under Salt Stress

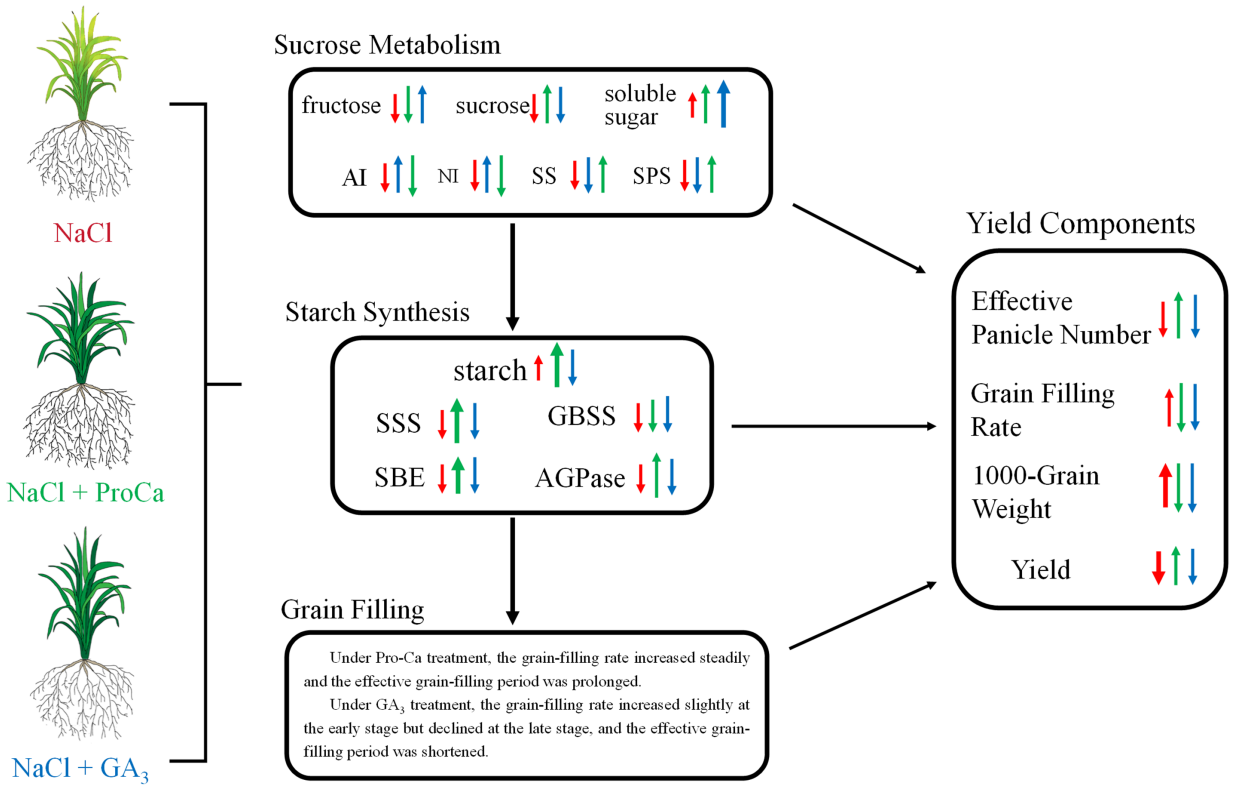


Figure 9

Comparative Analysis of the Regulation of Grain Filling and Physiological Metabolism by Foliar-Applied Pro-Ca and GA_3 in Rice under Salt Stress. Red arrows indicate the effects of NaCl stress; Green arrows represent the effects of prohexadione-calcium (Pro-Ca) + NaCl treatment; Blue arrows represent the effects of gibberellin (GA_3) + NaCl treatment. The size of the arrows indicates the strength of promoting or inhibitory effects of different treatments on various parameters of rice seedlings.