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RESEARCH PAPERS

The Physiological Basis of Genotypic Variations in Low-Oxygen Stress Tolerance in the Vegetable Sweet Potato

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Abstract—Waterlogging caused by rainfall or improper irrigation is a serious threat that limits the growth and yield of crop plants by hypoxia stress. In the current study, the seedlings of vegetable sweet potato (*Ipomoea batatas* (L.) Lam.) variety 'NC1' (hypoxia-tolerant) and 'C211' (hypoxia-sensitive) were treated with oxygen content of 2 mg/L. It was found that the growth rate, net photosynthetic rate (P_N), transpiration rate (E), stomatal limitation (L_s), maximum net photosynthetic rate (A_{max}), diurnal respiratory rate (R_d), maximum Rubisco carboxylation rate (Vc_{max}), maximum electron transport rate (J_{max}), ABA (abscisic acid) and IAA (indole-3-acetic acid) content were significantly higher in 'NC1' than those of 'C211'. It is concluded that under hypoxia stress, tolerant genotype could maintain higher photosynthetic efficiency by maintaining higher stomatal conductance and Rubisco efficiency, to maintain the growth of the root system and the canopy. This study provides the basis for further dissection of the mechanism of low-oxygen stress in sweet potato.

Keywords: *Ipomoea batatas*, hypoxia stress, photosynthesis, chlorophyll fluorescence, leaf water potential

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INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is considered a staple crop in many developing countries, mainly due to its high adaptation to low fertility soils and its nutritional and health-related values [1]. Both sweet potato roots and leaves are edible with protein contents in the leaves and roots range from 4.0 to 27.0% and 1.0 to 9.0%, respectively, in addition to higher contents of several anthocyanin types that are beneficial to human health [2, 3].

Sweet potato is more susceptible to waterlogged than drought condition. Early study reported that sweet potato plants were reduced about 30 and 70% of yield in the dry weight of roots and shoots, respectively, under waterlogging for 7 days [4]. Sweet potato is frequently cultivated in lowlands in tropical and subtropical regions. The crop water demand is around 406 mm/crop season, whereas the rainfall recorded in a crop region for each season crop ranged from 450 to 690 mm or even more than 1200 mm annually, representing a threatful conditions for farmers cultivating sweet potato. In addition, plants grown under heavy rainfall conditions produce higher number of tubers with small size and low quality categorized as unmarketable tuber [5]. Compared to the harvested underground sweet potato varieties, vegetable sweet potato is widely cultivated in areas of high rainfall, such as Southeast Asia. In addition, vegetable sweet potato needs a large amount of water during its growth period, and irrigation usually causes waterlogging stress in uneven lands.

Water stress caused by excess, or deficit have increased in agricultural regions over the past 50 years. Both flooding and waterlogging (WL) affects around 17 million km^2 of land surface and 10–12% of crop area is estimated to undergo water excess conditions [6]. Waterlogging effects are harmful to plants, mainly due to oxygen lack in the soil pores, in addition to the reduced nitrogen availability [7]. Modern crop technology such as heavy traffic and puddling usually induces waterlogging through soil structure modifications. The damage of plants caused by waterlogging mainly comes from the rapid depletion of oxygen (hypoxia or anoxic stress), rapid carbohydrate accumulation, nutrient deficiencies, and accumulation of toxic secondary metabolites [8]. Hence, effects in

plants are perceived such as, root respiration reduction, increase root damage and decrease root growth, water and nutrient uptake is limited whereon triggering chlorosis, poor growth, and yield and even death. Waterlogged plants show a light-induced damage associated with stomatal closure, associated with stomatal closure, abscisic acid production, ethylene, and reactive oxygen species [9]. Photosynthesis parameters, including CO_2 exchange have been reported to be reduced in waterlogged plants, such as, rice [10], barley [11] and cotton [12]. The factors affecting the photosynthetic rate could be mainly divided into two types, stomatal and nonstomatal inhibition [13]. The deficiency of CO_2 is the rate-limiting factor for photosynthesis because of stomatal closure, and then an imbalance between photochemical and biochemical reaction rates in the leaves. Other photosynthesis parameters such as net photosynthetic rate (P_N), photosynthetically active radiation (PAR) and gas exchange are common variables taken into consideration when assessing photosynthesis [14]. The relation between P_N and PAR are essential for evaluating photosynthesis and plant environment. Assimilation rate (A) and intracellular CO_2 content C_i yield A/C_i curves draws comprehension insight about the photosynthetic performance. In addition, waterlogged plants show damage in photosynthetic apparatus which result in lower ratio of variable to maximal fluorescence (F_v/F_m) [15]. Decreasing the F_v/F_m indicates that there is either reversible photoprotective downregulation or an irreversible inactivation of photosystem II (PSII) during waterlogging. In soybean flood stress reduced chlorophyll content by 18–34% [16].

Phytohormones are also involved in waterlogging response, since ethylene accumulation is a common factor under waterlogging conditions it is suggested that ethylene plays an important role [17]. Besides, waterlogging triggers the expression of *SPEEDY HYPONASTIC GROWTH* transcription factor and thus, activates the expression of *ACC Oxidase5* and *ACS* genes, directly or indirectly, leading to increased ethylene biosynthesis. Exogenous ethylene applications in soybean can considerably induce adventitious roots initiation [16]. Moreover, tomato plants treated with auxin transport inhibitor NPA, failed to produce adventitious roots (ARs), similarly inhibition of ethylene synthesis or perception resulted in low levels of ARs production [18]. GA (gibberellic acid) is also involved in waterlogging response. *OsEIL1* binds to the promoter of *SEMIDWARF1* (SD1), which predominantly catalyzes the biosynthesis of GA, therefore, promoting the internode elongation process, suggesting the implication of GA in plant submergence response pathway [19]. Furthermore, hypoxia stress promotes the transportation and accumulation of IAA in the stem base portion [20]. Although, the precise role and mechanism of phytohormones remains ambiguous, insights about the role of several phytohormones in adventitious root production in sweet potato has been published [21]. Nevertheless, the combination of exogenous hormones or substance to improve waterlogging resistance is a hot topic in molecular biology that still need more research [22].

At present, only few studies focusing on the response of photosynthetic parameters on sweet potato under hypoxia stress have been published. Therefore, the current study aimed to analyze the dynamic response of photosynthetic related parameters, including light response curve, carbon dioxide response curve, stomatal morphology, chlorophyll fluorescence parameters and multiple phytohormones content to hypoxia stress among two sweet potato genotypes possess contrasting hypoxia stress tolerance phenotypes, to help understand the genetic basis and mechanisms and improving of waterlogging tolerance in sweet potato.

MATERIALS AND METHODS

Plant materials and stress experiments. The seedlings of two vegetable sweet potato varieties, including 'C211' (identified as waterlogging sensitive) and 'NC1' (identified as waterlogging tolerant), were planted in pots (8 × 10 cm, diameter × height) filled with river soil in the greenhouse of Yangtze University (30°36′ N, 112°15′ E). Identical seedlings of 10-days old were transplanted into the Hoagland nutrient solution in hypoxia treatment plastic boxes (160 × 110 × 100 cm, length × width × height). Air pump was used to provide sweet potato with optimal aeration for 2 days to adapt to the hydroponic system. For the hypoxia treatment, the solution oxygen content was maintained at 1.5 mg/L with an automatic dissolved oxygen meter (Quantum, United States) by the discontinuous bubbling of N_2 gas. As control, the solution oxygen content was kept above 8.0 mg/L by an air pump [12]. The experiment was carried out in May, 2018. The day (16 h) temperature was 24°C d and the night (8 h) temperature was 22°C. The relative humidity (RH) was between 50–65%. The three-replicated completely randomized design (CRD) was applied, with 40 individual plants in each treatment of a replication.

Evaluation of morphological related characteristics. Morphological characteristics related to waterlogging stress were collected at 0, 2, 4, 6 and 8 d of hypoxia stress. A rectilinear scale was used to measure the stem length. Stem diameter was measured by a vernier caliper in the middle stem. The leaf area was recorded by ImageJ@1.51J8 from the photos with scale.

Leaf water potential and Leaf gas exchange. Leaf water potential was determined using a dew point water potential meter (Wescor, United States). Leaf water potential (ψ) was evaluated by a pressure chamber model (Pressure-volume (P–V)). Leaf gas exchange and photochemistry, including Net photosynthetic rate (P_N), stomatal conductance (g_s), inter-

cellular CO_2 concentration (C_i), transpiration rate (E) and the stomatal limitation value (L_s), were measured by a photosynthesis instrument (Li-6400, Li-COR, United States) coupled with a modulated fluorometer (6400-40 LCF, Li-COR) at 0, 2, 4, 6, and 8 d. The measurements were performed from 10:00 am to 1:00 pm at a photosynthetic photon flux density (PPFD) of 1500 μmol/m^2·s and air CO_2 concentration of 380 μmol/mol. For each plant, the last third mature leaves were used for data collection.

Stomatal morphology observation. Stomatal observation was carried out at 10:00–11:00 am at 0, 2, 3, 4, 5 and 6 d. The third leaf of each plant was used. First, the widest part of the leaf was cut off, and then the epidermis was carefully removed and put on a glass slide, covering with a coverslip. Then, it was observed and photographed under the microscope (ECLIPSE E600, Japan). The picture was imported into ImageJ software to measure stomatal area, stomatal density, stomatal aperture, stomatal length, stomatal width, and stomatal perimeter.

Determination of light-response curve. The light-response curves were created and recorded by LI-6400 photosynthesis measurement system (Li-COR), which was equipped with a 6400-02B Red Blue light source. The third fully expanded leaf was implemented to perform the measurements between 08:00 am and 11:00 am. The equipment was programmed to provide instantaneous photosynthetically active radiation (PAR) values of 400, 200, 100, 50, 25, 0, 600, 800, 1200, 1500 (μmol photon)/(cm^2 s) at a chamber temperature of 25°C and CO_2 concentration of 394.92 ± 2.69 μmol/mol.

CO_2 response curve. The leaves were induced by dark environment for more than 30 min until the photosynthetic rate and stomatal conductivity were relatively stable. The CO_2 concentration in the reference chamber was controlled by Li-6400 liquefied cylinder for 400, 300, 200, 50, 450, 600, 800, 1000, 1200 μmol/mol. Three leaves, from third leaf to sixth leaf, were measured for each plant. Curve order and O_2 exposure order were randomized to reduce bias. Chamber conditions were set up at flow rate of 600 μmol/s, fan speed of 10000 rpm, overpressure of 0.2 kPa, leaf temperature (T_{leaf}) of 25°C and a previously determined saturating irradiance of 1000 (μmol photons)/(m^2 s). Then, data were tested for Farquhar-von Caemmerer-Berry (FvCB) biochemical models.

Determination of chloroplast fluorescence parameters. Chloroplast fluorescence parameters (MINI-PAM-11/B, Germany) with a fluorescent leaf chamber was used according to the instrument manual. Before the test, the leaves were adapted to dark for 30 min to detect initial fluorescence value (F_0) and PSII maximum photochemical quantum yield (F_v/F_m). Then the photochemical active light PFD was set up to 1400 μmol m^2/s as a saturation pulse and an actinic light of 320 μmol m^2/s to test photochemical quenching (q_P), non-photochemical quenching (NPQ), non-photochemical quenching coefficient (q_N), PSII electron transfer rate (ETR) and effective quantum efficiency of photosystem II (ΦPSII) after the plants were treated for 30 min of light.

Determination of hormone content. Fresh leaf samples were ground using liquid nitrogen, weighed (0.2 g) and placed in a capped plastic tube. The samples were extracted with 1.0 mL mix of methanol : water : acetic acid (10 : 89 : 1, v/v/v), and overnight on a shaker at 4°C under darkness. Then, the samples were centrifuged for 10 min at 12000 *g*, and the supernatant was dried in a N2 stream. The dry extracts were suspended in 200 μL of methanol for next step. A chromatographic analysis was carried out using a UPLC Acquity chromatographer (Waters, United States) coupled with a TQD mass spectrometer (Micromass Waters, United Kingdom) with ESI source. We used a Waters Acquity BEH C18 column (100 × 2.1 mm, 1.7 μm) equipped with a VanGuard BEH C18 precolumn (5 × 2.1 mm, 1.7 μm) with both columns kept at 30°C and a full loop precision (10.0 μL) of injection volume. Milli-Q purified water with 0.1% (v/v) of formic acid (A) and acetonitrile (B) were used as solvents. The gradient started with 75% A changing to 65% in 6 min, then ramping to 0% A in 8 min and returning to the initial conditions for re-equilibration until 10 min, at a constant flow rate of 0.2 mL/min. Source and desolations temperatures were set to 150 and 350°C, respectively. The mass spectra of ABA IAA GA and *t*Z were acquired by ESI ionization in the negative ion mode using selected reaction monitoring (SRM) and individually optimized using Intellistart Waters software. In total, four compounds were evaluated: Auxin (IAA), gibberellin (GA), abscisic acid (ABA) and zeaxanthin (ZA). Ethylene (ETH) was detected by an ELISA kit following protocol.

Statistical analysis. The experimental data were subjected to analysis of variance (ANOVA) by SPSS 19.0 and Excel 2010®. Figures were drawn by SigmaPlot 10.0. P_N-PAR curves and photosynthetic parameters were analyzed statistically and fitted nonlinearly by SPSS 19.0 software.

RESULTS

Morphological Changes in 'NC1' and 'C211' under Hypoxia Stress

Hypoxia abiotic stress affects plant growth and development and results in phenotypic changes. In general, significant differences were found between the two sweet potato varieties 'C211' and 'NC1' in stem length, stem diameter, leaf area and the number of new leaves. The stem length was significantly decreased by 29.0, 39.0, 53.5, and 56.5% at 2, 4, 6, and 8 d of hypoxia treatment in 'C211', while it was no obvious changes in 'NC1' during hypoxia treatment

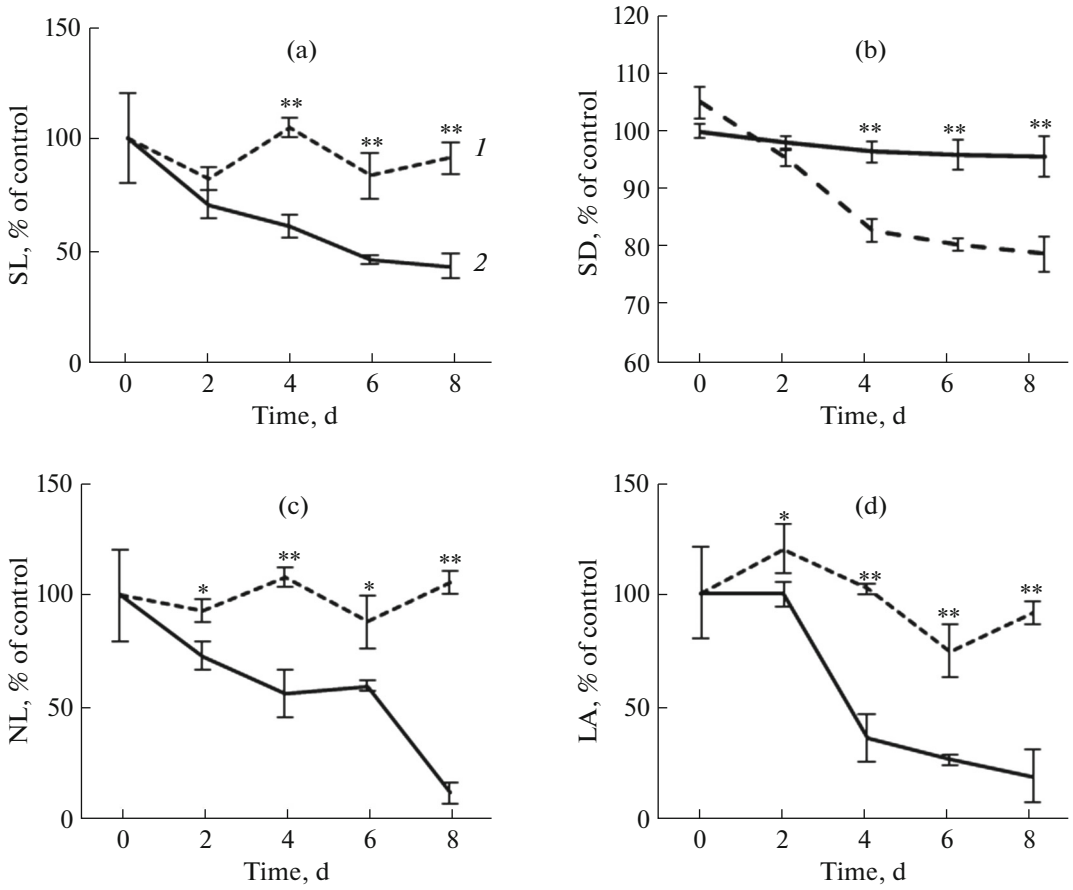

Fig. 1. Morphology of 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. (a) Stem length; (b) Total surface area; (c) New leaves number; (d) Relative leaf area. X-axis stands for the hypoxia stress duration time. All data calculated by value = treatment data/control data × 100%. "*" and "**" stands for the significant level of $P < 0.05$ and $P < 0.01$, respectively.

(Fig. 1a). Besides, stem diameter was significantly decreased by 21.1% in 'C211', and it was decreased by 4.3% in 'NC1' at 8 d of hypoxia stress (Fig. 1b). These results suggested that the growth rate of 'C211' was significantly decreased, but no effect on 'NC1'. The new leaf number and leaf area was significantly decreased by 87.4 and 81.5% in 'C211' at 8 d of hypoxia stress, compared to control. However, the new leaf number was almost same under hypoxia condition, despite the leaf area was significantly decreased by 8.1% in 'NC1' at 6 d, compared to that of control (Figs. 1c, 1d). The results suggested that hypoxia stress slightly inhibit the growth rate of new leaf in hypoxia-tolerant sweet potato variety, but significantly restrained germination of new leaf in hypoxia-sensitive sweet potato variety.

The Difference between 'NC1' and 'C211' in Leaf Gas Exchange and Photochemistry

Reducing stomatal conductance to avoid water loss is a common approach for plant to resist abiotic stresses. In this study, leaf water potential was significantly higher in 'NC1' compared to that of 'C211' under whole hypoxia treatment (Fig. 2). Interestingly, stomatal conductance (g_s) was significantly reduced (72.4%) in the sensitive variety 'C211' after hypoxia stress treatment for 8 d, while in the tolerant variety 'NC1', it was slightly reduced (Fig. 3a). The stomata morphological observation also showed that stomatal closure and apoptosis was found in 'C211' at 5 d of hypoxia induction, however, there was no significant change in 'NC1' variety (Fig. 4). After hypoxia treatment for 8 d, the intercellular CO_2 concentration (C_i) was decreased by 31.3% in 'C211' and 14.0% in 'NC1', leading to a higher reduction of the net photosynthetic rate (P_N) in 'C211' (53.9%) than 'NC1' (26.3%). Transpiration rate (E) was drastically reduced during the early period (within 4 d of hypoxia treatment) compared to the later period of hypoxia treatment in 'C211'. Meanwhile, in the hypoxia-tolerant variety 'NC1'', the E value was decreased by 23.1% at 8 d of hypoxia treatment (Figs. 3d–3f). These results indicated that in the hypoxia-sensitive variety, stomatal conductance and intercellular carbon dioxide concentration decreased rapidly at early stage of hypoxia stress.

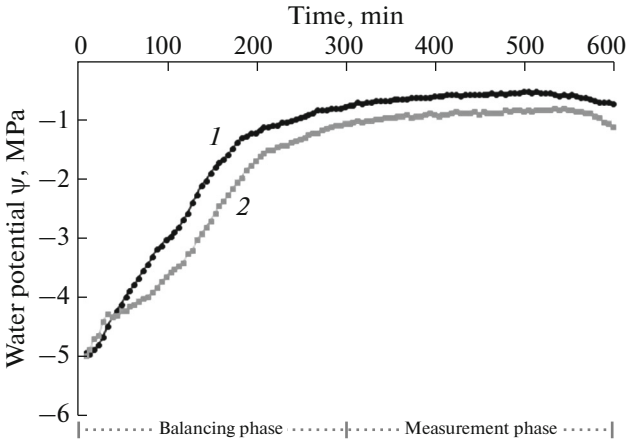

Fig. 2. The dynamic changes of water potential in 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. The whole process was divided into balance stage (0–300 min) and measurement stage (300–600 min).

Thereby inhibiting photosynthesis rate in hypoxia-sensitive variety. However, there was a low decrease of stomatal conductance in hypoxia tolerant variety, which provide an explanation for its quicker growth rate.

Light Response Curve of 'NC1' and 'C211' under Hypoxia Stress

The two sweet potato varieties showed a significantly different response of the net photosynthetic rate (P_N) to the photosynthetically active radiation (PAR) under hypoxia stress (Fig. 5a). The P_N-PAR curves could be divided into three stages, among which the first and second stages showed a similar trend between the two varieties 'NC1' and 'C211'. In the first stage, where PAR < 200 μmol (photon)/(m^2 s), the P_N was linearly increased as PAR increased in 'NC1'. The P_N reached saturation at a PAR value of 1200 μmol (photon)/(m^2 s), and the P_N of the variety 'NC1' was slowly decreased and then maintained at a higher level without obvious photoinhibition. The P_N of 'C211' variety was significantly decreased with the increase of the PAR and conspicuous photoinhibition was observed. The P_N reached saturation at low PAR value. Besides, A_{max} and R_d were significantly higher in 'NC1' compared to that of 'C211', suggesting that 'NC1' has a stronger metabolic rate of organic matter (Table 1).

A/C_i Curve of 'NC1' and 'C211' under Hypoxia Stress

Under hypoxia stress, the assimilation rate (A) of both vegetable sweet potato varieties were gradually increased in response to the increase in the intercellular CO_2 concentration (C_i), and finally tended to be gentle (Figs. 5b, 5c). When $C_i \le C_{i\_cj}$ (the Rubisco- and RuBP regeneration-limited states), the A was almost linearly increased with the increase of C_i, which indicated that photosynthesis of 'NC1' was limited by the activity of Rubisco enzyme. The increase in the A of 'NC1' was significantly higher than that of 'C211'

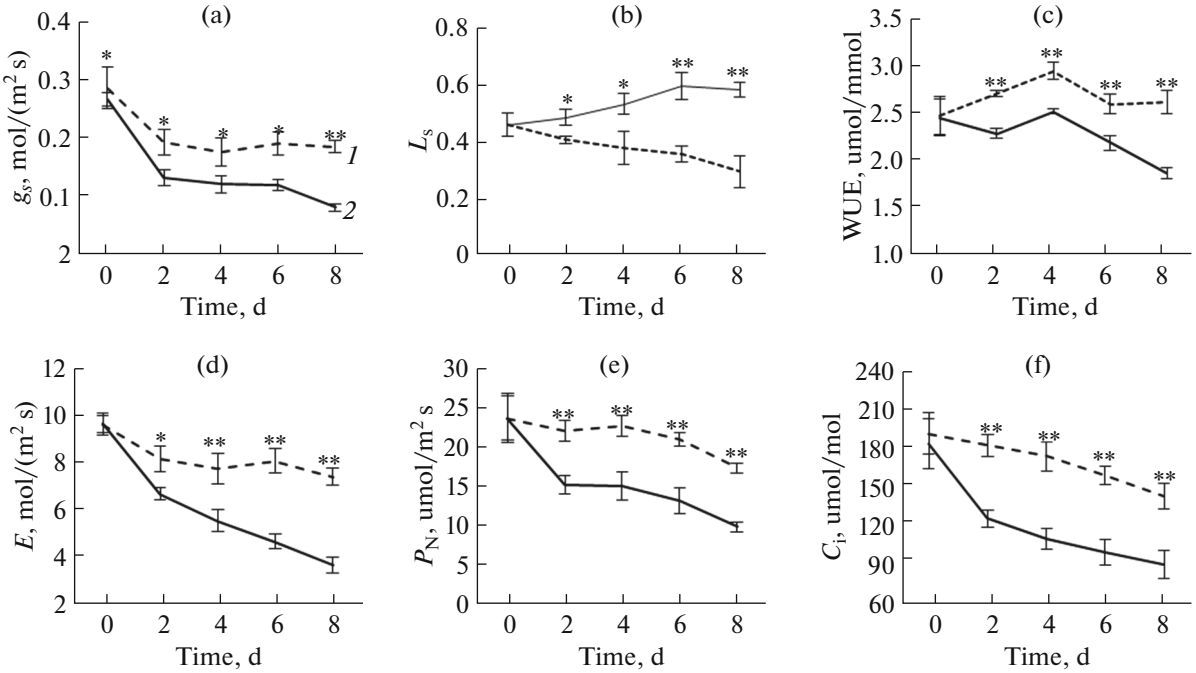

Fig. 3. Photosynthesis characteristics of 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. (a) g_s; (b) L_s; (c) WUE; (d) E; (e) P_N; (f) C_i. X-axis stands for the hypoxia stress duration time. "*" and "**" stands for the significant level of $P < 0.05$ and $P < 0.01$, respectively.

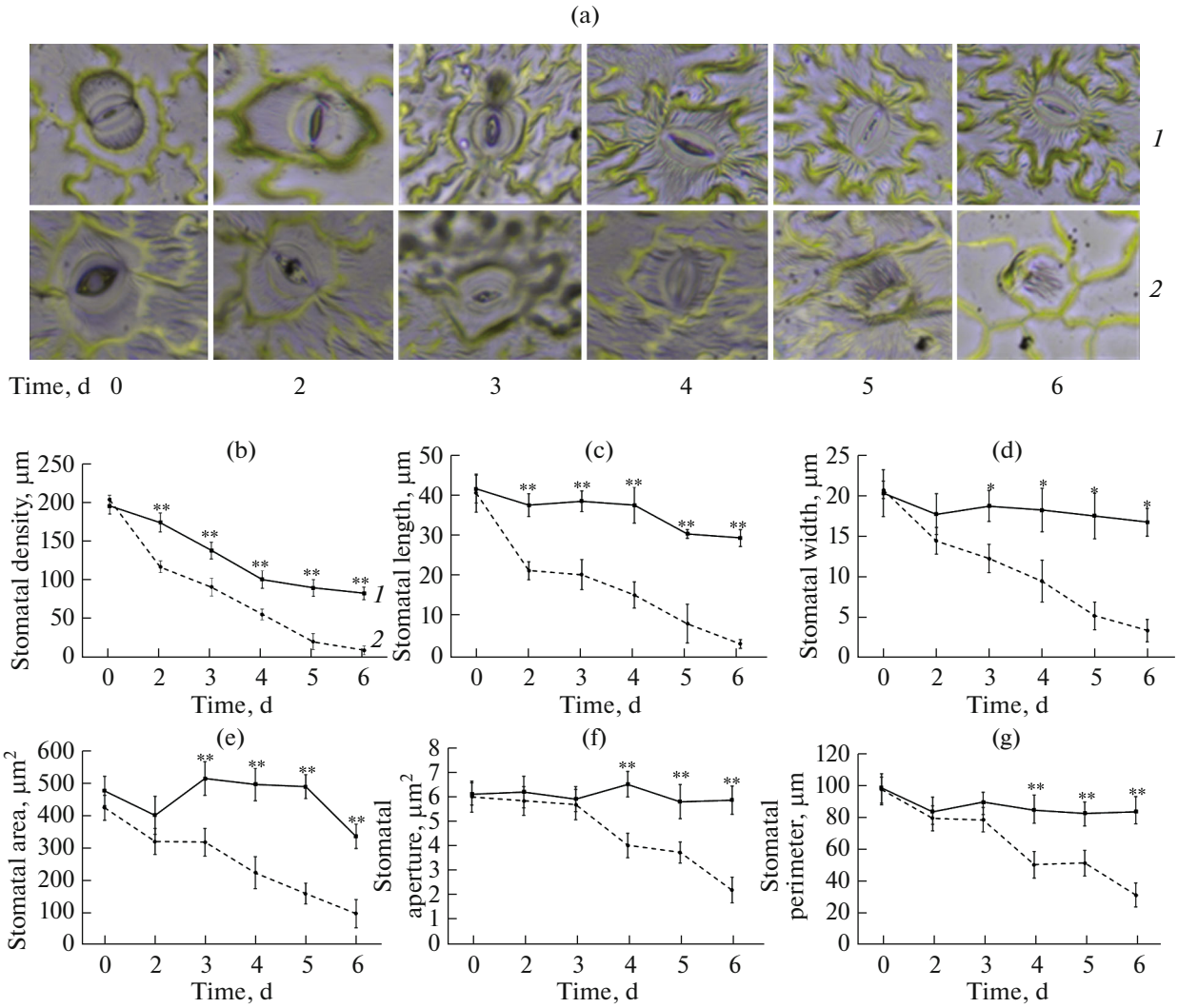

Fig. 4. Dynamic characteristics of stomatal morphology of 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. (a) stomal microstructure; (b) stomatal density; (c) stomatal length; (d) stomatal width; (e) stomatal area; (f) stomatal aperture; (g) stomatal perimeter. "*" and "**" stands for the significant level of $P < 0.05$ and $P < 0.01$, respectively.

variety, indicating that the sensitivity of the A to the C_i in 'C211' was lower than that of 'NC1' variety. When $C_i > C_i$_cj, the increment in A was reduced, indicating that photosynthesis is limited by the regeneration rate of Rubisco enzyme. The fitting effect of F_vCB model was analyzed by determinant the coefficient (R^2). The R^2 of CO_2 response curve fitting in 'NC1' and 'C211' was 0.9567 ~ 0.9911. The C_i_cj and the maximum electron transport rate (J_{max}) of 'C211' leaves were significantly lower than those of 'NC1' variety. Moreover, the C_i_cj of 'C211' leaves were 18.3%, which were significantly higher than those of 'NC1' (46.9%), and the maximum Rubisco carboxylation rate (Vc_{max}) of 'C211' leaves were significantly lower than those of 'NC1' variety (Table 2).

Table 1. Effects of hypoxia stress on photosynthesis-light response parameters of sweetpotato

Treatment	A_{max}, μmol/(m^2 s)	AQY, mmol/mol	LCP, μmol/(m^2 s)	R_d, μmol/(m^2 s)	R^2
'NC1'	17.80 ± 0.55a	0.09 ± 0.002a	7.54 ± 0.60a	0.594 ± 0.006a	0.9895 ± 0.0001
'C211'	12.26 ± 0.42b	0.09 ± 0.001a	7.11 ± 0.06a	0.417 ± 0.003b	0.9255 ± 0.0002

Different lowercase letters indicate significant differences at level $P < 0.05$

Chlorophyll Fluorescence Characteristic Changes in 'NC1' and 'C211' under Hypoxia Stress

Chlorophyll fluorescence parameters were used to reveal the response of PSII for the change in the photosynthetic efficiency. The maximum photochemical quantum yield (F_v/F_m) was significantly decreased in 'C211' at 2–8 days post hypoxia stress treatment, while there were no obvious changes in the F_v/F_m in 'NC1' (Fig. 6a). These results indicated that the hypoxia-tolerant varieties had stronger electron transfer activities and light energy-utilization ability (Fig. 6b). Besides, the photochemical quenching (q_P) was significantly increased (up to 31.9%) in 'NC1', while it was significantly decreased in 'C211' variety under hypoxia stress (Fig. 6c). NPQ was significantly reduced at 0–8 days post hypoxia stress treatment in 'NC1', while it was slightly decreased from 2–6 days post hypoxia stress treatment in 'C211' variety (Fig. 6d). Interestingly, non-photochemical quenching coefficient (q_N) was reduced in both varieties in response to hypoxia stress treatment (Fig. 6e). The effective quantum efficiency of photosystem II (ΦPSII) was significantly reduced in both varieties in response to the hypoxia stress treatment, however, 'NC1' had a significantly higher ΦPSII values compared to 'C211' variety under hypoxia stress condition (Fig. 6f). These results indicate that more thermal energy was lost in the hypoxia-sensitive plants than that of hypoxia-tolerant plants, which might be an important factor for growth inhibition.

Endogenous Hormone Changes in 'NC1' and 'C211' under Hypoxia Stress

Multiple phytohormones are involved in the plant response to waterlogging stress, especially in the regulation of stomatal morphology on the leaves. IAA, ABA and ethylene (ETH) were increased by 50, 40 and 260%, respectively, in 'NC1' at 0–4 days after hypoxia stress treatment. However, all these three phytohormones were slightly decreased in 'C211' variety. GA showed two peaks at the 2nd and 6th day of hypoxia stress treatment in 'NC1', while it was steadily decreased in 'C211' plants. *trans*-Zeatin (*t*Z) was reduced at the 4th day and recovered at the 8th day of

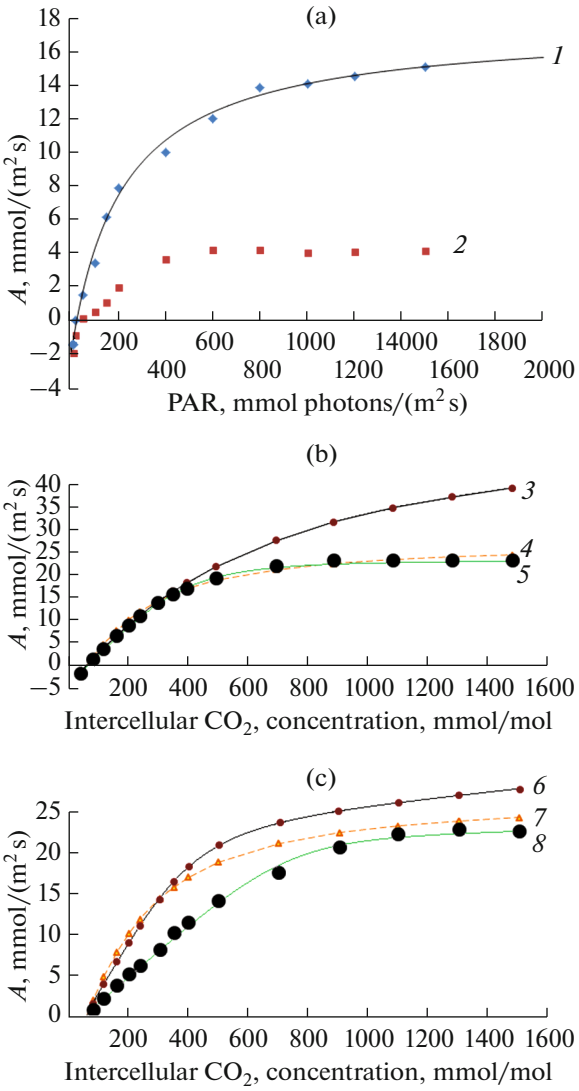

Fig. 5. Light response and CO_2 response curve of 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. (a) Light response curve of 'NC1' and 'C211'. The X-axis represents the light intensity, provided by a red-blue light source. (b) CO_2 response curve of NC1, (*3*) Ac; (*4*) Aj; (*5*) *A*; (c) CO_2 response curve of 'C211', (*6*) Ac; (*7*) Aj; (*8*) *A*. X-axis represents intercellular CO_2 concentration. Y-axis represents minimum of Rubisco carboxylation-limited rate (A_c), RUBP regeneration-limited (or electron transport-limited) rate (Aj), and net photosynthetic rate (A).

Table 2. Effects of hypoxia stress on photosynthesis-A/C_i response parameters of sweetpotato

Treatment	A, μmol/(m² s)	g_{sc}, mol/(m² s)	C_i/P_a	Vc_{max}, μmol/(m² s)	J_{max}, μmol/(m² s)	C_{i_cj}/P_a
'NC1'	15.74 ± 0.19a	0.25 ± 0.02a	108.14 ± 0.33a	37.12 ± 1.84a	31.56 ± 0.94a	397.3 ± 16.36a
'C211'	12.85 ± 0.14b	0.12 ± 0.02b	103.16 ± 1.85b	27.78 ± 1.15b	28.25 ± 0.66b	901.2 ± 20.62b

Different lowercase letters indicate significant differences at level $P < 0.05$

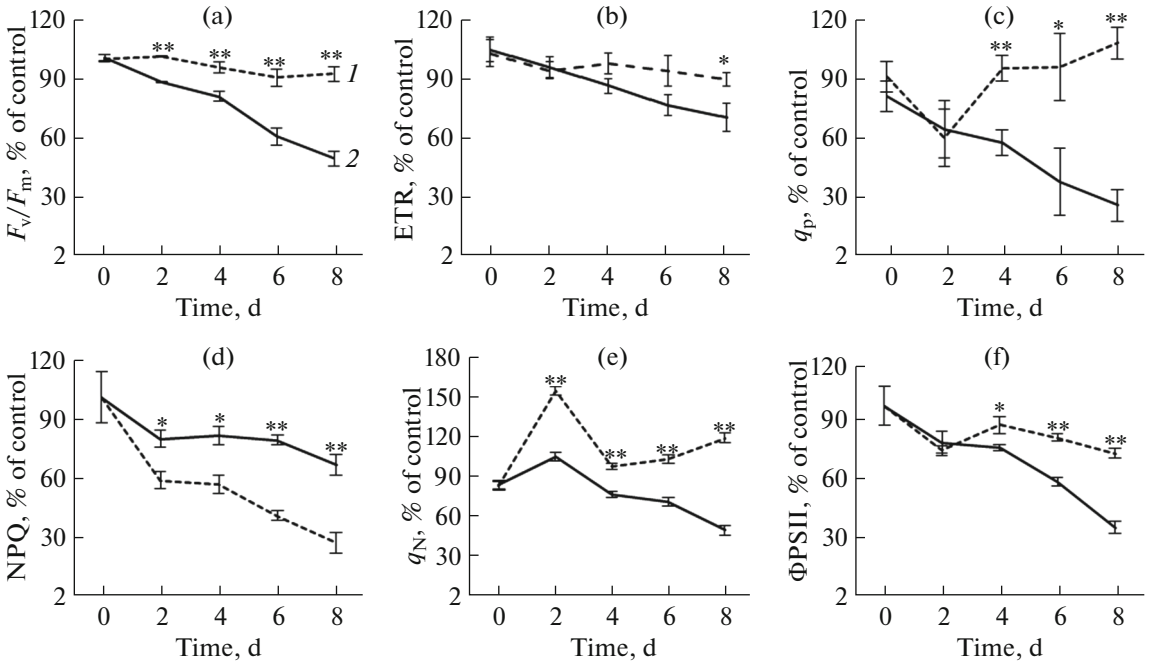

Fig. 6. Chlorophyll fluorescence characteristics of 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. (a) F_v/F_m; (b) ETR; (c) q_P; (d) NPQ; (e) q_N; (f) ΦPSII. The X-axis stands for treatment time. All data calculated by Value = treatment data/control data × 100%. "*" and "**" stands for the significant level of $P < 0.05$ and $P < 0.01$, respectively.

hypoxia stress treatment in 'NC1', but it was decreased from 0 to 8 days after hypoxia stress treatment in 'C211' plants (Figs. 7a–7e). These results suggested that multiple phytohormones worked together in the hypoxia-tolerant sweet potato to survive under hypoxia stress. However, in the hypoxia-sensitive sweet potato these phytohormones are not induced.

DISCUSSION

Flooding damages plants mainly through the production of low oxygen environment [8]. Under hypoxia stress, root cells rapidly changed from aerobic respiration to anaerobic respiration, leading to the decrease in the water absorption rate. Besides, the toxic effects of ethanol and acetaldehyde produced by anaerobic respiration on cell membrane were further aggravated [22]. The water loss of leaves was occurred due to transpiration, which caused a significant increase in ABA content in leaves (Fig. 2). Previous reports showed that ABA played an important role in stoma regulation, as an effective means to prevent water loss. In the present study, hypoxia-tolerant sweet potato plants produced high ABA content. However, it showed high stomatal conductance, which can be explained by the change of ethylene content that was significantly higher in hypoxia-tolerant sweet potato compared to that of hypoxia-sensitive sweet potato plants. In *Arabidopsis*, ethylene showed an inhibition effect on ABA-induced stomatal closure [23]. Moreover, ethylene was also involved in eliminating the reactive oxygen species (ROS) by inducing the expression of glutathione synthesis gene [21]. In rice, GA and IAA impose positive impacts on stem elongation and leaf growth [24]. With the effects of multiple phytohormones, hypoxia-tolerant sweet potato plants maintained stomatal opening and stem and leaf growth.

The efficiency of photosynthesis is affected by stomatal and non-stomatal factors. Our results suggest that the reduction in efficiency of photosynthetic carbon assimilation of both sweet potato varieties is directly related to the reduction in stomatal conductance, which is consistent with previous studies [12]. Under stress conditions, the reduction of stomata conductance in plant leaves is considered as an adaptive behavior, which effectively restrains water loss caused by transpiration and increases water use efficiency. However, it also increases the diffusion resistance of CO_2 into the leaves, which restricts plants photosynthesis [12]. Under hypoxia stress, the rate of gas exchange in 'C211' plants was significantly lower than that of 'NC1' plants. It is an effective way for plants to resist hypoxia stress by keeping stomata opening to maintain high photosynthetic rate and other physiological processes (Figs. 3a–3f). The increase of intercellular CO_2 concentration (C_i) and accompanied with the reduction of the stomatal limitation value (L_s) in the leaves of 'NC1' plants were significantly higher than that of 'C211' plants after 6 days of hypoxia stress induction, suggesting that the reduction in photosynthetic efficiency was non-stomata factors (Figs. 3b–3f), such as the reduction in the photosynthetic carbon

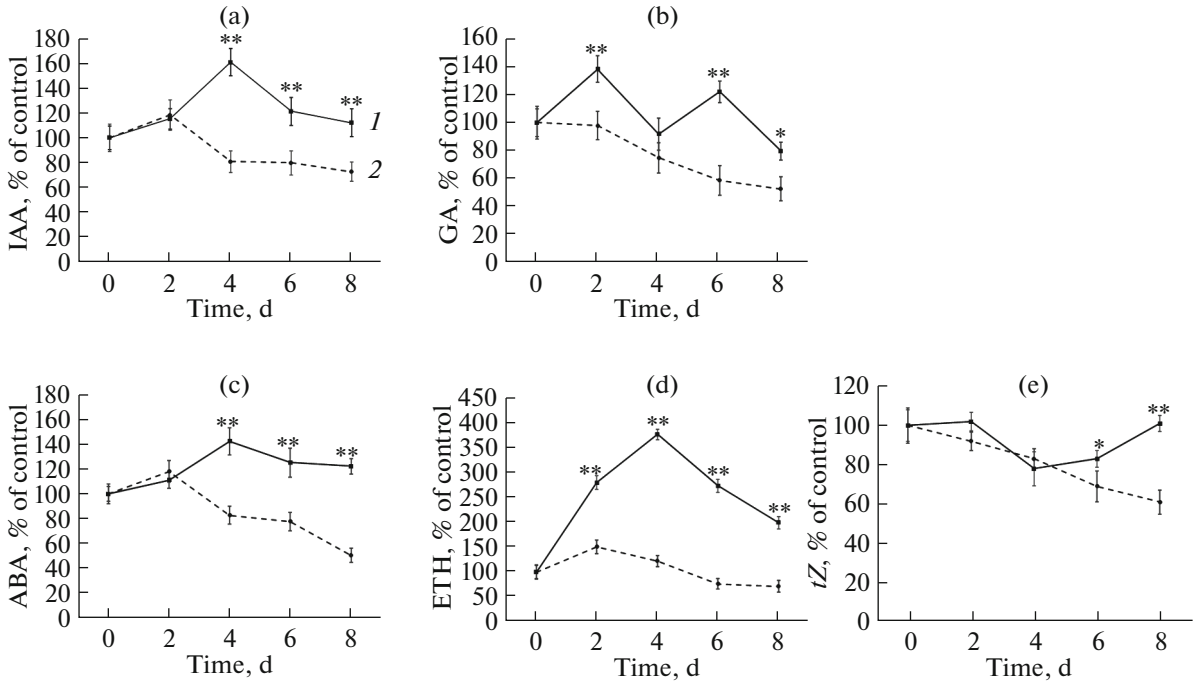

Fig. 7. The content of endogenous hormones in the leaves of 'NC1' (*1*) and 'C211' (*2*) varieties under hypoxia stress. (a) IAA content; (b) GA content; (c) ABA content; (d) ETH content; (e) *t*Z content. X-axis stands for treatment time. All data calculated by Value = treatment data/control data × 100%. "*" and "**" stands for the significant level of $P < 0.05$ and $P < 0.01$, respectively.

assimilation enzymes or damage of mesophyll cells occurred at a higher rate in 'C211' plants exposed to long-term hypoxia stress. In addition, the reduction of the maximum Rubisco carboxylation rate (Vc_{max}) and the maximum electron transport rate (J_{max}) after 8 days of hypoxia stress in both varieties indicates that hypoxia inhibited the arboxylation reaction of Rubisco enzyme and electron transfer process, resulting in reduction in the maximum assimilation rate (A_{max}), dark respiration (R_d) and net photosynthetic rate (P_N). The reduction in the Vc_{max} might be caused by the decrease in the Rubisco enzyme activity by hypoxia stress [25]. Dark respiration (R_d) of 'NC1' leaves was significantly increased under hypoxia stress, suggesting that the plant may respond to hypoxia damage by increasing the respiration to obtain more energy. the reduction in the stomatal conductance (g_s) and the intercellular CO_2 concentration (C_i) in 'C211' leaves indicate an increase in the leaves pore resistance which might be associated with a reduction in the density of pores and the number of open pores under hypoxia stress.

Under hypoxia stress, both stomatal and non-stomatal factors affect carbon assimilation and the plant response to light and CO_2. Non-stomatal factors play an important role in particular during the advanced stages of hypoxia stress [12]. The reduction in PSII photochemistry and the oxidative damage by reactive oxygen are considered the main non-stomatal limiting factors [26]. The chlorophyll fluorescence technique is widely used to estimate the physiological response of PSII function under varies stresses. The PSII maximum photochemical quantum yield (F_v/F_m), particularly, provides abundant information about the PSII primary photochemical reaction, which can directly analyze the injured sites of photosynthesis [27]. In our study, both of F_v/F_m and ΦPSII were significantly higher in 'NC1' leaves compared to 'C211' during hypoxia stress. The extent of reduction at the advanced stages of hypoxia stress was also lower in 'C211' compared to 'NC1', suggesting that PSII in 'NC1' leaves was significantly less sensitive to hypoxia stress than that of 'C211' (Figs. 6a–6c). It was one of the reasons why 'NC1' plants exhibited a higher photosynthetic capacity. The q_P, which had a positive correlation with the electron activity of PSII, reflects to some extent the opening degree of the PSII reaction center [28]. The q_P measures the proportion of open PSII reaction centers and represents the energy consumed in photosynthesis. The reduction in the q_P values indicates an increase the fraction of primary quinones in reduced state of PSII, suggesting that increasing the susceptibility to photoinhibition [29]. 'NC1' plants exhibited higher q_P values under hypoxia stress, and kept more PSII centers in an open state so that more excitation energy can be used for electron transport compared to 'C211' plants (Fig. 6d). Moreover, NPQ was positively correlated with the heat dissipation [30]. Variations of NPQ in 'NC1' plants were low across the hypoxia

treatment, whereas 'C211' plants exhibited significant increase in the NPQ during 2 d to 8 d of hypoxia stress, indicating that after hypoxia stress treatment, the light energy utilization ability of hypoxia sensitive sweet potato decreased sharply, which may be caused by the closure of the reaction center. The normal physiological function of PSII could be protected in 'NC1' plants through xanthophyll cycle-dependent energy dissipation. This protective mechanism was less active in 'C211' plants under hypoxia stress, and more likely leading to a significant suppression of the function of the PSII reaction center in 'C211' leaves at the advanced stages of hypoxia stress (Fig. 6c) as well as resulting in a significant reduction in the photosynthetic gas exchange capacity. The Non-photochemical quenching (q_N) of chlorophyll fluorescence is thought to be an indicator of an essential regulation and photoprotection mechanism against high-light stress in plants. 'NC1' Plants showed higher values of q_N during hypoxia stress (Fig. 6e), suggesting an efficient removal of excess excitation energy via thermal dissipation and thereby a better protection of leaves from photo-damage [45]. Regarding the photochemistry, 'NC1' higher photosynthetic capacity consumes more NADPH and ATP stimulating photochemical activity and causing higher PSII electron transfer rate (ETR) (Fig. 6f).

In conclusion, stomatal closure is a short-term limiting factor under hypoxia stress, while the damage on RuBP and Rubisco enzymes caused plant growth retardation or death under long-term waterlogging. Summarizing these results, 'NC1' variety exhibited a waterlogging resistance performance better than 'C211' variety and therefore, providing useful insights regarding sweet potato breeding.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

AUTHORS CONTRIBUTIONS

H. Han, R. Pan contributed equally to this work.

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