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Effects of Elevated Surface Ozone Concentration on Photosynthetic Fluorescence Characteristics and Yield of Soybean Parents and Offspring

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Abstract: Global climate change presents a significant threat to food security. Analyzing the effects of elevated ozone (O_3) concentration on photosynthetic fluorescence characteristics and yield addresses the damage of climate change on crops, which would serve food security. With open-top chambers (OTCs) and Tiefeng-29 soybeans, we investigated the responses of chlorophyll concentration, fluorescence characteristics, net photosynthetic rate (*Pn*) and yield components to different O_3 concentrations, which included CK (ambient concentration approximately 45 nL·L^{-1}, T1 (80 ± 10) nL·L^{-1} and T2 (120 ± 10) nL·L^{-1} O_3. The parent soybeans (S1) were planted in the current year, and O_3 fumigation commenced 20 days after seedling emergence. Aeration was stopped at maturity, and the offspring soybeans (S2) were retained after harvest for further experiments. In the following year, S1 and S2 soybeans were planted, and O_3 fumigation began 20 days after seedling emergence. The results show that leaf chlorophyll a (*chla*) and chlorophyll b (*chlb*) significantly decreased with longer O_3 fumigation time both in parents and offspring, causing damage to the light-trapping ability while the offspring suffered an earlier decrease. The elevated O_3 damaged the electron transfer process by significantly reducing the original and actual photochemical efficiencies of *PS*II both in parents and offspring. The electron transfer rate (*ETR*) of the parents and offspring decreased, while the difference between them was not significant after O_3 treatment. The non-photochemical quenching coefficient (*NPQ*) showed an increasing trend along time but showed no significant difference between parents and offspring. An elevated concentration of O_3 significantly reduced *Pn*, while the differences in *Pn* between the parents and the offspring were not significant. Elevated O_3 resulted in reduced yields in both parent and offspring soybeans. Although it was found that the offspring soybeans exhibited higher yields than the parents, their reduction in yield was more significant. Therefore, elevated O_3 concentration reduced soybean yield through damaging photosynthetic process and electron transfer capacity by impairing energy conversion and material accumulation capacity. The offspring had relatively higher light energy conversion efficiency than the parents, resulting in a higher yield than the parents under all treatments.

Keywords: soybeans; ozone fumigation; OTCs; photosynthetic characteristics; chlorophyll fluorescence; yield

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1. Introduction

In recent years, due to the swift advancement of the worldwide economy and the constant enlargement of metropolitan regions, near-surface ozone (O_3) contamination has emerged as a significant contributor to atmospheric pollution. O_3 is a highly reactive and potent oxidizing gas that brings about significant negative impacts on crop production and ecosystem carbon and nitrogen cycles [1–4]. It is important to explore crop sensitivity and response mechanisms to O_3 [5]. Since 2013, the number of statistical days with O_3 being the primary pollutant in Northeast China has been increasing yearly, and O_3 has

been consistently maintained at high pollution concentration levels [6]. It is estimated that the average surface O_3 concentration will increase by 23% by 2050, and the superposition of extreme climatic events will further increase the damage caused by O_3 [7], posing a great threat to grain production safety [8].

Soybeans, which are the world's largest cultivated dicotyledonous plant, are the main source of fats and dietary protein for many people [9]. Climate change is the main reason for reduced soybean yields [10], and according to statistics, soybean production losses in China were about 10,900–1,840,000 metric tons during 2010–2017 [11]. Understanding the mechanism behind soybean response to elevated O_3 levels is crucial for ensuring global food security, particularly in developing nations [12]. Existing studies have shown that the effects of O_3 on soybeans are complex and that soybeans react differently to elevated O_3 concentrations at varying developmental stages and according to soybean genotype [13]. An elevation in O_3 levels triggers the rapid generation of reactive oxygen species in soybeans, inducing oxidative stress and heightened stomatal resistance, ultimately causing leaf closure [14]. These factors result in reduced photosynthesis and accelerated senescence [15]. Recent research indicates that elevated levels of O_3 may also impact the interactions between soil microorganisms and plants [16]. Moreover, intracellular O_3 accumulation within soybean leaves impedes gas exchange by restricting external CO_2 from entering the leaf cells, thus affecting the gas exchange process of soybean leaves. When O_3 fumigation was continuously carried out, the source of damage shifted from stomatal limiting factors in the pre-fumigation period to non-stomatal limiting factors, resulting in severe damage to the photosynthetic organs of soybean leaves. This led to a reduction in photosynthetic pigment content, hampering normal photosynthesis and damaging the electron transfer pathway of soybean leaves [17,18]. Elevated O_3 concentration also inhibits the activity of relevant enzymes in soybean leaves, reduces the content of amino acids and proteins [19], and damages the important pathways of soybean material accumulation, energy transfer and conversion, and carbon and nitrogen element distribution, resulting in a significant decrease in plant biomass, leading to a reduction in soybean yields [20,21].

Although the impacts of elevated concentrations of O_3 on crops have been extensively studied, the majority of these studies have focused solely on crop parents. Given that soybeans exhibit genetic variability between parents and offspring generations [22], and considering that soybeans are one of the most sensitive crops to changes in O_3 concentration [23,24], more research is needed to examine the acclimation of offspring soybeans that have been propagated from parents grown under O_3 stress. The test site of Shenyang area from June to July experienced O_3 pollution for approximately 51% of the year [6]. This critical growth period coincides with the crucial development stage of spring-sown soybeans, leading to significant damage to soybeans [25]. To investigate the photosynthetic response mechanism of different generations of soybean to elevated O_3 concentration, in this study, we employed open-top chambers (OTCs) to examine the effects of elevated O_3 on photosynthetic and fluorescence characteristics, as well as yield-related indices of soybean parents and offspring. Our objective is to provide both theoretical and practical guidance for the sustainable growth of soybean production within the context of elevated O_3 levels.

2. Materials and Methods

2.1. Selection of Test Material

The soybean cultivar "Tiefeng 29" was utilized as the parent material, with its offspring being the seeds obtained from the fumigated parent material in the first year. This cultivar has a growth period of approximately 133 days, making it well-suited for cultivation in soils with medium to high fertility levels.

2.2. Experimental Design

This experiment was conducted at the Shenyang National Field Scientific Observatory for Farmland Ecosystems, Chinese Academy of Sciences (41°31′ N, 123°24′ E). This area

experiences an annual rainfall of 700 mm, a frost-free period of 147–164 days, and primarily comprises meadow brown loam soil.

In the fumigation test, OTCs fitted with a ventilation system, an O_3 generator (BGY-Q8, BBL, Beijing, China), an O_3 concentration sensor (S900, Aeroqual, Auckland, New Zealand), an automatic inflation control system, and an analysis system were employed. The OTCs had octagonal cross-sections with side lengths of 1.15 m and heights of 2.4 m and were made of glass walls. These chambers were arranged outdoors at intervals of 4 m and featured automatic measurement systems for temperature, wind speed, and humidity indoors and outdoors. Real-time data storage ensured that the test had a stable O_3 concentration and that the environmental conditions, such as temperature, humidity, and illumination, were consistent both inside and outside the chambers.

The experiment was conducted with three O_3 concentration treatments, namely CK: ambient concentration of O_3 at around 45 nL·L^{-1}, T1: O_3 concentration at (80 ± 10) nL·L^{-1}, and T2: O_3 concentration at (120 ± 10) nL·L^{-1}. PVC buckets measuring 34 cm in diameter and 30 cm in height were used for potting. The parents were sowed in 2019, with 20 potting buckets placed in each air chamber and 10 seeds sown in each bucket. After seedling emergence, five plants were retained in each potting bucket and then incubated for another 20 days and treated with O_3 fumigation from 9 a.m. to 5 p.m. daily. No additional impacts were recorded due to weeds, pests, diseases, fertility, etc. during this period. Aeration was stopped when the soybeans matured, and the offspring plants (S2) were kept for subsequent experiments after harvesting.

There was simultaneous sowing of the parents (S1) and their corresponding offspring (S2) in 2020, and 10 buckets sown with 10 S1 seeds and 10 buckets sown with 10 S2 seeds were placed in each chamber. After seedling emergence, five plants were retained in each potting bucket and then incubated for another 20 days and treated with O_3 fumigation from 9 a.m. to 5 p.m. daily. No additional impacts due to weeds, pests, diseases, fertility, etc. were recorded during this period.

2.3. Measurement Items and Methods

2.3.1. Determination of Chlorophyll Content

Leaf samples were taken at the branching, flowering, and podding stages, and six representative plants with uniform growth were selected from each of the parent–offspring treatments in each air chamber for the determination of chlorophyll content using the 95% ethanol method.

2.3.2. Measurement of Fluorescence Parameters

A Pulse-modulated Fluorometer (FMS-2, Hansatech, Norfolk, UK) was used to make measurements at the branching, flowering, and podding stages. On a sunny day with suitable light, six plants with uniform and representative growth were selected from 9:00 a.m. to 12:00 p.m. in each of the air chamber parent–offspring treatments. The same leaves were light-responsive and fully dark-adapted for 20 min, then the initial fluorescence (F_0), maximum fluorescence (Fm), PSII primary photochemical efficiency (Fv/Fm), PSII actual photochemical efficiency $\Phi PS\text{II} = (Fm' - s)/Fm'$, PSII maximum photochemical efficiency (Fv'/Fm'), PSII electron transfer rate (ETR), photochemical quenching coefficient $qP = (Fm' - Fs)/(Fm' - F_0')$, and non-photochemical quenching coefficient $NPQ = (Fm - Fm')/(Fm - F_0')$ were determined.

2.3.3. Determination of Net Photosynthetic Rate

Portable photosynthesis systems (CIRAS-3, pp systems, Amesbury, MA, USA) were utilized to ascertain the net photosynthetic rate (Pn). A suitable sunny day with adequate illumination was chosen, and the light intensity inside the leaf chamber was set to a constant rate of 1200 μmol·m^{-2}·s^{-1}. The temperature was maintained at 25 °C and the CO_2 concentration was set at 390 μmol·mol^{-1}, to determine Pn of the leaves. Six plants with uniform and representative growth were selected from 9:00 a.m. to 12:00 p.m. in each

of the air chamber parent–offspring treatments and measured at the branching, flowering, and podding stages.

2.3.4. Determination of Yield Components

At harvest time, in each air chamber, 10 parent plants and 10 corresponding offspring plants with uniform growth were collected in mesh bags and naturally dried and considered test species, and then yield components such as pod number per plant, seed number per plant, mass per 100 seeds, and seed mass per plant were investigated [26].

2.4. Data Analysis

Data processing was conducted using Microsoft Excel 2016 and plotting was performed using Origin2021. One-way ANOVA was performed using SPSS 26.0 to analyze the effects of different treatments on *Pn*, chlorophyll content, fluorescence parameters, and soybean yield. The significance was determined using the least significant difference (LSD) method, and correlation analysis was conducted using the Pearson index. The experimental data are presented as mean ± standard deviation.

3. Results

3.1. Effect of Increased O_3 Concentration on Soybean Leaf Chlorophyll Content

Throughout the growth stages, there was a general downward trend in the chlorophyll a (*chla*) content for each treatment (as shown in Figure 1a). At the branching stage, there was no significant difference between T2S1 and CKS1, while both CK and T1 treatments showed S1 < S2. Under the T2 treatment, S2 decreased significantly by 39.1% compared to S1. At the flowering stage, there was a significant decrease of 21.5% in S2 compared to CKS2 under T1 treatment. T2 treatment showed a significant decrease of 21.2% in S1 and 10.1% in S2 compared to CKS1 and CKS2, respectively. There was a significant difference between S2 and S1, where S2 had a higher value. At the podding stage, both T1S2 and T2S2 were significantly lower than CKS2, but there was no significant difference between the parents and offspring under each treatment. The high O_3 concentration significantly affected *chla* content in soybean leaves. In terms of the differences between parents and offspring, there were significant differences under the T2 treatment at the branching and flowering stages, while there were no significant differences in the other treatment periods.

Under the T2 treatment at the branching stage, S1 > S2 but the difference was not significant (Figure 1b). At the flowering stage, there was a significant decrease of 16.4% in S2 compared to S1 under CK treatment, while under T1 treatment, there were significant decreases of 29.9% in S1 and 20.2% in S2 compared to CKS1 and CKS2, respectively. T2 treatment showed a significant decrease of 29.4% in S1 compared to CKS1. There were no significant differences between parents and offspring under any treatments at the podding stage, while S2 was significantly higher than CKS2 by 133.6% under T2 treatment. The chlorophyll b (*chlb*) content for S1 showed a general trend of decreasing and then increasing during all growth periods, with no significant differences observed between S1 and S2 under T1 and T2 treatments.

The ratio of chlorophyll a and b (*chla*/*chlb*) remained relatively unchanged at the branching and flowering stages (as shown in Figure 1c), and S1 < S2 under both CK and T2 treatments at the podding stage. There were no significant differences between S1 and S2 at any periods and treatments, or among treatments.

Elevated O_3 concentrations caused an overall decrease in the total chlorophyll content of growing soybean (as shown in Figure 1d). At the branching stage, under T2 treatment, S1 was significantly higher than T1S1 by 46.4%, while S2 was significantly lower than CKS2 by 40.1%, and the values for offspring were significantly lower than those for parents by 62.3%. At the flowering stage, under T1 treatment, S1 and S2 were significantly lower than CKS1 and CKS2 by 20.4% and 21.1%, respectively, and the difference between S2 and S1 was not significant. Under T2 treatment, S1 was significantly lower than CKS1 by 23.9%, and S2 was lower than CKS2, but S2 was significantly higher than S1 by 16.1%. At the

podding stage, there were no significant differences between S1 and S2 compared to CK under T1 treatment, while under T2 treatment, S2 was significantly greater than CKS2 by 55.5%. In all growth periods, there were significant differences between S1 and S2 under T2 treatment.

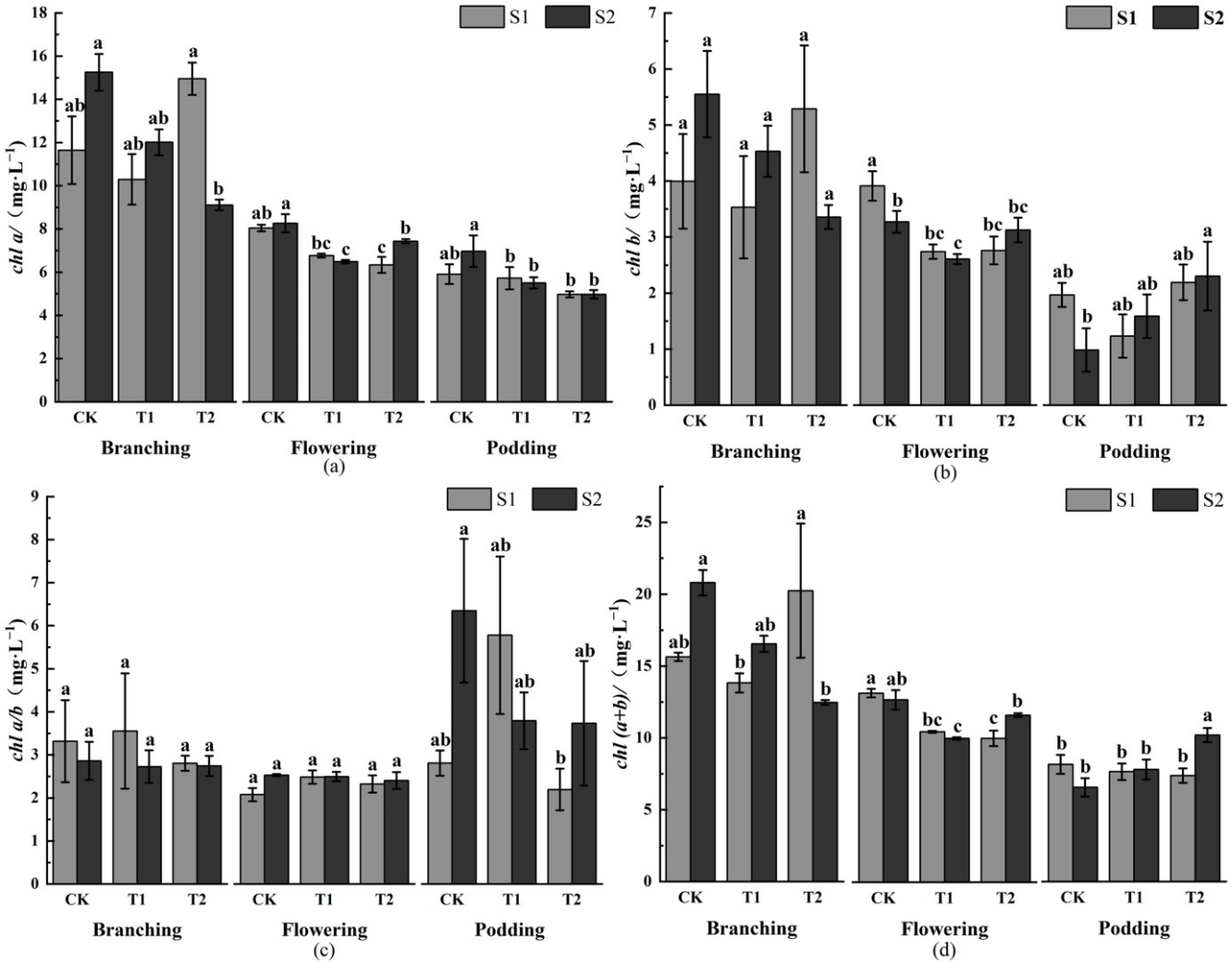

Figure 1. (**a**) Chlorophyll a Content in Soybean Leaves under Elevated O_3 Concentration; (**b**) Chlorophyll b Content in Soybean Leaves Under Elevated O_3 Concentration; (**c**) Chlorophyll a/b Ratio in Soybean Leaves under Elevated O_3 Concentration; (**d**) Total Chlorophyll in Soybean Leaves under Elevated O_3 Concentration. Different lowercase letters in the figure indicate that the difference among different treatments reaches a significant level ($p < 0.05$), with the same below.

3.2. Effects of Elevated O_3 Concentration on Fluorescence Parameters of Soybean Leaves

3.2.1. Primary Photochemical Efficiency of *PS*II and Actual Photochemical Efficiency of *PS*II

Throughout the growth of the soybeans, there was little overall change in the *Fv*/*Fm* of *PS*II (as indicated in Table 1). At the branching and flowering stages, there were no significant differences between parents and offspring, while S1 was significantly lower than S2 by 11.4% under the T1 treatment at the podding stage. There were no significant differences between the treatments of the parents and offspring at the branching stage and flowering stage, while at the podding stage, S1 was significantly lower than CKS1 by 11.7%.

Table 1. Fluorescence Kinetic Parameters of Soybean Leaves under Elevated O_3 Concentration.

Period	Treatment	*Fv*/*Fm*	Φ*PS*II	*Fv*′/*Fm*′	*ETR*	*qP*	*NPQ*
Branching stage	CKS1	0.788 ± 0.016 a	0.510 ± 0.050 b	0.662 ± 0.031 a	4.861 ± 0.075 a	0.761 ± 0.044 a	1.087 ± 0.205 a
	CKS2	0.738 ± 0.015 ab	0.574 ± 0.024 a	0.692 ± 0.016 a	5.311 ± 0.095 a	0.827 ± 0.018 a	0.765 ± 0.081 a
	T1S1	0.783 ± 0.019 ab	0.453 ± 0.097 c	0.633 ± 0.041 a	4.643 ± 1.032 a	0.701 ± 0.115 a	1.277 ± 0.265 a
	T1S2	0.698 ± 0.045 b	0.488 ± 0.089 bc	0.628 ± 0.046 a	5.210 ± 0.919 a	0.763 ± 0.092 a	1.039 ± 0.424 a
	T2S1	0.779 ± 0.008 ab	0.469 ± 0.077 bc	0.631 ± 0.051 a	4.611 ± 0.939 a	0.729 ± 0.074 a	1.173 ± 0.265 a
	T2S2	0.708 ± 0.046 b	0.541 ± 0.029 ab	0.641 ± 0.028 a	5.258 ± 0.260 a	0.853 ± 0.011 a	0.949 ± 0.081 a
Flowering stage	CKS1	0.782 ± 0.009 a	0.476 ± 0.046 a	0.637 ± 0.021 a	5.330 ± 0.571 a	0.742 ± 0.051 a	1.087 ± 0.205 a
	CKS2	0.783 ± 0.009 a	0.490 ± 0.046 a	0.644 ± 0.025 a	5.040 ± 0.247 a	0.757 ± 0.057 a	0.765 ± 0.081 a
	T1S1	0.794 ± 0.010 a	0.520 ± 0.061 a	0.666 ± 0.047 a	3.869 ± 0.484 b	0.777 ± 0.041 a	1.092 ± 0.366 a
	T1S2	0.805 ± 0.012 a	0.486 ± 0.038 a	0.618 ± 0.022 a	3.158 ± 0.423 bc	0.784 ± 0.040 a	1.230 ± 0.235 a
	T2S1	0.810 ± 0.004 a	0.392 ± 0.079 b	0.659 ± 0.029 a	2.906 ± 0.138 bc	0.590 ± 0.110 a	1.293 ± 0.242 a
	T2S2	0.802 ± 0.017 a	0.348 ± 0.024 b	0.572 ± 0.018 a	2.582 ± 0.073 c	0.609 ± 0.028 a	1.265 ± 0.099 a
Podding stage	CKS1	0.775 ± 0.021 a	0.430 ± 0.057 b	0.598 ± 0.032 ab	3.654 ± 0.276 b	0.629 ± 0.048 ab	1.084 ± 0.172 b
	CKS2	0.735 ± 0.025 ab	0.427 ± 0.070 b	0.632 ± 0.036 ab	4.760 ± 0.416 a	0.659 ± 0.079 ab	1.050 ± 0.138 b
	T1S1	0.683 ± 0.021 b	0.324 ± 0.052 c	0.535 ± 0.059 b	1.972 ± 0.368 c	0.635 ± 0.047 ab	1.440 ± 0.196 a
	T1S2	0.772 ± 0.031 a	0.513 ± 0.044 a	0.672 ± 0.025 a	2.898 ± 0.205 bc	0.761 ± 0.038 a	1.482 ± 0.143 a
	T2S1	0.785 ± 0.020 a	0.371 ± 0.041 bc	0.646 ± 0.019 ab	2.291 ± 0.148 c	0.574 ± 0.057 ab	1.547 ± 0.884 a
	T2S2	0.794 ± 0.027 a	0.338 ± 0.032 c	0.632 ± 0.034 ab	1.835 ± 0.068 c	0.537 ± 0.060 b	1.409 ± 0.473 a

Different lowercase letters in the table indicate that the difference among different treatments reaches a significant level ($p < 0.05$).

Additionally, Φ*PS*II displayed a declining trend during the soybean developmental period, and all values were lower than *Fv*/*Fm*. In terms of parent–offspring relationships, S1 significantly differed from S2 under CK treatment at the branching stage and T1 treatment at the podding stage, and the Φ*PS*II of S2 was higher than S1 under all treatments throughout the branching stage. At the flowering stage, S2 showed lower values than S1 under the T1 and T2 treatments, in contrast to the results of CK treatment. At the podding stage, T1S1 was significantly lower than CKS1 by 24.5%, T1S2 was significantly higher than CKS2 by 19.9%, and S2 was significantly lower than CKS2 by 20.9% under T2 treatment.

3.2.2. Maximum Photochemical Efficiency of *PS*II

Table 1 reveals that Fv'/Fm' did not significantly vary during all stages of soybean growth. At the branching and flowering stages, there were no significant differences between the parents and offspring under all treatments, while the differences between the parents and offspring were noteworthy under the T1 treatment at the podding stage. This indicates that low O_3 concentration should be monitored during the podding stage, as high O_3 concentration did not prove to significantly affect Fv'/Fm'.

3.2.3. Photosynthetic Electron Transfer Rate

ETR generally demonstrated a decreasing trend during all stages of soybean growth and development (as denoted in Table 1). At the branching stage, there was no significant difference in *ETR* between the parents and offspring under each treatment. At the flowering stage, there was no significant difference between the parents and offspring under each treatment, but T1S1 and T2S1 decreased significantly by 27.4% and 45.5%, respectively, compared with CKS1, while T1S2 and T2S2 decreased significantly by 37.3% and 48.8%, respectively, compared with CKS2. No significant difference in *ETR* was observed between parents and offspring under each treatment and among treatments at the podding stage. Overall, it is evident that at the flowering stage, elevated O_3 concentration had a significant impact on the *ETR* of soybean leaves.

3.2.4. Photochemical Quenching and Non-Photochemical Quenching

From Table 1, it is evident that the overall trend of *qP* in leaves decreased during all stages of soybean development. At the branching stage, there were no significant differences between parents and offspring under each treatment and among treatments. At the flowering stage, there was no significant difference in *qP* among the parents and

offspring under each treatment, while T2S1 and T2S2 showed significant decreases of 20.4% and 19.6%, respectively, compared with CKS1 and CKS2. At the podding stage, there was no significant difference between the parents and offspring under each treatment, while T2S2 was significantly lower than T1S2 by 29.4%. At the flowering stage, the *qP* of soybean leaves under the high-O_3 treatments was significantly altered, but none of the differences between parents and offspring were significant.

In comparison, *NPQ* tended to gradually increase during all stages of soybean growth. However, no significant differences were observed under each treatment in each period, either between parents and offspring or between treatments.

3.3. Effects of Elevated O_3 Concentration on Net Photosynthetic Rate

Elevated O_3 concentration significantly decreased *Pn* in the branching and flowering stages with no significant difference between the parents and offspring (Figure 2). At the branching stage, S2 was lower than S1 under both T1 and T2 treatments. Specifically, under T1 treatment, S1 and S2 were significantly lower than CKS1 and CKS2 by 36.2% and 43.1%, respectively. Similarly, under T2 treatment, S1 and S2 were significantly lower than CKS1 and CKS2 by 59.1% and 71.7%, respectively. At the flowering stage, S2 was significantly higher than S1 by 35.4% under the CK treatment. Under both T1 and T2 treatments, S1 was greater than S2, but the difference was not significant under T2 treatment. At the podding stage, *Pn* displayed a decreasing-then-increasing trend with increasing O_3 concentration. The *Pn* of S2 was significantly higher than that of S1 by 44.3% under CK treatment. However, under T1 and T2 treatments, the difference between the parents and offspring was not significant with regard to *Pn*, except for the flowering stage.

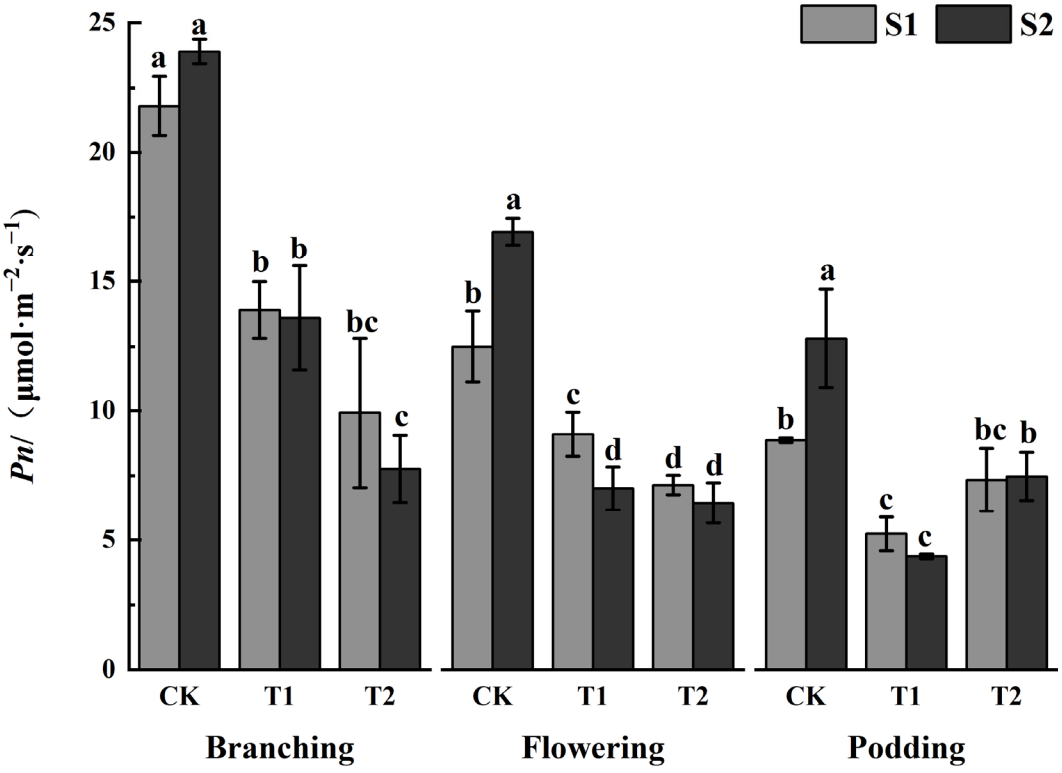

Figure 2. Net Photosynthetic Rate of Soybean Leaves under Elevated O_3 Concentration.

3.4. The Effect of Elevated O_3 Concentration on Soybean Yield Component Factors

With the elevation of O_3 concentration, the pod number per plant, seed number per plant, mass per 100 seeds, and the seed mass per soybean plant all declined, resulting in significantly lower soybean yield indicators in comparison to the CK treatment in both T1 and T2 treatments (refer to Table 2). From the point of view of the parents and offspring,

the pod number per plant, seed number per plant, mass per 100 seeds, and the seed mass per plant of S2 were higher than those of S1, but the treatments did not significantly affect the pod number per plant and the seed mass per plant of the parents and offspring, and the seed number per plant of S2 was significantly higher than that of S1 by 7.3% under high O_3 concentration, while only the mass per 100 seeds of S1 was significantly different from that of S2 under the T1 treatment.

Table 2. Soybean Yield under the Condition of Elevated O_3 Concentration.

Treatment	Pods Number per Plant	Seed Number per Plant	Mass per 100 Seeds/g	Seed Mass per Plant/g
CKS1	38.67 ± 3.86 b	58.67 ± 4.03 b	25.60 ± 0.37 b	15.43 ± 0.95 b
CKS2	58.33 ± 9.93 a	76.33 ± 7.17 a	30.76 ± 0.63 a	23.80 ± 1.40 a
T1S1	29.33 ± 2.05 c	42.33 ± 3.68 c	23.50 ± 0.28 c	12.20 ± 1.37 c
T1S2	35.00 ± 2.64 b	60.00 ± 3.05 b	23.76 ± 0.26 c	15.46 ± 1.32 b
T2S1	25.67 ± 3.40 d	36.67 ± 1.25 c	23.57 ± 0.85 c	9.30 ± 1.07 c
T2S2	22.00 ± 1.73 d	39.33 ± 5.82 c	26.00 ± 0.35 b	10.23 ± 0.38 c

Different lowercase letters in the same line indicates significant difference ($p < 0.05$).

3.5. Correlation Analysis of Photosynthetic and Fluorescence Parameters with Yield

Based on the findings in Figure 3, the *Pn* of the parent soybean leaves exhibited a significant positive correlation with the Φ*PS*II and *ETR*. Additionally, *ETR* displayed a significant positive correlation with *Pn* and *chla* and a highly significant positive correlation with *chlb* and Φ*PS*II. Furthermore, both *Pn* and *ETR* demonstrated a positive correlation with yield.

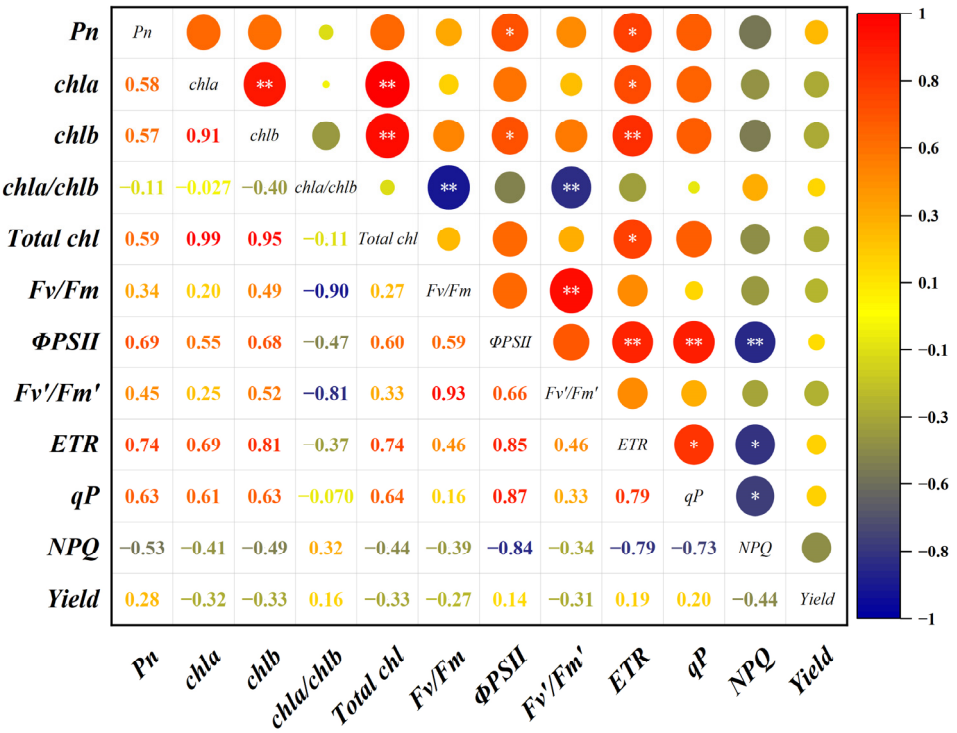

Figure 3. Correlation Analysis between Photosynthesis, Fluorescence and Yield of Parent Soybeans under Elevated O_3 Concentrations. *: significant correlation at the $p < 0.05$ level (both sides); **: significant correlation at the $p < 0.01$ level (both sides).

In Figure 4, *Pn* of the offspring soybean leaves displays a highly significant positive correlation with *chla*, a highly significant negative correlation with *NPQ*, and a significant positive correlation with yield. Moreover, *ETR* exhibited a significant positive correlation with *Pn*, *chla*, Φ*PS*II, and *qP*.

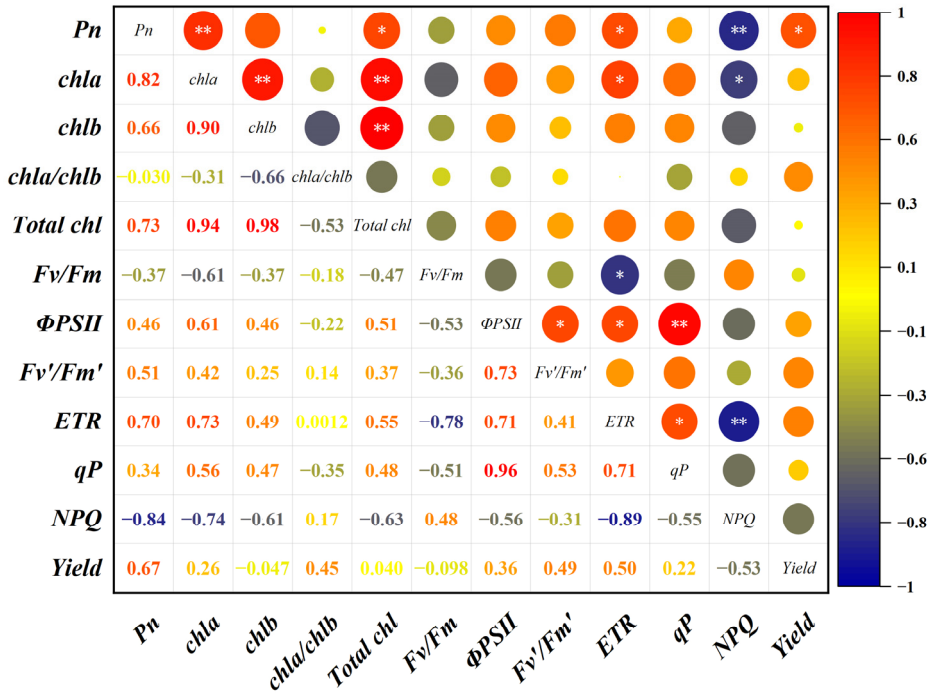

Figure 4. Correlation Analysis between Photosynthesis, Fluorescence and Yield of Offspring Soybeans under Elevated O_3 Concentrations. *: significant correlation at the $p < 0.05$ level (both sides); **: significant correlation at the $p < 0.01$ level (both sides).

4. Discussion and Conclusions

Elevated O_3 concentrations exacerbated the peroxidation of membranes in both parents and offspring soybeans, leading to damage to the membrane system and degradation of leaf chlorophyll, resulting in a significant decrease in chlorophyll content [27]. Our studies show that elevated O_3 concentrations caused a declining trend in chlorophyll content in both parent and offspring soybeans, which is consistent with previous research on parent soybeans [28]. Studies on plant crops like catalpa, rice, and others also suggest a similar conclusion in relation to their response to O_3 stress [29,30]. High concentrations of O_3 can lead to significant damage to the leaves and cell membrane system of soybean, thereby reducing stomatal conductance and limiting stomatal movement. Our preliminary experiments of the current study and the findings of Bailey et al. [31] support this statement. Xiongfei Guo et al. [32] also found similar results in studies of autumn maple, cottonwood, and other plant seedlings.

Green plants perform energy conversion through photosynthesis and light energy absorbed by chlorophyll can drive photosynthesis and release chlorophyll fluorescence [33]. It is of significant importance to investigate the utilization, transfer, dissipation, and distribution of light energy in plants through chlorophyll fluorescence detection techniques. [34]. *Fv*/*Fm* reflects a plant's adaption to long-term light intensity, while *Fv*′/*Fm*′ represents the actual photosynthetic reaction center's energy transfer efficiency [35,36]. We demonstrated that O_3 treatment reduced both *Fv*/*Fm* and *Fv*′/*Fm*′ in both parents and offspring generations of soybeans, probably due to the more significant effect of O_3 damage on the photoreaction centers of soybean leaves. During the podding stage, the photochemical efficiencies of the offspring were found to be higher compared to the parents under the low concentration treatment. This could possibly be attributed to the fact that the low concentration of O_3 damaged the *PS*II of the parents, but appeared to have promoted *PS*II of the offspring at the late stage of soybean growth. *ETR*, *qP*, and *NPQ* are parameters that reflect electron transfer. Both parents and offspring exhibited a decrease in *ETR* as the concentration of O_3 increased, with high O_3 concentrations having a greater impact on the offspring during the soybean podding stage. *qP*, which reflects the electron transfer

activity of *PS*II, shows a gradual decrease under conditions of increasing O_3 concentration. *NPQ* is a self-protective mechanism for photosynthetic organs and is the portion of light energy not undergoing photosynthetic electron transfer that is dissipated in the form of heat energy [37]. In the current study, there were no significant differences observed in *qP* and *NPQ* between parent and offspring soybeans. However, *NPQ* exhibited an increasing trend under conditions of increasing O_3 concentration due to the requirement of excess light energy dissipation.

The *Pn* of leaves is closely related to stomatal conductance [38]. As O_3 concentrations increased, the *Pn* of soybean leaves decreased, affecting both parents and offspring leaves. The results of our previous study indicate that non-stomatal limiting factors caused the decrease in *Pn*, which aligns with the findings of a study conducted by Weiwei Zhang et al. on parent soybeans [39]. The *Pn* of the offspring was higher than that of the parents under all periods of CK treatment, which may be attributed to the continued decrease in the expression of genes involved in photosynthesis in old leaves under O_3 stress, while young leaf tissue dominated changes in the expression of defense genes [40].

The yield components of the offspring soybeans, including the seed number per plant, mass per 100 seeds, and the seed mass per plant of soybeans, all exhibited superior results compared to those of the parent generation. The high concentration of O_3 had a detrimental effect on photosynthesis and electron transport pathways in soybean leaves, causing a decline in both *Pn* and *ETR*. A correlation analysis indicated a significant positive correlation between the *Pn* and *ETR* of both the parents and offspring soybeans, as well as the yield. Consequently, the various yield indices of the parents and offspring soybeans witnessed a significant decrease. These findings are consistent with previous research conducted by Ming Zhang et al., Rongjun Wu et al., Lianxin Yang et al., and others on the impact of elevated O_3 concentrations on the yield of parent soybeans [41–43]. Under high O_3 concentration treatment, the number of pods per plant of the offspring was slightly lower than that of the parents, while the number of seeds per plant was significantly higher than that of the parents. However, this could be attributed to the higher rate of empty pods produced by offspring soybeans under O_3 stress.

In summary, elevated O_3 concentration significantly reduces *Pn* and chlorophyll content of the parents and offspring soybean leaves. High O_3 concentration harms the electron transfer pathway, ultimately impeding the energy conversion ability of the parents and offspring soybean leaves. Nevertheless, the offspring exhibit higher light energy conversion efficiency and a higher seed yield than the parent generation. Simultaneously, the offspring yield had a higher reduction compared to the parents under stress. The current investigation was carried out on soybeans, and additional exploration is necessary in forthcoming studies to ascertain if comparable phenomena are demonstrated in other crops. Additionally, the mechanism of genetic variation in O_3 tolerance among crop varieties requires further study in multigenerational long-term experiments. Measures to mitigate the risks of grain production security caused by global climate change should continue to be pursued through agricultural practices, such as the cultivation of resilient crop varieties and the implementation of exogenous conditioning techniques.

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Abbreviations

Abbreviation	Full Name
O_3	Ozone
OTCs	Open-top chambers
Pn	Net photosynthetic rate
chla	Chlorophyll a
chlb	Chlorophyll b
chla/*chlb*	Ratio of Chlorophyll a and b
ETR	Electron transfer rate
F_0	Initial fluorescence
Fm	Maximum fluorescence
Fv/*Fm*	*PS*II primary photochemical efficiency
Φ*PS*II	*PS*II actual photochemical efficiency
Fv′/*Fm*′	*PS*II maximum photochemical efficiency
qP	Photochemical quenching coefficient
NPQ	Non-photochemical quenching coefficient

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