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MOLECULAR MECHANISMS AND MULTI-OMICS INSIGHTS INTO OXYGEN NANOBUBBLE-ENRICHED DEFICIT FERTIGATION FOR ENHANCING RESOURCE-USE EFFICIENCY AND FRUIT QUALITY IN GREENHOUSE CUCUMBER

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ABSTRACT

Oxygen nanobubble-enriched fertigation is a newly developed strategy for improving available oxygen in the rhizosphere and crop performance under limited irrigation; however, its physiological and molecular effects in greenhouse cucumber remain unclear. This study tested the effect of oxygen nanobubble-enriched deficit fertigation on growth, yield, resource-use efficiency, fruit quality, oxidative stress regulation, and multi-omics responses of cucumber (Cucumis sativus L.) under greenhouse conditions. A total of five fertigation treatments were compared: full irrigation with conventional fertigation (control); 75% deficit irrigation with conventional fertigation; full irrigation with oxygen nanobubble fertigation; 75% deficit irrigation with oxygen nanobubble fertigation; and 60% deficit irrigation with oxygen nanobubble fertigation. Oxygen nanobubble enrichment significantly raised the root-zone dissolved oxygen and alleviated the negative impacts of deficit irrigation on photosynthesis, root growth, and yield. Among treatments, 75% deficit irrigation combined with oxygen nanobubble fertigation was the most promising, as it retained yields near full-irrigation levels while enhancing water productivity, nitrogen-use efficiency, marketable yield, fruit firmness, soluble solids, vitamin C, phenolics, and antioxidant activity. Additionally, this treatment lowered nitrate accumulation in fruit, hydrogen peroxide, malondialdehyde, and electrolyte leakage, and increased antioxidant enzyme activities. The gene expression analysis indicated that genes related to aquaporins, nutrient transporters, antioxidants, oxygen-responsive factors, and stress-regulatory factors were upregulated. Additional metabolomic and rhizosphere analyses revealed improved carbon metabolism, Osmo protective adjustments, accumulation of antioxidant metabolites, and increased abundance of beneficial microbes. Overall, oxygen nanobubble-enriched fertigation at 75% irrigation optimizes cucumber yield, fruit quality and resource-use efficiency through coordinated physiological, biochemical, molecular, metabolic, and rhizosphere regulation.

KEYWORDS: Cucumber; oxygen nanobubbles; deficit fertigation; water-use efficiency; fruit quality; oxidative stress; multi-omics.

1. INTRODUCTION

Cucumber (Cucumis sativus L.) is a widely cultivated vegetable crop that requires substantial irrigation and fertilization to achieve adequate fruit yield and quality; however, the scarcity of water resources and high input costs have intensified pressure for resource-efficient cultivation methods [1-5]. Although it has been widely suggested that deficit irrigation is a practicable means of saving water, inadequate irrigation can limit root activity, photosynthesis, nutrient uptake, biomass accumulation, and fruit development. Moreover, in most protected cultivation systems, reduced water availability may further exacerbate rhizosphere hypoxia, oxidative stress, and nitrate accumulation, ultimately affecting fruit yield and quality. Technologies that help maintain key root-zone functions under reduced irrigation are thus needed for sustainable greenhouse vegetable production [4, 6-13].

Currently, the potential to improve root-zone conditions and crop performance through oxygen enrichment of irrigation or fertigation solutions has emerged as a viable alternative [14]. Previous studies indicate that crop responses to oxygen-enriched nutrient solutions depend on species, substrate, growth stage, and the root zone's oxygen-limitation status, with yield benefits when root-zone oxygen availability reaches limiting levels [15, 16]. Its derivative, nanobubble-based irrigation, has been a focus in recent years, as it is essentially based on the premise that nanobubbles can remain suspended in water for extended periods and may increase the transport of dissolved oxygen to the rhizosphere [17-19]. Moderate oxygen nanobubble concentrations in lettuce, for example,

when using precision nanobubble irrigation, result in yield promotion and water savings; however, excessive concentrations may negate these effects, reinforcing the necessity of optimized application [20]. Micro-Nano bubble oxygenation with drip irrigation also increased alfalfa yield, root vitality, soil enzyme activity, microbial abundance, and water-use efficiency, indicating that oxygenated irrigation can positively influence crop production by enhancing root and rhizosphere functions [11, 17, 21-25].

Notwithstanding these improvements, the physiological and molecular underpinnings of how oxygen nanobubble-amended deficit fertigation regulates greenhouse cucumber performance remain poorly elucidated. Reference basis for choice: Plant adaptability to water-limited environments involves collaborative responses in aquaporins, nutrient transporters, antioxidant enzymes, stress-responsive transcription factors, Osmo protective metabolites and phylogenetically related microbial associates in the rhizosphere [25-29]. This integration can now provide novel insights into regulatory links among physiology, metabolism, transcriptomics, and stress adaptation (multi-omics), as demonstrated in abiotic stress studies in tomato and other crops [30]. Thus, the present study aimed to assess whether fertigation with oxygen nanobubbles can effectively enhance cucumber productivity and fruit quality under deficit irrigation and to elucidate the underlying physiological, biochemical, transcriptomic, metabolomic, and rhizosphere mechanisms responsible for increased resource-use efficiency.

2. MATERIALS AND METHODS

2.1 Experimental Site, Crop Establishment, and Greenhouse Conditions

Under a regulated greenhouse production regime, an experiment was conducted to evaluate the effects of oxygen-nanobubble-enriched deficit fertigation on multi-omics responses, nutrient use efficiency, fruit quality, growth, physiological traits, and oxidative stress regulation in cucumber (Cucumis sativus L.). The plant material was a commercial greenhouse cucumber hybrid. All seedlings were raised under nursery conditions and transplanted into a drip-fertigated greenhouse system at the 3–4 true-leaf stage. The crop was raised for 90 days after transplantation. The entire experiment was conducted in accordance with standard Cucumber greenhouse management practices, including vertical training, pruning, pest control, and removal of senescent leaves. The greenhouse temperature, relative humidity, and quality of irrigation water were recorded regularly throughout the growing period. Irrigation and fertigation were carried out according to the assigned standard regime for all treatments, with fertigation supplied through a drip irrigation system.

2.2 Experimental Design and Treatment Structure

The experiment was laid out in a randomized complete block design with five fertigation treatments and four blocks. Each treatment was represented once in each block, giving a total of 20 treatment × block experimental plots. For plant-level fruit quality assessment, 12 plants were sampled from each treatment within each block, giving 240 sampled plants in total. The five fertigation treatments were:

- **T1:** Full irrigation with conventional fertigation, coded as FI-CF
- **T2:** 75% deficit irrigation with conventional fertigation, coded as DI75-CF
- **T3:** Full irrigation with oxygen nanobubble-enriched fertigation, coded as FI-ONB
- **T4:** 75% deficit irrigation with oxygen nanobubble-enriched fertigation, coded as
- DI75-ONB
- **T5:** 60% deficit irrigation with oxygen nanobubble-enriched fertigation, coded as DI60-ONB

The full irrigation treatments received 100% of crop evapotranspiration requirement, equivalent to 90.0 L plant^{-1} over the crop cycle. The 75% deficit treatments received 67.5 L plant^{-1}, while the 60% deficit treatment received 54.0 L plant^{-1}. Fertilizer input was adjusted according to fertigation volume. The full irrigation treatments received 210 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 280 kg K_2O ha^{-1}. The 75% deficit treatments received 157.5 kg N ha^{-1}, 67.5 kg P_2O_5 ha^{-1}, and 210 kg K_2O ha^{-1}. The 60% deficit treatment received 126 kg N ha^{-1}, 54 kg P_2O_5 ha^{-1}, and 168 kg K_2O ha^{-1}.

2.3 Oxygen Nanobubble-Enriched Fertigation

An oxygen nanobubble generation system was installed in the fertigation line before treatment application to prepare oxygen-nanobubble-enriched fertigation water. For the conventional fertigation treatments without nanobubble enrichment, the nutrient solution had a dissolved oxygen concentration of approximately 7.1–7.2 mg L^{-1}. Nutrient solution enriched with oxygen nanobubbles was applied to the oxygen nanobubble treatments prior to being delivered through the drip system. Mean dissolved oxygen concentrations of oxygen nanobubble-enriched fertigation water were kept around 18.5 mg L^{-1} for FI-ONB, 18.0 mg L^{-1} for DI75-ONB, and 17.8 mg L^{-1} for DI60-ONB. Each concentration maintained a nanobubble density of approximately 1.20×10^8 particles mL^{-1} for FI-ONB, 1.10×10^8 particles mL^{-1} for DI75-ONB, and 1.00×10^8 particles mL^{-1} for DI60-ONB. Liquid rhizosphere analysis of the rhizosphere solution was used to periodically measure rootzone dissolved oxygen and confirm each treatment's oxygen enrichment.

2.4 Growth and Root Trait Measurements

The measurements of plant growth at the fruiting stage: Plant height was measured from the base of the plant to the apical growing point using a measuring tape. A digital caliper was used to assess the stem diameter. Leaf surface area (cm^2 plant^{-1}) was measured for fully expanded leaves. Chlorophyll status was measured from recently matured leaves using a SPAD chlorophyll meter. At the conclusion of the experiment, plants were divided into shoots and roots. All roots were thoroughly washed to eliminate substrates, dried, and weighed. To assess dry biomass, shoot and root samples were dried at 70 °C until reaching a constant weight. Root-to-shoot ratio was computed as the ratio of root dry weight to shoot dry weight. The triphenyl tetrazolium chloride reduction method

was used to quantify root viability, expressed as absorbance g^{-1} fresh weight. Root hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1}) was determined.

2.5 Physiological Measurements

Leaf gas exchange parameters were measured in fully expanded leaves during the active photosynthetic period. The net photosynthetic rate was expressed as μmol CO_2 m^{-2} s^{-1}. Stomatal conductance was expressed in mol H_2O m^{-2} s^{-1}, and transpiration rate (E) in mmol H_2O m^{-2} s^{-1}. Instantaneous water-use efficiency was evaluated as the ratio of net photosynthetic rate to transpiration rate (μmol CO_2 $mmol^{-1}$ H_2O).

2.6 Yield and Resource-Use Efficiency

Throughout the production period, fruits were harvested at commercial maturity. Data on the number of fruits per plant, average fruit weight, total yield, and marketable yield were collected. Total yield was reported in kg $plant^{-1}$ and converted to t ha^{-1}. All fruits with visual defects, poor shape, damage or marketability score below acceptable commercial standards were excluded from the analysis of marketable yield. Water productivity was calculated as marketable yield per unit of applied irrigation water, expressed in kg m^{-3}. Partial factor productivity of nitrogen was computed as yield per unit of nitrogen input (kg yield kg^{-1} N), and nitrogen-use efficiency (NUE) was expressed as an index of % values relative to the full-irrigation conventional fertigation control.

2.7 Fruit Quality Measurements

Fruit quality was evaluated on 12 sampled plants per treatment within each block. Fruit length was measured with a measuring scale, and fruit diameter was measured with a digital caliper. Fruit firmness was measured in Newtons by a penetrometer. Digital refractometer readings expressed in °Brix were used to assess total soluble solids. Titratable acidity was titrated and reported as percentage acidity. Vitamin C concentrations were measured spectrophotometrically, and all values were expressed as mg 100 g^{-1} of fresh weight. The Folin–Ciocalteu method was used to determine total phenolic content, expressed as mg gallic acid equivalents per 100 g^{-1} fresh weight. Methods: The antioxidant activity was determined on the DPPH radical and expressed as percentage inhibition. Spectrophotometric determination of fruit nitrate concentration was expressed as mg kg^{-1} FW. Color coordinates L, a, and b* were measured for fruit color.

2.8 Oxidative Stress and Antioxidant Enzyme Analysis

The leaf samples were collected from each treatment × block plot, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent biochemical analysis. Hydrogen peroxide concentrations were spectrophotometrically determined and expressed as μmol g^{-1} fresh weight. The content of malondialdehyde (MDA) was determined according to thiobarbituric acid reactive substances (TBARS) and was expressed in nmol g^{-1} fresh weight. Electrolyte leakage was measured from leaf discs and expressed as a percentage of conductivity. Proline content was analyzed by the acid ninhydrin method and expressed as μmol g^{-1} fresh weight. Soluble sugar was quantified by the anthrone method and expressed as mg g^{-1} of fresh weight. Fresh leaf extracts were directly analyzed in the laboratory and expressed as specific activities of various antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase) in U mg^{-1} protein. The activity of nitrate reductase was determined, and values are expressed as μmol NO_2^- g^{-1} fresh weight h^{-1}. Leaf pigment extracts were used to quantify chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll.

2.9 RNA Extraction and qRT-PCR Analysis

For gene expression analysis, samples were collected from each treatment-by-block plot. Total RNA was extracted using a plant RNA extraction protocol, and RNA quality was assessed for purity and integrity. Complementary DNA (cDNA) was synthesized by reverse transcription from purified RNA. These primers were used for quantitative real-time PCR (qRT-PCR), with an internal reference gene, CsACTIN. The expression of 16 cucumber genes involved in water transport, nutrient uptake, carbon metabolism, oxygen signaling, stress response, and antioxidant regulation was analyzed. For **CsPIP1;1**, **CsPIP2;1**, **CsNRT1.1**, **CsNRT2.1**, **CsPHT1;4**, **CsSUT1**, **CsRBCS**, **CsDREB2A**, **CsNAC72**, **CsWRKY33**, **CsCAT1**, **CsAPX1**, **CsCuZnSOD**, **CsERF-VII**, **CsHRE1-like**, and **CsAOX1a**. Data analysis was conducted using the $2^{-}\Delta\Delta Ct$ method, with the full-irrigation conventional fertigation treatment as the calibrator.

2.10 RNA-Seq Analysis

RNA-seq was performed in triplicate per treatment (Giving 15 RNA-seq libraries in total) for Transcriptomic profiling. Raw sequence reads were filtered to exclude low-quality reads, adapter sequences, and reads including ambiguous bases. Clean reads were aligned to the cucumber reference genome, and then the mapping rate, uniquely mapped reads, GC content, and Q30 values were used as quality control parameters. Five comparisons of differential gene expressions were performed (DI75-CF vs FI-CF, FI-ONB vs FI-CF, DI75-ONB vs FI-CF, DI60-ONB vs FI-CF, and DI75-ONB vs DI75-CF). Differentially expressed genes were identified using significance thresholds adjusted for multiple testing using false discovery rates and absolute log_2 fold-change thresholds. Functional annotation was completed using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses. Biological functions and pathways enriched for reactive oxygen species (ROS), water transport, nitrogen metabolism, carbon fixation, plant hormone signaling, MAPK signaling, and oxygen response were identified.

2.11 Metabolomic Profiling

Metabolomic profiling was performed for leaf and fruit samples collected from each treatment × block plot. The samples were rapidly frozen in liquid nitrogen, lyophilized, homogenized to a fine powder and extracted with a methanol-based solvent system. Primary metabolites, secondary metabolites, osmoprotectants, organic acids, amino acids, and phytohormone-related metabolite abundance. They included glucose, fructose, sucrose, malate, citrate, glutamate, γ-aminobutyric acid, proline, putrescine, spermidine, phenylalanine, chlorogenic acid, caffeic

acid, rutin, quercetin, luteolin, ascorbate, dehydroascorbate, α-ketoglutarate, succinate, trehalose, glycine betaine, jasmonic acid, zeatin, abscisic acid within the metabolite panel. Normalization of metabolite values was performed prior to multivariate analysis.

2.12 Rhizosphere Microbiome Analysis

At the fruiting stage of the crop, rhizosphere samples were collected from each treatment-by-block plot. Soil attached to roots was collected, homogenized, and stored at −80°C until microbial analysis. Alpha diversity, including Shannon index, Chao1 richness, and Simpson index, was used to evaluate microbial diversity. Relative abundance of other major bacterial groups, including Proteobacteria, Firmicutes, Actinobacteriota, and Bacteroidota, was measured. Quantified were plant-beneficial genera, such as Bacillus, Pseudomonas, Azospirillum, Sphingomonas, and Rhizobium. The relative abundance of Fusarium was retrieved, and an indicator of rhizosphere microbial balance was calculated as the beneficial-to-Fusarium ratio.

2.13 Multi-Omics Integration

A series of growth, physiological, yield, biochemical, qRT-PCR, transcriptomic, metabolomic, and microbiome datasets were integrated to identify coordinated plant responses to oxygen nanobubble-enriched deficit fertigation. Principal component analysis was used to visualize the separation of treatments based on standardized physiological and molecular variables. Associations between root-zone dissolved oxygen, photosynthetic performance, water productivity, fruit quality, oxidative stress markers, antioxidant enzyme activities, gene expression, metabolite abundance, and rhizosphere microbial traits were determined by Pearson correlation analysis. Heatmap clustering was used to visualize treatment-specific patterns of gene expression, metabolite accumulation, and microbial abundance. Description: Integrated interpretation based on the progress made on our current understanding of root function, ROS homeostasis, aquaporin, nutrient transporter, carbon metabolism, antioxidant defense, and fruit quality under deficit fertigation.

2.14 Statistical Analysis

The statistical analyses were conducted using IBM SPSS Statistics, version 26.0. The experimental unit for growth, physiological, yield, biochemical, qRT-PCR, metabolomic, and microbiome analyses was the plot level. To avoid pseudo replication, inferences were drawn from summary data aggregated at the treatment × block level. The Shapiro–Wilk test assessed normality, and Levene's test assessed homogeneity of variance. Where necessary, percentage data were transformed prior to analysis, and untransformed means were presented for interpretational purposes. Using the following model, an analysis of variance was performed in a randomized complete block design.

$$\mathbf{Y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}}$$

where Y_{ij} is the observed value, μ is the overall mean, T_i is the fixed effect of the ith fertigation treatment, B_j is the effect of the jth block, and ε_{ij} is the residual error. Treatment means were separated using Tukey's honestly significant difference test at $P \leq 0.05$.

The relative expression values by qRT-PCR were compared within factors to analyze gene-wise using one-way ANOVA under a randomized block design. To clarify the relationships among the variables, data on physiological, yield, quality, biochemical, and molecular traits were subjected to Pearson correlation analysis. We performed principal component analysis (PCA) using standardized variables. For RNA-seq and omics-level enrichment outputs, false discovery rate correction was done using the Benjamini–Hochberg method. Unless stated otherwise, results were expressed as mean ± standard error.

3. RESULTS

3.1 Root-Zone Oxygenation, Vegetative Growth, and Photosynthetic Performance

Oxygen nanobubble-enriched fertigation markedly increased root-zone dissolved oxygen compared with conventional fertigation treatments (Table 1, Figure 1). Root-zone dissolved oxygen was highest in FI-ONB and DI75-ONB, with both treatments showing substantially greater oxygen availability than FI-CF and DI75-CF. The severe deficit oxygen nanobubble treatment also maintained high root-zone oxygen, although its value was slightly lower than FI-ONB and DI75-ONB. Deficit irrigation without oxygen enrichment reduced vegetative growth and photosynthetic activity. DI75-CF recorded lower plant height, leaf area, SPAD index, photosynthetic rate, stomatal conductance, and root dry weight than FI-CF. In contrast, oxygen nanobubble-enriched fertigation mitigated these reductions. DI75-ONB maintained plant height, leaf area, SPAD index, photosynthetic rate, and root dry weight at levels statistically comparable with FI-ONB and, in several cases, FI-CF. Instantaneous water-use efficiency was significantly enhanced under DI75-ONB and DI60-ONB, indicating improved carbon gain per unit of transpired water under oxygen-enriched deficit fertigation.

Table 1: Effect of Fertigation Treatments on Root-Zone Oxygenation, Growth, and Physiological Traits of Greenhouse Cucumber

Variable	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
Root-zone DO (mg L^{-1})	7.30 ± 0.04c	7.18 ± 0.06c	18.34 ± 0.13a	17.93 ± 0.18a	17.66 ± 0.13b	2823.70	<0.001
Plant height (cm)	222.8 ± 1.9a	200.3 ± 4.6b	237.1 ± 3.5a	230.3 ± 7.9a	212.2 ± 5.1b	12.51	<0.001

Leaf area (cm² plant⁻¹)	6242 ± 160b	5135 ± 254c	6916 ± 170a	6331 ± 185a	5628 ± 146b	21.06	<0.001
SPAD index	43.45 ± 0.66b	38.45 ± 0.77d	45.80 ± 0.43a	45.98 ± 0.71a	41.05 ± 0.61c	59.77	<0.001
Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	19.31 ± 0.35a	14.82 ± 0.41c	20.36 ± 0.57a	20.73 ± 0.41a	17.41 ± 0.60b	28.56	<0.001
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.336 ± 0.013a	0.213 ± 0.014b	0.359 ± 0.005a	0.314 ± 0.011a	0.241 ± 0.017b	26.69	<0.001
Instantaneous WUE (µmol CO_2 $mmol^{-1}$ H_2O)	3.56 ± 0.09b	3.50 ± 0.18b	3.49 ± 0.07b	4.76 ± 0.13a	4.71 ± 0.26a	17.95	<0.001
Root dry weight (g plant⁻¹)	21.27 ± 0.83b	18.20 ± 0.50c	24.41 ± 0.73a	25.58 ± 0.68a	22.68 ± 0.39b	27.64	<0.001

Values are mean ± SE. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

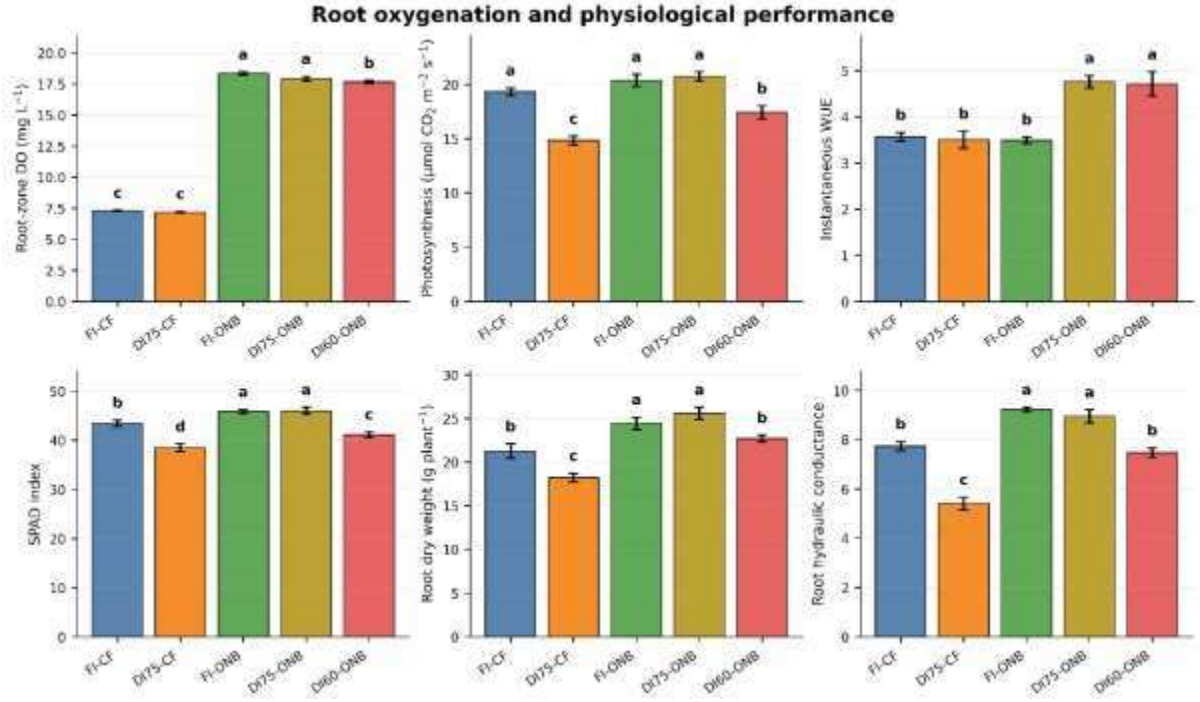

Figure 1: Effects of conventional and oxygen nanobubble-enriched deficit fertigation on root-zone dissolved oxygen, plant growth, photosynthetic performance, and water-use efficiency of greenhouse cucumber.

3.2 Yield, Marketable Yield, and Resource-Use Efficiency

Fertigation treatment significantly affected fruit production and resource-use efficiency (Table 2, Figure 2). DI75-CF reduced fruit number, average fruit weight, total yield, and marketable yield compared with FI-CF. Oxygen nanobubble enrichment improved yield performance under both full and deficit irrigation. DI75-ONB produced total yield statistically comparable with FI-ONB and FI-CF and significantly higher than DI75-CF. Compared with DI75-CF, DI75-ONB increased total yield by 25.17% and marketable yield percentage by 18.16%. Water productivity, partial factor productivity of nitrogen, and nitrogen-use efficiency were highest under DI75-ONB and DI60-ONB. Although DI60-ONB improved resource-use indices, its total and marketable yields were lower than DI75-ONB, indicating that moderate deficit fertigation combined with oxygen nanobubbles provided the most balanced response between productivity and input saving.

Table 2: Effect of Fertigation Treatments on Yield and Resource-Use Efficiency of Greenhouse Cucumber

Variable	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
Fruits plant⁻¹	18.4 ± 0.2a	16.9 ± 0.4b	19.8 ± 0.3a	19.5 ± 0.5a	17.7 ± 0.5b	11.03	0.001

Average fruit weight (g)	313.0 ± 7.7a	285.3 ± 4.7b	319.6 ± 4.0a	313.1 ± 5.8a	286.7 ± 4.5b	20.35	<0.001
Total yield (kg plant^{-1})	5.83 ± 0.09a	4.84 ± 0.17b	6.29 ± 0.07a	6.05 ± 0.13a	4.88 ± 0.20b	37.51	<0.001
Marketable yield (kg plant^{-1})	5.16 ± 0.07b	3.73 ± 0.13c	5.65 ± 0.07a	5.52 ± 0.11a	4.00 ± 0.16c	105.14	<0.001
Marketable yield (%)	88.5 ± 0.7a	77.2 ± 1.4b	89.8 ± 0.2a	91.3 ± 2.1a	82.1 ± 0.4b	26.38	<0.001
Water productivity (kg m^{-3})	64.78 ± 1.04b	71.67 ± 2.50b	69.95 ± 0.79b	89.70 ± 1.96a	90.32 ± 3.66a	45.28	<0.001
PFP-N (kg yield kg^{-1} N)	694.05 ± 11.17b	767.88 ± 26.79b	749.40 ± 8.44b	961.12 ± 21.00a	967.77 ± 39.20a	45.29	<0.001
NUE index (% control)	100.5 ± 1.6b	111.2 ± 3.9b	108.5 ± 1.2b	139.2 ± 3.1a	140.2 ± 5.7a	45.54	<0.001

Values are mean ± SE. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

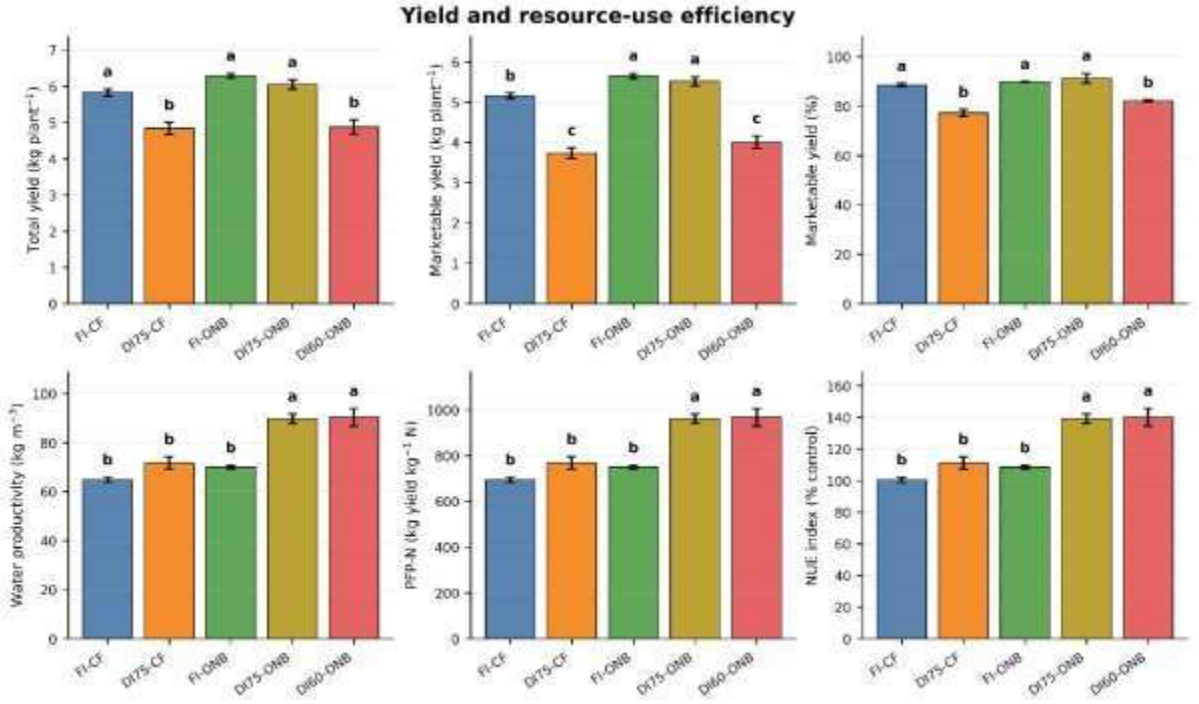

Figure 2: Influence of oxygen nanobubble-enriched deficit fertigation on fruit yield, marketable production, water productivity, and nitrogen-use efficiency in greenhouse cucumber.

3.3 Fruit Quality Attributes

Fruit quality traits responded strongly to fertigation regime (Table 3, Figure 3). DI75-CF increased total soluble solids and vitamin C compared with FI-CF but also increased fruit nitrate accumulation and reduced marketability. Oxygen nanobubble-enriched fertigation improved fruit firmness, soluble solids, vitamin C, phenolics, antioxidant activity, and marketability while reducing nitrate accumulation. DI75-ONB produced a favorable fruit quality profile, with higher firmness, TSS, vitamin C, total phenolics, DPPH antioxidant activity, and marketability than conventional deficit fertigation. Fruit nitrate concentration was highest in DI75-CF, whereas FI-ONB and DI75-ONB showed substantially lower nitrate accumulation. DI60-ONB produced the highest TSS, vitamin C, phenolics, and antioxidant activity, but this occurred with reduced yield compared with DI75-ONB.

Table 3: Effect of Fertigation Treatments on Fruit Quality Attributes of Greenhouse Cucumber

Variable	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
Fruit firmness (N)	23.96 ± 0.18c	23.05 ± 0.10d	25.57 ± 0.16a	26.13 ± 0.25a	25.01 ± 0.18b	39.57	<0.001
TSS (°Brix)	3.80 ± 0.03d	4.07 ± 0.03c	4.17 ± 0.04c	4.46 ± 0.05b	4.69 ± 0.05a	58.86	<0.001

Titratable acidity (%)	0.183 ± 0.002d	0.199 ± 0.001c	0.196 ± 0.001c	0.209 ± 0.002b	0.219 ± 0.002a	63.79	<0.001
Vitamin C (mg 100 g^{-1} FW)	8.39 ± 0.10e	9.11 ± 0.08d	9.95 ± 0.07c	10.43 ± 0.08b	10.99 ± 0.13a	150.10	<0.001
Total phenolics (mg GAE 100 g^{-1} FW)	43.6 ± 0.7e	52.9 ± 0.1d	58.3 ± 0.5c	63.6 ± 0.1b	68.8 ± 0.7a	405.43	<0.001
DPPH activity (%)	31.1 ± 0.7e	36.5 ± 0.3d	42.4 ± 0.3c	45.5 ± 0.3b	49.4 ± 0.1a	291.62	<0.001
Nitrate (mg kg^{-1} FW)	350.4 ± 2.2b	401.0 ± 3.2a	298.2 ± 2.0e	313.5 ± 4.7d	331.1 ± 1.4c	201.47	<0.001
Marketability score (1–9)	7.10 ± 0.10b	6.71 ± 0.08c	7.80 ± 0.05a	7.93 ± 0.06a	7.01 ± 0.07b	48.50	<0.001

Values are mean ± SE. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

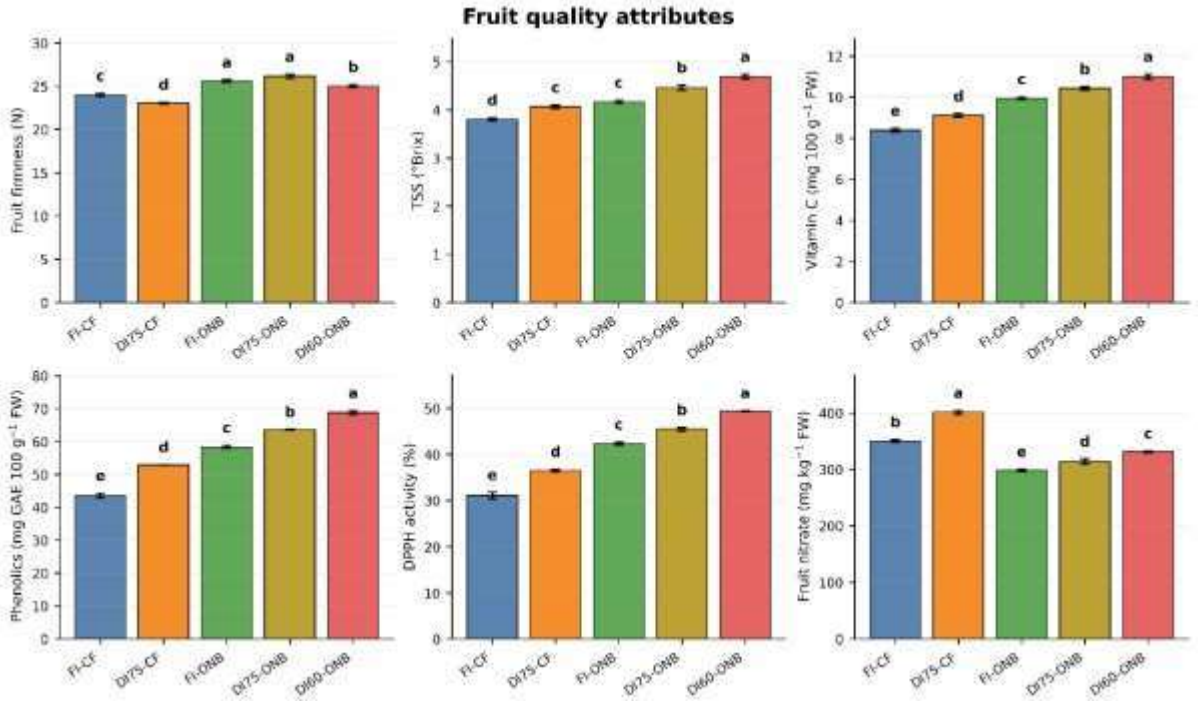

Figure 3: Changes in cucumber fruit firmness, soluble solids, vitamin C, phenolic compounds, antioxidant activity, nitrate accumulation, and marketability under different fertigation treatments.

3.4 Oxidative Stress Markers and Antioxidant Defense

Deficit irrigation under conventional fertigation intensified oxidative stress, as indicated by increased H_2O_2, MDA, electrolyte leakage, and proline content in DI75-CF (Table 4, Figure 4). Oxygen nanobubble enrichment reduced oxidative injury under deficit fertigation. Compared with DI75-CF, DI75-ONB reduced H_2O_2 by 44.31% and MDA by 38.14%, indicating lower reactive oxygen species accumulation and lipid peroxidation. Antioxidant enzyme activity was significantly enhanced by oxygen nanobubble-enriched fertigation. SOD, CAT, APX, and POD activities were higher in DI75-ONB than in FI-CF and DI75-CF. DI60-ONB showed the highest antioxidant enzyme activity, reflecting stronger stress acclimation under severe deficit conditions. Nitrate reductase activity was greatest in DI75-ONB, supporting improved nitrogen assimilation under oxygen-enriched moderate deficit fertigation.

Table 4: Effect of Fertigation Treatments on Oxidative Stress and Antioxidant Enzyme Activity

Variable	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
H_2O_2 (µmol g^{-1} FW)	3.50 ± 0.24b	5.89 ± 0.06a	2.80 ± 0.15c	3.28 ± 0.14c	4.23 ± 0.20b	53.85	<0.001
MDA (nmol g^{-1} FW)	6.24 ± 0.28b	9.55 ± 0.26a	4.87 ± 0.14c	5.91 ± 0.05b	6.50 ± 0.11b	104.66	<0.001
Electrolyte leakage (%)	11.0 ± 0.8c	20.3 ± 0.7a	9.8 ± 0.2c	11.0 ± 0.7c	15.4 ± 0.5b	50.03	<0.001

Proline (µmol g^{-1} FW)	26.02 ± 2.83c	47.55 ± 1.27a	30.22 ± 1.32b	34.56 ± 0.93b	45.91 ± 1.02a	30.70	<0.001
Soluble sugars (mg g^{-1} FW)	27.39 ± 1.49b	28.86 ± 0.19b	29.04 ± 1.78b	35.12 ± 2.04a	36.37 ± 0.86a	13.77	<0.001
SOD (U mg^{-1} protein)	102.8 ± 2.4d	136.3 ± 2.3c	126.8 ± 2.0c	149.9 ± 1.7b	161.3 ± 3.4a	102.16	<0.001
CAT (U mg^{-1} protein)	42.1 ± 0.9d	49.7 ± 0.7c	52.7 ± 0.7c	61.7 ± 1.2b	66.4 ± 1.0a	112.04	<0.001
APX (U mg^{-1} protein)	8.4 ± 0.5c	13.2 ± 0.7a	10.4 ± 0.5b	14.7 ± 0.5a	14.8 ± 0.3a	45.74	<0.001
POD (U mg^{-1} protein)	86.0 ± 2.8c	111.5 ± 0.4b	102.2 ± 1.7b	129.6 ± 5.8a	132.8 ± 2.4a	45.11	<0.001
Nitrate reductase (µmol NO_2^- g^{-1} FW h^{-1})	8.27 ± 0.24c	7.78 ± 0.18c	9.93 ± 0.44b	11.03 ± 0.27a	9.93 ± 0.10b	30.37	<0.001

Values are mean ± SE. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

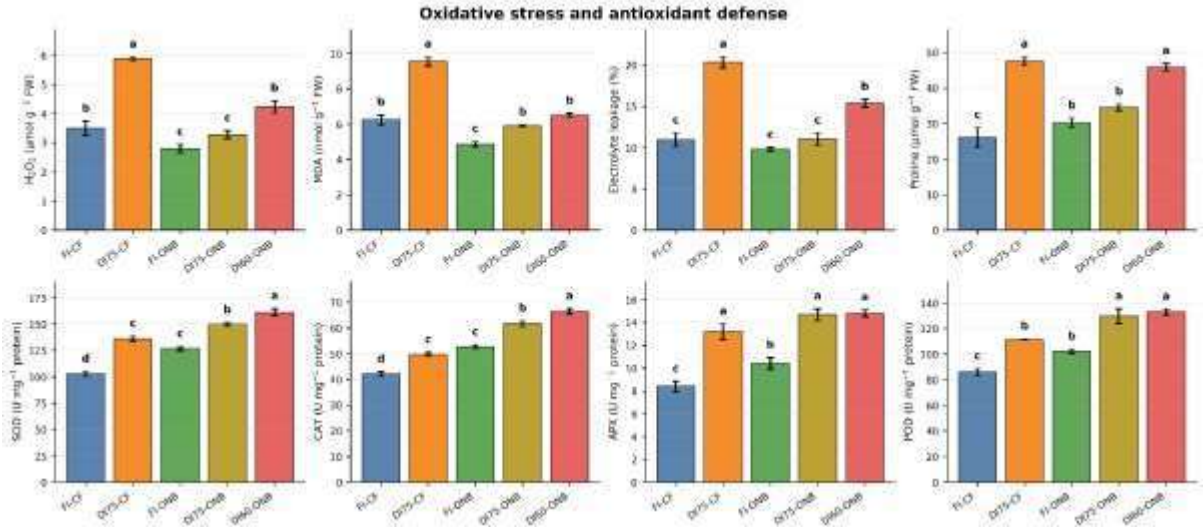

Figure 4: Response of oxidative stress indicators (H_2O_2, MDA, electrolyte leakage) and antioxidant defense enzymes (SOD, CAT, APX, and POD) to oxygen nanobubble-enriched deficit fertigation.

3.5 qRT-PCR-Based Gene Expression Responses

Fertigation treatments significantly altered the expression of genes involved in water transport, nutrient uptake, antioxidant defense, oxygen response, and stress signaling (Table 5. Figure 5). Aquaporin genes CsPIP1;1 and CsPIP2;1 were upregulated under oxygen nanobubble-enriched fertigation, with DI75-ONB showing the strongest or among the strongest expression responses. Nutrient transporter genes CsNRT1.1, CsNRT2.1, and CsPHT1;4 were also upregulated in DI75-ONB relative to DI75-CF, consistent with improved nitrogen and phosphorus acquisition under oxygen-enriched deficit fertigation. Antioxidant-related genes, including CsCAT1, CsAPX1, and CsCuZnSOD, showed higher expression in oxygen nanobubble treatments, particularly under deficit fertigation. Oxygen-responsive genes CsHRE1-like and CsAOX1a were strongly induced under oxygen nanobubble treatments, suggesting activation of oxygen-sensing and mitochondrial respiratory adjustment pathways. Stress-responsive genes such as CsDREB2A and CsWRKY33 were most strongly expressed under DI60-ONB, indicating greater stress signaling under severe deficit conditions.

Table 5: Relative Expression of Selected Cucumber Genes Under Different Fertigation Treatments

Gene	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
CsPIP1;1 (aquaporin)	0.99 ± 0.12b	0.81 ± 0.11b	1.32 ± 0.10a	1.75 ± 0.14a	1.74 ± 0.13a	11.33	<0.001
CsPIP2;1 (aquaporin)	0.99 ± 0.04b	0.84 ± 0.04c	1.84 ± 0.12a	2.21 ± 0.19a	1.45 ± 0.07b	26.08	<0.001
CsNRT1.1 (nitrate transport)	0.93 ± 0.05b	0.74 ± 0.02b	1.20 ± 0.12a	1.52 ± 0.13a	1.16 ± 0.08a	10.20	0.001

CsNRT2.1 (nitrate transport)	0.82 ± 0.02c	0.72 ± 0.01c	1.39 ± 0.15b	1.90 ± 0.06a	1.25 ± 0.14b	19.49	<0.001
CsPHT1;4 (phosphate transport)	0.90 ± 0.07b	0.72 ± 0.07b	1.32 ± 0.11a	1.53 ± 0.04a	1.00 ± 0.02b	20.89	<0.001
CsCAT1 (catalase)	1.13 ± 0.10c	1.49 ± 0.05b	1.73 ± 0.07b	1.99 ± 0.13a	2.33 ± 0.14a	22.19	<0.001
CsAPX1 (ascorbate peroxidase)	1.10 ± 0.05b	1.59 ± 0.09b	1.60 ± 0.13b	2.19 ± 0.17a	2.38 ± 0.09a	20.36	<0.001
CsCuZnSOD (SOD)	0.96 ± 0.09b	1.35 ± 0.05b	1.58 ± 0.07a	2.37 ± 0.29a	2.29 ± 0.30a	11.87	<0.001
CsHRE1-like (oxygen signaling)	1.05 ± 0.03c	1.33 ± 0.12b	1.99 ± 0.13b	2.47 ± 0.25a	3.21 ± 0.28a	22.18	<0.001
CsAOX1a (alternative oxidase)	1.10 ± 0.03b	1.15 ± 0.05b	1.67 ± 0.12a	1.91 ± 0.07a	1.99 ± 0.14a	24.82	<0.001
CsDREB2A (stress response)	1.03 ± 0.10c	1.91 ± 0.11a	1.31 ± 0.10b	1.60 ± 0.08b	2.30 ± 0.15a	17.83	<0.001
CsWRKY33 (stress signaling)	0.94 ± 0.05c	1.33 ± 0.07b	1.13 ± 0.06b	1.36 ± 0.10b	1.98 ± 0.16a	23.56	<0.001

Values are mean ± SE of relative expression. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

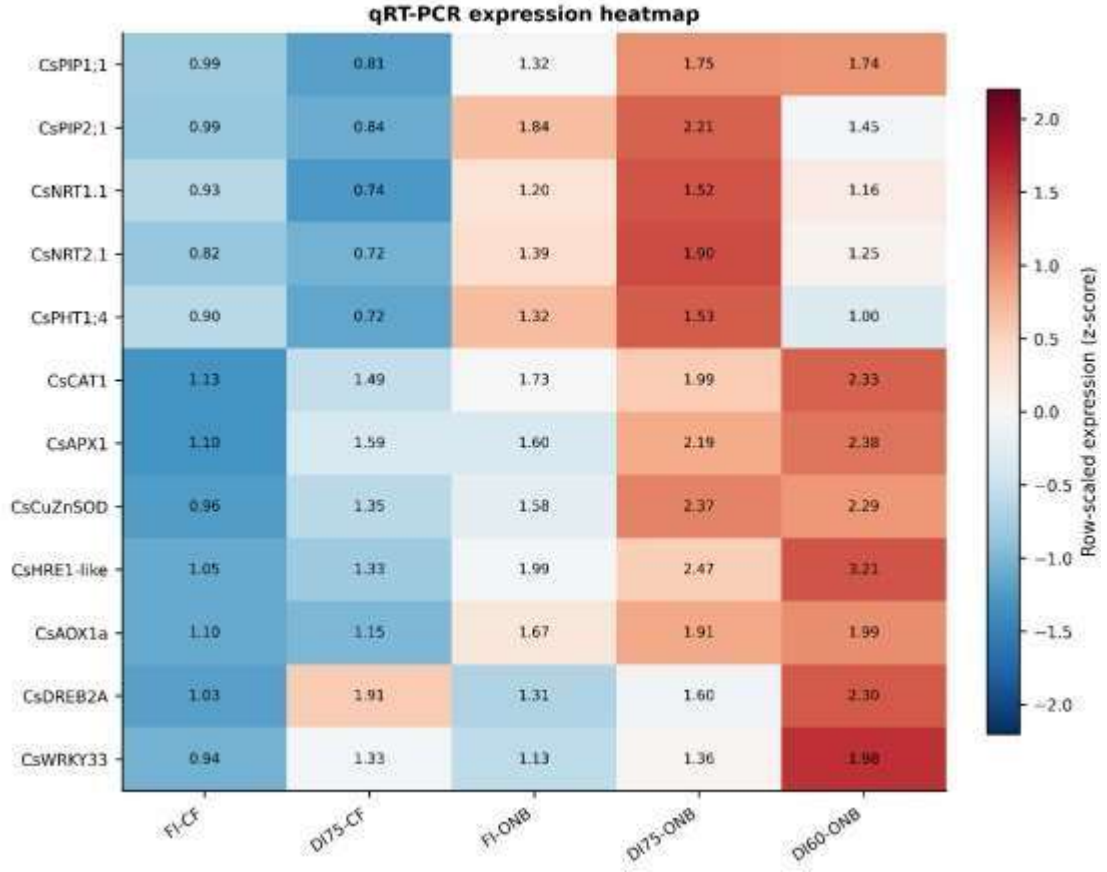

Figure 5: Heatmap visualization of relative expression patterns of genes associated with water transport, nutrient uptake, oxygen signaling, stress responses, and antioxidant defense under different fertigation regimes.

3.6 Metabolomic Shifts Associated with Oxygen Nanobubble-Enriched Deficit Fertigation

Metabolomic profiling showed significant treatment-dependent changes in carbon metabolites, osmoprotectants, antioxidants, and hormone-related metabolites (Table 6, Figure 6). DI75-CF reduced sucrose and increased stress-associated metabolites such as proline, GABA, glycine betaine, and ABA. Oxygen nanobubble-enriched fertigation increased sucrose, chlorogenic acid, rutin, and ascorbate, indicating improved carbon allocation and

antioxidant metabolite accumulation. DI75-ONB showed higher sucrose, ascorbate, chlorogenic acid, rutin, and glycine betaine than DI75-CF, while ABA accumulation was lower than in DI75-CF and DI60-ONB. This response indicates partial relief of deficit-induced stress under oxygen-enriched fertigation. DI60-ONB had the highest proline, GABA, glycine betaine, and ABA, reflecting stronger osmotic and stress adaptation under severe deficit.

Table 6: Selected Metabolomic Responses Under Different Fertigation Treatments

Variable	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
Sucrose	2446.62 ± 63.06a	2076.32 ± 80.99b	2706.95 ± 136.56a	2836.22 ± 58.67a	2613.78 ± 156.90a	9.04	0.001
Proline	1787.20 ± 25.49c	3347.90 ± 208.06a	2205.65 ± 69.32b	2794.70 ± 173.73b	3480.62 ± 91.92a	26.65	<0.001
GABA	6404.65 ± 246.02c	10161.52 ± 124.70a	7453.50 ± 85.63c	9283.95 ± 513.27b	11588.05 ± 409.52a	35.37	<0.001
Chlorogenic acid	5796.65 ± 191.72b	7270.70 ± 151.25b	8467.02 ± 211.57a	8776.52 ± 688.72a	9710.47 ± 610.04a	15.89	<0.001
Rutin	3011.97 ± 91.32b	3223.00 ± 189.30b	3828.18 ± 115.07b	4732.43 ± 357.72a	5319.32 ± 205.94a	19.80	<0.001
Ascorbate	7322.98 ± 285.59c	8278.60 ± 226.28b	9050.10 ± 510.05b	10285.75 ± 229.27a	9873.25 ± 114.76a	23.34	<0.001
Glycine betaine	4066.47 ± 154.71c	5351.27 ± 157.23b	4790.42 ± 128.96b	6056.48 ± 129.40a	6519.73 ± 240.94a	32.71	<0.001
ABA	7488.50 ± 220.19c	15313.80 ± 485.82a	7981.02 ± 384.23c	11147.50 ± 365.16b	15698.83 ± 784.13a	61.02	<0.001
Zeatin	1208.33 ± 7.22b	1080.95 ± 87.96b	1639.38 ± 51.29a	1589.80 ± 86.71a	1236.40 ± 49.65b	13.57	<0.001

Values are mean ± SE. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

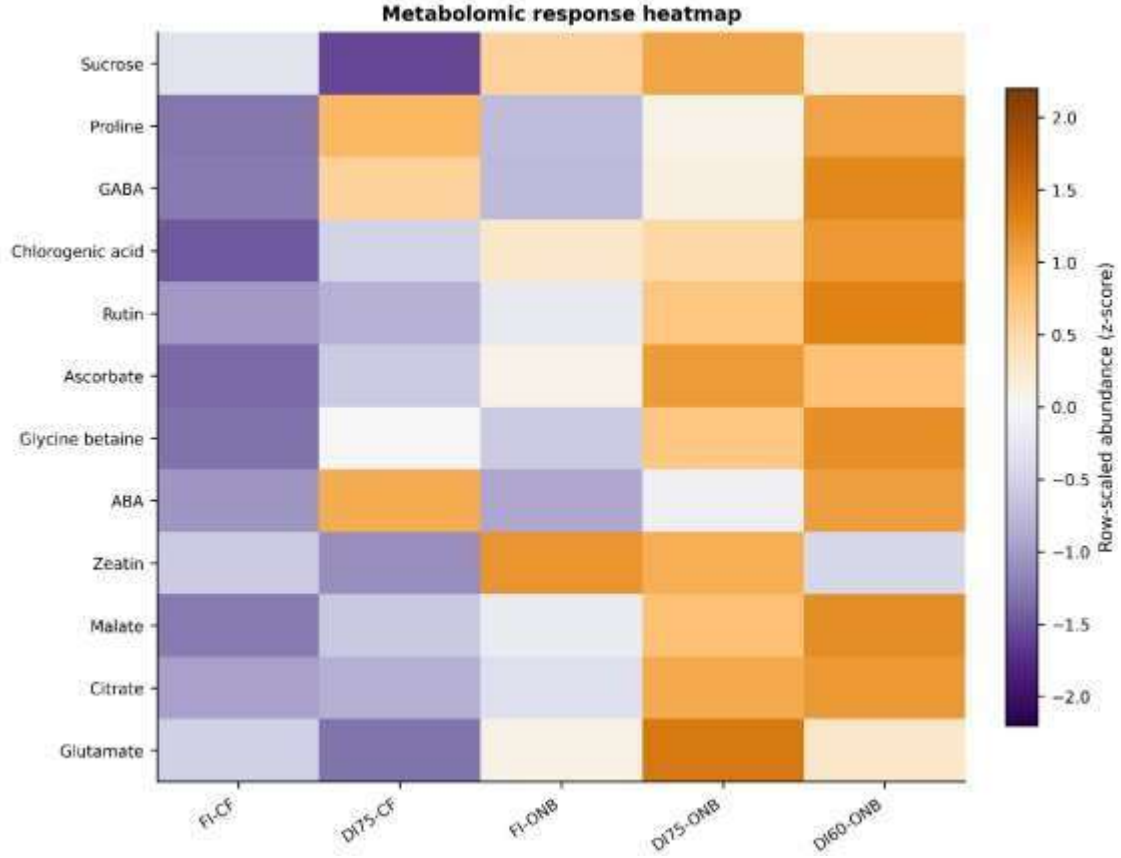

Figure 6: Heatmap showing standardized relative abundance patterns of selected primary and secondary metabolites involved in carbon metabolism, osmotic adjustment, antioxidant activity, and stress adaptation.

3.7 Rhizosphere Microbial Responses

Rhizosphere microbial indices were significantly influenced by fertigation treatment (Table 7). DI75-CF reduced Shannon diversity and the relative abundance of beneficial genera while increasing Fusarium abundance. Oxygen nanobubble-enriched fertigation increased Shannon diversity and promoted beneficial microbial groups, including

Bacillus, Pseudomonas, and Azospirillum. DI75-ONB produced the highest beneficial-to-Fusarium ratio, indicating a more favorable rhizosphere microbial balance under moderate deficit fertigation when oxygen nanobubbles were applied. Although DI60-ONB maintained higher beneficial microbial abundance than DI75-CF, its beneficial-to-Fusarium ratio was lower than DI75-ONB, suggesting that severe deficit partially constrained the microbial benefits of oxygen enrichment.

Table 7: Effect of Fertigation Treatments on Selected Rhizosphere Microbial Indices

Variable	FI-CF	DI75-CF	FI-ONB	DI75-ONB	DI60-ONB	F	P
Shannon index	4.36 ± 0.12a	3.85 ± 0.11b	4.60 ± 0.09a	4.79 ± 0.08a	4.56 ± 0.10a	12.64	<0.001
Bacillus (%)	5.02 ± 0.16b	3.22 ± 0.47c	6.90 ± 0.31a	8.22 ± 0.30a	6.41 ± 0.32b	28.46	<0.001
Pseudomonas (%)	4.54 ± 0.20b	2.80 ± 0.27b	7.83 ± 0.51a	7.91 ± 0.43a	6.28 ± 0.65a	25.16	<0.001
Azospirillum (%)	2.03 ± 0.20b	1.22 ± 0.18c	3.07 ± 0.34a	3.88 ± 0.13a	2.74 ± 0.10b	22.06	<0.001
Fusarium (%)	1.46 ± 0.05b	3.10 ± 0.21a	1.06 ± 0.10b	1.08 ± 0.17b	1.61 ± 0.09b	34.97	<0.001
Beneficial ratio	11.12 ± 0.49b	3.15 ± 0.24c	22.69 ± 2.18a	25.97 ± 3.48a	12.48 ± 0.77b	33.06	<0.001

Values are mean ± SE. Different lowercase letters within a row indicate significant differences according to Tukey's HSD test at $P \leq 0.05$.

3.8 Transcriptomic Responses

RNA-seq analysis revealed distinct transcriptomic reprogramming across fertigation treatments (Table 8, Figure 7). DI75-CF vs FI-CF identified 1,174 differentially expressed genes, indicating a clear deficit-induced stress response. FI-ONB vs FI-CF identified 932 DEGs, mainly associated with oxygenation-related regulation, root respiration, nutrient transport, and antioxidant priming. DI75-ONB vs FI-CF identified 1,286 DEGs, reflecting integrated activation of oxygen response, water transport, nutrient uptake, and antioxidant defense pathways. The largest transcriptional contrast was observed between DI75-ONB and DI75-CF, with 1,648 DEGs, including 1,010 upregulated and 638 downregulated genes. This comparison indicates that oxygen nanobubble enrichment strongly reprogrammed the molecular response of cucumber under equal deficit fertigation. DI60-ONB vs FI-CF showed 1,518 DEGs, consistent with strong stress acclimation under severe deficit despite oxygen support.

Table 8: Summary of Differentially Expressed Genes Under Fertigation Treatment Comparisons

Comparison	Contrast	Total DEGs	Upregulated	Downregulated	Dominant response
T2 vs T1	DI75-CF vs FI-CF	1174	712	462	Stress induction; repression of photosynthesis and nutrient uptake
T3 vs T1	FI-ONB vs FI-CF	932	548	384	Root respiration, nutrient transport, and antioxidant priming
T4 vs T1	DI75-ONB vs FI-CF	1286	771	515	Integrated oxygen, water transport, and antioxidant adaptation
T5 vs T1	DI60-ONB vs FI-CF	1518	930	588	Stress acclimation with partial growth penalty
T4 vs T2	DI75-ONB vs DI75-CF	1648	1010	638	Stress mitigation and resource-use reprogramming

DEGs were summarized using FDR ≤ 0.05 and absolute $\log_2$ fold-change ≥ 1.

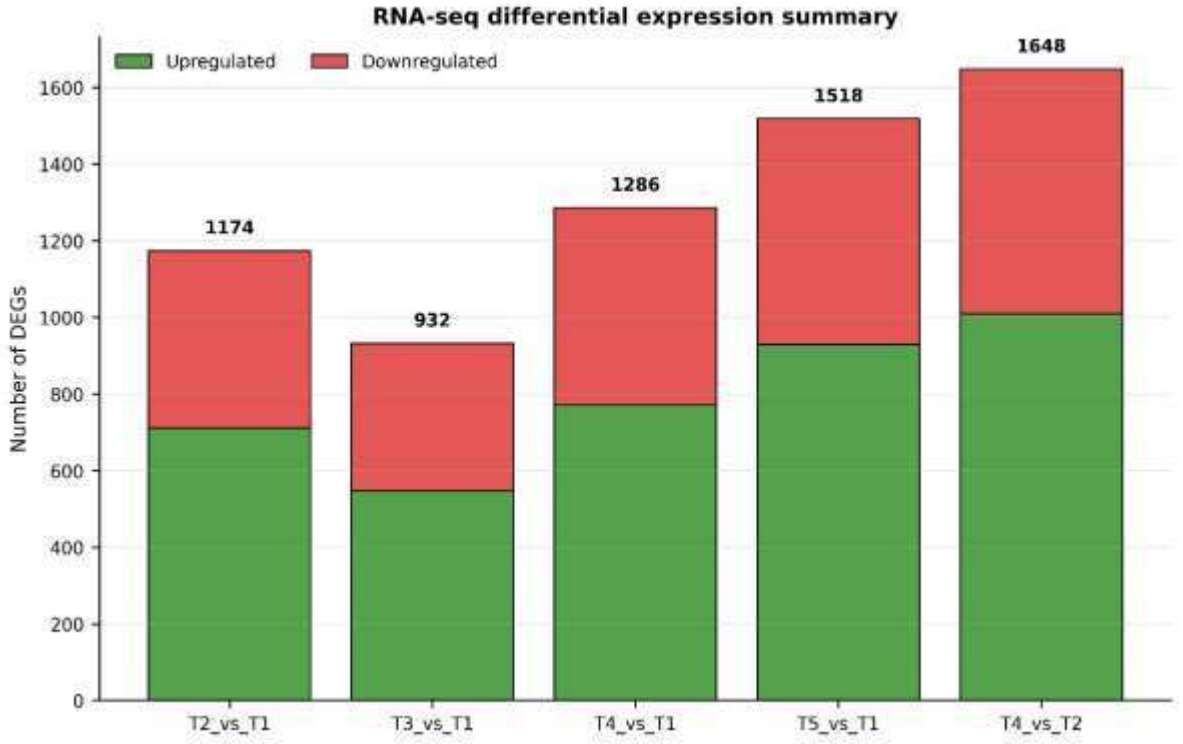

Figure 7: Overview of differentially expressed genes, volcano plot distribution, and enriched Gene Ontology and KEGG pathways associated with oxygen nanobubble-mediated stress mitigation.

3.9 Principal Component and Correlation Analyses

Principal component analysis separated the fertigation treatments based on integrated physiological, biochemical, fruit quality, and rhizosphere variables (Table 9, Figure 8). PC1 explained 52.72% of the total variation, while PC2 explained 36.61%, giving a cumulative explanation of 89.32% for the first two components. PC1 was positively associated with root-zone dissolved oxygen, vitamin C, beneficial-to-Fusarium ratio, photosynthetic rate, and root hydraulic conductance, and negatively associated with fruit nitrate, MDA, and H_2O_2. PC2 contributed mainly to the separation of yield-related and quality-related responses. Pearson correlation analysis supported the integrated response pattern. Root-zone dissolved oxygen was positively correlated with TSS, vitamin C, instantaneous WUE, and beneficial-to-Fusarium ratio, but negatively correlated with fruit nitrate and MDA (Table 10, Figure 9). Total yield was negatively associated with H_2O_2 and positively associated with beneficial-to-Fusarium ratio. Fruit nitrate showed a strong negative relationship with beneficial-to-Fusarium ratio, suggesting that improved rhizosphere balance was associated with lower nitrate accumulation.

Table 9: Principal Component Analysis Eigenvalues and Explained Variance

Principal component	Eigenvalue	Variance explained (%)	Cumulative variance (%)
PC1	7.38	52.72	52.72
PC2	5.13	36.61	89.32
PC3	0.58	4.17	93.50
PC4	0.44	3.11	96.61
PC5	0.17	1.20	97.82

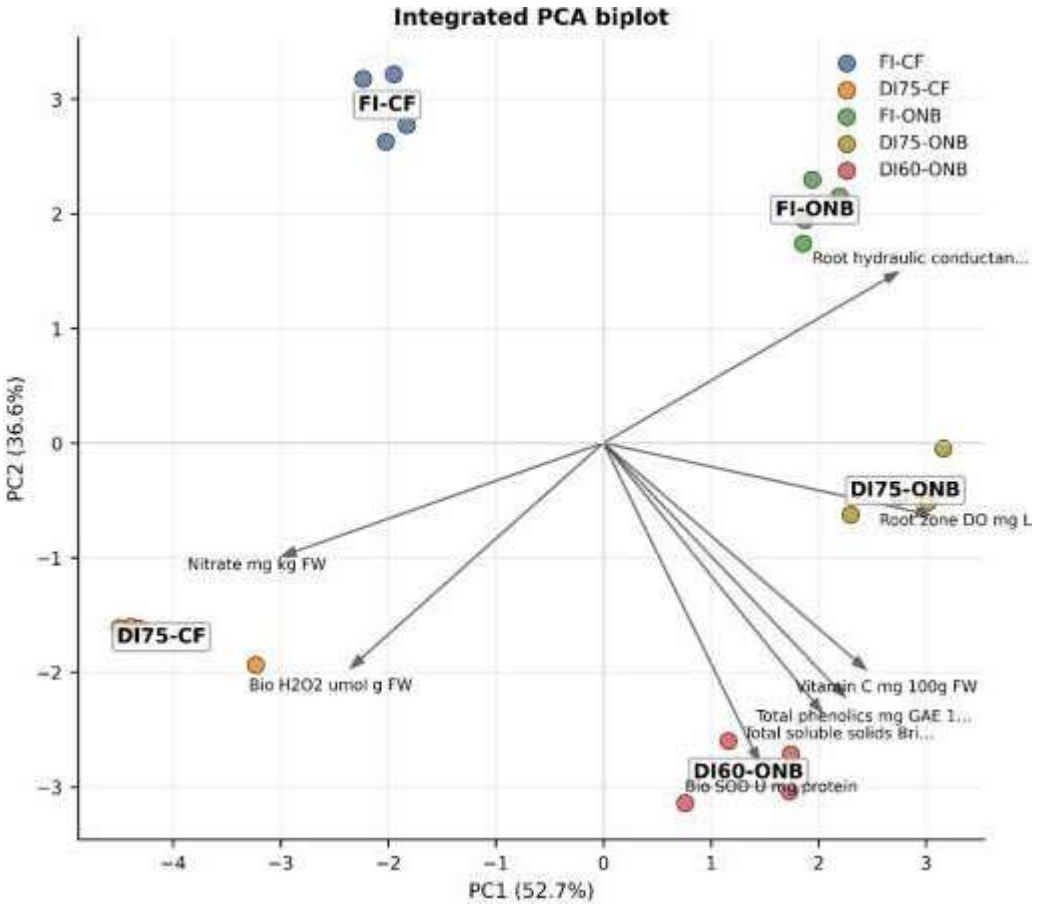

Figure 8: Integrated principal component analysis (PCA) biplot showing treatment separation based on combined physiological, yield, fruit quality, oxidative stress, and rhizosphere-related traits under different fertigation regimes.

Table 10: Selected Pearson Correlation Coefficients Among Integrated Response Variables

Variable 1	Variable 2	n	r	P	FDR significant
Root-zone DO	TSS	20	0.749	<0.001	Yes
Root-zone DO	Vitamin C	20	0.871	<0.001	Yes
Root-zone DO	Fruit nitrate	20	-0.851	<0.001	Yes
Root-zone DO	Instantaneous WUE	20	0.557	0.011	Yes
Root-zone DO	MDA	20	-0.671	0.001	Yes
Root-zone DO	Beneficial ratio	20	0.749	<0.001	Yes
Total yield	H_2O_2	20	-0.761	<0.001	Yes
Total yield	Beneficial ratio	20	0.727	<0.001	Yes
TSS	Vitamin C	20	0.939	<0.001	Yes
Fruit nitrate	Beneficial ratio	20	-0.849	<0.001	Yes

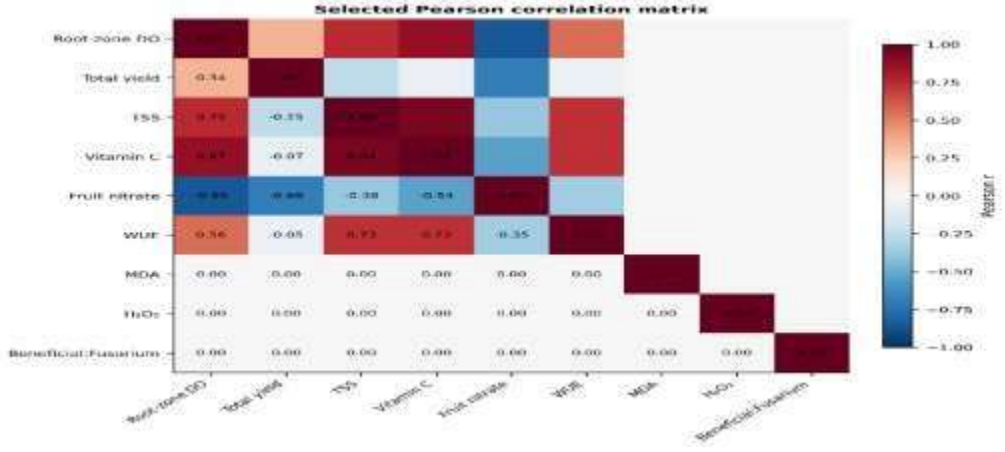

Figure 9: Pearson correlation matrix showing relationships among integrated physiological, yield, fruit quality, oxidative stress, and rhizosphere microbial traits under different fertigation treatments. Color intensity represents correlation strength, with red indicating positive and blue indicating negative associations.

3.10 Overall Treatment Response

Across growth, yield, physiological, biochemical, molecular, metabolomic, and rhizosphere indicators, DI75-ONB provided the most balanced response. This treatment maintained yield at a level statistically comparable with full irrigation while substantially improving water productivity, nitrogen-use efficiency, fruit quality, antioxidant defense, and molecular markers of water and nutrient transport. DI60-ONB further increased some quality and stress-adaptive metabolites but reduced yield performance, indicating that 60% irrigation imposed a stronger stress burden. Overall, oxygen nanobubble-enriched fertigation under 75% irrigation achieved the best compromise between productivity, resource-use efficiency, fruit quality, and stress mitigation in greenhouse cucumber.

4. DISCUSSION

Oxygen nanobubble-enriched deficit fertigation improved cucumber performance by enhancing root-zone oxygen availability and sustaining physiological activity during reduced irrigation, as indicated by the present results. Deficit fertigation with no oxygen enrichment created impairment for canopy function and root performance, as diminished photosynthetic rate, stomatal conductance, leaf area, root dry weight and yield-related traits were recorded. In comparison, DI75-ONB maintained photosynthesis and root-related traits at levels like well-irrigated treatments while significantly increasing instantaneous water-use efficiency. This answer is consistent with the response of Morón-López, Varela [20], which reported that moderately diluted oxygen nanobubbles improved lettuce growth, biomass accumulation, and water savings of approximately 23%. This is a relevant comparison because both studies argue that oxygen nanobubbles also benefit plant performance under water-saving irrigation. However, Morón-López, Varela [20] also found negative responses at the highest nanobubble concentration, confirming that both the nanobubble dose and the crop context are crucial. Analogously, in the current study, DI75-ONB yielded the most favorable response, whereas DI60-ONB improved certain stress-adaptive traits without maintaining yield.

The positive yield and resource-use response under DI75-ONB is also consistent with previous work on oxygen-enriched fertigation and micro-nano bubble irrigation. Bonachela, Acuña [15] showed that oxygen enrichment of nutrient solution increased melon yield on rockwool by approximately 7%. However, the response was crop- and substrate-dependent and absent in sweet pepper and melon grown on perlite. This supports the interpretation that oxyfertigation benefits are strongest when root-zone oxygen becomes limiting. The results extend this concept to cucumber under deficit fertigation, where oxygen enrichment increased root-zone dissolved oxygen and improved water productivity and nitrogen-use efficiency. Cao, Zheng [21] similarly reported that micro-nano-bubble-oxygenated subsurface drip irrigation increased alfalfa dry matter yield by 8.3–30.1% and improved root vitality, soil enzyme activity, microbial abundance, and water-use efficiency. The agreement between these findings and the DI75-ONB response suggests that oxygenated irrigation may enhance crop productivity by improving root function and rhizosphere biological activity, rather than acting solely through direct water-supply effects.

This decrease in oxidative injury in DI75-ONB sheds light on a mechanism for enhanced physiologic stability. Conventional deficit irrigation significantly elevated H_2O_2, MDA, electrolyte leakage, proline, and ABA-related stress responses, indicating increased oxidative and osmotic stress. Results showed that increasing the concentration of oxygen nanobubbles applied via fertigation significantly decreased H_2O_2 and MDA levels. In contrast, SOD, CAT, APX, and POD activities increased significantly, indicating better regulation of reactive oxygen species. These findings are consistent with Ramesh, Hebbar [26], who showed that coconut seedlings with contrasting water-use efficiency under water deficit exhibited differential antioxidant enzyme responses, lipid peroxidation status, aquaporin regulation, and stress-responsive gene expression. In our study, upregulation of **CsCAT1**, **CsAPX1**, **CsCuZnSOD**, **CsPIP1;1**, and **CsPIP2;1** supports a similar drought-adaptive mechanism involving antioxidant defense and water transport regulation. The higher vitamin C, phenolics, rutin, chlorogenic acid, and DPPH activity under oxygen nanobubble treatments further indicate that improved oxidative balance contributed to better fruit nutritional quality.

The transcriptomic and metabolomic results corroborate the perspective that DI75-ONB drove concerted molecular reprogramming rather than a single physiological adaptation. The largest contrast in DEG was found for the pair DI75-ONB vs DI75-CF, indicating that oxygen nanobubble enrichment had a strong impact on the plant response under the same level of deficit irrigation. This is consistent with the findings of Shahriari, Soltani [27] on a comprehensive transcriptomic characterization of soybean in response to drought stress, including regulation of photosynthesis, amino acid metabolism, hormone signaling, transcription factors, and ROS-associated pathways. The drought metabolic pattern is like that of other studies. Goufo, Moutinho-Pereira [28] demonstrated that cowpea adaptation to drought was associated with Osmo protective and antioxidant metabolites, including proline, as well as flavonoid derivatives related to yield performance. However, in the present study, DI75-ONB exhibited upregulation of sucrose, ascorbate, rutin, chlorogenic acid, and glycine betaine, while DI60-ONB showed greater accumulation of proline, GABA, and ABA. This shows that a moderate deficit with oxygen enrichment enhanced productive metabolic adjustment, while a severe deficit led to greater stress-protective metabolism at a yield cost.

Responses of rhizosphere microbes provide further evidence for the proposed mechanism. The DI75-ONB enhanced Shannon diversity, the prevalence of beneficial bacterial genera (Bacillus, Pseudomonas, and Azospirillum), and the beneficial-to-Fusarium ratio. These results are consistent with Sun, Liu [29], who demonstrated that root-zone aeration increased soil oxygen content, altered bacterial and fungal community structure, boosted beneficial microorganisms involved in nitrogen fixation and potassium solubilization, and

improved root growth and nutrient status in peach. Oxygen nanobubble fertigation could also improve cucumber rhizosphere function, as suggested by the present results, by promoting aerobic and beneficial plant-associated microbial groups. This interpretation was confirmed by PCA and correlation analysis: root-zone dissolved oxygen was positively correlated with WUE, vitamin C, photosynthetic performance, and beneficial microbes balance, and negatively correlated with fruit nitrate, H_2O_2, and MDA. This unified response is consistent with the multi-omics framework described in Pascual, Serna [30], which demonstrated that tomato adaptation to multifactorial stress is mediated by transcriptomic, metabolic, hormonal, and physiological mechanisms. In summary, the present findings suggest that DI75-ONB was the best treatment, as water-saving has improved root oxygenation, antioxidant regulation, nutrient-use efficiency, and altered rhizosphere structure and fruit quality.

5. CONCLUSION

The significantly enhanced root-zone dissolved oxygen, sustainable photosynthetic activity, and strengthened root function and water and nitrogen use efficiency under reduced irrigation by oxygen nanobubble-enriched deficit fertigation resulted in markedly improved greenhouse cucumber performance. Among the tested treatments, 75% deficit irrigation plus oxygen nanobubble fertigation produced the most balanced response, maintaining yield near full-irrigation conditions, while increasing marketable yield, water productivity, fruit firmness, soluble solids, vitamin C, phenolics, antioxidant activity, and decreasing nitrate accumulation and oxidative injury. The decrease in H_2O_2, MDA, and electrolyte leakage, even with increased SOD, CAT, APX, and POD activities, reflected that ROS homeostasis was improved by oxygen nanobubbles under water-deficiency. Molecular and multi-omics analyses also showed coordinated upregulation of aquaporin molecules, nutrient transporters, antioxidants, oxygen-responsive genes, and stress-regulatory genes, as well as favorable shifts in metabolites associated with carbon metabolism, Osmo protection, and antioxidant defense. More specifically, the higher abundance of beneficial microbiota and higher beneficial-to-Fusarium ratio in the rhizosphere suggest that the root microbial environment was also improved by oxygen nanobubble fertigation. The results suggest that harvesting inoculated potencies offers a reliable means of minimizing inputs while augmenting yield and fruit quality, thus serving as a model for sustainable and efficient greenhouse vegetable propagation.

Funding

Not applicable.

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