Chou and Ting *Chem. Biol. Technol. Agric.* (2023) 10:146
https://doi.org/10.1186/s40538-023-00497-2

Chemical and Biological Technologies in Agriculture

RESEARCH

Open Access

Plasma-activated water regulated transcriptome gene expression leading to high germination and growth of mung beans

Yu-Jou Chou[1] and Yuwen Ting[1*]

Abstract

Background Plasma-activated water (PAW) is a solution created by exposing water to cold atmospheric plasma discharge, resulting in a biocidal agent with unique biochemical properties attributed to highly reactive oxygen and nitrogen species (RONS). Plasma-activated water (PAW) has been the subject of research for its potential in promoting seed germination. While it has shown promising results, the exact mechanism by which PAW promotes seed growth remains unclear. This study aims to investigate the role of PAW in promoting mung bean germination, including its effects on vitality improvement and the triggering of plant stress responses to promote crop growth. Through the utilization of next-generation sequencing, we aim to explore the interaction between the properties of PAW and gene expression in mung beans. By deciphering the nature of PAW and analyzing gene expression patterns, we hope to uncover the underlying mechanisms that govern their interaction.

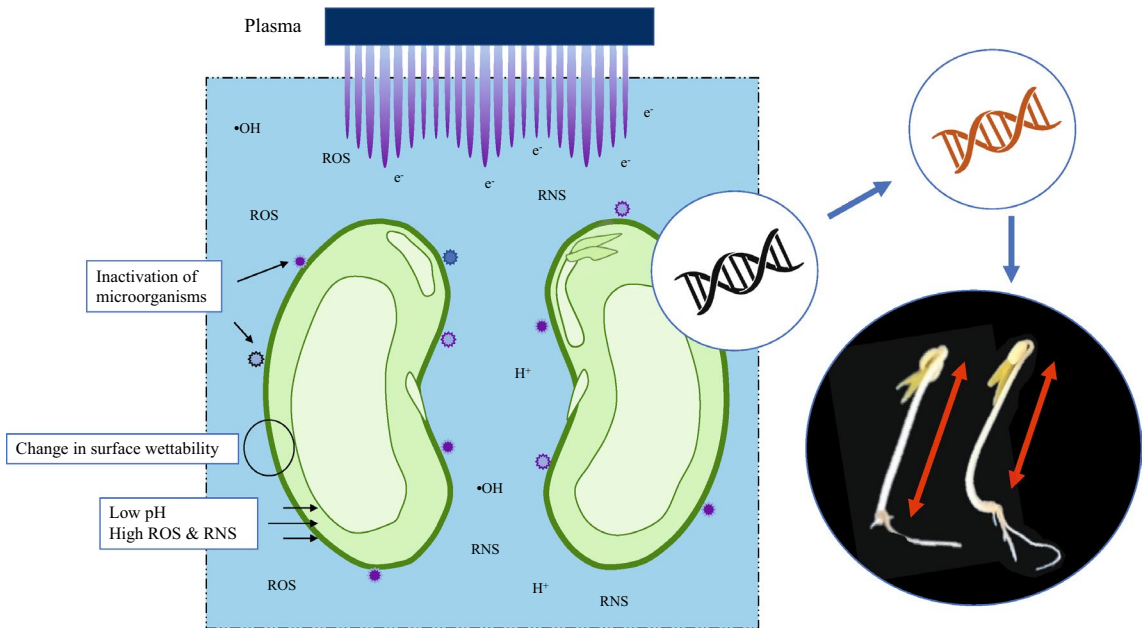
Results The results revealed that nitrogen plasma-activated water (NPAW) treatment improves the vitality and hypocotyl length of mung beans and leads to a good overall growth state. Moreover, we identified numerous differentially expressed genes (DEGs), including genes related to stress responses, growth regulation, and metabolic processes, that were upregulated or downregulated in response to PAW treatment. As a result of APAW treatment, 168 genes were upregulated and 90 genes were downregulated. Furthermore, 179 genes were upregulated in the NPAW compared to 125 genes that were downregulated in the control group. Gene expression analysis revealed involvement in stress signaling and metabolic processes.

Conclusions PAW treatment can promote crop growth and serve as a reference for other seeds. This research provides insights into the regulatory mechanisms and benefits of PAW in sustainable agriculture.

Keywords Mung beans, Plasma-activated water (PAW), Phenylpropanoids, GABA, Differentially expressed genes (DEGs)

*Correspondence:
Yuwen Ting
pywting@ntu.edu.tw
Full list of author information is available at the end of the article

Graphical Abstract



Background

It is well known that plant growth is influenced by environmental aspects, including basic elements such as sunlight, air, and water. Environmental factors such as temperature, soil type, and nutrient content also have a profound effect on plant growth and development [1, 2]. High temperature, cold, salt, and oxidative stress can all affect crop growth and induce plant stress responses to enhance their survival and tolerance [3]. Most current research focuses on the molecular mechanisms of environmental stress on the *Arabidopsis thaliana* plant [4], with limited insights into other important food crops. Notably, improving the abiotic stress tolerance of food crops can enhance agricultural productivity.

Plasma technology has gradually been applied as a technique to promote plant growth, mainly targeting microorganism-induced biological stresses that can be removed by plasma, disrupting the epidermis to facilitate rapid water absorption and inducing oxidative stress in plants to enhance growth [5]. Plasma-activated water technology, which can prolong the effects of plasma, has been particularly effective; numerous studies have addressed the application of plasma-activated water to enhance the growth of crops, including tomatoes [6], soybeans [7], and wheat [8]. However, most of these studies have mainly focused on the effects of plasma-activated water on the properties of crops, such as their germination rate, dry weight, and vitality, with limited investigations into its own properties and impact on crops.

Mung beans are a common Asian food source that can be consumed raw or processed into winter noodles, bean sprouts, etc. They are characterized by a short life cycle and high nutritional value and exhibit a close relation to many other crops, making them relatively simple to study when investigating other complex genomes. However, research studies on this topic are sparse; to address this gap, this study comprehensively examines the properties of plasma excitation and the generation of plasma-activated water and explores the gene expression changes in mung beans. In doing so, the study aims to provide a more complete understanding of how crops respond to plasma-activated water treatment and generate more in-depth insights into the potential applications of plasma technology in agriculture.

Materials and methods

Materials

Mung beans (*Vigna radiata* L.) were obtained from a local market in Taipei, Taiwan. All chemical reagents used were of analytical grade and purchased from Merck (Burlington, MA, USA). The Quantitative PCR analysis kit was purchased from GeneDireX, Inc.

Atmospheric cold plasma conditions

PAW is a water solution generated by applying plasma to water as a conductive medium, resulting in the formation of high-activity RONS. Different PAW formulations can be produced by using different equipment, gases, and treatment durations. A 1:20 ratio of mung bean to water (treated using reverse osmosis [RO]) was used for the test, and 5 slm of air and nitrogen gas were introduced to induce the generation of plasma-activated water (PAW). In this study, air and nitrogen gas sources were used to generate air plasma-activated water (APAW) and nitrogen plasma-activated water (NPAW), respectively, as the experimental conditions. The APAW and NPAW were treated at 30 W of input power for 10 min, and physical and chemical analyses were conducted.

Plasma properties

Optical emission spectroscopy (OES) was employed for the analysis of excited reactive species using an optical emission spectrometer (USB2000 + UV–VIS-ES, Ocean Optics, Inc., FL, USA) and a UV–VIS collimating lens with a focal length of 10 mm. The spectral data obtained were analyzed using the Ocean Optics SpectraSuite spectrometer operating software. The probe of the spectrum analyzer was aimed at the plasma torch at a distance of 3 cm; the integration time was set to 100 ms, and the detection light emission wavelength range was 200–880 nm.

PAW analysis

The pH value, oxidation–reduction potential (ORP) and the temperature, detected by a pH meter (HI 9017, Hanna Instruments, Fondata, Italy); and the electrical conductivity, measured using a handheld conductivity meter (Eutech Cond 6 +, Thermo Scientific, England).

We referred to previous studies in evaluating the long-life reactive oxygen/nitrogen species (RONS) content in PAW, such as the hydrogen peroxide [9], oxygen [10], and nitrite [11] contents.

Germination of mung beans

The treated mung beans were soaked for 6 h to pre-germinate them, after which the excess water was removed via filtration. The mung beans were then placed into a test tube with PAW, and the germination rate was observed for 8–72 h. The rate of mung bean germination was calculated according to Eq. 1:

$$\text{Germination ratio} = (X/A) \times 100\%, \quad (1)$$

where X is the number of germinated mung beans, and A is the total mung bean count.

Physical appearance of mung bean sprouts

After cultivating the mung bean sprouts for 72 h, their hypocotyl diameter, length, and root width were measured, and their growth was observed and recorded.

γ-Aminobutyric acid (GABA) content analysis

The mung beans were freeze-dried and ground into a powder. The 0.2 g sample was then mixed with 2 mL 70% ethanol, extracted using ultrasonic waves at 30 °C for 10 min, centrifuged at 100 × *g* and 4 ℃, and then filtered and concentrated. Derivatization was performed after dissolving the sample in 500 mL of 0.1 M ammonium acetate. The prepared solution was analyzed for γ-aminobutyric acid (GABA) content using the UltiMate 3000 Standard HPLC system (Thermo Fisher Scientific, Waltham, MA, USA), including a degasser (LPG-3400SD), a diode array detector (DAD-3000), an autosampler (WPS-3000TSL), data processing software (LC-Solution), and a column (ReproSil Saphir 100 C18 4.6*250 mm 5 μm). Quantification was carried out using an external standard method with standard solutions of GABA. Mobile phase A (acetonitrile) and mobile phase B (0.1% formic acid) were used to elute the sample at a flow rate of 0.5 mL per minute and detect its absorbance at 330 nm. Mobile phase A was increased from 35 to 40% in the first 5 min, increased to 55% during the subsequent 5 min, and then reduced to 35% to complete the analysis for a total of 35 min.

RNA sequencing (transcriptome)

After germinating the mung beans, they were immediately moved to a 1.5-mL tube and frozen with liquid nitrogen. The sample was ground to a powder in liquid nitrogen using a pestle and mortar, and the RNA was extracted; carrying out the process in liquid nitrogen prevented RNA degradation. The RNA concentrations were determined using Nanodrop (TM1000, Thermo Scientific, England), and 2 μg of RNA was subsequently sent to Biotools Co., Ltd., Taiwan for RNA sequencing analysis.

The analysis was performed by the NovaSeq 6000 Sequencing System (Illumina, San Diego, CA, USA) with paired-end sequencing, a read length of 150 bp, and a sequencing depth of 6 G (20 M reads). During analysis, clean reads are first obtained from the sequenced offline data raw reads using Trimmomatic (v0.38) [12] for quality filtering, also cutting off adapters. Clean reads use HISAT2 (v2.1.0) [13, 14] to compare the Vradiata_ver6 reference genome. The quality of FASTQ files was confirmed by the FastQC and MultiQC programs [15], and only clean reads were used for the subsequent analysis. Additionally, reads mapped by transcripts are counted. The read numbers were counted using FeatureCounts

(v1.6.0) [16], and DEGseq2 (v1.36.1) [17] analysis was performed with biological replicates. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were then conducted using clusterProfiler (v3.10.1) [18].

Quantitative PCR (cCR) analysis

RNA samples were reverse transcribed into cDNA using the GScript First Strand Synthesis Kit (GeneDireX, USA). Quantitative PCR analysis was then conducted to amplify the promoter region of the DNA fragment. Specific primers were utilized to detect the probable WRKY transcription factor 30 and probable bifunctional TENA-E protein (Additional file 1). To standardize the results, the relative abundance of TUB was determined and used as the internal standard [19]. Fold enrichment and percentage of specific genes were collected and calculated. For detailed calculation methods, please refer to Hsieh et al. [20].

Microorganisms

Microorganisms were counted using the standard plate count method. Here, 0.5 mL of PAW after mung bean incubation was taken, a tenfold serial dilution with sterile PBS was performed, and 1 mL was then added to the sterile culture medium, conducting counts after culturing at 35 °C for 48 h.

Statistical methodology

The experiments for each sample were carried out using three to five repeats, and the calculated and measured values were expressed as the mean ± standard deviation. All data were analyzed with a one-way ANOVA using SAS (Version 9.00). The significance level of the comparison using the Tukey's honestly significant difference (HSD) test in the significant difference analysis was 0.05 ($p < 0.05$).

Results

Germination rate and physical properties of mung beans

The mung beans were cultivated for 3 days, and the germination rate was observed to elucidate the vigor of the mung bean seeds. After 72 h, the control and PAW treatment groups reached a 100% germination rate, indicating that the viability of the sample seeds was good. The initial germination rate was approximately 97.5% for both the control and NPAW treatment groups in Table 1. The initial germination rate of 90.80 ± 6.68 was marginally lower in the APAW treatment group.

Changes were also observed in the physical properties of mung beans cultured in PAW in Fig. 1b, c and Table 1. NPAW significantly increases the hypocotyl length of the bean sprouts, which is 3.35 ± 0.99 cm, while the lengths in the control (Fig. 1a) and APAW groups are 2.58 ± 0.94 and 2.72 ± 0.82 cm, respectively. The root lengths of the control, APAW, and NPAW groups are 3.39 ± 2.07, 3.25 ± 1.67, and 3.05 ± 1.78 cm, respectively, with respective diameters of 2.49 ± 0.32, 2.42 ± 0.31, and 2.39 ± 0.35 cm. Regardless of the gas-induced PAW used, the cultured bean sprout shoots are higher (and significantly higher for the NPAW group) than those in the control group.

GABA

To understand whether the GABA of the mung bean sprouts increases indirectly through their rapid growth or is directly stimulated by the properties of the plasma-activated water and increases, the GABA content during the growth of the bean sprouts was monitored. After 8 h of germination, the GABA contents of the NPAW and APAW groups are 85.6 ± 8.7 and 105.6 ± 31.0 mg/kg (dry basis), respectively, while that of the control group is 31.3 ± 25.6 mg/kg (dry basis) in Table 1. Moreover, after three days of germination, the GABA contents of the NPAW and APAW groups are 360.0 ± 8.8 and 333.8 ± 67.8 mg/kg (dry basis), respectively, 1.61 and 1.49 times higher than that of the control group, which is 224.1 ± 14.9 mg/kg (dry basis).

Plasma properties

To understand the influence of plasma on mung beans, its composition should first be considered. Plasma includes short-life reactive oxygen/nitrogen species (RONS) and long-life RONS, where OES is used to observe short-life ions in PAW. The emission spectra of APAW and NPAW (shown in Fig. 2) are notably similar because air comprises approximately 78% nitrogen. The spectral data can be used to determine the composition of PAW, which

Table 1 Physical properties of mung bean and mung bean sprout

	Initial germination rate (%)	Hypocotyl (cm)	Root (cm)	Diameters (mm)	GABA (mg/kg) after 8 h	GABA (mg/kg) after 72 h
Control	97.50 ± 4.52^{a}	2.58 ± 0.94^{b}	3.39 ± 2.07^{a}	2.49 ± 0.32^{a}	31.3 ± 25.6^{b}	224.1 ± 14.9^{b}
Nitrogen	97.50 ± 4.52^{a}	3.35 ± 0.99^{a}	3.05 ± 1.78^{a}	2.39 ± 0.35^{a}	85.6 ± 8.7^{ab}	360.0 ± 8.8^{a}
Air	90.80 ± 6.68^{b}	2.72 ± 0.82^{b}	3.25 ± 1.67^{a}	2.42 ± 0.31^{a}	105.6 ± 31.0^{a}	333.8 ± 67.8^{a}

Different superscripts (a and b) in the same row indicate a significant difference ($p < 0.05$)

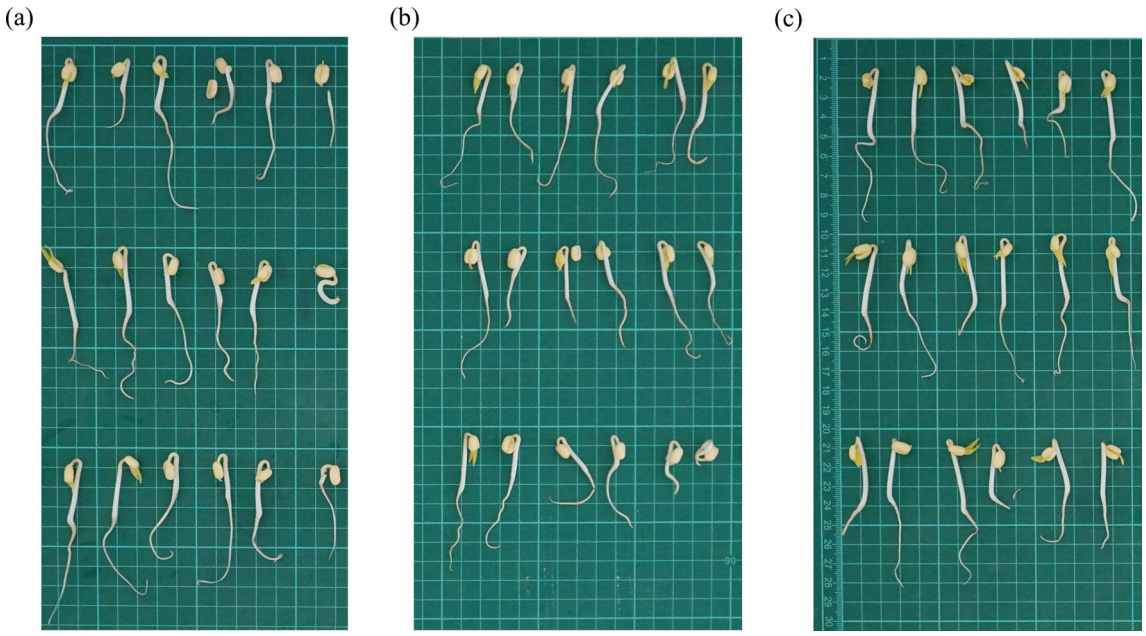


Fig. 1 An illustration of a mung bean sprout **a** control **b** APAW **c** NPAW treatment

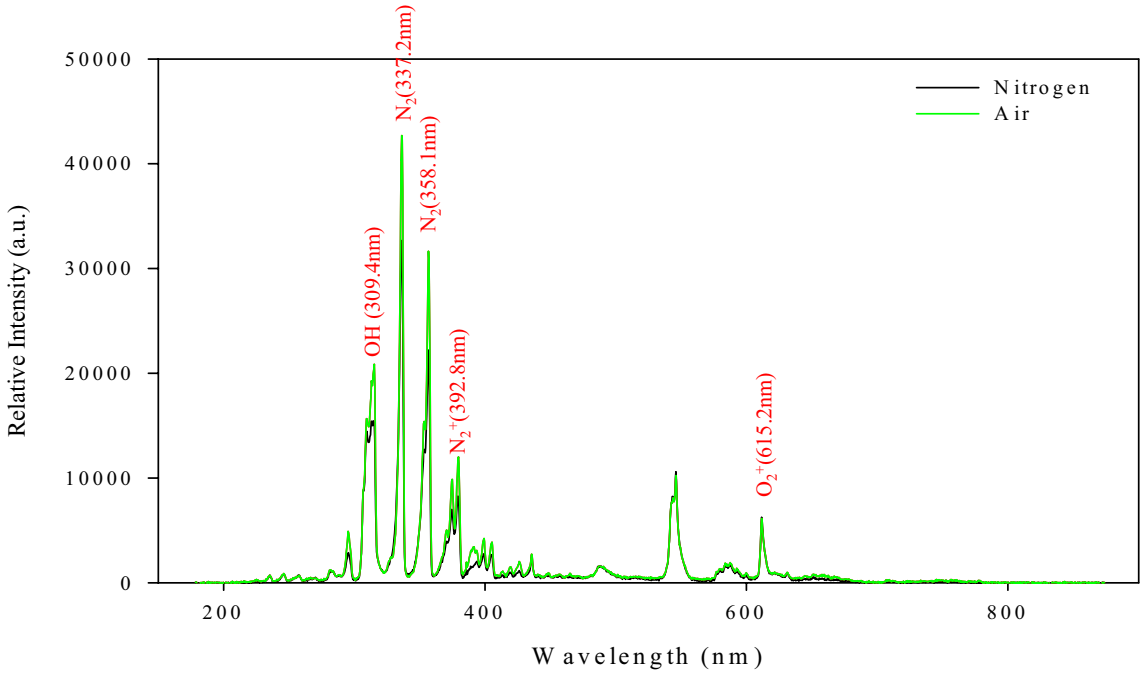


Fig. 2 Optical emission spectra of air and nitrogen plasma

Table 2 Physical properties of plasma-activated water

	pH	TDS(μS/cm)	ORP (mv)	NO_2^- (μg/mL)	NO_3^- (μg/mL)	H_2O_2 (μM)	Ozone (ppb)
Control	6.81 ± 0.10^a	3.03 ± 0.00^c	-1.80 ± 3.14^c	ND	ND	6.09 ± 5.52^b	ND
Nitrogen	6.28 ± 0.53^b	25.41 ± 2.23^b	18.39 ± 10.93^b	2.12 ± 0.33^a	5.55 ± 0.79^b	63.79 ± 22.20^a	149.22 ± 32.90^b
Air	4.66 ± 0.12^c	33.80 ± 4.97^a	106.93 ± 26.24^a	2.64 ± 0.54^a	22.16 ± 0.63^a	52.68 ± 3.23^a	269.10 ± 35.94^a

Different superscripts (a–c) on the same row indicate a significant difference ($p < 0.05$)

comprises N_2 (337.2, 358.1, 380.5, 392.8, 405.9, and 427.8 nm), •OH (309 nm), and O (616 and 780 nm) as the main species. At 309 nm, APAW has a higher hydroxyl radical content than NPAW because air contains oxygen atoms that can react with water molecules to produce more hydroxyl radicals.

Properties of plasma-activated water

The properties of PAW, including the analytical results of long-life and short-life RONS, are given in Table 2. The pH value of activated water after plasma excitation drops significantly, and the temperature also rises from room temperature to 42 ℃. In the experimental procedure, the water temperature of the control group was raised to equal that after plasma activation to reduce the variables. The oxidation–reduction potential in the PAW significantly increases. The total dissolved solids (TDS) generate many active ions due to plasma addition, with a notable increase from 3.03 ± 0.00 to 33.80 ± 4.97 in APAW. Nitrite, nitrate, hydrogen peroxide, and ozone in PAW all increase significantly: nitrite and nitrate increase to 2.12–2.64 and 5.55–22.16 μg/mL, respectively; hydrogen peroxide increases from 6.09 μM to 52.68–63.79 μM; and ozone increases to 149.22–269.10 ppb.

Microorganisms

During the growth phase, the seeds were subjected to biotic and abiotic stress. Pesticides or bleach were added to the crops to remove biotic stresses from the seeds and improve their survival rate by removing surface microorganisms and pests. After treating the seeds with PAW, the total bacterial count is reduced, which can promote the growth of the seeds. The aerobic plate count in the control group is 3.42 ± 0.17 log CFU/g, which decreases to 2.43 ± 0.25 and 2.61 ± 0.67 log CFU/g after nitrogen and air plasma-activated water treatment, respectively.

Differential gene expression analysis

Despite the increasing number of studies on the promotion of seed growth by plasma, the mechanism of action of plasma in this regard remains unknown. Thus, in this study, the mung beans were sequenced after plasma treatment, and the changes in the beans after plasma treatment as well as the relationship between plasma and seed growth were thoroughly investigated.

Principal component analysis (PCA), as shown in Fig. 3, was used to visualize the relationship between genotypes, where the differences between samples can be observed. The total variation of PC1 and PC2 is determined as 39.96% and 29.67%, respectively. PC1 distinctly distinguishes between the plasma treatment and control groups, that is, the control group occupies a space distinct from those of the air and nitrogen plasma treatment groups.

The Illumina sequencing technique was used to find a total of 20,846 expressed genes. DEGseq2 was used with biological repeats to filter the number of differentially expressed genes (DEGs) between groups, where the filtering conditions include genes with Fold Change ≥ 2 and corrected *p*-adjust ≤ 0.05. The volcano plot in Fig. 4a and b shows the distribution and significance of the differences in gene expression. A total of 258 genes, including

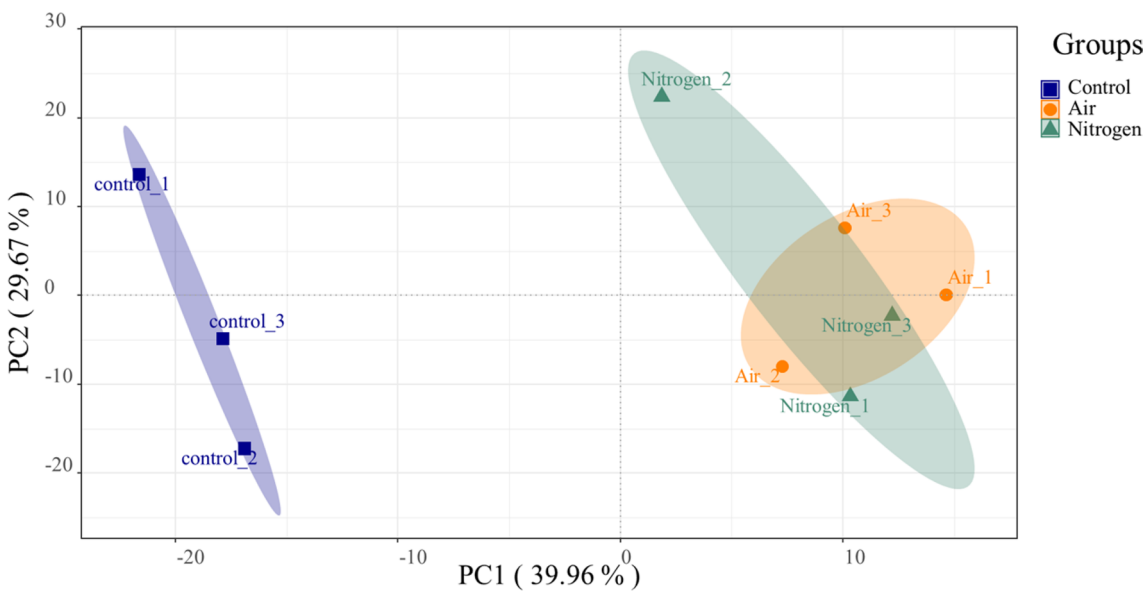


Fig. 3 Principal component analysis (PCA) plot of mung beans from the control and plasma-activated water treatment groups

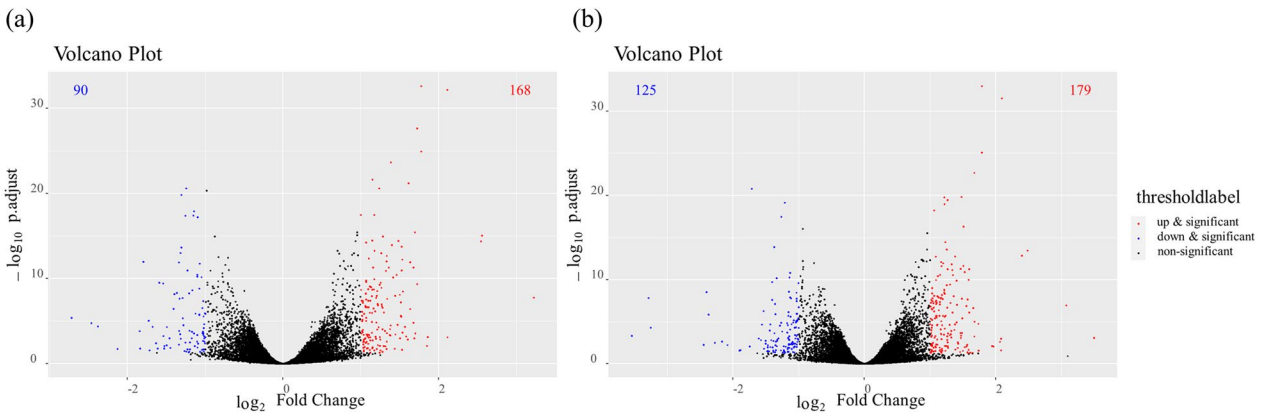


Fig. 4 Volcano plot analysis of differentially expressed genes (DEGs) using RNA-seq. **a** 90 downregulated and 168 upregulated genes between the control and APAW, and **b** 125 downregulated and 179 upregulated genes between the control and NPAW

168 upregulated and 90 downregulated genes, show differential expression after APAW treatment compared to the control; comparing the NPAW and control groups reveals a total of 304 DEGs, of which 179 exhibit upregulation and 125 downregulation. No DEGs are present in the comparison between the APAW and NPAW groups.

Gene Ontology classification and pathway enrichment analysis of DEGs

We conducted a GO analysis using *Vigna radiata* as a reference to assess the observed variations in gene expression profiles toward specific activities and understand the biological function of the upregulated and downregulated DEGs derived from various comparison combinations. The three basic categories of GO analysis are cell composition (CC), biological process (BP), and molecular function (MF).

In this study, 491 GO items in APAW and 462 in NPAW are significantly enriched according to DEGs; some of the most enriched pathways include the BP (stimulus, transport, metabolism, etc.) and MF (transcription factor activity, structural constituents of the cytoskeleton, etc.) categories. In the PAW-treated mung beans, the downregulated genes are more affected; only a few significant changes are present regarding upregulation, but the number of downregulated genes is as many as 30. The top 10 GO enrichment analysis revealed differences in gene expression between APAW and NPAW, and their common point mainly involves the upregulation of DNA-binding transcription factor activity (GO:0003700), as shown in Fig. 5a, b, c and d. Moreover, responses to auxin (GO:0009733) are significantly upregulated in APAW. However, the downregulation of affected genes has a wider scope. In APAW, the main functions affected are BP and CC, while in argon plasma, these are CC and MF. Cellular carbohydrate metabolism procedures, carbohydrate transport, and the extracellular region are impacted by the downregulated genes of APAW and NPAW.

The core gene can be identified using Fig. 6a, b, c and d, which displays the top three GO genes that are significantly altered in APAW and NPAW. In the upregulated genes, the genes LOC106754125, Vradi01g07160, LOC106755105, LOC106757330, LOC106761370, LOC106764361, Vradi07g03920, LOC106769811, Vradi08g09150, LOC106771839, Vradi08g22260, Vradi11g01350, and LOC106776583, which affect DNA-binding transcription factor activity and NPAW, are additionally regulated by two genes, Vradi0307s00010 and Vradi05g11250. This GO functional term (GO:0003700) is engaged in the regulation of gene-specific transcription [21]. In response to auxin (GO:0009733), APAW impacts the genes LOC106780333, LOC106757520, Vradi05g19460, Vradi06g02360, and Vradi08g06820. NPAW exhibits the highest impact among the downregulated genes, where the extracellular area (GO:0005576); hydrolase activity, hydrolyzing O-glycosyl compounds (GO:0004553); and cell periphery (GO:0071944) are the top three factors. APAW represents the monocarboxylic acid metabolic process (GO:0032787), carbohydrate transport (GO:0008643), and extracellular region. Notably, Vradi11g03830 and LOC106762408, two important genes, simultaneously control all the top three GOs in NPAW.

KEGG pathway analysis with the DEGs

We annotated the enriched pathways using the scatter feature of the KEGG database to clarify the function of the DEGs. As a useful resource for genome sequencing, the KEGG database incorporates data on genomes, biochemistry, biological systems, and other topics and may

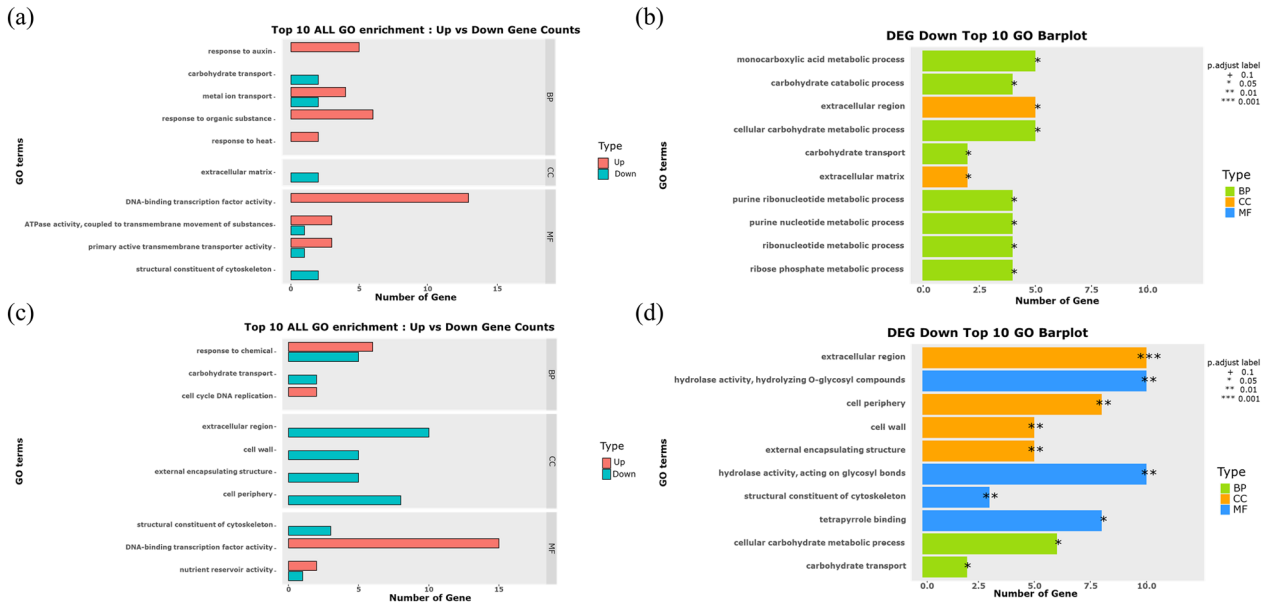


Fig. 5 Ranking of top 10 GO enriched categories of the PAW treatment dataset. **a** Top 10 all GO enrichment between the control and APAW; **b** DEG down top 10 barplot between the control and APAW; **c** top 10 all GO enrichment between the control and NPAW; and **d** DEG down top 10 barplot between the control and NPAW

be used to predict the pathways in which a particular gene is enriched.

Regardless of the type of gas-induced PAW that affects the KEGG pathway after seed treatment, the significant changes occur in phenylpropanoid biosynthesis; plant hormone signal transduction; plant–pathogen interactions; the connection between mitogen-activated protein kinase (MAPK) signaling pathways and plant development; photosynthetic antenna proteins; flavonoid metabolism; stilbenoid, diarylheptanoid, and gingerol biosynthesis; biosynthesis of unsaturated fatty acids; fatty acid elongation; steroid biosynthesis; and protein processing in the endoplasmic reticulum, while NPAW exhibits more of the thiamine metabolism pathway, as shown in Fig. 7a and b (only the top 10 factors are presented).

Quantitative PCR analysis

The qPCR results were used to verify the reliability and accuracy of Next-Generation Sequencing (NGS) findings. The genes LOC106754125 (the probable WRKY transcription factor 30) and LOC106767562 (probable bifunctional TENA-E protein) were chosen for observation due to their significant differences in expression and their major impact on plant growth. The results can be seen in Fig. 8, where Fig. 8a and b shows the expression of genes influenced by WRKY and TENA-E after qPCR, respectively. Figure 8c and d represents the relative expression levels of these two genes after NGS.

Discussion

Plasma technology has been extensively researched to break seed dormancy and promote the growth and germination of crops such as buckwheat [22]; radishes, tomatoes, and sweet peppers [5]; and mung beans [23, 24]. Compared to direct treatment, PAW exhibits greater advantages, including its ability to treat uneven seed surfaces and retain more long-lasting ions for prolonged effects on crops such as wheat [8] and soybeans [7]. However, few studies exist on the impact of plasma on crop gene expression. In this study, we selected the mung bean as a model crop due to its importance as a food source, small genome size, short life cycle, and close relation to several other crops. This was carried out to investigate the effects of PAW on crops, provide a comprehensive understanding of the potential impact of plasma on crop genetics, and identify the optimal way to use plasma technology in agriculture.

Properties of PAW

It is important to note that the effects of plasma on crop genetics likely depend on a variety of factors, including the type and intensity of plasma used, the stage of plant development, and the specific crop species being treated. At the start of the study, we made use of air and nitrogen plasma, where the peak difference in the spectra is rather small due to air containing a nitrogen content of 78%; notably, the peak height is the primary distinction. As one can observe, air plasma has a higher relative intensity

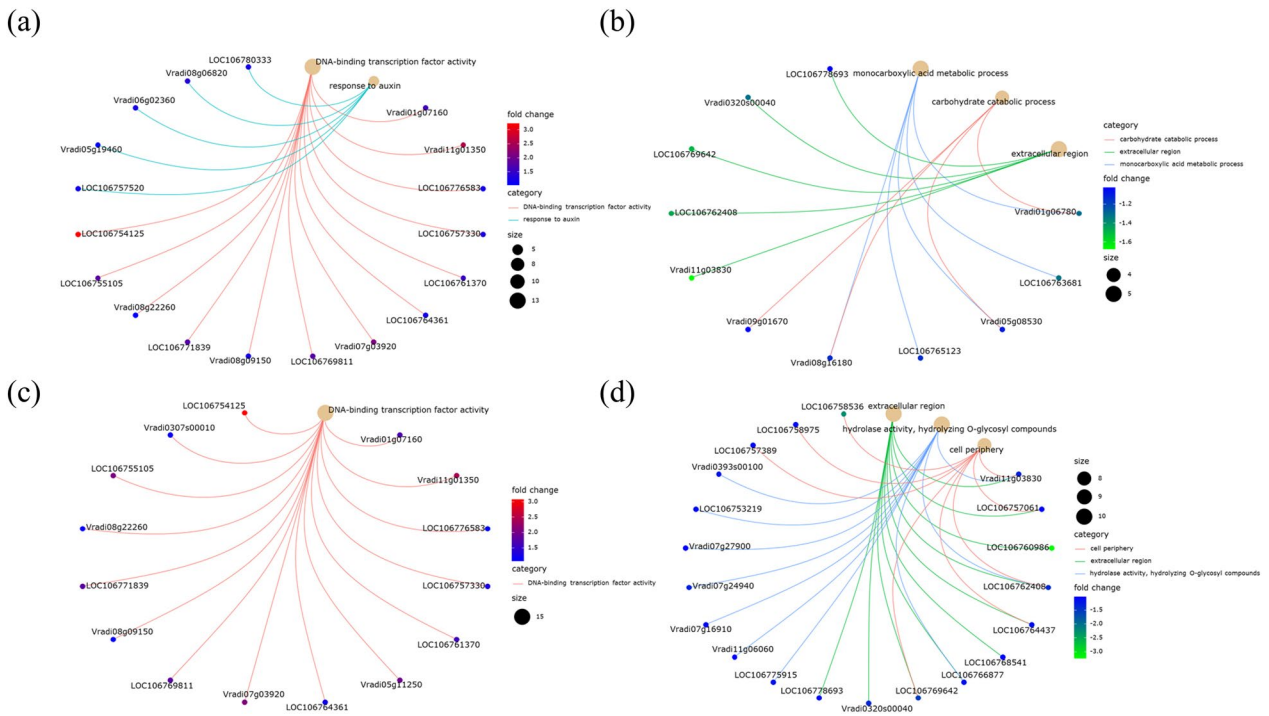


Fig. 6 Conceptual networks of GO genes for **a**, **c** upregulated and **b**, **d** downregulated genes, showing **a**, **b** control versus APAW and **c**, **d** control versus NPAW. In each network analysis, the top three terms are listed, and the related genes are connected

than nitrogen plasma. Further, PAW is composed of a complex and diverse series of chemical reactions. As shown in Fig. 2, plasma excites many active species, such as HO• radicals and N_2^+ and O_2^+ ions, and produces several substances during water activation. An increase in the TDS indicates the successful plasma activation of water, generating many chemical substances. The ORP significantly increases due to the high-energy electrons and oxygen molecules generated by the plasma, and the H_2O_2 and ozone levels in the water also increase significantly, which is related to the production of HO• radicals. The H_2O_2 generated in PAW is transported to the gas–liquid interface by HO• radicals [25]. In addition, the pH value of the aqueous solution significantly decreases, which is mainly attributed to the formation and dissolution of nitrite and nitrate [26]. The key differences between the two types of gas-induced PAW are their pH and nitrate and ozone contents. The pH value of APAW is lower than that of NPAW, as evidenced by the nitrite results. The nitrogen oxides of active substances generated from air plasma are higher in quantity than those arising from nitrogen plasma; air plasma also generates more nitrite in plasma water than its nitrogen counterpart. This is consistent with the results of previous studies indicating that the generation of nonthermal plasma in a nitrogen-containing carrier gas leads to a large number of nitrogen oxides [26]. The APAW includes more ions than NPAW, which is reflected in the difference in the TDS. The experimental findings demonstrate that APAW has a greater ion concentration and, thus, a higher content of TDS.

A study comparing the effects of the same concentrations of PAW and hydrogen peroxide water on mung bean growth revealed that the former promoted better growth than the latter, indicating that PAW exhibits other properties that promote plant growth in addition to its hydrogen peroxide content. On the other hand, high concentrations of hydrogen peroxide inhibit plant growth, where the relationship between H_2O_2 concentration and crop growth is inversely proportional [27]. Using 0–500 μM H_2O_2 to improve mung bean growth and increase antioxidant enzyme activity, other scholars found that 100 μM H_2O_2 displayed the optimal growth-promoting effect [28]. The PAW concentration used in this study ranged from 50 to 60 μM, which constitutes a low concentration range that promotes crop growth [27]. Nitrogen is a necessary nutrient for crop growth, but the excessive use of nitrogen fertilizers can lead to nitrogen metabolism disorders in legume plants, inhibiting mung bean nodule formation and symbiotic nitrogen fixation [29]. Furthermore, high nitrate concentrations can inhibit germination rates. Therefore, appropriate nitrogen levels are beneficial for crop growth. Previous studies have shown that within the 0–8 μg/mL nitrogen concentration

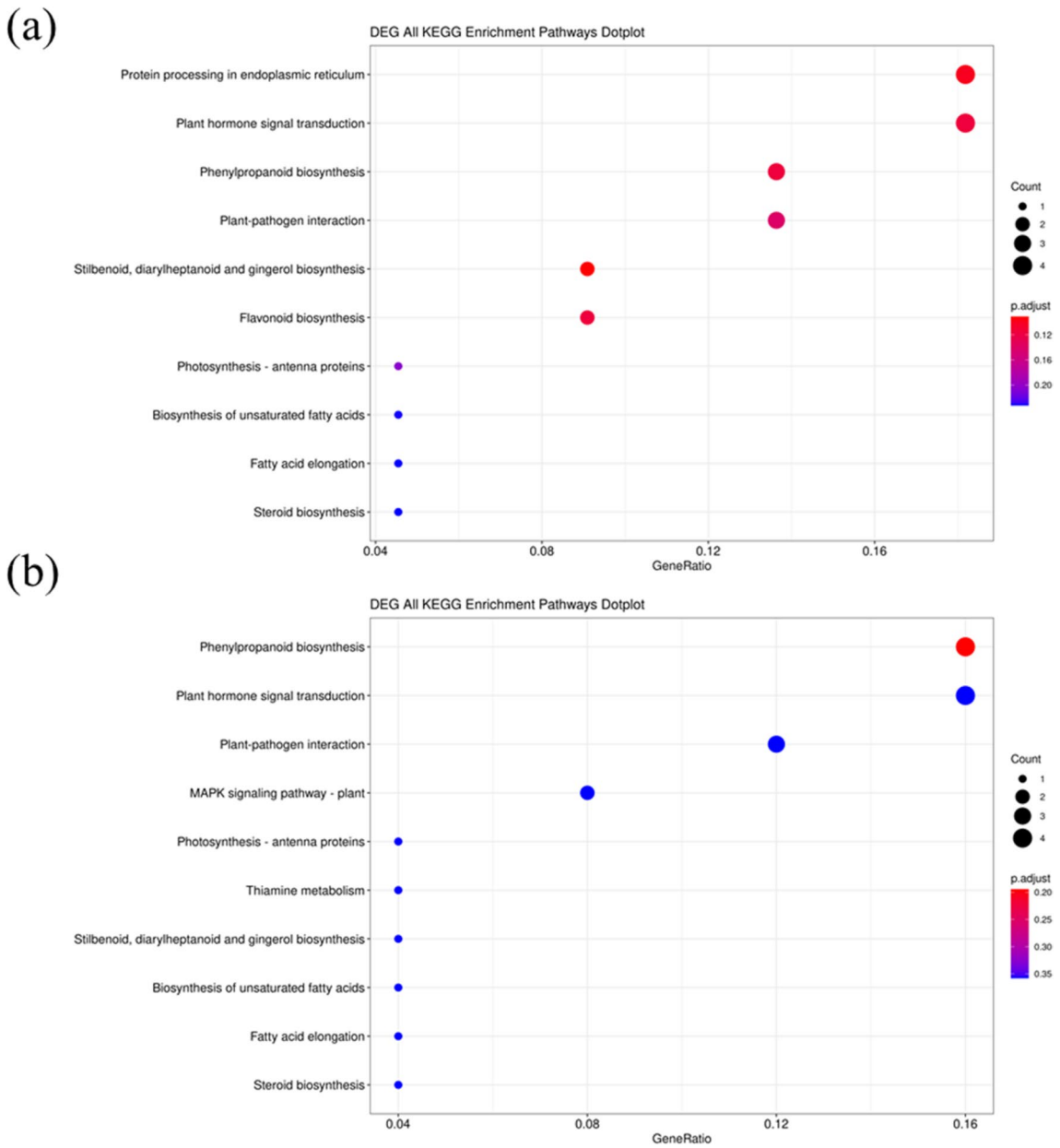


Fig. 7 KEGG enriched pathway dot plot of the top 10 DEGs affected by plasma-activated water: **a** control versus APAW and **b** control versus NPAW

range, 4 μg/mL can effectively improve germination characteristics. In this study, the nitrogen concentrations ranged from 5 to 22 μg/mL. It is evident that nitrate ions in the air may be harmful to mung beans, and in terms of mung bean vitality, NPAW notably exhibits better germination rates and growth conditions than APAW. Ozone has a significantly brief half-life, ranging from a few seconds to a few minutes [30]. Optimized parameters can enhance germination, or involve using ozone as a sterilizing agent, and can also generate ethylene, breaking seed dormancy [31]. In this study, the concentration of ozone employed ranged from 149–269 ppb. It is noteworthy that exposure to high levels of ozone can have detrimental effects on seed vigor. For instance, scholars have found that acute concentrations of ozone exceeding 500 ppb significantly reduce the number or quality of soybean nodules, by at least 10% [32]. Thus, careful regulation of the concentration of ozone used is essential to avoid detrimental impacts on seed quality. The ozone concentration in APAW is significantly higher than that in NPAW, which may have caused damage to the mung beans and reduced the initial germination rate. However,

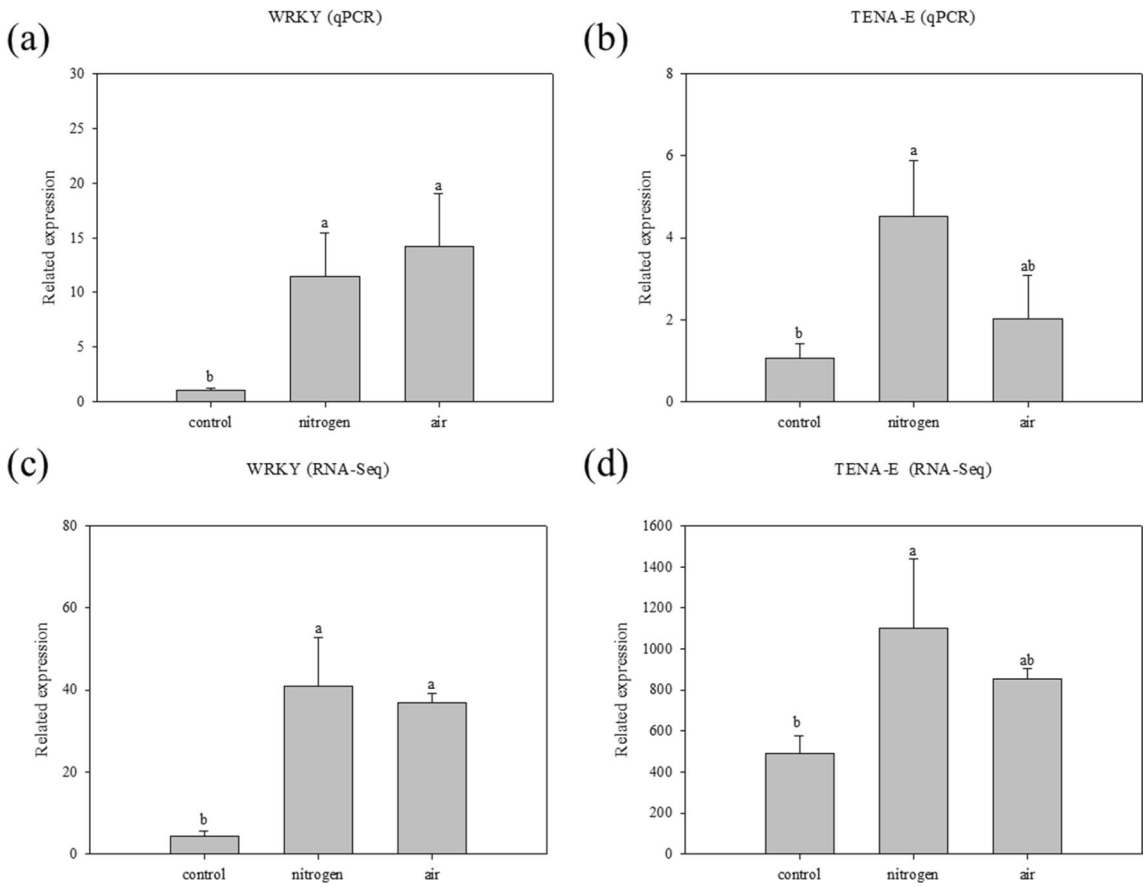


Fig. 8 Expression intensity quantified by RNA sequencing (RNA-Seq) and quantitative polymerase chain reaction (qPCR). **a** WRKY expression in qPCR. **b** TENA-E expression in qPCR. **c** WRKY expression in RNA-Seq **d** TENA-E expression in RNA-Seq

after treatment, the concentration of ozone decreases significantly, subsequent cultivation was not carried out using PAW, and the germination rate using APAW subsequently improved. One can speculate that the effect of PAW on seeds mainly occurs in the early stage of plasma activation and initial growth. The impact of plasma-generated reactive species on crops is crucial and varies with the type of instrument and method used for the generation of the species. Therefore, determining suitable parameters and understanding the effects of different species on crops as well as the appropriate concentration levels are important for optimal results. Achieving a synergistic effect is the ultimate goal of plasma treatment, where the combined effect of reactive species generates a more substantial impact than individual treatments. Thus, further research is necessary to identify suitable plasma treatment conditions for different crops and the appropriate concentration levels of reactive species to maximize the associated benefits.

Vitality and growth of mung beans

The results indicate that PAW treatment only has an inhibitory effect on the germination rate of mung beans in the initial stage, while subsequent germination is not significantly affected. In comparison to the control group, no discernible difference exists between different periods or groups; all groups can grow properly. In terms of growth, the hypocotyl length of the mung beans treated with NPAW shows a significant 1.30-fold increase, indicating the positive effect of NPAW on mung bean growth. This result is consistent with several previous research studies indicating that plasma treatment has no significant effect on the germination rate but has a positive effect on subsequent growth [24]. Although the hypocotyl length also increases from APAW treatment, no significant statistical difference is present. Under the experimental conditions, one can note that NPAW is more suitable for the growth of mung beans than APAW. Comparing the active substances and properties of the two PAW types, one can conclude that their

difference in intensity is the main factor affecting growth. Air plasma constitutes excessive pressure for crops, leading to a trend of growth inhibition; as mentioned in Sect. "Properties of PAW", this may also result from higher concentrations of ozone and nitrate salts. In this study, mung beans were treated with plasma at a higher power, where plasma stimulated by an excessively high power was found to inhibit germination. According to previous research results, excessively high or low plasma treatments can exert an inhibitory effect on crop growth [22]. Scholars have attributed the majority of the detrimental crop effects to its greater acidity [33]. Therefore, identifying suitable conditions and exploring key points of influence is crucial.

Biotic stress

Crop growth is affected by both biotic and abiotic stresses, and the main reasons for the promotion of crop growth by plasma can be simply divided according to these two categories, depending on the type and degree of stress and the varying degree of response by different crops. The first reason involves removing surface microorganisms from mung beans and reducing the biotic stress on crops such that the mung beans can grow with less interference. Several studies have used plasma as a technique for microorganism removal, and plasma machines are currently available on the market for this purpose, indicating that plasma is a common and feasible technology in this regard. The PAW used in this study can successfully reduce microorganisms by 0.82–0.99 log CFU/g. Liu et al. used plasma water generated by different voltages to treat apples, finding that it can reduce the microorganism content from 1.05 ± 0.07 to 0.28 ± 0.04 log CFU/g [34]. The main cause of microbial inactivation is the active ions in PAW, including reactive oxygen and nitrogen species (RONS) and reactive nitrogen species (RNS) that react with water twice. Powerful oxidants such as $HO\bullet$, H_2O_2, O_3, etc., can kill bacteria. The major ions include ONNOH, $HO\bullet$, H_2O_2, O_3, O_2NOOH, $HO_2\bullet$, NO_2^-, NO_3^-, etc., among which RONS can lower the pH value and also provide an environment for inactivating microorganisms [35]. The extremely low pH characteristics and active ions in PAW, mentioned in Sect. "Properties of PAW", can effectively eliminate bacteria.

Abiotic stress

In terms of abiotic pressure, plasma can be regarded as a source of pressure in addition to low ion bombardment, causing surface etching and improving hydrophilicity [23]; providing nutrient sources, such as nitrogen and other essential elements, to facilitate biological growth; breaking seed dormancy; and promoting mung bean growth. As highly active substances in PAW, RONS can stimulate crops, affect enzyme activity in their growth, and even change their gene expression. The existing literature focuses on the amylase and protease activity that affect seed growth [36]; the antioxidant capacity of plants to resist the active substances in plasma [37]; auxins, cytokinins, MAPK, hormone-like salicylic acid, jasmonic acid, abscisic acid, gibberellic acid, ethylene, and other plant hormones [6]; and the promotion of phenolic substances [33].

In this study, the changes in the GABA level in plants were also measured. Previous research has confirmed that GABA metabolism is regulatory and helps plants combat external stress, where an increased GABA content can aid in subsequent metabolic processes [23]. To understand GABA metabolism during growth, GABA levels were measured continuously over a certain period; regardless of the time point, the PAW group had higher GABA levels than the control, with a 2.73–3.73-fold increase during the initial growth and a 1.49–1.61-fold enhancement in subsequent growth. The PAW-treated mung beans were only treated in the initial stages, while untreated water was used for growth in later stages. One can speculate that as initial growth can impact subsequent growth, differences are still observed in later stages. This confirms that plasma treatment is also a form of non-biological stress for plants and, under appropriate conditions, can promote growth.

However, the growth process of plants is rather complex, and its prediction using only a single factor is difficult. In this study, the treated mung beans were further sequenced to fully understand the genetic expression of the effect of plasma-activated water on seed growth. Using transcriptomic analysis, one can understand the abundance and types of genes expressed in a species, which can be used to infer phylogenetic relationships among individual plants using transcriptomic data.

Functional analysis

Sections "Differential gene expression analysis", "Gene ontology classification and pathway enrichment analysis of DEGs" and "KEGG pathway analysis with the DEGs" discuss the factors that influence the changes in gene expression after PAW treatment. As mentioned earlier, PAW primarily induces oxidative stress in plants, which can affect gene expression; therefore, the study focused on investigating the effects of oxidative stress on the latter. PCA was used to explain the differences in gene expression patterns before and after plasma therapy and those between the two types of gas plasmas. The results suggest that the composition of mung beans becomes somewhat closer after plasma-activated water treatment. Significant differences in gene expression patterns were observed between the experimental and control groups,

with NPAW exerting a greater effect by regulating a total of 304 genes. The upregulation effects of both treatments include changes in cell composition, including, for example, organelles, as well as specific biochemical activities involving, for example, enzymes, receptors, and transporters. On the other hand, downregulation effects by APAW were mainly observed in biological processes related to cell division, protein synthesis, and metabolism in BP.

Transcription factors play a crucial role in regulating gene expression in response to environmental and physiological signals. The treatment groups show the most significant upregulation in genes with DNA-binding transcription factor activity (GO:0003700), including the WRKY, heat stress, and ethylene-responsive transcription factors, among others. These are involved in the molecular regulation, physiological metabolism, and growth and development of plants under various stress conditions, such as cold weather [38], droughts [39], oxidative stress [40], and salt stress [41]. LOC106754125, which is significantly upregulated, is the top-ranked gene affecting DNA-binding transcription factor activity among the significantly altered GO genes in APAW and NPAW. The qPCR results support the significant upregulation of this gene. This indicates that after PAW treatment, this gene may be involved in the regulation of plants under external stress. Studies have found that plants exhibit similar transcriptional expressions under different stresses, as gene regulation is complex and involves multiple factors acting simultaneously [40]. The properties of PAW also include oxidative stress, which may cause changes in transcription factors in mung beans under PAW stimulation to resist external pressures. PAW, as an abiotic pressure source, can induce physiological responses in plants to counteract external pressure. Notably, the top 10 GO terms are different after PAW treatment, but overall, they are similar. This may be related to the properties of PAW mentioned earlier, where the main difference between the properties of APAW and NPAW is the degree of oxidative stress. Proper oxidative pressure can have positive effects on plants and vice versa. In a study using PAW generated for 15–60 min to treat tomatoes, researchers found that prolonged exposure to PAW generated more RONS and caused oxidative stress in seedlings, with better treatment effects at 15 and 30 min. In addition, changes in calcium ion-related functional genes have also been identified. It is known that Ca^{2+} signaling is related to plant responses to many abiotic stresses, including low temperature, osmotic stress, heat, oxidative stress, hypoxia, mechanical damage, etc., indicating that calcium regulation plays a role in this regard, helping protect plants and allowing them to adapt to new environmental conditions [42]. This study found that it regulates calcium ion binding (GO:0005509), calcium ion transmembrane transporter activity (GO:0015085), calcium ion transmembrane transport (GO:0070588), and calcium ion transport (GO:0006816), among which calcium receptor and calcium-binding proteins form important components of plant abiotic stress responses [4].

KEGG enrichment analysis

The alterations in the KEGG pathways depicted in Fig. 7 reflect how plants react to external stimuli. The primary elements of plants' defense against oxidative stress are phenols. The phenylpropanoid pathway produces polyphenols and flavonoids, and the biosynthetic precursors of polyphenols are often acquired from the by-products of the pentose phosphate and glycolysis processes [43]. A change in the way that cells process carbohydrates was also discovered from the GO enrichment analysis. It mainly affects the performance of cinnamic acid 4-hydroxylase [EC1.14.14.91] and plant peroxidase [EC1.11.1.7] enzymes, which play an important role in the metabolism of phenylpropanoids. Additionally, downstream genes related to flavonoid biosynthesis, such as LOC106765809, LOC106767486, and LOC106771868, were also regulated, indicating alterations in their expression that contribute to the regulation of growth.

When plants are stimulated to respond to external shocks, the signal transduction of their hormones changes. This study found that PAW upregulates GH3 and SAUR in the IAA signal transduction pathway, ERF1/2 in the ethylene signaling pathway, and JAZ in the jasmonic acid signaling pathway. The upregulation of GH3 and SAUR expression is beneficial for cell elongation and expansion, while the enhancement of ERFs can increase GA signal transcription, which may further promote the growth of tea tree seedlings [44]. Jasmonic acid transcription factors are involved in plants' responses and adaptation to the environment. They co-regulate with signaling molecules of ethylene, salicylic acid, and abscisic acid and can also induce the transcription of the ERF and WRKY genes to jointly respond to environmental stress. The JAZ2 protein interacts with COI1 and release transcription factors to activate downstream gene expression and regulate jasmonic acid [45]. Protein processing in the endoplasmic reticulum can have an impact on signaling molecules. Further, an increase in the expression of sHSF has been linked to an increase in plant stress tolerance [46]. MAPK signaling pathways play a key role in various cellular processes in plants, including stress responses, cell differentiation, and growth regulation [47]. Photosynthetic antenna proteins affect the production of ATP and NADPH. Studies have also shown

that the impact of salt stress on sweet sorghum leaves is primarily observed in photosynthesis and carbohydrate metabolism [48]. Other pathways affect plant growth and metabolism and are resistant to external environmental stresses. For example, the pathways of stilbenoid, diarylheptanoid, and gingerol biosynthesis are related to antioxidant function [49]. Unsaturated fatty acids, fatty acid elongation, and steroid biosynthesis all play significant roles in the structure and function of cell membranes, energy storage in plants, and plant responses to environmental stimuli. Unsaturated fatty acids are particularly crucial in this regard. For instance, temperature and light variations have an impact on the fatty acid composition of plant membranes, which impacts the fluidity and functionality of the membranes [50]. Thiamine is a cofactor that is crucial for plants regarding both biotic and abiotic stress. Under conditions of abiotic stress, increased thiamine content attained by improving gene expression can improve seed germination and seedling vigor [51]. This study found that thiamine metabolism genes significantly increases following NPAW therapy, and the expression of the thiamine metabolism gene LOC106767562—a regulatory bifunctional TENA-E protein—was also boosted. Furthermore, qPCR results showed an increase in gene expression after NPAW treatment, suggesting a potential correlation. Studying the KEGG pathways can help researchers understand the mechanisms underlying plant hormone signaling and identify potential targets for genetic engineering or chemical manipulation to improve plant growth and stress tolerance.

Conclusion

This study induced plasma-activated water production using different gases to promote mung bean growth, revealing that NPAW was the most effective in enhancing the hypocotyl length. The properties of plasma-activated water and the mechanisms behind its effects on crops were explored by studying biological and non-biological stress responses. The use of next-generation sequencing helped elucidate the changes in mung bean growth induced by plasma-activated water, revealing that gene changes primarily countered oxidative stress. This included changes in genes related to phenylpropanoids, MAPK, plant hormones, and calcium ions, which functioned to balance growth, given that plasma-activated water inherently involves oxidative stress. Overall, this study sheds light on and promotes more comprehensive insights into the changes in crops after plasma-activated water treatment, deepening the understanding of the complex regulatory networks behind the mechanism of mung bean growth in response to plasma-activated water.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00497-2.

Additional file 1. Figure S1. Trimmomatic trim result summary. **Table S1.** Data quality control statistics table. **Table S2.** Reference genome mapping statistics table. **Table S3.** Primer sequence.

Acknowledgements
Not applicable.

Author contributions
YJC contributed to the conceptualization, methodology, software development, visualization, formal analysis, as well as writing the original draft and editing the manuscript. YJC also contributed to data curation, investigation, and validation. YT contributed to the conceptualization, visualization, allocation of resources, as well as writing and editing the manuscript. YT provided supervision, project administration, and was involved in funding acquisition. Both authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Data will be made available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

Author details
[1]Graduate Institute of Food Science and Technology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei City, Taiwan.

Received: 28 July 2023 Accepted: 25 October 2023
Published online: 13 December 2023

References

1. de Oliveira AB, Alencar NLM, Gomes-Filho E. Comparison between the water and salt stress effects on plant growth and development. Responses Org Water Stress. 2013;4:67–94.
2. Onwuka B, Mang B. Effects of soil temperature on some soil properties and plant growth. Adv Plants Agric Res. 2018;8(1):34–7.
3. Prasad PVV, Staggenborg SA, Ristic Z. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Ahuja LR, Reddy VR, Saseendran SA, Qiang Yu, editors. Response of crops to limited water: understanding and modeling water stress effects on plant growth processes. Madison, WI: American Society of Agronomy and Soil Science Society of America; 2008. p. 301–55.
4. Priest HD, Fox SE, Rowley ER, Murray JR, Michael TP, Mockler TC. Analysis of global gene expression in Brachypodium distachyon reveals extensive network plasticity in response to abiotic stress. PLoS ONE. 2014;9(1): e87499.
5. Sivachandiran L, Khacef A. Enhanced seed germination and plant growth by atmospheric pressure cold air plasma: combined effect of seed and water treatment. RSC Adv. 2017;7(4):1822–32.
6. Adhikari B, Adhikari M, Ghimire B, Park G, Choi EH. Cold atmospheric plasma-activated water irrigation induces defense hormone and gene expression in tomato seedlings. Sci Rep. 2019;9(1):16080.

7. Guragain RP, Pradhan SP, Baniya HB, Pandey BP, Basnet N, Sedhai B, et al. Impact of plasma-activated water (PAW) on seed germination of soybean. J Chem. 2021;2021:1–12.
8. Wang J, Cheng J-H, Sun D-W. Enhancement of wheat seed germination, seedling growth and nutritional properties of wheat plantlet juice by plasma activated water. J Plant Growth Regul. 2022. https://doi.org/10.1007/s00344-022-10677-3.
9. Lukes P, Dolezalova E, Sisrova I, Clupek M. Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H2O2 and HNO2. Plasma Sources Sci Technol. 2014;23(1): 015019.
10. Bader H, Hoigné J. Determination of ozone in water by the indigo method. Water Res. 1981;15(4):449–56.
11. Shen J, Tian Y, Li Y, Ma R, Zhang Q, Zhang J, et al. Bactericidal effects against *S. aureus* and physicochemical properties of plasma activated water stored at different temperatures. Sci Rep. 2016;6(1):1–10.
12. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
13. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357–60.
14. Sahraeian SME, Mohiyuddin M, Sebra R, Tilgner H, Afshar PT, Au KF, et al. Gaining comprehensive biological insight into the transcriptome by performing a broad-spectrum RNA-seq analysis. Nat Commun. 2017;8(1):59.
15. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics. 2016;32(19):3047–8.
16. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014;30(7):923–30.
17. Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics. 2010;26(1):136–8.
18. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics J Integr Biol. 2012;16(5):284–7.
19. Bai X, Todd CD, Desikan R, Yang Y, Hu X. N-3-Oxo-decanoyl-l-homoserine-lactone activates auxin-induced adventitious root formation via hydrogen peroxide- and nitric oxide-dependent cyclic GMP signaling in mung bean. Plant Physiol. 2011;158(2):725–36.
20. Hsieh C-C, Yu S-H, Kuo H-C, Cheng K-W, Hsu C-C, Lin Y-P, et al. Alleviation of PM2.5-induced alveolar macrophage inflammation using extract of fermented *Chenopodium formosanum* Koidz sprouts via regulation of NF-κB pathway. J Ethnopharmacol. 2024;318: 116980.
21. Wu J, Zhao Q, Sun D, Wu G, Zhang L, Yuan H, et al. Transcriptome analysis of flax (*Linum usitatissimum* L.) undergoing osmotic stress. Ind Crops Prod. 2018;116:215–23.
22. Šerá B, Gajdová I, Černák M, Gavril B, Hnatiuc E, Kováčik D, et al., editors. How various plasma sources may affect seed germination and growth. 2012 13th International Conference on Optimization of Electrical and Electronic Equipment (OPTIM); 2012: IEEE.
23. Chou YJ, Cheng KC, Hsu FC, Wu JSB, Ting Y. Producing high quality mung bean sprout using atmospheric cold plasma treatment: better physical appearance and higher γ-aminobutyric acid (GABA) content. J Sci Food Agric. 2021;101(15):6463–71.
24. Adhikari B, Adhikari M, Park G. The effects of plasma on plant growth, development, and sustainability. Appl Sci. 2020;10(17):6045.
25. Locke BR, Shih K-Y. Review of the methods to form hydrogen peroxide in electrical discharge plasma with liquid water. Plasma Sources Sci Technol. 2011;20(3): 034006.
26. Bradu C, Kutasi K, Magureanu M, Puač N, Živković S. Reactive nitrogen species in plasma-activated water: generation, chemistry and application in agriculture. J Phys D Appl Phys. 2020;53(22): 223001.
27. Zhou R, Zhou R, Zhang X, Zhuang J, Yang S, Bazaka K, et al. Effects of atmospheric-pressure N 2, He, Air, and O 2 microplasmas on mung bean seed germination and seedling growth. Sci Rep. 2016;6:32603.
28. Khan TA, Yusuf M, Fariduddin Q. Seed treatment with H2O2 modifies net photosynthetic rate and antioxidant system in mung bean (Vigna radiata L. Wilczek) plants. Israel J Plant Sci. 2015;62(3):167–75.
29. Entesari M, Sharif-Zadeh F, Zare S, Farhangfar M, Dashtaki M. Effect of seed priming on mung been (*Vigna radiata*) cultivars with salicylic acid and potassium nitrate under salinity stress. Int J Agric Res Rev. 2012;2(Special issue):926–32.
30. Hoigné J. The chemistry of ozone in water. Process technologies for water treatment. 1988:121–43.
31. Waskow A, Howling A, Furno I. Mechanisms of plasma-seed treatments as a potential seed processing technology. Front Phys. 2021. https://doi.org/10.3389/fphy.2021.617345.
32. Hewitt DKL, Mills G, Hayes F, Norris D, Coyle M, Wilkinson S, et al. N-fixation in legumes—an assessment of the potential threat posed by ozone pollution. Environ Pollut. 2016;208:909–18.
33. Fan L, Liu X, Ma Y, Xiang Q. Effects of plasma-activated water treatment on seed germination and growth of mung bean sprouts. J Taibah Univ Sci. 2020;14(1):823–30.
34. Liu C, Chen C, Jiang A, Sun X, Guan Q, Hu W. Effects of plasma-activated water on microbial growth and storage quality of fresh-cut apple. Innov Food Sci Emerg Technol. 2020;59: 102256.
35. Mošovská S, Medvecká V, Klas M, Kyzek S, Valík Ľ, Mikulajová A, et al. Decontamination of *Escherichia coli* on the surface of soybean seeds using plasma activated water. LWT. 2022;154: 112720.
36. Ling L, Jiafeng J, Jiangang L, Minchong S, Xin H, Hanliang S, et al. Effects of cold plasma treatment on seed germination and seedling growth of soybean. Sci Rep. 2014. https://doi.org/10.1038/srep05859.
37. Sajib SA, Billah M, Mahmud S, Miah M, Hossain F, Omar FB, et al. Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (*Vigna mungo* L.). Plasma Chem Plasma Process. 2020;40(1):119–43.
38. Hu H, Feng N, Shen X, Zhao L, Zheng D. Transcriptomic analysis of Vigna radiata in response to chilling stress and uniconazole application. BMC Genomics. 2022;23(1):205.
39. Kumar S, Ayachit G, Sahoo L. Screening of mungbean for drought tolerance and transcriptome profiling between drought-tolerant and susceptible genotype in response to drought stress. Plant Physiol Biochem. 2020;157:229–38.
40. Davletova S, Schlauch K, Coutu J, Mittler R. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. Plant Physiol. 2005;139(2):847–56.
41. Ding ZJ, Yan JY, Li CX, Li GX, Wu YR, Zheng SJ. Transcription factor WRKY46 modulates the development of Arabidopsis lateral roots in osmotic/salt stress conditions via regulation of ABA signaling and auxin homeostasis. Plant J. 2015;84(1):56–69.
42. Knight H. Calcium signaling during abiotic stress in plants. In: Jeon KW, editor. International review of cytology. 195. Cambridge: Academic Press; 1999. p. 269–324.
43. Wang L, Li X, Gao F, Liu Y, Lang S, Wang C, et al. Effect of ultrasound combined with exogenous GABA treatment on polyphenolic metabolites and antioxidant activity of mung bean during germination. Ultrason Sonochem. 2023;94: 106311.
44. Di T, Zhao L, Chen H, Qian W, Wang P, Zhang X, et al. Transcriptomic and metabolic insights into the distinctive effects of exogenous melatonin and gibberellin on terpenoid synthesis and plant hormone signal transduction pathway in Camellia sinensis. J Agric Food Chem. 2019;67(16):4689–99.
45. Ruan J, Zhou Y, Zhou M, Yan J, Khurshid M, Weng W, et al. Jasmonic acid signaling pathway in plants. Int J Mol Sci. 2019;20(10):2479.
46. Maheswari M, Varalaxmi Y, Sarkar B, Ravikumar N, Vanaja M, Yadav SK, et al. Tolerance mechanisms in maize identified through phenotyping and transcriptome analysis in response to water deficit stress. Physiol Mol Biol Plants. 2021;27(6):1377–94.
47. Wu P-M, Leng Y, Li S-W. Transcriptome profiling provides new insights into ABA-mediated genes and pathways in leaves, stems, and roots of mung bean seedlings. Plant Growth Regul. 2022;98(3):569–87.
48. Sui N, Yang Z, Liu M, Wang B. Identification and transcriptomic profiling of genes involved in increasing sugar content during salt stress in sweet sorghum leaves. BMC Genomics. 2015;16(1):1–18.
49. Jiang Y, Liao Q, Zou Y, Liu Y, Lan J. Transcriptome analysis reveals the genetic basis underlying the biosynthesis of volatile oil, gingerols, and diarylheptanoids in ginger (*Zingiber officinale* Rosc.). Bot Stud. 2017;58:1–12.

50. Sui N, Wang Y, Liu S, Yang Z, Wang F, Wan S. Transcriptomic and physiological evidence for the relationship between unsaturated fatty acid and salt stress in peanut. Front Plant Sci. 2018;9:7.
51. Yazdani M. Genetic engineering of plant seeds to increase thiamin (vitamin b 1) content. University of Nevada, Reno; 2015.

Publisher's Note