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### Research Article

## Gene Expression of *ycf2* and *ndhD* in Two Rapeseed Varieties under Salt Stress with Ascorbic Acid, Ultraviolet Radiation and Allelopathic Effects on Soybean

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#### ABSTRACT

The present study investigated the expression patterns of chloroplast-encoded genes *ycf2* and *ndhD* in two rapeseed varieties (Swedish and Iranian) under treatments of ascorbic acid, ultraviolet radiation, and salt stress, alongside the allelopathic effects of rapeseed residues on soybean growth. Chloroplast genes play a crucial role in photosynthesis and stress tolerance; however, their regulation under combined abiotic stresses remains insufficiently understood. Therefore, this study aimed to elucidate how antioxidant application and radiation interact with salinity to influence gene expression and plant performance. Results revealed that the combined application of ascorbic acid and radiation markedly enhanced *ycf2* expression, reaching over 8.11-fold in the Swedish variety and 1.84-fold in the Iranian variety. Similarly, *ndhD* expression peaked under the same combined treatment, with 4-fold and 3.13-fold increases in Swedish and Iranian varieties, respectively. In contrast, salt stress alone consistently resulted in the lowest expression levels of both genes in both varieties. Combined stress treatments involving salt generally suppressed gene expression, although partial mitigation was observed when ascorbic acid was included. Overall, the Swedish variety exhibited a stronger transcriptional response compared to the Iranian variety. Furthermore, allelopathic assays demonstrated that rapeseed leaf residues significantly reduced plant height, leaf area, chlorophyll content, and relative water content in soybean plants, indicating inhibitory effects on growth and physiology. In conclusion, the synergistic application of ascorbic acid and radiation enhances chloroplast gene expression and may improve stress tolerance, whereas salinity exerts a suppressive effect. Moreover, rapeseed residues exhibit notable allelopathic potential, adversely affecting subsequent crop growth.

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#### Introduction

One of the world's best oil crops and a great source of vegetable oils is rapeseed (*Brassica napus* L.). With an estimated 71.15 million tons produced worldwide, it ranks third in terms of production, following palm oil and soybeans and approximately 37.77 million hectares were

under cultivation worldwide (USDA, 2022). Rapeseed oil is characterized by a low percentage of saturated fatty acids (6%) and a high percentage of unsaturated fatty acids, namely oleic acid (80%) and linoleic acid (20%), compared to other oil crops which make the oil of high quality (Bocianwski et al., 2012; Anonymous, 2015).

The growth and production of plants are affected when exposed to ionizing radiations such as ultraviolet (UV) rays. It has been discovered that subjecting plants to UV radiation for a predetermined amount of time positively affects the activation of chemical and biological reactions in plants generally, which results in improved growth and production (Ibrahim et al., 1990). One of the most prevalent water-soluble antioxidants in both plants and animals is ascorbic acid (ASA), also known as vitamin C. It controls a number of physiological processes that govern growth, development, and stress tolerance in plants as well as oxidation and reduction processes (Hossain et al., 2017). Furthermore, the presence of salt ions prevents several vital elements from being absorbed, necessary for plant growth, salinity especially sodium ions, is one of the biggest challenges to crop production worldwide. It also leads to ionic poisoning of the cell as a result of the accumulation of high levels of sodium, chloride and sulfate ions beyond the cell's capacity (Amerian et al., 2024; Hamza, 2024).

Gene expression is the process by which the genetic information stored in the gene (DNA) is converted into a functional product such as protein or RNA that contributes to performing a specific function within the cell. In other words, it is a series of steps that includes copying the gene into mRNA, then translating this mRNA into a protein, or in some cases, RNA remains the final product and performs a specific function without being translated into a protein (Alberts et al., 2022).

Soybean (*Glycine max* L.) belongs to the legume family Leguminosae and is one of the important crops in the world and occupies the first place in terms of cultivated area and global production among oilseed crops (Mishra et al., 2024).

Allelopathy is a common biological phenomenon resulting from the release of chemical compounds from a particular organism, such as a plant, and its various parts into the environment. These compounds affect the growth and development of other organisms, whether the effect is positive or negative, beneficial or harmful, depending on the nature and concentration of the chemical compounds (Abid-Aljabar and Saeed, 2019). Allelopathic compounds are secondary metabolites produced by plants, fungi, algae, and viruses, which originate from either Shikimic acid or Acetate acid pathways (Saeed et al., 2013; Hameed and Hamed, 2023). The decomposition of plant waste is responsible for most of the chemicals added to the soil. When a

plant dies, its remains decompose and allelopathic compounds are released into the environment (Salih et al., 2018). Many factors influence this process, including the nature of plant residues, soil quality, and decomposition conditions. These factors can limit the effectiveness of compounds released from plants on other living organisms, depending on whether they are highly toxic, non-toxic, or stimulant to plants. (Saffar and Al-Shalal, 2020).

The objective of the present study was to assess whether the treatment with ascorbic acid and ultraviolet radiation modify the gene expression of the *ycf2* and *ndhD* genes in rapeseed under salt stress, reflecting on the plant's salt tolerance efficiency, and subsequently affecting the allelopathic activity of plant residues towards soybeans.

## Materials and Methods

### The first experiment

A greenhouse experiment was carried out in the Department of Biology, College of Science, University of Mosul. The effect of ascorbic acid at a concentration of 100 ppm was tested on the germination and growth of rapeseed of two varieties (Swedish and Iranian), as well as when their seeds were exposed to ultraviolet radiation before planting at a wavelength of 200-28 nm, for 30 min, at a distance of approximately 25 cm and under a salt stress level of 150 mmol/L. The gene expression levels of several genes associated with chloroplast activity in rapeseed (four replicates per treatment) were analyzed using RT-PCR. The quantitative estimation of gene expression levels of the genes (*ycf2-ndhD*- and *H.K*) associated with the photosynthetic pathway in chloroplasts involved several stages.

### RNA extraction

After crushing the plant tissue, it was mixed with 1 ml of Trizol. The mRNA was then extracted using an analytical kit supplied by Trans. A Biodrop device was used to measure the extracted RNA's concentration and purity. This was done by taking 1  $\mu$ l of the extracted RNA sample and placing it in a microcentrifuge for 5 sec until the liquid droplets that form on the wall fall off. The droplets are then placed in the designated area of the device, and the concentration and purity were read on the device's screen.

### Measuring the concentration and purity of RNA

The concentration and purity of the extracted RNA were estimated using a Biodrop device. This is done by taking

1 µl of the extracted RNA sample and placing it in a microcentrifuge for 5 sec until the liquid droplets that form on the wall fall off. The drops are then placed in the designated area of the device, and the concentration and purity displayed on the device's screen are read.

#### The procedure for creating a cDNA molecule from the extracted mRNA molecule

Once the mRNA extraction procedure was finished, the reverse transcriptase enzyme was used to transform it into cDNA, with the analytical kit supplied by Trans. The random primer was prepared by placing it at a temperature of 25°C for 10 min, after which the EasyScript RT/RI Enzyme Mix was added to the extracted RNA sample and kept at a temperature of 42°C for 15 min for the purpose of conducting RT-PCR reactions. The mixture was incubated at a temperature of 85°C for 5 sec to stop the activity of the enzyme.

#### RT-PCR reaction

The housekeeping gene primers (L18a) and the gene-specific primers were employed to conduct the quantitative test of gene expression levels as indicated in Table 1.

Table 1. Primers for *ycf2* and *ndhD* genes in the chloroplast photosynthetic pathway and housekeeping genes used in RT-PCR.

| Primer | Sequence                     |
|--------|------------------------------|
| ndhD-F | CGTTGACCGGGAGATGTTGAAGCTGC   |
| ndhD-R | GGGAATCAAGCCTATCCGGTCACACTCG |
| Ycf2-F | CACAAATAAGCGCGTTGCGCGTTCC    |
| Ycf2-R | GCCAAGCCGACACAATAGGCATTGCC   |
| L18a-F | AGCAAGAACAGCAGCTATGG         |
| L18a-R | CATTCAAGAACATCAAACCGTTCC     |

The RT-PCR settings were as follows: 1 cycle of initial denaturation at 95°C for 10 min; 40 cycles of denaturation at 95°C for 15 sec; 40 cycles of annealing at 60°C for 1 min; and a melting curve between 60 and 95°C.

#### Calculation of gene expression rate

The gene expression rate of the genes under study was calculated based on the CT value of the target gene and the standard housekeeping gene for both control and treated plant samples, using the rules of Haimes et al. (2013):

$$1- \Delta CT (\text{test}) = CT (\text{target, test}) - CT (\text{ref, test})$$

$$\Delta CT (\text{control}) = CT (\text{target, control}) - CT (\text{ref, control})$$

CT (target, test) indicates the number of mRNA cycles of the *ycf2-ndhD* genes in the study samples.

CT (ref, test) indicates the number of mRNA cycles of the housekeeping gene in the study samples.

CT (target, control) indicates the mRNA cycles of the *ycf2-ndhD* genes in the control samples.

(ref, control) indicates the mRNA cycles of the housekeeping gene for control samples.

2- The equation for  $\Delta CT$  of the treated sample relative to  $\Delta CT$  of the control sample is given by the following formula:

$$\Delta \Delta CT = \Delta CT(\text{test}) - \Delta CT(\text{control}).$$

3- The gene expression value was calculated according to the following equation:

$$\text{Gene Expression folding} = 2^{-\Delta \Delta CT}$$

#### The second experiment

##### Addition of plant residues

The residues of rapeseed leaves were mixed with soil at levels of 0, 10, and 20 g/kg, then placed in plastic pots and their openings were sealed with perforated aluminum foil and incubated for two weeks. After that, 10 soybean seeds were planted in each pot. After two weeks of planting, the number of seedlings was reduced to five seedlings, and watering was done with regular water for all treatments. After 60 days of planting, some morphological and physiological characteristics were studied.

The experiment was conducted in a greenhouse using a completely randomized design (CRD) with three replicates per treatment.

##### Plant height (cm)

The plant height was measured from above the soil surface to the highest point of the plant for five normal seedlings.

##### Leaf area

Leaf area was calculated by dividing the dry weight of discs of known area by the total dry weight of the plant's leaves, using the method of Roy et al. (1981).

##### Estimation of relative water content

It was estimated according to the method of Turner (1981).

##### Estimation of Total Chlorophyll Content

Total chlorophyll was estimated by weighing 100 mg of fresh leaves from each soybean plant sample, then crushing them with 10 ml of 80% acetone using a ceramic mortar and centrifuging them for 15 min at a speed of 3000 rpm. The filtrate was collected and the absorbance was measured at wavelengths of 645 and 663 nm using a spectrophotometer (Arnon, 1949).

#### Results

The expression analysis of the gene *YCF2* in chloroplasts of the Swedish variety revealed that a maximum of more

than 8.11 times upregulation occurred under joint treatment of ascorbic acid and radiation followed by ascorbic acid (5.69-times) and radiation (2.09-times) as shown in Table 2 and Figure 1. Ascorbic acid, radiation, and salt, when applied in combination, caused a relatively poorer expression level of 1.85-times as compared to the control.

On the other hand, numerous treatments resulted in a downregulation of *YCF2* expression. The combined treatment of ascorbic acid and salt showed a 0.51-fold reduction in expression, followed by the joint treatment of salt and radiation which was 0.40-fold. The minimum expression level of 0.19-fold was noticed under sole salt stress treatment as compared to the control.

In case of Iranian variety, the maximum expression of *YCF2* gene (1.84-fold) was also observed with the combined treatment of ascorbic acid and radiation (Table 3; Figure 2). Majority of the treatments showed higher degrees of gene expression as compared to the control, excepting the salt alone treatment, which exhibited a minimum level of 0.20-fold expression.

As for as the expression of the *ndhD* gene is concerned, which was found to be involved in cyclic electron transport around Photosystem I (PSI), the maximum expression level of 4-fold was observed with combined treatment of ascorbic acid and radiation in the Swedish variety (Table 4; Figure 3). Conversely, the expression levels in ascorbic acid and radiation alone treatments were significantly lower corresponding to 1.71-fold and 1.60-folds respectively. Similarly, the consortium

treatment of ascorbic acid, radiation, and salt led to moderate degree of expression of 1.10-fold which showed no significant difference from that of the control treatment. Likewise, the ascorbic acid and salt treatment showed expression levels similar to the control treatment. On the contrary, the combined treatment of salt and radiation resulted in 0.32-fold reduced level of expression, whereas the minimum expression level of 0.30-fold was found with sole salt stress treatment.

A similar pattern in *ndhD* expression was observed in the Iranian variety (Table 5; Figure 4). The combined treatment of ascorbic acid and radiation resulted in the highest expression level equivalent to 3.13-fold. This was followed by the ascorbic acid and radiation alone treatments, and the combined treatment of ascorbic acid + radiation + salt resulting in 1.67-fold, 1.45-fold and 1.40-fold respectively as compared to the control treatment. On the other hand, the rest of the treatments caused a reduction in expression, with combined treatments of ascorbic acid + salt and salt + radiation resulting in 0.52-fold and 0.29-fold declines respectively. The minimum expression level of 0.22-fold was again observed with the treatment where only salt was applied.

Moreover, significant reductions were observed in plant height, leaf area, total chlorophyll and relative water contents in the two soybean varieties, with all applications of leaf residues of rapeseed as compared to untreated control plants as evident from the results shown in Table 6.

Table 2. Gene expression analysis of *YCF2* in the Swedish rapeseed variety.

| Sample                    | No | CT target gene (mean) | CT housekeepin g gene (mean) | $\Delta$ CT target gene (mean) | $\Delta$ CT control (mean) | $\Delta \Delta$ CT (mean) | Gene Expression folding (mean $\pm$ SD) | p- value  |
|---------------------------|----|-----------------------|------------------------------|--------------------------------|----------------------------|---------------------------|---|-----------|
| control                   | 4  | 25.35                 | 27.28                        | -1.93                          | -1.93                      | 0                         | 1                                       |           |
| Ascorbic                  | 4  | 30.93                 | 30.35                        | 0.58                           | -1.93                      | 2.51                      | 5.69 $\pm$ 0.106                        | p< 0.0001 |
| salt                      | 4  | 27.91                 | 32.22                        | -4.31                          | -1.93                      | -2.38                     | 0.19 $\pm$ 0.025                        | p< 0.0001 |
| Radiation                 | 4  | 28.92                 | 29.78                        | -0.86                          | -1.93                      | 1.07                      | 2.09 $\pm$ 0.029                        | p< 0.0001 |
| Ascorbic * salt           | 4  | 25.64                 | 28.52                        | -2.88                          | -1.93                      | -0.95                     | 0.51 $\pm$ 0.038                        | p< 0.0001 |
| Ascorbic * Radiation      | 4  | 30.74                 | 29.65                        | 1.09                           | -1.93                      | 3.02                      | 8.11 $\pm$ 0.025                        | p< 0.0001 |
| Salt * Radiation          | 4  | 25.46                 | 28.68                        | -3.22                          | -1.93                      | -1.29                     | 0.40 $\pm$ 0.029                        | p< 0.0001 |
| Ascorbic * Radiation*salt | 4  | 28.06                 | 29.10                        | -1.04                          | -1.93                      | 0.89                      | 1.85 $\pm$ 0.043                        | p< 0.0001 |

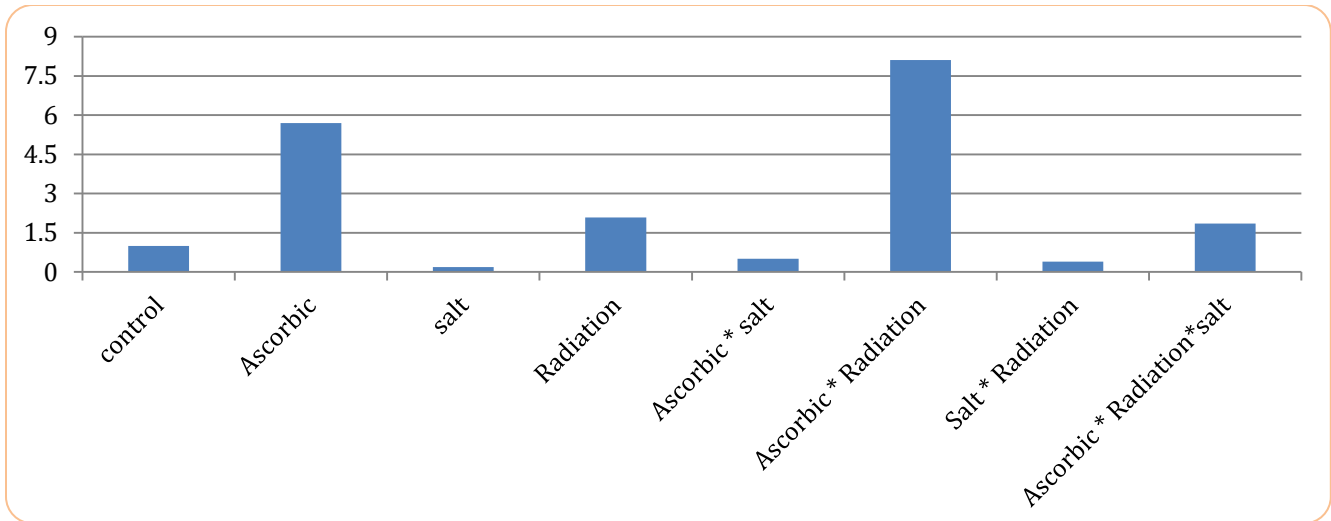


Figure 1. Gene expression analysis of the YCF2 gene in the Swedish variety of rapeseed.

Table 3. Gene expression analysis of the YCF2 gene in the Iranian variety of rapeseed.

| Sample          | NO | CT target gene (mean) | CT housekeeping gene (mean) | $\Delta$ CT target gene (mean) | $\Delta$ CT control (mean) | $\Delta \Delta$ CT (mean) | Gene Expression folding | p-value   |
|-----------------|----|-----------------------|-----------------------------|--------------------------------|----------------------------|---------------------------|-------------------------|-----------|
| Control         | 4  | 28.88                 | 30.11                       | -1.23                          | -1.23                      | 0                         | 1                       |           |
| Ascorbic        | 4  | 28.06                 | 28.43                       | -0.37                          | -1.23                      | 0.86                      | 1.81±0.043              | p< 0.0001 |
| salt            | 4  | 25.18                 | 28.72                       | -3.54                          | -1.23                      | -2.31                     | 0.20±0.016              | p< 0.0001 |
| Radiation       | 4  | 28.21                 | 28.89                       | -0.68                          | -1.23                      | 0.55                      | 1.46±0.022              | p< 0.0001 |
| Ascorbic * salt | 4  | 26.91                 | 27.85                       | -0.94                          | -1.23                      | 0.29                      | 1.22±0.035              | p< 0.0001 |
| Ascorbic *      | 4  | 28.97                 | 29.32                       | -0.35                          | -1.23                      | 0.88                      | 1.84±0.034              | p< 0.0001 |
| Radiation       |    |                       |                             |                                |                            |                           |                         |           |
| Salt *          | 4  | 28.19                 | 29.38                       | -1.19                          | -1.23                      | 0.04                      | 1.02±0.0179             | 0.026     |
| Radiation       |    |                       |                             |                                |                            |                           |                         |           |
| Ascorbic *      | 4  | 28.30                 | 29.10                       | -0.80                          | -1.23                      | 0.43                      | 1.34±0.051              | p< 0.0001 |
| Radiation*salt  |    |                       |                             |                                |                            |                           |                         |           |

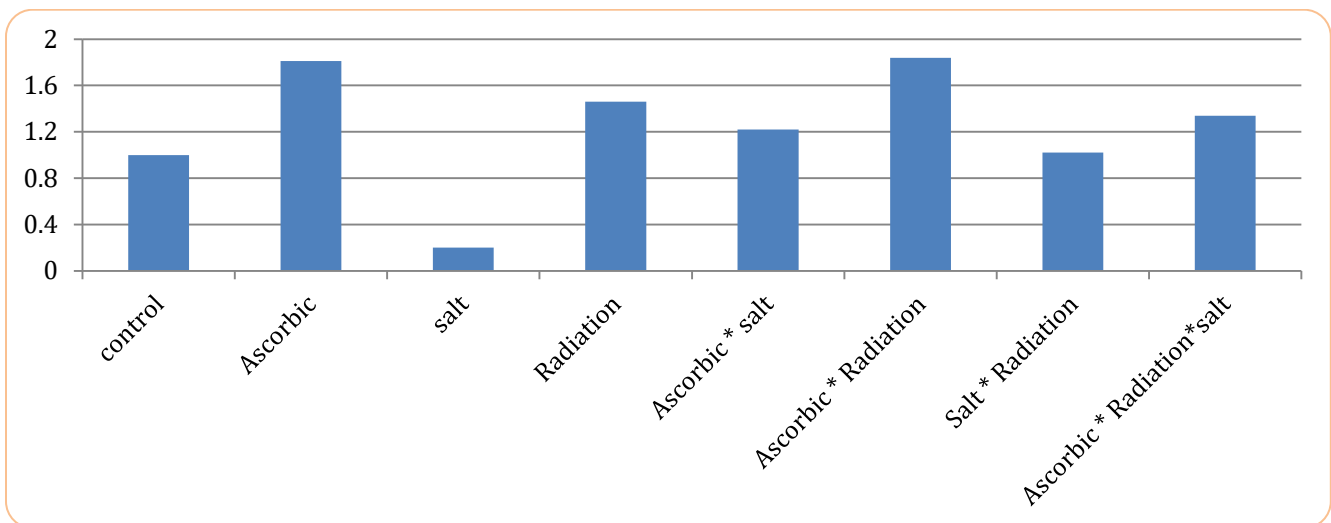


Figure 2. Gene expression analysis of the ycf2 gene in the Iranian rapeseed variety.

Table 4. Gene expression analysis of the ndhD gene in the Swedish rapeseed variety.

| Sample                      | No | CT target gene (mean) | CT housekeeping gene (mean) | Δ CT target gene (mean) | Δ CT control (mean) | Δ Δ CT (mean) | Gene Expression folding | p-value   |
|-----------------------------|----|-----------------------|-----------------------------|-------------------------|---------------------|---------------|-------------------------|-----------|
| Control                     | 4  | 24.2                  | 27.28                       | -3.08                   | -3.08               | 0             | 1                       |           |
| Ascorbic salt               | 4  | 28.05                 | 30.35                       | -2.3                    | -3.08               | 0.78          | 1.71±0.0187             | p< 0.0001 |
| Radiation                   | 4  | 23.28                 | 28.06                       | -4.78                   | -3.08               | -1.7          | 0.30±0.038              | p< 0.0001 |
| Ascorbic * salt             | 4  | 24.28                 | 26.68                       | -2.4                    | -3.08               | 0.68          | 1.60±0.032              | p< 0.0001 |
| Ascorbic *                  | 4  | 26.7                  | 29.78                       | -3.08                   | -3.08               | 0             | 1                       | 1         |
| Radiation                   | 4  | 28.57                 | 29.65                       | -1.08                   | -3.08               | 2.0           | 4±0.187                 | p< 0.0001 |
| Salt *                      | 4  | 27.5                  | 32.22                       | -4.72                   | -3.08               | -1.64         | 0.32±0.042              | p< 0.001  |
| Ascorbic * Radiation * salt | 4  | 24.59                 | 27.52                       | -2.93                   | -3.08               | 0.15          | 1.10±0.158              | 0.2       |

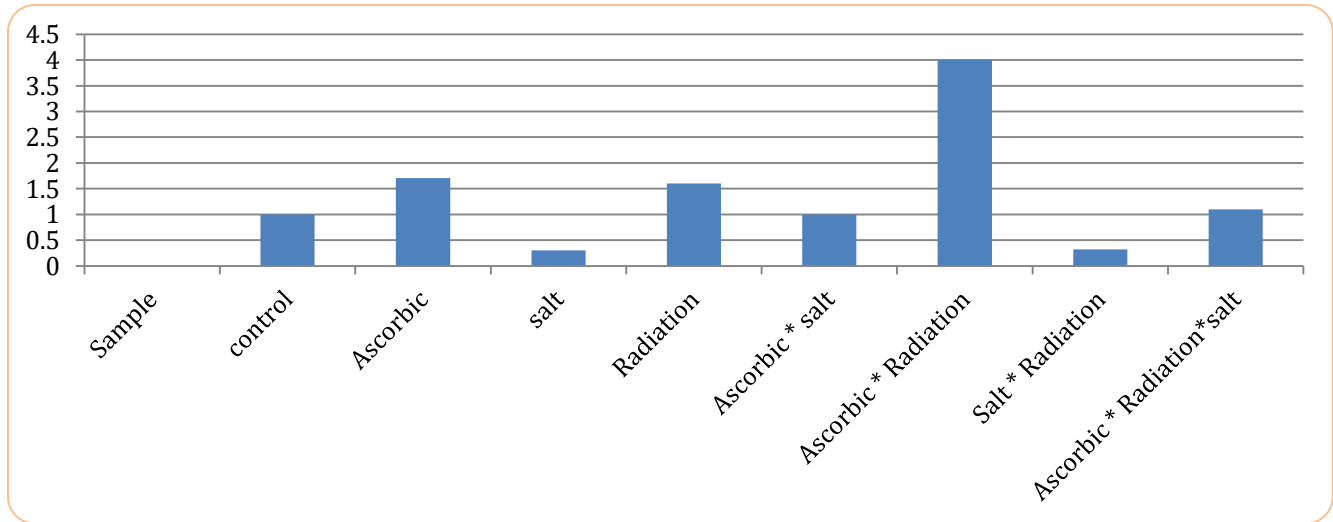


Figure 3. Expression analysis of the ndhD gene in the Swedish variety of rapeseed.

Table 5. Expression analysis of the ndhD gene in the Iranian variety of rapeseed.

| Sample                      | No | CT target gene (mean) | CT housekeeping gene (mean) | Δ CT target gene (mean) | Δ CT control (mean) | Δ Δ CT (mean) | Gene Expression folding | p-value   |
|-----------------------------|----|-----------------------|-----------------------------|-------------------------|---------------------|---------------|-------------------------|-----------|
| Control                     | 4  | 26.3                  | 30.11                       | -3.81                   | -3.81               | 0             | 1                       |           |
| Ascorbic salt               | 4  | 25.36                 | 28.43                       | -3.07                   | -3.81               | 0.74          | 1.67±0.0255             | p< 0.0001 |
| Radiation                   | 4  | 25.67                 | 31.62                       | -5.95                   | -3.81               | -2.14         | 0.22±0.0259             | p< 0.0001 |
| Ascorbic * salt             | 4  | 25.68                 | 28.95                       | -3.27                   | -3.81               | 0.54          | 1.45±0.0332             | p< 0.0001 |
| Ascorbic *                  | 4  | 24.35                 | 29.1                        | -4.75                   | -3.81               | -0.94         | 0.52±0.051              | p< 0.0001 |
| Radiation                   | 4  | 27.16                 | 29.32                       | -2.16                   | -3.81               | 1.65          | 3.13±0.068              | p< 0.0001 |
| Salt *                      | 4  | 23.17                 | 28.72                       | -5.55                   | -3.81               | -1.74         | 0.29±0.0158             | p< 0.0001 |
| Ascorbic * Radiation * salt | 4  | 25.57                 | 28.89                       | -3.32                   | -3.81               | 0.49          | 1.40±2.0235             | p< 0.0001 |

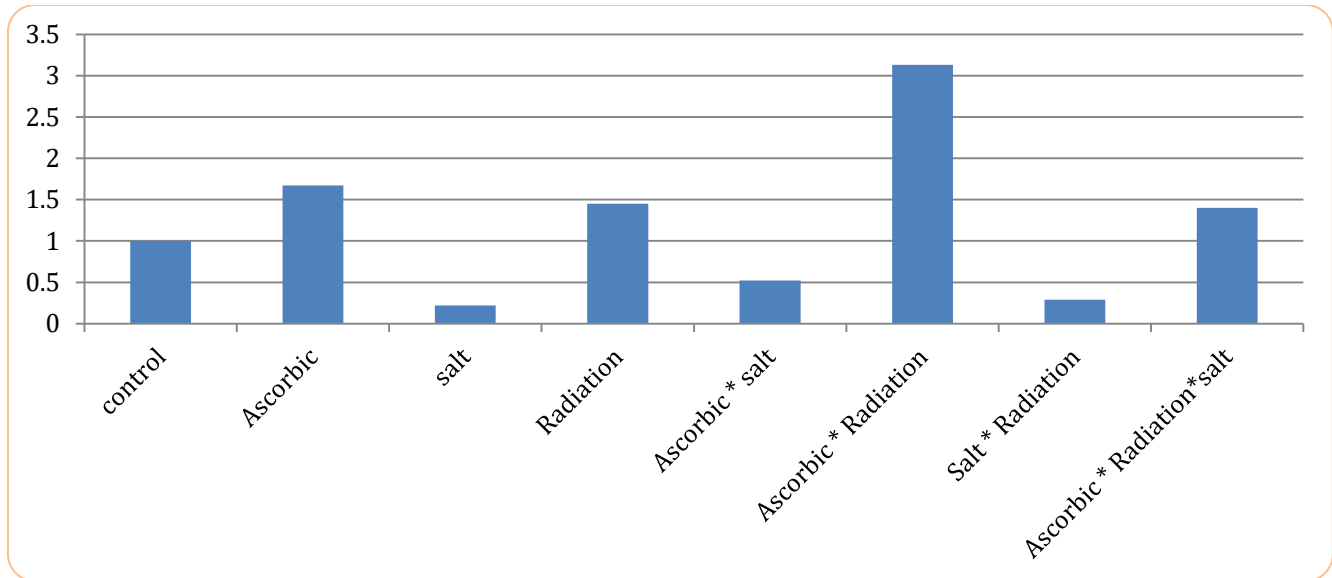


Figure 4. Expression analysis of the ndhD gene in the Iranian rapeseed variety.

Table 6. Effect of rapeseed leaf residues on morphological and physiological traits of two soybean varieties.

| Varieties | Plant residues | Plant height | Leaf area | Relative water content | Total chlorophyll |
|-----------|----------------|--------------|-----------|------------------------|-------------------|
| Al Shaima | Control        | 15.7 a       | 16.0 a    | 70.4 a                 | 1.90 a            |
|           | 10 g           | 14.6 b       | 14.3 b    | 64.2 b                 | 1.73 b            |
|           | 20 g           | 13.0 c       | 12.8 c    | 59.3 c                 | 1.55 c            |
| Indian    | Control        | 15.0 a       | 15.2 a    | 68.5 a                 | 1.78 a            |
|           | 10 g           | 13.9 b       | 14.1 b    | 62.8 b                 | 1.56 b            |
|           | 20 g           | 12.7 c       | 12.5 c    | 54.6 c                 | 1.40 c            |

## Discussion

The YCF2 gene is a large gene in chloroplasts and is found in plants and some algae. It encodes a protein that forms a part of the chloroplast genome and is believed to play a role in the chloroplast function (Drescher et al., 2000). The results in Table 2 and Figure 1 for the Swedish variety showed that treatment with ascorbic acid and exposure to radiation gave the highest rate of increase in gene expression of the ycf2 gene. This is due to the activation of chloroplast repair mechanisms resulting from oxidative stress and the stimulation of transporter protein function, thus leading to an increase in the gene expression of the YCF2 gene, which plays a prominent role in maintaining chloroplast activity after exposure to stress. It is important to note that ascorbic acid is a multifunctional metabolite in plants that can alter gene expression and control the activity of enzymes (Tóth et al., 2013). The effect of radiation causes mutations in regulatory genes, and consequently, leads to the changes in chromosome content (Britt, 1996;

Hamed et al., 2021). This is confirmed by the current study, as an increase was observed in the gene expression of the YCF2 gene, which plays a prominent role in plastid activity (that duly plays an important role in pigment uptake). The lowest rate of gene expression was recorded in salt-treated samples that were not exposed to radiation, compared to the control group. This is due to the fact that many biosynthetic pathways of compounds in the cell are negatively affected when exposed to salt stress, and therefore, a decrease in these compounds (pigments) leads to a reduction in the efficiency of photosynthesis and consequently a decrease in the gene expression rate of the YCF2 gene (Lü et al., 2010). Regarding other treatments, bilateral and triple interactions played a role in increasing or decreasing the gene expression rate of the YCF2 gene, depending on the effect of each treatment factor and its relationship to other factors.

The results in Table 3 and Figure 2 regarding the Iranian variety demonstrate that in comparison to the control,

the majority of treatments increased the YCF2 gene expression rate. This is attributed to the ascorbic acid that contributes to the activity of certain enzymatic reactions as well as the synthesis of some chemical defense chemicals and stress-resistant proteins (Agami, 2014; Moghadam, 2017).

The chloroplasts import thousands of primary proteins encoded in the nucleus that are made in the cytosol via transporters on the outer and inner membranes TOC and TIC. Thus, AAA ATPase acts as an import engine that interacts directly with various transporter proteins, which are made up of a protein complex encoded by the chloroplast gene, including the gene *ycf2* (Kikuchi et al., 2018).

Ultraviolet radiation also affects the gene expression rates and leads to an increase in the gene expression value of the YCF2 gene. This is attributed to the activity of antioxidant enzymes. In addition, the growth-related gene expression might play a role in this increase (Garbeles et al., 2024). The remaining treatments that recorded an increase in gene expression rates were the result of the bilateral and trilateral interactions between the study factors. Regarding the treatment that recorded the lowest rate of gene expression for the YCF2 gene, the salt treatment and not exposed to radiation, the decrease is attributed to the decrease in protein synthesis under salt stress, hence leading to a decrease in the protein content, amino acids, and mutagenesis of the enzymes involved in protein synthesis (Hassanpour et al., 2013). As a result, the expression of YCF2 gene naturally declines.

As for the *ndhD* gene, the NDH complex, which takes part in the cyclic electron flow around PSI in chloroplasts, includes the H subunit of the NAD(P)H-quinone oxidation-reduction enzyme. This enzyme uses NAD(P)H to aid in the reduction of plastoquinone. This helps create a protein gradient across the thylakoid membrane, which is necessary for the creation of ATP (Peltier et al., 2016; Strand et al., 2017).

The results in Tables 4, 5 and Figures 3, 4 for the Swedish and Iranian varieties, indicated that treatment with ascorbic acid and exposure to radiation recorded the highest rate of gene expression of the *ndhd* gene compared to the control treatment. This is due to the fact that the ascorbic acid plays a key role in cell activity as it removes the reactive oxygen species (ROS) and reduces oxidative damage to cells. Recent studies indicate its role in photosynthesis as an alternative electron donor to the

photosynthetic system under abiotic stress conditions. It also plays a key role in protecting the photosynthetic apparatus in chloroplasts by controlling the activity of reactive oxygen species (Venkatesh and Park, 2014; Ramadan et al., 2020). Regarding the effect of radiation, it leads to a selective gene response within the chloroplast that focuses on enhancing the electron transport pathways to counteract oxidative stress. Thus, this response protects the photosynthetic system and maintains the production of bioenergy ATP and NADPH during the period of exposure to stress (Jazayeri et al., 2024). As for the remaining treatments that recorded an increase in the gene expression rate of the *ndhD* gene compared to the control treatment and for the Swedish and Iranian varieties, the increase was due to the bilateral and tertiary interactions between the study factors, which stimulated an increase in the gene expression of the *ndhD* gene, except one treatment in the Swedish variety i.e., the treatment with ascorbic acid, which was not exposed to radiation. Regarding the treatment with salt, no increase in gene expression of the *ndhD* gene was recorded. As for the treatments that showed a decrease in the expression of the *ndhD* gene compared to the control in both the Swedish and Iranian varieties, these included treatments involving salt and radiation exposure, salt without irradiation, and the combined application of ascorbic acid and salt. For the Iranian variety, the non-irradiated treatment showed a decrease in gene expression of the *ndhD* gene compared to the control. This is attributed to the fact that high salinity concentrations lead to a decrease in ion transport and gene expression associated with chloroplast development, hence resulting in ion accumulation and damage to photosynthetic structures (Al-Hassani, 2020; Lu et al., 2023).

In Table 6, the addition of 20 g caused the greatest reduction in plant height, leaf area, relative water content, and total chlorophyll compared to the control. This is due to the fact that the rapeseed plant containing active allelopathic compounds can affect the studied traits. These compounds include benzoic acid, caffeic acid, chlorogenic acid, vanillic acid, and syringic acid. They are either stimulators or inhibitors depending on the additives (Hussain et al., 2023). In addition, most of the allelopathic compounds decompose through a series of intermediate compounds that exhibit allelopathic effects, and therefore the partial transformation of one compound may lead to the formation of several compounds that exhibit allelopathic potentials (Ali et al., 2021).

### Conclusions

From what has been so far stated, it is concluded that the expression level of the genes *ycf2* and *ndhD* significantly increased in treatments with ascorbic acid at a concentration of 100 ppm and ultraviolet radiation at a wavelength of 200-280 nanometers for both the Swedish and Iranian varieties compared to the control. Contrariwise, the expression level of the genes *ycf2* and *ndhD* when treated with salt, it significantly decreased compared to the control. Moreover, the results outline a definite relationship between the molecular changes in gene expression within rapeseed plants and the allelopathic effect of rapeseed residues on soybean plants, where salt stress, ascorbic acid treatment, and ultraviolet radiation lead to changes in cellular metabolism, which is reflected in the nature of the compounds accumulated in plant tissues and duly in their allelopathic efficiency.

### Author's contributions

NGS contributed to the conception and design of the study, conducted data collection and analysis, and drafted the initial version of the manuscript. AHA supervised the research project, provided guidance on the experimental design and data interpretation, and critically reviewed and revised the manuscript for important intellectual content. Both authors contributed to the refinement of the manuscript, approved the final version for publication, and agree to be accountable for all aspects of the work.

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### Conflict of Interest

The authors declare no conflict of interest.

### Sustainable Development Goals Targeted

SDG 2: Zero Hunger  
SDG 13: Climate Action  
SDG 15: Life on Land

### Policy Addressed

National Agricultural Research Policy

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