

RESEARCH

Open Access



Assessment of the effect of paclobutrazol treatment on the activity of genes related to flavonoid synthesis in *Brassica napus* under salt stress conditions

Alireza Mirzaei^{1*}, Jamshid Fooladi¹ and Bahman Fazeli-Nasab^{2*}

Abstract

The samples were under the influence of the treatment with pyrrolizine.

Salinity adversely impacts oilseed production by leading to excessive salt accumulation and degrading soil structure. In rapeseed, salinity specifically hinders germination and the growth of aerial organs while also reducing the production of flavonoids and anthocyanins. Recent research has demonstrated that paclobutrazol treatment can enhance cellular activities under environmental stress. Accordingly, this study investigated the effects of paclobutrazol on the molecular mechanisms related to the synthesis of flavonoids, as one of the most important natural polyphenolic compounds. In this study, after applying salinity stress, the samples were under the influence of the treatment with paclobutrazol. The results of the expression evaluation of flavonoid synthesis-related genes production (MYB111 and MYB12) showed that paclobutrazol treatment had a significant effect on increasing the expression of these genes. The findings of this study also indicated that paclobutrazol treatment, in addition to reducing the negative effects of salinity, significantly increased the production of anthocyanins and the growth of plant aerial organs. MDA measures membrane damage from stress. SOD and POD are key antioxidant enzymes that protect plant cells from oxidative harm. Bioinformatics analyses of this study also showed that MYB111 and MYB12 genes have negative hydropathicity while interacting with a network of genes and transcription factors. These findings indicate that these genes, in addition to creating resistance to salinity stress, are also effective in reducing the effects of drought stress. This study's results indicate that applying paclobutrazol to oilseeds effectively prevents damage from salinity stress and reduces sodium ion accumulation in the plant structure.

Keywords Paclobutrazol, Salt stress, Hydropathicity, Flavonoids, Rapeseed

*Correspondence:

Alireza Mirzaei

amirzaei25@gmail.com

Bahman Fazeli-Nasab

fazelinsb@rizabol.ac.ir; bfazelinasab@gmail.com

¹Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran

²Department of Agronomy and Plant Breeding, Agriculture Institute, Research Institute of Zabol, Zabol, Iran



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Introduction

Salinity stress poses a significant challenge to agricultural production globally, threatening food security. It not only damages the physiological and biochemical mechanisms within plants but also disrupts various processes essential for plant growth and development [1]. When the amount of solutes in the root activity area increases, water absorption is disturbed [2]. Electrical conductivity of 4 dS/m is the boundary of separating saline soils from non-saline soils [3]. Saline soils contain soluble salts, including chlorides, sulfates, sodium bicarbonates, magnesium, and calcium [4]. In saline soils, in addition to the increase in osmotic pressure caused by the accumulation of soluble salts, the toxicity of ions such as sodium, chlorine, and boron is also a concern [5]. The imbalance between evaporation, transpiration, and rainfall in these regions results in the accumulation of soluble salts in the soil. After periods of water stress, the concentration of these salts becomes a major limiting factor for plant growth. Key sources of salts in saline soils include precipitation, the decomposition of primary and secondary minerals, residual salts from ancient marine or lake sediments, fossil salts, rising groundwater tables, and improper fertilization. Salinity disrupts several processes: it hinders the transfer of nutrients from the soil to the plant, impedes the release of elements from the solid state into the soil solution, affects the movement of nutrients toward the roots, and interferes with the plant's ability to absorb and utilize these elements in various organs [6, 7]. Saline soils also require more energy from the plant to absorb water and regulate biochemical activities, which ultimately hurts plant growth. Today, emerging technologies such as genetic engineering, along with the use of markers and probes, have had a significant impact on molecular modification and dynamic tracking of plant growth under salt stress conditions. These developments represent a significant step in understanding the processes related to salinity stress and its control [8].

Paclobutrazol, as a plant growth regulator, plays an important role in plant reproductive growth. This substance limits the production of this hormone by inhibiting the biosynthesis pathway of gibberellic acid and is therefore known as a vegetative growth inhibitor [9–11]. In addition, paclobutrazol has a significant effect on reducing environmental stresses in plants, as research has shown that the resistance of plants such as peach, grape, pear, and other species to various environmental stresses increases with the use of this substance [12]. In 2025, Hajihoseinlou et al. reported that paclobutrazol enhances antioxidant activity, especially that of superoxide dismutase, in *Triticum aestivum*. Furthermore, paclobutrazol helps plants cope with salt stress by reducing the absorption and accumulation of chloride and sodium ions in their tissues. Overall, the application of

paclobutrazol minimizes membrane damage, increases cellular water content, improves photosynthesis rates, and elevates the concentrations of essential pigments such as chlorophyll and carotenoids under salt stress conditions [13]. These results led to the selection of paclobutrazol as an effective treatment option in the present study to investigate its effects on the molecular mechanisms of rapeseed in more detail.

In winter rapeseed (*Brassica napus* L.), salt tolerance is achieved through various molecular mechanisms. These include the accumulation of osmolytes such as proline and glycine betaine, the activation of antioxidant enzyme systems like superoxide dismutase, catalase, and peroxidase, and the upregulation of genes involved in ion transport, such as NHX and SOS1. Among these mechanisms, flavonoids, particularly anthocyanins, play a crucial role in stress adaptation as secondary metabolites. Their strong antioxidant activity helps neutralize reactive oxygen species (ROS) generated under salt stress, thereby preventing oxidative damage to cellular macromolecules. Research has shown that salinity conditions lead to increased levels of anthocyanin accumulation in canola leaves and stems, which directly correlates with improved plant tolerance by enhancing the degradation of hydrogen peroxide and protecting cell membranes. Thus, anthocyanins serve not only as pigments but also as signaling and protective molecules within the intricate network of salt stress responses in canola [14–18]. Flavonoids are crucial plant secondary metabolites that significantly contribute to cardiovascular health and cell immunity through their antioxidant, anti-inflammatory, and anticancer properties [19–21]. These compounds are generally synthesized in plants in two main steps, and their synthesis process is influenced by numerous genes and enzymatic factors. The initial step of this pathway begins with the production of precursors such as phenylalanine through the shikimic acid metabolic pathway [22–24]. In the subsequent steps, flavonoid synthesis is carried out through the activity of specific enzymes such as chalcone synthase (CHS). Enzymes such as cinnamate and picumarate also play a vital role in this process [25]. MYB111 and MYB12 genes are among the genes related to flavonoid synthesis, which are involved in flavonoid biosynthesis by binding to the promoter regions of key enzymes such as chalcone synthase (CHS) and flavonol synthase 1 (FLS1). These genes are activated under salt stress conditions and play an effective role in coping with this type of stress. In addition, MYB111 and MYB12 genes, together with other genes such as MYB12, TT4, TT5, and TT6, form a complex that is effective in regulating the synthesis and activation of flavonoids [26, 27]. Given the significant role of these genes in flavonoid synthesis and their function in reducing the effects of salt stress, a detailed examination of these genes has been

considered in the upcoming research. Finally, the aim of this study is to evaluate the effect of paclobutrazol treatment on the expression of MYB111 and MYB12 genes in rapeseed under salt stress conditions.

Materials and methods

Plant materials, growth conditions, and stress

In this study, seeds of the SLM046 cultivar were sourced from the Oilseeds Cultivation Development Company. The seeds were initially sterilized in a 3% sodium hypochlorite solution for one minute. After disinfection, they were rinsed thoroughly with autoclaved distilled water to ensure cleanliness. Subsequently, the seeds were cultured in glass jars and irrigated with autoclaved water. Throughout the growth period, which lasted 25 days, the glass jars were maintained under sterile and controlled conditions, including a temperature of 25 °C, with a light cycle of 16 h of light and 8 h of darkness in the growth room of the Biotechnology Research Institute. Regular irrigation with autoclaved water continued until the plants developed 2 to 3 leaves. Once this growth stage was reached, salinity stress was applied, and the final irrigation utilized a solution containing NaCl at a concentration of 150 mM.

Cultivation conditions

Initially, seeds of the winter canola (*Brassica napus* L.) cultivar SLM046 were immersed in 70% ethanol for one minute. Subsequently, they were rinsed multiple times with autoclaved distilled water. Following this, the seeds were subjected to a 3% sodium hypochlorite solution for one minute and again thoroughly rinsed several times with autoclaved distilled water, thereby completing the seed sterilization protocol. The sterilized seeds were placed on sterile filter paper within glass jars and irrigated with autoclaved water to ensure complete saturation of the filter paper. The jars were maintained under strictly aseptic conditions in a growth chamber at the Biotechnology Research Institute, set at a temperature of 25 °C with a photoperiod of 16 h of light and 8 h of darkness. Regular irrigation with autoclaved water was conducted for a period of 25 days until the plants reached the growth stage of 2 to 3 true leaves, at which point the salinity stress treatment was initiated. This was performed by administering the final irrigation with a 150 mM NaCl solution. All harvested samples were immediately flash-frozen in liquid nitrogen and transferred to an ultra-low temperature freezer at -80 °C for long-term storage.

Experimental and treatment design

Paclobutrazol is a biosynthetic gyberlin regulator that plays a significant role in regulating physiological processes, especially under drought stress conditions. In this

research, 100 ppm of paclobutrazol was first prepared in distilled water, and then, according to the concentration of treatments, an appropriate volume was added to the environment. This study was carried out in a completely randomized design with three replications, and a total of 12 samples were examined. Of these, three samples were considered as controls and nine other samples as treatment groups. The control samples did not receive any paclobutrazol treatment. However, in the group of samples that were considered as treatments, the first three samples were affected by paclobutrazol treatment with a concentration of 10 PPM, the second three samples were treated with a concentration of 20 PPM paclobutrazol, and finally, the remaining three samples were treated with a concentration of 40 PPM paclobutrazol. In this research, the analysis of variance for the data was performed using the SAS software, and mean comparisons were calculated based on the Duncan test at the 5% significance level.

Measurement of physiological parameters

To measure chlorophyll and carotenoids in rapeseed, the first step was to separate the leaves from the other vegetative parts of the plant. Next, 10 g of leaves were thoroughly ground in a porcelain mortar using a mixture of 16 ml of acetone and 4 ml of water. The resulting solution was then centrifuged at 2700 rpm for 10 min. After centrifugation, light absorption was measured at wavelengths of 663, 646, and 470 nm using a spectrophotometer. The amounts of chlorophyll a, chlorophyll b, and carotenoids were calculated using the following formulas [28].

$$\text{Chlorophyll a} = (12.25 \times A_{663} - 2.79 \times A_{646})$$

$$\text{Chlorophyll b} = (21.21 \times A_{646} - 5.1 \times A_{663})$$

$$\text{Carotenoid} = (1000 \times A_{470} - 1/8 \times \text{chl a} - 85.02 \times \text{chl b}) / 198$$

Expression study and real-time PCR analysis

After salt stress, leaves from both paclobutrazol-treated plants and control samples were collected. The samples were rapidly placed in liquid nitrogen and stored in a -80 °C freezer. Next, RNA extraction was conducted using the DENAzist kit, followed by cDNA synthesis with the appropriate Fermentas kit. Four primers were designed by Primer premier software (Table 1). The primers were checked by Primer Blast software. Finally, a real-time PCR reaction was carried out using the KTG Holdings proprietary kit and a Bioer device manufactured in China, employing primers for the Elongation Factor 1 gene as an internal control.

Table 1 Primers designed from MYB111 and MYB12

	Primer	TM	Product Size
MYB111-F	CTCAGCCGCAAAATCTACGC	60°C	198 bp
MYB111-R	GGCCGTCAACATTGCTTGT	60°C	198 bp
MYB12-F	CCGGGAGAACAGACAACGAA	60°C	178 bp
MYB12-R	TTGGGTTTCATGCCGATCT	59.7°C	178 bp

Table 2 Molecular identification and analysis of MYB111 and MYB12 genes

Name	MYB111	MYB12
ORGANISM	<i>Brassica napus</i>	<i>Brassica napus</i>
Accession number nucleotide	XM_013803650.3	XM_013892082.3
Accession number protein	XP_013659104.2	XP_013747536.2
Gene symbol	LOC106364001	LOC106450432
Chromosome	A9	C4
Location	2,656,760..2659586	258,022..261701
GenelD	106,364,001	106,450,432
nucleotide length	1554 bp	1803 bp
protein length	349 aa	390 aa
Molecular weight (Da)	39,383.54	43,618.15
Isoelectric point	5.24	5.27
Exon	3	3
GC	40.7%	43.3%

Molecular and bioinformatics analyses

In this study, we first conducted a detailed analysis of the MYB gene family, which is crucial for flavonoid biosynthesis. We focused specifically on the MYB111 and MYB12 genes due to their effectiveness and common usage in this process. Structural and molecular analyses of these genes were performed using the Expasy database, which included measurements of isoelectric points, calculations of molecular weights, determination of GC percentages, and precise gene localization. The results of these analyses are summarized in Table 2. Next, we examined the three-dimensional structures and stability of the proteins using the Swiss Model database, and we conducted protein network analysis with the String database. Additionally, we performed a phylogenetic analysis of the protein sequences using MEGA5 software. To assess the similarity of these protein sequences with

others, we carried out a protein BLAST analysis using the Circoletto database. Finally, we conducted a hydropathicity analysis using the ProtScale database and examined the secondary structures of the MYB111 and MYB12 proteins using the Proteus database.

Results

Analysis of physiological indices

In this study, we measured the levels of chlorophyll a, chlorophyll b, and carotenoids in both control and treated leaves exposed to a concentration of 40 PPM paclobutrazol, using a spectrophotometer. The results, illustrated in Fig. 1, indicate that the treated plants contain higher levels of chlorophyll and carotenoids compared to the control plants. This significant difference confirms that paclobutrazol treatment positively enhances the physiological indices of the plants. Additionally, we found that chlorophyll and carotenoid levels decreased under salt stress conditions compared to the control. Concurrently, physiological analyses revealed significant increases in malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), and electrolyte leakage (EL) under salt stress compared to the control. These analyses were conducted at the stages of stem formation, flowering, and grain filling, as shown in Fig. 2.

Determination of MDA content

The generation of reactive oxygen species (ROS) under salinity stress induces lipid peroxidation of the cell membrane, leading to the formation of intermediate compounds such as short-chain hydrocarbons, ketones, and malondialdehyde (MDA), with MDA being the most significant. These intermediates can react with thiobarbituric acid (TBA) to produce a chromogenic compound, collectively termed thiobarbituric acid reactive substances (TBARS), which serve as an estimate of the extent of membrane lipid peroxidation. Membrane lipid peroxidation represents the most deleterious impact of oxygen free radicals on plants under stress conditions, ultimately culminating in cell death. Quantifying the degree of membrane lipid peroxidation alone can be

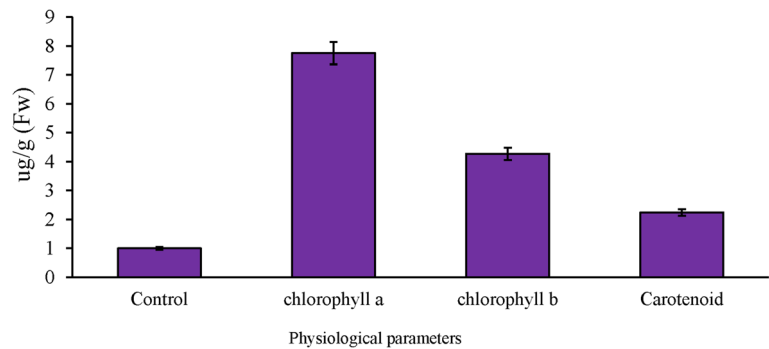


Fig. 1 Analysis of physiological indices in *Brassica napus* leaves under the influence of paclobutrazol treatment

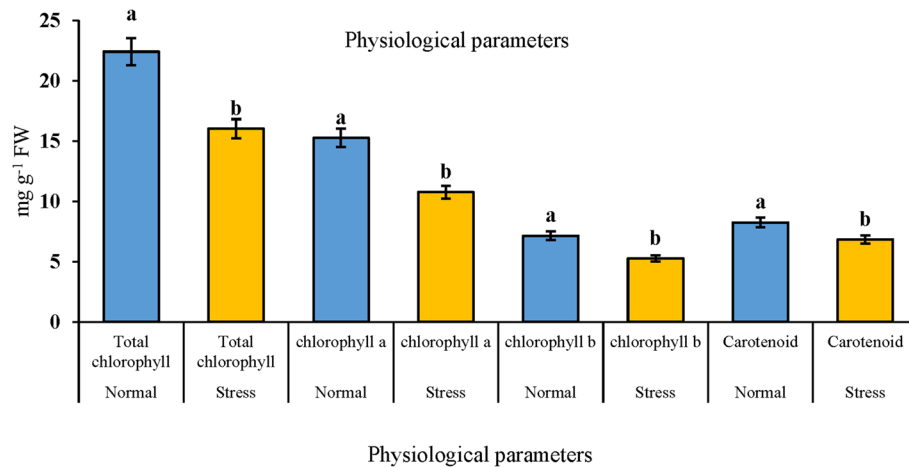


Fig. 2 Determination of some physiological parameters

employed as a reliable indicator of cellular damage. The extent of membrane lipid peroxidation reflects the severity of stress imposed on the plant, and malondialdehyde concentration can be utilized as a biochemical index to assess membrane stability and the magnitude of membrane injury (Fig. 3A).

Determination of SOD content

Superoxide dismutase (SOD) is one of the most powerful intracellular antioxidants and is often considered the strongest known antioxidant. It protects many plants from oxidative damage caused by oxygen free radicals and enhances their resilience to various environmental stressors. Researchers have identified multiple isozymic forms of this enzyme in plants, which are categorized into three groups based on their metallic cofactors: Mn-SOD is found in mitochondria and peroxisomes, Cu/Zn-SOD is located in chloroplasts and the cytosol, and Fe-SOD is present in chloroplasts. SOD catalyzes the dismutation of two superoxide molecules through a concerted reaction involving protonation, converting them into two molecules of hydrogen peroxide. This hydrogen peroxide is then scavenged by other antioxidant systems. Additionally, by keeping superoxide concentrations low, SOD helps minimize the formation of hydroxyl radicals. This study revealed that salinity stress in rapeseed significantly increases superoxide dismutase activity, highlighting the critical role of this enzyme in detoxifying oxygen free radicals (Fig. 3B).

Determination of POD content

Another critical antioxidant is peroxidase (POD), which exists in diverse isoforms and is responsible for scavenging hydrogen peroxide from biological systems. Owing to its capacity to oxidize guaiacol, it is also termed guaiacol peroxidase (GPOX). Peroxidases are recognized for their

distinct physiological roles compared to other antioxidants (Fig. 3C).

Relative electrolyte leakage (EL)

This index was measured according to the Sairam method. According to this method, 0.1 g of leaf is placed in 10 ml of double distilled water. After that, the sample is placed in 40° C water for 30 min and its electrical conductivity (EC) is read with the help of an EC meter. (C1) Then, the sample is placed in a bain-marie bath at 100° C for 15 min and its electrical conductivity is read for the second time (C2). And the desired indices are calculated based on the following relations (Fig. 4).

$$EL = C_1/C_2$$

Analysis of MYB111 and MYB12 gene expression profiles

In this study, rapeseed cultivar SLM046, which is relatively tolerant to salinity, was used. In this research, we tried to investigate the effects of paclobutrazol treatment on the changes in the expression profile of MYB111 and MYB12 genes under salt stress. For this reason, RNA extraction and cDNA synthesis were done from the stressed and control (no paclobutrazol treatment) samples, and then the samples were placed in the real-time PCR machine to check the actual expression of the genes under salt stress. The results showed that the paclobutrazol treatment increased the expression of MYB111 and MYB12 genes compared to the control (no paclobutrazol treatment) state. It was also found that with increasing treatment concentration, the expression level of these genes also increased. Comparison of the expression profiles showed that MYB12 gene expression under the influence of paclobutrazol is at a higher level than MYB111. Changes in the expression levels of MYB111 and MYB12

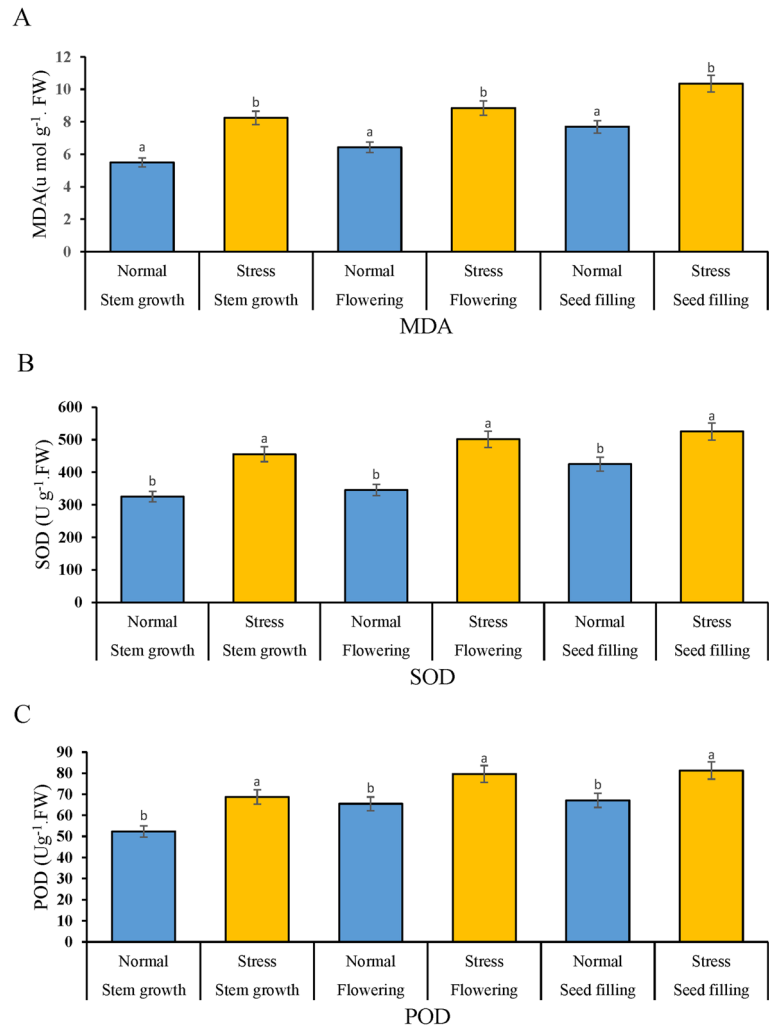


Fig. 3 Determination of (A) MDA, (B) SOD, and (C) POD content

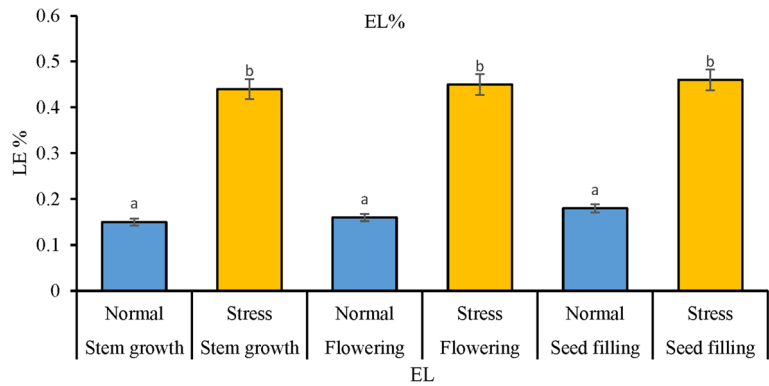


Fig. 4 Determination of EL percent

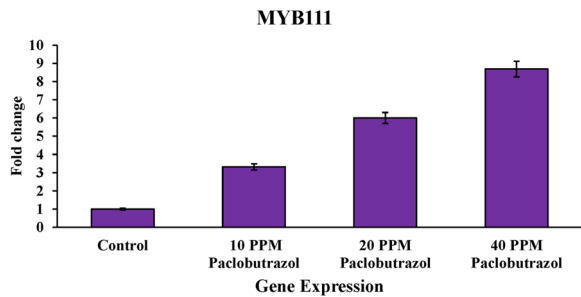


Fig. 5 Analysis of MYB111 gene expression under the influence of different concentrations of paclobutrazol treatment

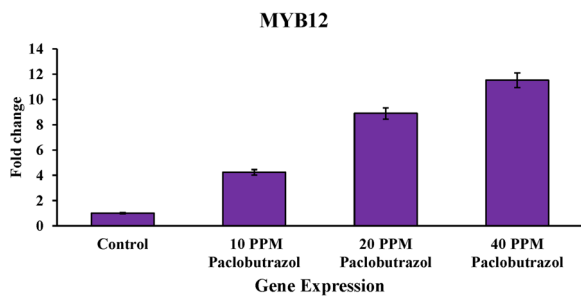


Fig. 6 Analysis of MYB12 gene expression under the influence of different concentrations of paclobutrazol treatment

genes are clearly shown in Figs. 5 and 6 under the influence of different treatment concentrations.

Network analysis and determination of the three-dimensional structure and stability of MYB111 and MYB12 proteins

Network analysis of the MYB111 and MYB12 genes using the STRING database has shown that these genes interact with a wide network of genes and transcription factors. This communication network generally leads to the production of antioxidants and increased plant resistance to stresses such as salinity and drought. Figure 7 depicts the network structure of the MYB111 and MYB12 genes. The three-dimensional structure and stability of the MYB111 and MYB12 proteins were also examined using the Swiss Model database. The results of the three-dimensional structure analysis of the MYB111 protein in rapeseed showed that this structure is 81.63% similar to that of its similar protein in *Arabidopsis thaliana*. In addition, the three-dimensional structure of the MYB12 protein in rapeseed also showed 79.11% similarity to the MYB12 protein in *Arabidopsis thaliana* (Fig. 8). Also, the Ramachandran plot was calculated for the MYB111 and MYB12 proteins with respect to the ϕ and ψ angles. This analysis showed that the stability and energy levels of these proteins are 85.04% and 73.45%, respectively. Based

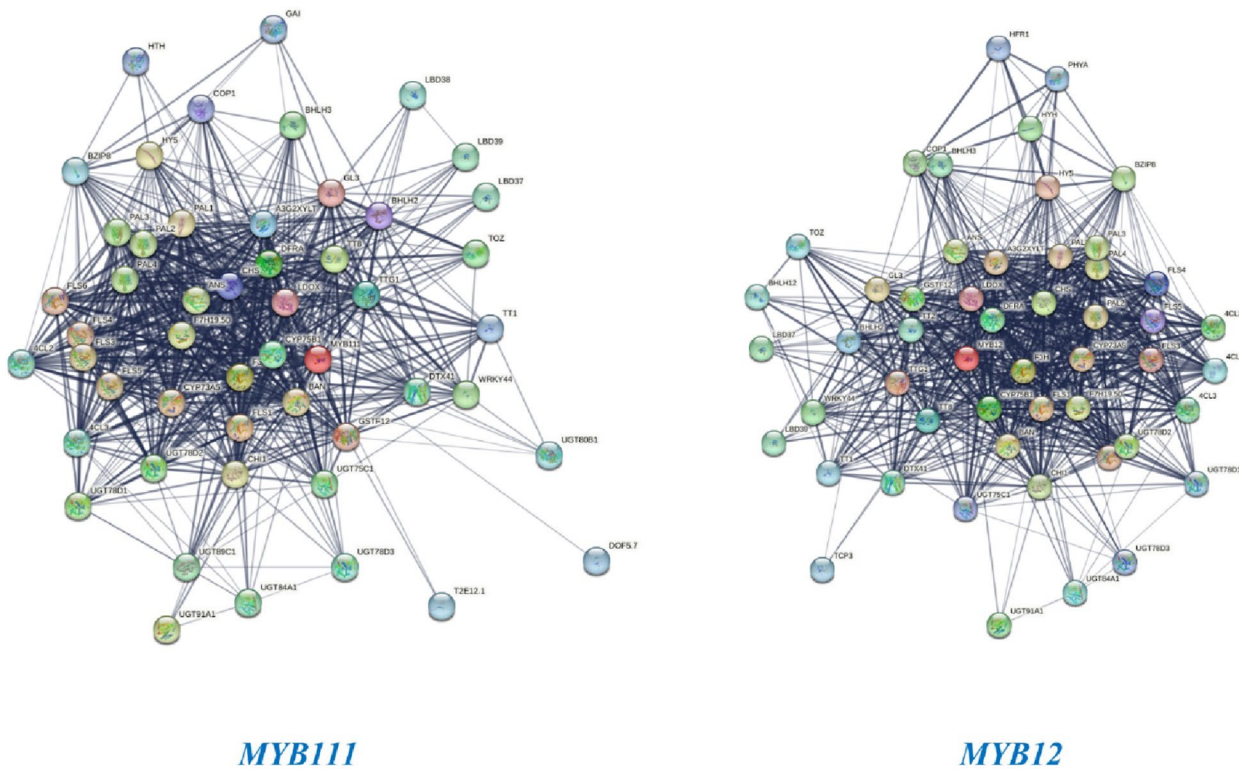


Fig. 7 Results from network analysis of MYB111 and MYB12 genes. Red circles indicate the target genes MYB111 and MYB12, while other circles represent 50 genes related to these genes

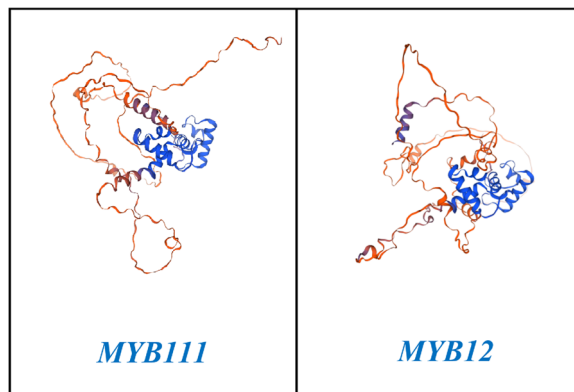


Fig. 8 Three-dimensional structure of MYB111 and MYB12 proteins

on these values, it can be concluded that both proteins are at an acceptable level of stability, but the MYB111 protein is more stable than the MYB12 protein. Figure 9 shows the Ramachandran plot in terms of the ϕ and ψ angles in the MYB111 and MYB12 proteins.

BLAST analysis and phylogenetic tree study of MYB111 and MYB12 proteins

BLAST analysis, both at the nucleotide and protein levels, is widely used for identifying and comparing sequences across different species. Such studies can lead to the discovery of previously unknown genes and proteins in various organisms. In this study, the Circoletto database was used to compare protein sequences. First, the protein sequences of *Brassica carinata* and *Eruca vesicaria* species that had more than 50% similarity to the MYB111 protein sequence in *Brassica napus* were extracted from the NCBI database. Then, BLAST analysis was performed using the Circoletto database on the protein sequences. The results of BLAST analysis showed that a total of 24 protein bands were formed. These bands

showed a minimum similarity of 53.85% and a maximum similarity of 88.05%. Also, for the MYB12 protein sequence, proteins with more than 50% similarity to *Brassica napus* were extracted from *Arabidopsis thaliana* and *Brassica carinata* species, which ultimately led to the formation of 24 bands with a minimum similarity of 32.91% and a maximum similarity of 77.08%. Figures 10 and 11 present the results of the BLAST analysis for the MYB111 and MYB12 protein sequences, respectively. Phylogenetic tree analysis is a valuable tool in bioinformatics that classifies sequences based on their similarity, enabling researchers to analyze them more effectively. The phylogenetic tree analyses for the MYB111 and MYB12 protein sequences are illustrated in Figs. 12 and 13, respectively.

Secondary structure and hydropathicity analysis of the MYB111 and MYB12 proteins

The analysis of protein secondary structure provides valuable insights into the spatial arrangement and folding of peptides in specific regions. In this study, we determined the secondary structure of the MYB111 and MYB12 proteins using the PROTEUS2 database. The results of this analysis are presented in Table 2. Additionally, hydropathicity analysis revealed that both MYB111 and MYB12 proteins exhibit a negative average hydropathicity, classifying them as hydrophilic proteins. Figures 14 and 15 display the hydropathicity analysis results for MYB111 and MYB12 proteins, respectively (Table 3).

Discussion

In recent years, researchers have conducted extensive studies to understand the mechanisms of resistance to abiotic stresses, particularly salt stress. A clear understanding of these mechanisms can enhance plant resilience to saline conditions. Additionally, the use of

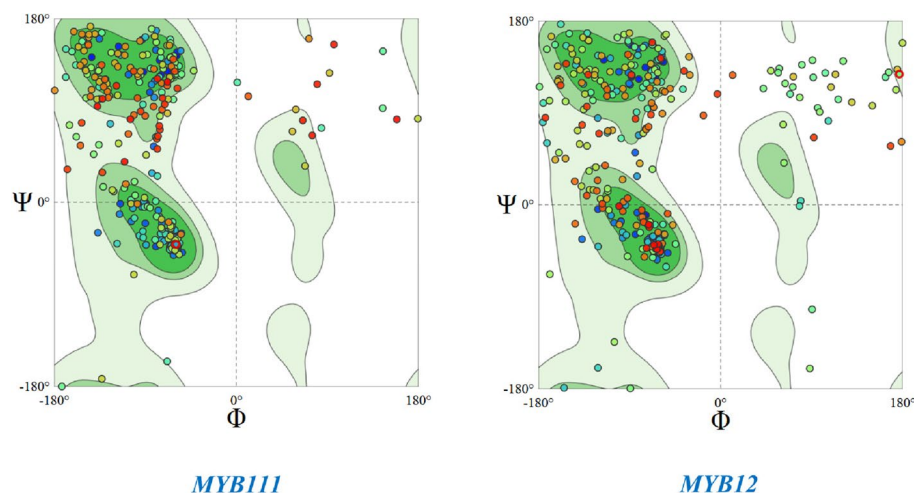


Fig. 9 Ramachandran plot of MYB111 and MYB12 proteins

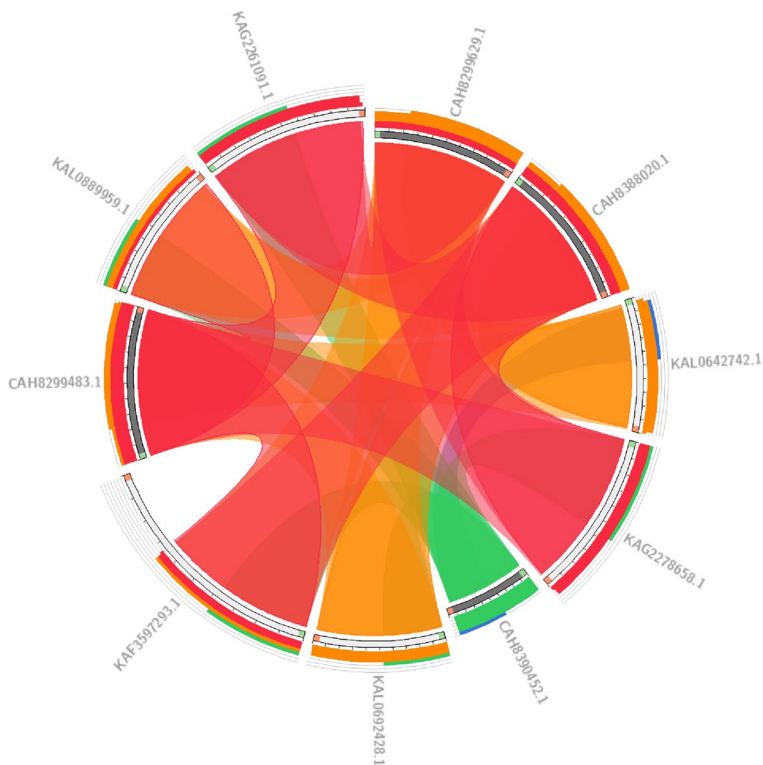


Fig. 10 Blast analysis of MYB111 and MYB12-related proteins in *Brassica carinata* and *Eruca vesicaria*. Blue bands indicate less than 25% similarity, green bands indicate less than 50% similarity, orange bands indicate less than 75% similarity, and red bands indicate more than 75% similarity

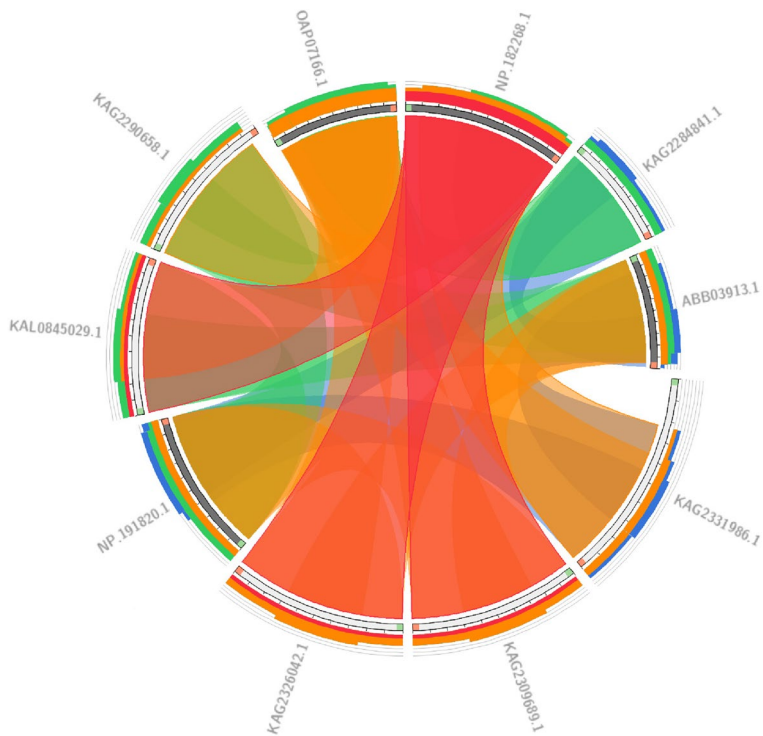


Fig. 11 Blast analysis of MYB111 and MYB12-related proteins in *Arabidopsis thaliana* and *Brassica carinata*. Blue bands indicate less than 25% similarity, green bands indicate less than 50% similarity, orange bands indicate less than 75% similarity, and red bands indicate more than 75% similarity

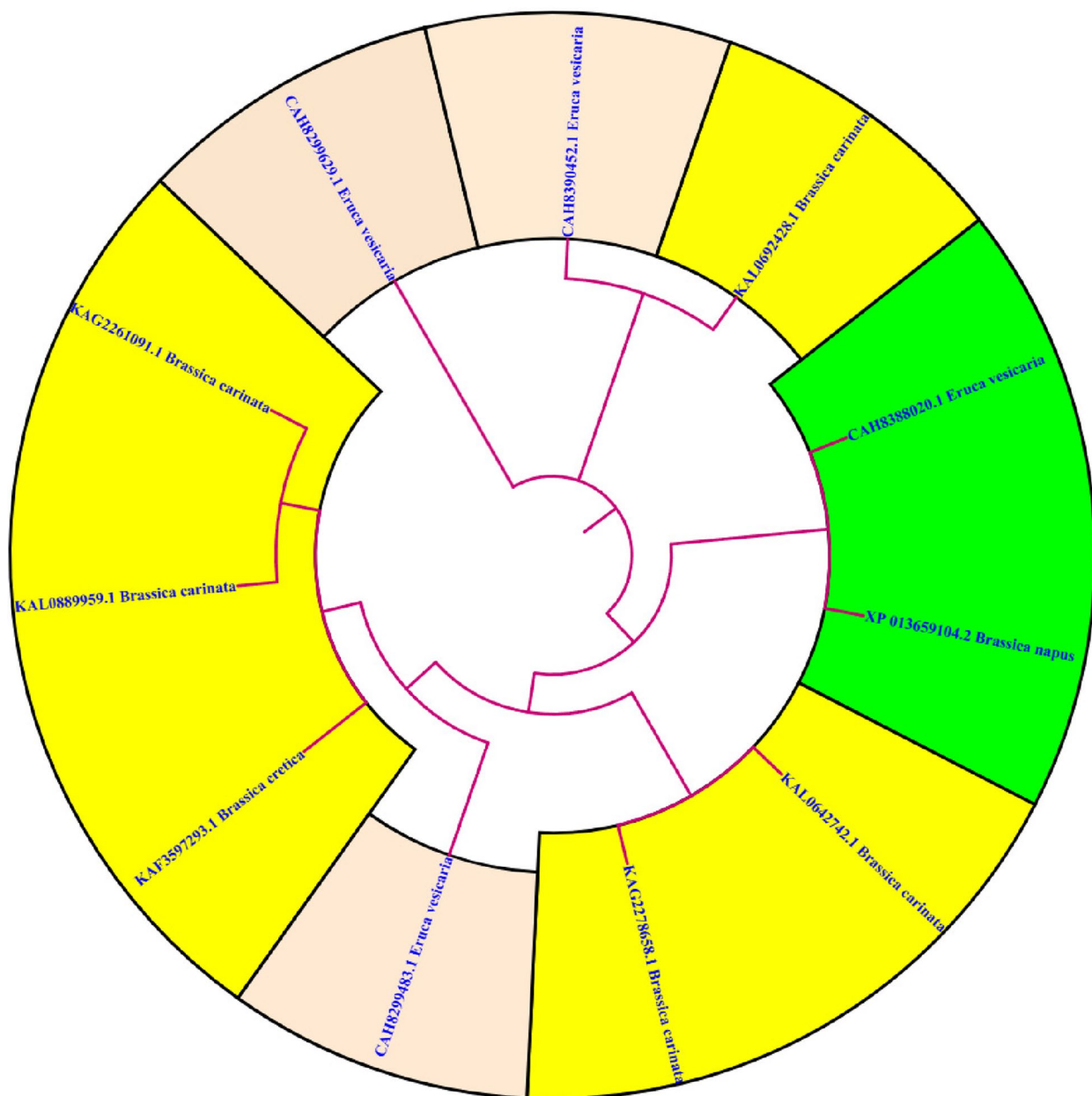


Fig. 12 Phylogenetic tree analysis of MYB111-related proteins in *Brassica carinata* and *Eruca vesicaria*. As can be seen, the sequence CAH8388020.1 (*Eruca vesicaria*) has the highest similarity to the (XP_013659104.2) protein

compounds and regulatory factors can effectively mitigate the harmful effects of salinity. While research in biology and genetic engineering has shown that sensitivity to salt stress occurs upstream of certain genes, with flavonoid production occurring downstream, the complex interactions between these factors remain unclear. Therefore, this study aimed to investigate the changes in antioxidants and the genes involved in this process by utilizing paclobutrazol treatment, a significant growth regulator. Our findings demonstrate that paclobutrazol treatment interacts with transcription

factors related to salt and drought stress, leading to changes in intracellular hormone levels [29, 30].

The results of this study demonstrated that paclobutrazol significantly increases the mineral content of rapeseed. Additionally, the gene expression analysis revealed that a concentration of 40 ppm of paclobutrazol enhances the expression of genes associated with flavonoid production. These findings suggest that paclobutrazol acts as a stimulant for flavonoid production, thereby increasing secondary metabolites in rapeseed. This aligns with the findings of Zhang et

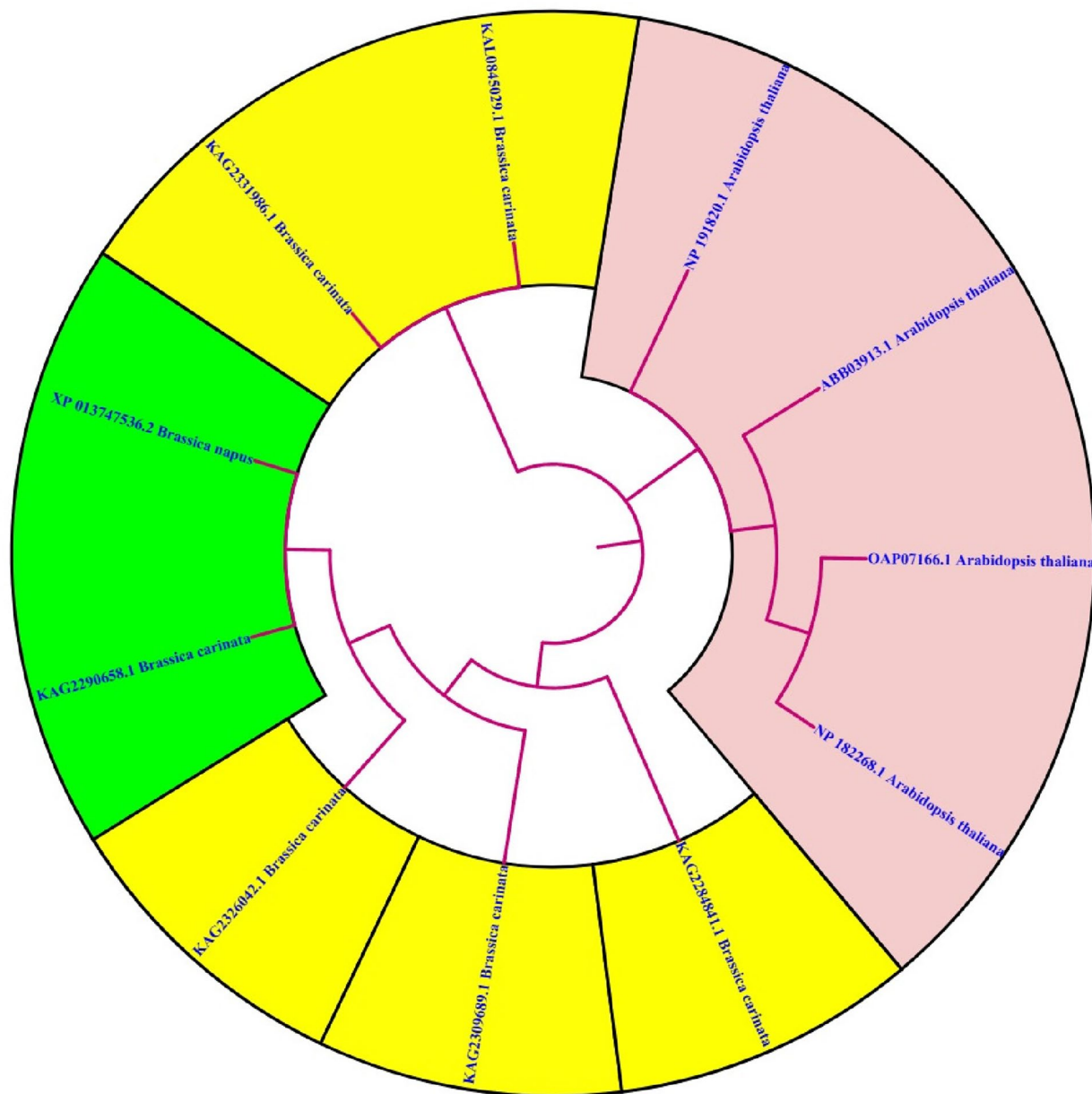


Fig. 13 Phylogenetic tree analysis of MYB12-related proteins in *Arabidopsis thaliana* and *Brassica carinata*. As can be seen, the sequence KAG2290658.1 (*Brassica carinata*) has the highest similarity to the (XP_013747536.2) protein

al. in 2025, who also reported that paclobutrazol stimulates antioxidant biosynthesis and increases levels of flavonoids, anthocyanins, and terpenoids [31].

While paclobutrazol (PBZ) is effective for controlling vegetative growth in fruit trees, it carries significant agronomic risks. Long-term use or improper dosing of this gibberellin biosynthesis inhibitor can result in excessive dwarfing, reduced leaf area, and diminished photosynthetic capacity. Additionally, since gibberellins play a crucial role in inducing flowering in certain species, inappropriate PBZ application may delay the transition to the reproductive stage, disrupt flower bud

formation, and lead to uneven crop maturity. Other risks include disturbances to the plant's hormonal balance and the buildup of persistent residues in the soil, which can negatively impact subsequent crop cycles. To optimize PBZ use and mitigate side effects, it is essential to determine the correct dose based on factors such as plant species, tree age, and environmental conditions. A key strategy is to target applications at specific growth stages—such as early in the growing season to manage vegetative growth—while avoiding use during sensitive periods, like near flowering. The choice of application method (soil or foliar) should

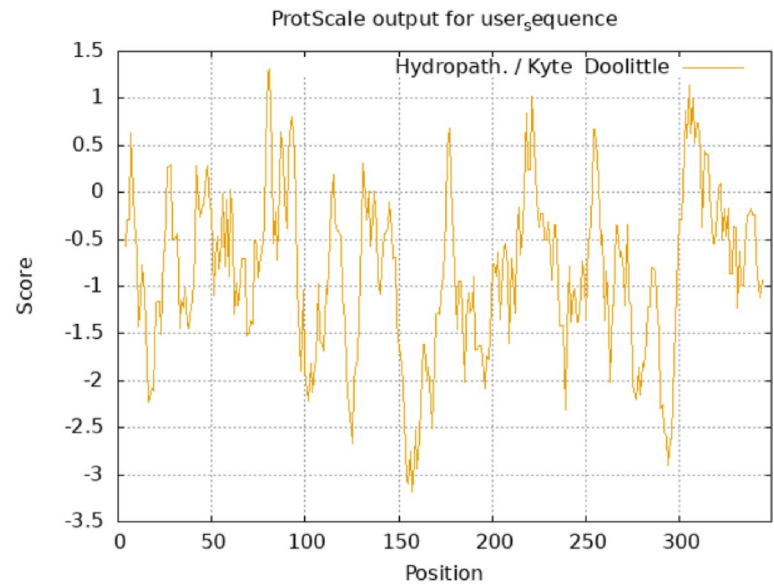


Fig. 14 Hydropathicity diagram of MYB111 protein. The average, minimum, and maximum hydropathicity of MYB111 protein are equal to AV = −0.915772201, MIN = −3.178, and MAX = 1.311

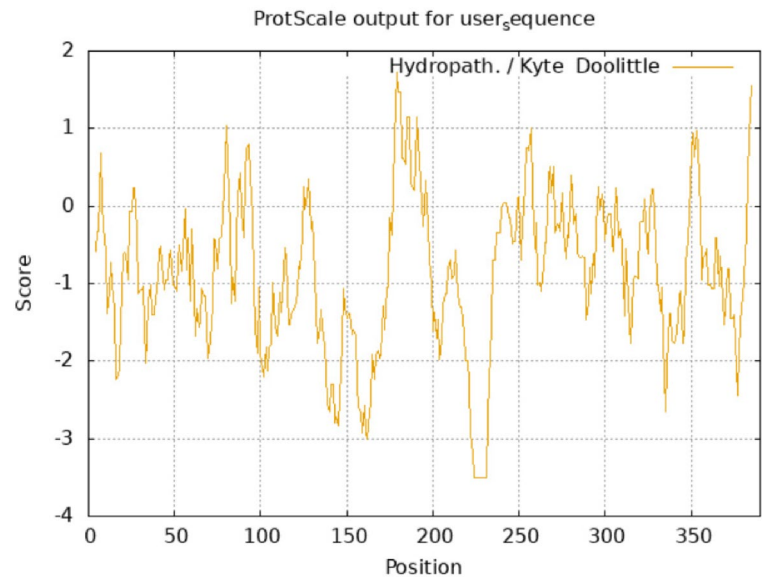


Fig. 15 Hydropathicity diagram of MYB12 protein. The average, minimum, and maximum hydropathicity of MYB12 protein are equal to AV = −0.885126667, MIN = −3.500, and MAX = 1.722

Table 3 Results of the secondary structure analysis for the proteins MYB111 and MYB12

Secondary structure analysis	MYB111	MYB12
Number of sequence alignments used for ab-initio predictions	49	49
Overall confidence value	74.8%	73.0%
Helix content	20%	24%
Beta sheet content	0%	2%
Coil content	80%	74%
Signal peptide content	0%	0%
membrane content	0%	0%

also align with the desired duration of effect and manageability. Overall, integrating PBZ with other growth management practices, such as pruning and regular soil monitoring to prevent residue buildup, is crucial for a balanced and sustainable management program [32–34].

Paclobutrazol activates the expression of the evolutionarily conserved transcription factors MYB111/12 by modulating intracellular hormonal balance. These transcription factors, in turn, increase flavonoid biosynthesis by binding to the promoters of key

constitutive genes (e.g. CHS, F3H, FLS). This integrated interpretation provides a strong bridge between bioinformatics analyses and the physiological consequences of PBZ treatment [35–39]. To complete this picture, future studies could focus on directly verifying the binding of MYB111/12 proteins to the promoters of target genes under PBZ treatment conditions, as well as directly measuring hormone levels.

Studies show that PBZ acts as a potent antioxidant and a metabolic regulator and uses several mechanisms to cope with salinity. Among others, this compound reduces the level of destructive reactive oxygen species (ROS) by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), preventing oxidative damage to membranes and macromolecules. PBZ also increases the accumulation of compatible osmolytes such as proline and glycine betaine, which contribute to the maintenance of cell osmotic potential and the integrity of protein structures. On the other hand, reports suggest that PBZ may selectively inhibit the uptake of sodium ions (Na^+) and, in turn, facilitate the uptake of potassium ions (K^+), leading to an improvement in the vital K^+/Na^+ ratio in plant tissues. These physiological and biochemical changes are ultimately evident in the form of significant improvements in growth indices such as plant height, leaf area, chlorophyll content, and dry weight in salt-stressed plants treated with PBZ (especially at optimal concentrations such as 100 mM) compared to stressed control plants. These findings strongly support the aforementioned proposition that PBZ plays a mitigating role on the negative effects of salt stress [40–43].

Bioinformatics analyses, particularly the evaluation of the MYB111 and MYB12 genes, revealed significant interactions with transcription factors and regulatory elements related to reactive oxygen species (ROS). These findings suggest that increased expression of these genes enhances the activity of ROS scavengers. As a result, elevated flavonoid production in plants not only boosts antioxidant activity but also reduces the damaging effects of ROS. In a related study, Li et al. (2019) demonstrated that the upregulation of MYB111 in *Arabidopsis* lowers ROS levels and improves the plant's tolerance to salinity. Their findings further confirm the crucial role of MYB111 in enhancing the plant's ability to withstand salt stress [44]. Bioinformatics analyses of this study also showed that flavonoid-synthesizing genes have negative hydrophobicity. This means that these genes are associated with transcription factors that increase drought stress resistance. As a result, it can be said that MYB111 and MYB12 genes, in addition to providing resistance to salinity, also increase drought stress resistance. As the

findings of Qi et al. in 2025 showed, the *Astragalus membranaceus* plant becomes more resistant to salinity and drought stress by increasing flavonoid production [45].

It has been investigated the accumulation of flavonoids and the reduction of reactive oxygen species production through MYB transcription factors in the *Schima superba* family, and their results indicated an increase in drought stress resistance in this family [46]. The results of this study, particularly the protein blast and phylogenetic analyses, indicate that the MYB111 and MYB12 genes are linked to R2R3-MYB transcriptional activators in both protoplasts and the nucleus. These activators promote genes associated with abscisic acid, enhancing plant resistance to abiotic stresses. Therefore, it can be concluded that flavonoid synthesis is crucial for improving plant resilience against such stresses.

Since previous studies have well proven the role of MYB111 and MYB12 genes in improving salt stress. In this study, when paclobutrazol treatment was used, the expression of these genes increased significantly. As a result, by combining the findings of this study and previous studies, it is concluded that paclobutrazol treatment has a positive effect on increasing salt resistance [36, 44, 47].

Conclusion

Salinity, as a factor that leads to excessive accumulation of salts and destruction of soil structure, has significant negative effects on oilseed production. In rapeseed, this environmental stress directly disrupts the germination process and shoot growth, and also reduces the production of flavonoids and anthocyanins. This study showed that the use of paclobutrazol treatment can improve cellular activities under environmental stresses. In addition, the expression of flavonoid synthesis-related genes production is also significantly increased under the influence of paclobutrazol. These mechanisms can significantly reduce the negative effects of salt stress. Overall, the results of this study indicate that the use of paclobutrazol in oilseeds can reduce the accumulation of sodium ions in the plant structure.

Ethical issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Authors' contributions

Conceptualization: A. A. Data curation: A. A. Formal analysis: A. A. Investigation: B. F-N. Project administration: A. A. Resources: B. F-N. Supervision: J. F. Validation: B. F-N. Visualization: B. F-N. Writing—original draft: A. A. Writing—reviewing & editing: All authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

All the data are embedded in the manuscript. The datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

No human or animals were used in the present research. The authors declare not used any patients in this research.

Consent for publication

All authors read and approved the final manuscript for publication.

Competing interests

The authors declare no competing interests.

Received: 8 February 2026 / Accepted: 7 April 2026

Published online: 20 May 2026

References

1. Bayoumi Y, et al. Growth, biochemical, physiological, yield and quality traits responses of ten tomato varieties to soil salinity stress. *Egypt J Soil Sci.* 2025;65:387–400.
2. Song Y, et al. Reducing the sodium adsorption ratio promotes cotton growth and development by enhancing antioxidant enzyme activities and the plant's potassium-sodium ratio under brackish-water irrigation. *Agronomy.* 2025;15:2092.
3. Osman KT. In *Management of Soil Problems* (ed Khan Towhid Osman) 255–298 (Springer International Publishing). 2018.
4. Chhabra R. In *Salt-affected Soils and Marginal Waters: Global Perspectives and Sustainable Management* (ed Ranbir Chhabra) 1–47 (Springer International Publishing). 2021.
5. Canavar Ö, et al. Physiological responses of sunflower (*Helianthus annuus* L.) to multiple combined prolonged drought stress, salinity stress and boron toxicity: insights from pre-and post-recovery stages. *J Agro Crop Sci.* 2025;211:e70047.
6. Bidalia A, Vikram K, Yamal G, Rao KS. In *Salt Stress, Microbes, and Plant Interactions: Causes and Solution: Volume 1* (ed Mohd Sayeed Akhtar) 273–297 (Springer Singapore). 2019.
7. Patel D et al. In *Climate Change and Soil Microorganisms for Environmental Sustainability* (eds Meenu Saraf, Dweepayan Goswami, & Dinesh Kumar Maheshwari) 265–292 (Springer Nature Singapore). 2025.
8. Ma X, et al. Visualizing plant salt stress with a NaCl-responsive fluorescent probe. *Nat Protoc.* 2025;20:902–33.
9. Jabir O, et al. Effects of gibberellin and gibberellin biosynthesis inhibitor (Paclobutrazol) applications on radish (*Raphanussativus*) taproot expansion and the presence of authentic hormones. *Int J Agric Biol.* 2017;19:1814–9596.
10. Aziz RB, et al. Characterization of main responsive genes reveals their regulatory network activated by multi-biological metabolic pathways in paclobutrazol (PAC)-modulated grape seed development (GSD) at the stone-hardening stage. *Int J Mol Sci.* 2025;26:1102.
11. Fazeli-Nasab B, Mirzaei N. Evaluation of total phenol and flavonoid content in a wide variety of native and imported plants. *Sci J Ilam Univ Med Sci.* 2018;26:141–54.
12. Selim C, Durak A. Use of paclobutrazol and trinexpac-ethyl on growth, development, and flowering characteristics of safflower as an ornamental plant. *BioResources.* 2025;20:6436–56.
13. Hajihoseinlou S, Rezayian M, Niknam V, Esfandiari E. Exogenous γ -aminobutyric acid and penconazole mitigated salt stress-induced impairments in *Triticumaestivum* plants. *Cereal Res Commun.* 2025;53:1413–25.
14. Dabravolski SA, Isayenkov SV. The role of anthocyanins in plant tolerance to drought and salt stresses. *Plants.* 2023;12:2558.
15. El-Badri AM, et al. Antioxidative and metabolic contribution to salinity stress responses in two rapeseed cultivars during the early seedling stage. *Antioxidants.* 2021;10:1227.
16. Ahammed GJ, Yang Y. Anthocyanin-mediated arsenic tolerance in plants. *Environ Pollut.* 2022;292:118475.
17. Ahmadzai AS, Hu C, Zhang C, Li Y. Mechanisms of anthocyanin-mediated salt stress alleviation and cellular homeostasis in plants. *Plant Growth Regul.* 2025;105:655–73.
18. Ahmad P, et al. Upregulation of antioxidant and glyoxalase systems mitigates NaCl stress in *Brassica juncea* by supplementation of zinc and calcium. *Journal of Plant Interactions.* 2018;13:151–62.
19. Bhatla SC, Lal MA. In *Plant Physiology, Development and Metabolism* (eds Satish C. Bhatla & Manju A. Lal) 765–808 (Springer Nature Singapore). 2023.
20. Roy A, et al. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *Biomed Res Int.* 2022;2022:5445291.
21. Fazeli-Nasab B. The effect of explant, BAP and 2,4-D on callus induction of *trachyspermumammi*. *Potravinarstvo Slovak Journal of Food Sciences.* 2018;12:578–86.
22. Paul A, Acharya K, Chakraborty N. In *Biology and biotechnology of environmental stress tolerance in plants* 27–66 (Apple Academic Press). 2023.
23. Yu W, Cai M, You C, Wei W, Liu H. Metabolomic and transcriptomic analyses unveil the accumulation of shikimic acid in the leaves of *Ginkgo biloba*. *Front Plant Sci.* 2025;16:1631197.
24. Marchiosi R, et al. Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochem Rev.* 2020;19:865–906.
25. Nunes AR, et al. Use of flavonoids and cinnamates, the main photoprotectors with natural origin. *Adv Pharmacol Pharmaceut Sci.* 2018;2018:5341487.
26. Li S. Transcriptional control of flavonoid biosynthesis: fine-tuning of the MYB-bHLH-WD40 (MBW) complex. *Plant Signal Behav.* 2014;9:e27522.
27. Wang XC, et al. Arabidopsis MYB4 plays dual roles in flavonoid biosynthesis. *Plant J.* 2020;101:637–52.
28. Lichtenthaler HK, Wellburn AR. (Portland Press Ltd., 1983).
29. Monje-Rueda MD, Pal'ove-Balang P, Márquez AJ, Betti M, García-Calderón M. Differential involvement of MYB13 and MYB15 transcription factors in isoflavonoid biosynthesis and tolerance to salt stress in *Lotus japonicus*. *Plant Stress.* 2025;17:100913.
30. Ni X, et al. The transcription factor GmbZIP131 enhances soybean salt tolerance by regulating flavonoid biosynthesis. *Plant Physiol.* 2025;197:kiaf092.
31. Zhang D-X, et al. Paclobutrazol induces triterpenoid biosynthesis via down-regulation of the negative transcriptional regulator SIMYB in *Sanguangporuslonicericola*. *Commun Biol.* 2025;8:551.
32. Desta B, Amare G. Paclobutrazol as a plant growth regulator. *Chem Biol Technol Agri.* 2021;8:1–15.
33. Orozco-Meléndez LR, et al. Paclobutrazol and its use in fruit production: a review. *Phyton.* 2022;91:1–12.
34. Liu W, et al. Optimizing paclobutrazol application for regulating dwarfing in *Ougan* (*Citrus reticulata* cv. Suavissima): comprehensive insights from growth, photosynthesis, and physiological responses. *Plants.* 2025;14:763.
35. Shi Y, et al. *Rosa rugosa* R2R3-MYB transcription factors RrMYB12 and RrMYB111 regulate the accumulation of flavonols and anthocyanins. *Front Plant Sci.* 2024;15:1477278.
36. Schilbert HM, Glover BJ. Analysis of flavonol regulator evolution in the Brassicaceae reveals *MYB12*, *MYB111* and *MYB21* duplications and *MYB11* and *MYB24* gene loss. *BMC Genomics.* 2022;23:604.
37. Pandey A, Misra P, Bhambhani S, Bhatia C, Trivedi PK. Expression of Arabidopsis MYB transcription factor, *AtMYB111*, in tobacco requires light to modulate flavonol content. *Sci Rep.* 2014;4:5018.
38. Ma Z, Hu L, Zhong Y. Structure, evolution, and roles of MYB transcription factors proteins in secondary metabolite biosynthetic pathways and abiotic stresses responses in plants: a comprehensive review. *Front Plant Sci.* 2025;16:1626844.
39. Dai G, et al. Molecular evolution analysis of MYB5 in Brassicaceae with specific focus on seed coat color of *Brassica napus*. *BMC Plant Biol.* 2024;24:52.
40. Ahmad I, Basra SMA, Wahid A. Exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide improves the productivity of hybrid maize at low temperature stress. *Int J Agric Biol.* 2014;16:825–30.
41. Naser Alavi SM, Arvin MJ, Manoochehri Kalantari K. Salicylic acid and nitric oxide alleviate osmotic stress in wheat (*Triticum aestivum* L.) seedlings. *J Plant Interact.* 2014;9:683–8.
42. Khatir K, Shekhawat G. Nitric oxide mitigates salt-induced oxidative stress in *Brassica Juncea* seedlings by regulating ROS metabolism and antioxidant defense system. *Biotech.* 2020;10:499.

43. Manaf HH. Beneficial effects of exogenous selenium, glycine betaine and seaweed extract on salt stressed cowpea plant. *Annals of Agricultural Sciences*. 2016;61:41–8.
44. Li B, et al. The Arabidopsis MYB transcription factor, MYB111 modulates salt responses by regulating flavonoid biosynthesis. *Environ Exp Bot*. 2019;166:103807.
45. Qi L, et al. The *AmMYB35-AmFLS* module mediates the accumulation of flavonol induced by drought stress in *Astragalusmembranaceus*. *Food Biosci*. 2025;68:106541.
46. Zhang G, Yu Z, Yao B, Teixeira da Silva JA, Wen D. SsMYB113, a *Schima superba* MYB transcription factor, regulates the accumulation of flavonoids and functions in drought stress tolerance by modulating ROS generation. *Plant and Soil*. 2022;478:427–44.
47. Lotkowska ME, et al. The Arabidopsis transcription factor MYB112 promotes anthocyanin formation during salinity and under high light stress. *Plant Physiol*. 2015;169:1862–80.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.