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RESEARCH

Drought and Salt Stressors Alter *NAC* and *WRKY* Gene Expression Profiles in Grafted *Citrullus lanatus*

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Abstract

Increasing drought and soil salinity pose significant threats to crop production around the world. One potential strategy to mitigate the impacts of these environmental changes is grafting, a horticultural technique that joins tissues from different plants. This study aimed to investigate and model changes in the expression of *NAC* and *WRKY* genes in grafted watermelon under varying salt and drought stress conditions (mild to extrem). The control groups were not restricted by any limitations. *Citrullus lanatus* (Thunb.) Matsum. & Nakai (watermelon) was used as the scion, and *Lagenaria siceraria* (Molina) Standl (bottle gourd) served as the rootstock in our experiments. During the 14-day treatment period, individuals from the grafted watermelon and bottle gourd plants were more successful to maintained water balance and growth rates, while ungrafted plants exhibited growth retardation and tissue damage, especially under salt stress. The analysis of gene expression revealed that grafted plants showed significantly increased expression of salt- and drought-sensitive genes, including *ClNAC2b*, *ClNAC69*, *ClNAC72*, *ClWRKY13*, *ClWRKY14*, and *ClWRKY23*, compared to ungrafted plants under low-stress conditions. These adaptations, such as stomatal closure and regulation of evaporation, enabled the improved grafted plants to respond more effectively to abiotic stress, supporting their survival and normal development. Our findings underscore that grafting is an environmentally friendly, rapid, and effective technique to enhance plant resilience against abiotic stresses, offering promising avenues to improve stable food crop production amidst increasing environmental challenges.

Keywords Drought · Graft · *NAC* · Salt · Watermelon · *WRKY*

Rita Márkus and Szilvia Czigle contributed equally to this study and share first authorship.

We dedicate our article to the memory of our tutor, Professor György Borbély, DSc. Academician, the Nestor of plant stress biology. Who passed away on April 23, 2024. Requiescat in pace.

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Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a popular crop grown worldwide, with a total area of 3.48 million hectares, making it among the five most consumed fresh fruits (FAO 2024). The edible fruits from the family Cucurbitaceae are rich in minerals (calcium, iron), nutrients (protein), flavonoids, and vitamins and thus provides numerous health benefits to the human body (Sharif et al. 2022). The limited availability of suitable growing areas, along with the demand for vegetable crops throughout the year, has led to the cultivation of crops under unfavorable conditions, such as extreme temperatures, drought, high soil salinity, and various diseases (Lee and Oda 2003). Against these issues, grafting presents an effective and eco-friendly solution, widely used in global crop production (Davis et al. 2008; Colla et al. 2010; Márkus et al. 2024). Grafting is a horticultural technique used in agriculture for woody plants for thousands of years, and it has gained ground in recent decades in herbaceous plants (Lee, 1994; Goldschmidt, 2014). The relationship between the rootstock and the scion goes beyond the sharing of nutrients and water; many molecules travel between the plant parts, thereby helping the growth regulation and adaptation of the graft (Liu et al. 2016). The role of auxin in regulating different organs (root, hypocotyl, shoot, leaf, tendril, flower, and fruit) and response to environmental (biotic and abiotic) stresses, as such drought, salinity, heavy metals, heat, cold, waterlogging, cold. Some auxins reversed the harmful effects of salinity stress, regulate the silicon-mediated salinity resistance. Auxin participation in regulating plants of the family Cucurbitaceae response to multiple stresses summarized Sharif et al. (2022).

The *NAC* [The family name of the NAC gene was derived from the names of three transcription factors: NAM (no apical meristem, Petunia, ATAF1–2 (*Arabidopsis thaliana* activating factor) and CUC2 (*Arabidopsis* cup-shaped cotyledon), which share the same DNA-binding domain)] and *WRKY* (transcription factor genes) gene families play an important role in the regulation of transcriptional reprogramming associated with plant stress responses (Yang et al. 2018; Nan et al. 2021). The *NAC* transcription factors stimulate successful adaptation to stresses by both activating and repressing the effects of transcription factors on specific target genes. Lv et al. (2016) identified 80 *ClNAC* (NAC gene identified in *Citrullus lanatus*) genes in watermelon and predicted their induced patterns and functions through genome-wide analysis.

WRKY family genes have been identified in numerous plant species, including watermelon, in the last decade. The *WRKY* gene family is among the largest families of transcription factors (TFs) in higher plants (Wei et al. 2012). Various research studies have demonstrated the significant biological functions of *WRKY* genes in plant responses to various biotic and abiotic stresses such as pathogens, cold, salt, and drought (Phukan et al. 2016; Jiang et al. 2017). Yang et al. (2018) identified a total of 63 *ClWRKY* (*WRKY* gene identified in *Citrullus lanatus)* genes in watermelon. The expression of *ClWRKY* genes in response to drought and salt stress suggests that they can affect the plant's tolerance to various abiotic stresses.

The role of the *NAC* and *WRKY* genes in the stress response of grafted plants has not been investigated before. In this research, we conducted a gradient experiment to compare the presence and function of selected *NAC* and *WRKY* genes in ungrafted, grafted watermelon, and bottle gourd plants. In addition to monitoring treatment responses, we compare the stress responses of grafted watermelons with those of ungrafted watermelons and bottle gourds.

The selected genes needed to be stably expressed in the examined plants and tissues, that their function was known, and that they played a role in protecting plants against stress, especially against salt and drought stress (Lv et al. 2016; Yang et al. 2018). Also, adequate background information about them was needed in the literature (Wu et al. 2009; Phukan et al. 2016).

The aim of our research was (1) to model the different degrees of drought and salt stress for plant groups and (2) to investigate the gene expression changes of selected members of the *NAC* and *WRKY* gene families between plant groups and treatment groups, focussing on ungrafted and grafted watermelon plants. We used *Citrullus lanatus*—watermelon as scion, and *Lagenaria siceraria* (Molina) Standl—bottle gourd as the rootstock.

Materials and Methods

Plant Material and Culture Conditions

Citrulus lanatus (Thunb.) Matsum. & Nakai (cv. *Lady*)—watermelon as the scion, and *Lagenaria siceraria* (Molina) Standl (cv. *Argentario*)—bottle gourd as rootstock. The seeds of *C. lanatus* and *L. siceraria* were planted in a commercial peat pot (6 cm × 6 cm × 6 cm seedlings) (Kekkilä BVB, Vantaa, Finland). At 3 weeks old the plants were grafted and were then kept in a healing chamber for another 2 weeks, under a controlled light conditions (in the first 48 h the healing chamber was covered with black plastic), at an average temperature of 28 °C ± 2 °C, with a relative humidity 90–95%. Each plant was occasionally watered with 100 mL of water. YaraLive Calcinit fertilizer was added to the irrigation water in greenhouse life stage. Except during sowing, when we watered all plants 100 mL supplemented with

Ferticare Starter 15–30 - 15 fertilizer, each time according to the distribution company's recipe. Seed sowing, seedling cultivation, and grafting began in April, and we started the treatment in June of the same year, which lasted 14 days. The voucher specimens of the seeds labelled with a unique code were deposited at the Institute of Biology, Faculty of Sciences, University of Pécs, Hungary (Voucher code: CL, LS).

The Applied Grafting Method and Fieldwork

We used the one-cotyledon grafting method (Hassell and Memmot 2008). After grafting, the plants were placed in a healing chamber, after 3 days, the chamber ventilation started.

After 4 to 8 days, we increased ventilation for longer and longer periods and slowly increased the number of illuminated hours. After 9 days, the plants were placed under a 13/11 day/night period. When the plants reached the stage of three to five leaves, they were removed from the healing chamber and transplanted into plastic pots filled with a mix of growing medium (Kekkilä peat) and arid sand. The plants were occasionally watered with 100 mL of water. At approximately 6 weeks old, the plants were placed in a greenhouse of the Botanical Garden of the University of Pécs (GPS 46°04′35.5″N 18°12′24.9″E). The plants for the treatment groups were chosen by random selection. After entering the greenhouse, the plants were given a 3-day adaptation period before starting the 14-day treatment phase.

We conducted a gradient experiment on watermelon and bottle gourd plants to model water deficit and salt stress. We established five salt-treated groups (25, 50, 100, 200, and 300 mmol/L NaCl) and three control groups, which were supplemented with nutrients and water according to the previous system. Each group consisted of five plants, totaling 75 plants (25 ungrafted watermelons, 25 grafted watermelons, 25 rootstocks) treated, with 15 plants (five ungrafted watermelons, five grafted watermelons, five rootstocks) included in the control group (Table 1).

For drought treatment, we created four groups to stimulate different levels of water shortage (40%, 50%, 60%, 80%). Each group contained five plants totaling 60 plants (20 ungrafted watermelons, 20 grafted watermelons, 20 rootstocks) and 15 control plants (five ungrafted watermelons, five grafted watermelons, five rootstocks) that participated in the experiment. They were further irrigated with water and nutrients (Table 2). The plants in each treatment group were watered on the first day of treatment and then weighed. The initial weight was considered their 100% weight for further comparisons. Plants were given water when their weight dropped below a certain percentage (Table 2). The chosen plants were of the same age and grown under the same conditions. We selected healthy plants with similar development. We monitor each plant affected by drought stress individually and provide them with water replacement as needed. The chosen plants were of the same age and grown under the same conditions. We selected healthy plants with similar development. The greenhouse experiment took place from June 10, 2020, to June 24, 2020.

Table 1 Salt treatment and plant groups

Abbreviation of plant groups	Description of treatment
sn25	Salt, ungrafted, 25 mmol/L
sn50	Salt, ungrafted, 50 mmol/L
sn100	Salt, ungrafted, 100 mmol/L
sn200	Salt, ungrafted, 200 mmol/L
sn300	Salt, ungrafted, 300 mmol/L
sg25	Salt, grafted, 25 mmol/L
sg50	Salt, grafted, 50 mmol/L
sg100	Salt, grafted, 100 mmol/L
sg200	Salt, grafted, 200 mmol/L
sg300	Salt, grafted, 300 mmol/L
sa25	Salt, rootstock (bottle gourd), 25 mmol/L
sa50	Salt, rootstock (bottle gourd), 50 mmol/L
sa100	Salt, rootstock (bottle gourd), 100 mmol/L
sa200	Salt, rootstock (bottle gourd), 200 mmol/L
sa300	Salt, rootstock (bottle gourd), 300 mmol/L

Table 2 Drought treatment and plant groups

Abbreviation of plant groups	Description of treatment
dn80	Drought, ungrafted, 80% mass retention
dn60	Drought, ungrafted, 60% mass retention
dn50	Drought, ungrafted, 50% mass retention
dn40	Drought, ungrafted, 40% mass retention
dg80	Drought, grafted, 80% mass retention
dg60	Drought, grafted, 60% mass retention
dg50	Drought, grafted, 50% mass retention
dg40	Drought, grafted, 40% mass retention
da80	Drought, rootstock (bottle gourd), 80% mass retention
da60	Drought, rootstock (bottle gourd), 60% mass retention
da50	Drought, rootstock (bottle gourd), 50% mass retention
da40	Drought, rootstock (bottle gourd), 40% mass retention

Quantitative RT-PCR Analysis

RNA isolation, cDNA synthesis from reserves, and quantitative RT-PCR (quantitative real-time polymerase chain reaction) analysis were performed according to the manufacturer's instructions. To carry out the tests, we collected leaf tissue samples. Total RNA was purified using the Direct-zol™ RNA MiniPrep Plus kit (Direct-zol™, Zymo Research Corporation, Irvine, CA, USA) following the manufacturer's protocol. Purified RNA samples were subjected to cDNA synthesis using the SuperScript™ IV Reverse Transcriptase (Invitrogen™, Thermo Fisher Scientific Inc., Waltham, MA, USA) system with random hexamers.

Quantitative Real-Time PCR analysis of cDNA samples was performed on the Biorad CFX Opus 96 Real-Time PCR System (Bio-Rad Laboratories, Vienna, Austria) using GoTaqR qPCR Master Mix (Promega Corporation, Madison, WIS, USA) with the following cycling conditions: polymerase activation at 95 °C for 2 min, then 45 cycles of denaturation at 95 °C for 3 s, annealing and denaturation at 60 °C for 30 s, used plate: MultiplateR PCR PlatesTM 96-well, clear (Bio-Rad Laboratories, Dubai, United Arab Emirates). Directly after PCR amplification, the amplicons were subjected to melting curve analyses in which samples were ramped from 65 °C, increasing the temperature of 0.5 °C increments up to 95 °C with fluorescence acquisition after 5 s incremental holding periods. After data acquisition, the melting curves of the amplicons were visually inspected in the CFX Maestro 2.3 software (Bio-Rad Laboratories, Dubai, United Arab Emirates). The *Taq* gene (*Thermus aquaticus* DNS polymerase gene) was used as an endogenous control. To identify the orthologous gene pairs of the studied genes, we used the following: PlantTFDB (https://planttfdb.gao-lab.org). The primers used are presented in Table 3.

Statistical Analysis

We processed the results using the GraphPad Prism program. The normality of the residuals was confirmed using the Kolmogorov–Szmirnov test, For the group comparison analysis, a two-way ANOVA model was applied. The group comparison analysis is based on the means and ± standard deviations of three independent determinations ($n = 3$); data in the same column with different superscripted symbols (*, **, ***) indicate significant differences between the grafted and ungrafted plant materials: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$***.

Results

We examined the function of six genes in a total of 12 treatment groups, along with control groups (Tables 2 and 3). During our research, we concentrated on the molecular changes that occur under the influence of salt and drought stress (Yetisir and Uygur 2010; Singh et al. 2020).

Drought Stress

During the 14-day treatment, we observed the development rate of the plants, particularly in cases of extreme water deficit, comparing the grafted and ungrafted watermelon groups. The impact of water withdrawal had a minimal effect on the bottle gourd and the growth of the grafted watermelon. In the case of grafted watermelons, we observed a partial growth slowdown only in the case of the strongest drought. The ungrafted plants showed a visible lag (lower plant height, thinner stems, fewer leaves) compared to the control group and the grafted group, in severe water shortage already halfway through the treatment (Fig. 1).

During mild (80% weight) drought treatment, the gene expression of the ungrafted and grafted plant groups showed a significant difference in the case of *ClNAC* genes (*ClNAC2b*, *ClNAC69*, *ClNAC72*) (Fig. 2). The largest difference in the genes expression of the studied between the two groups was observed in this lowest treatment group (Fig. 2).

Increased water deficit resulted in a significant difference in the expression of the tested *ClNAC* genes between grafted and ungrafted plant groups in the treatment groups (Figs. 3, 4, and 5). Compared to the control grafted watermelon group and treated grafted watermelon group *ClNAC* genes responses were significantly increased (Fig. 6).

During mild (80%) drought treatment the gene expression of the tested *ClWRKY* genes showed no significant difference between the grafted and ungrafted groups (Fig. 2).

Table 3 Used primers

Gene	Forward primer	Reverse primer
GAPDH	CTGGCAGTACTTTGCCAACA	TGACAGCTGTACCTGGCATC
ClCAC	AATTGTGGTTGATGCTGCAC	AGGATTGGAGAGGAGGTCGT
ClWRKY13	TTGAATCTCGGGTTGTTG	GACTTGAGCATCTGTAGTAAC
ClWRKY14	TTCACCAACGCTCAACTC	GCTTGGCAATCGGAGTC
ClWRKY23	AGAAGTAGAAGTAGAAGAATCATC	GAAATTAGCACCATGAATATCTTT
ClNAC2b	AGAAGCCGAAGATTTTCAC	GTGTAATCGCGGTATTGAA
ClNAC72	TCCACTAATTTCGACTGGG	AACCGTTGAAATCCACTC
ClNAC69	GCTGGGCTCTACTCAGT	GAAGGAACGTAGAGATCG



Fig. 1 Strong (50% weight retention) plants exposed to drought stress (bottle gourd, grafted watermelon, ungrafted watermelon). In the last phase of the 14-day experiment. The development lag is visible between the grafted and ungrafted plants

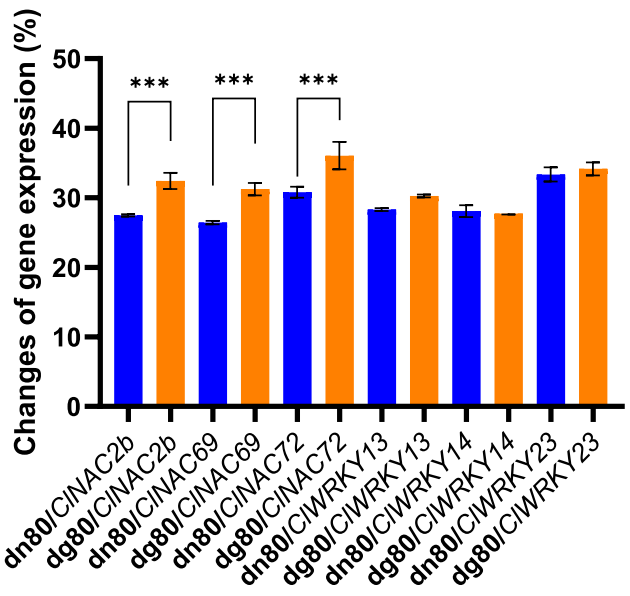


Fig. 2 Changes in gene expression in different plant types resulting from dn80/dg80 (drought treatment; ungrafted, 80% weight/drought treatment, grafted, 80% weight). There was a significant difference in the expression of three genes (*ClNAC2b*, *ClNAC69*, *ClNAC72*) between the grafted and ungrafted groups. Data are means ± SD of three independent determinations ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

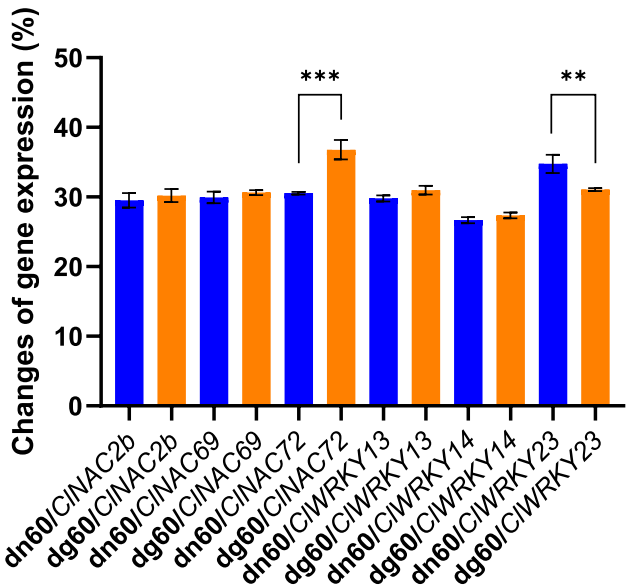


Fig. 3 Changes in gene expression in plant types resulting from dn60/dg60 (drought treatment, ungrafted, 60% weight/drought, grafted, 60% weight) treatment. There was a significant difference in the expression of two genes (*ClNAC72*, *ClWRKY23*) between the grafted and ungrafted groups. Data are means ± SD of three independent determinations ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

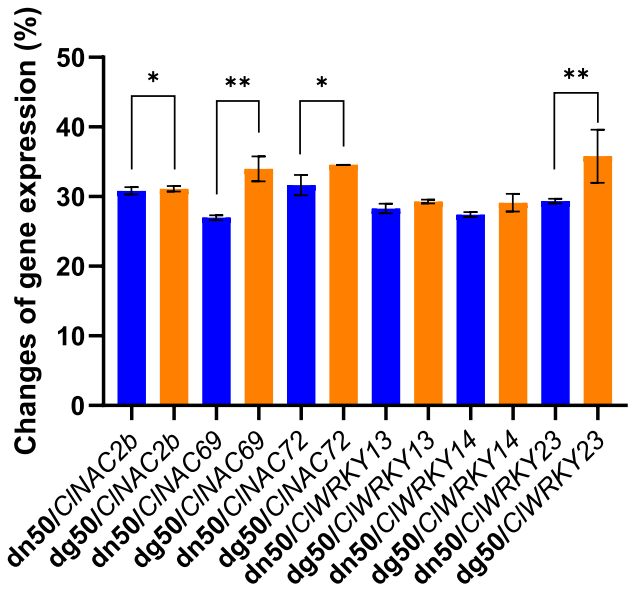


Fig. 4 Changes in gene expression in plant types resulting from dn50/dg50 (drought treatment, ungrafted, 50% weight/drought, grafted, 50% weight) treatment. There was a significant difference in the expression of two genes (*ClNAC69*, *ClWRKY23*) between the grafted and ungrafted groups. Data are means ± SD of three independent determinations ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

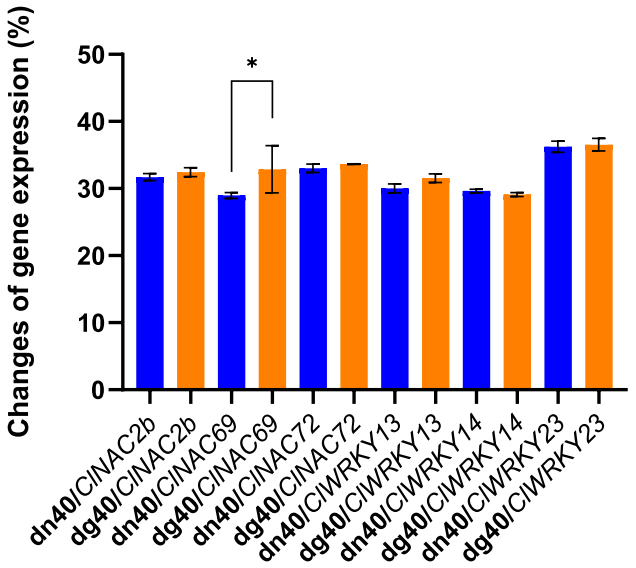


Fig. 5 Changes in gene expression in plant types resulting from dn40/dg40 (drought treatment, ungrafted, 40% weight/drought, grafted, 40% weight) treatment. There was a significant difference in the expression of one gene (*ClNAC72*) between the grafted and ungrafted groups. Data are means ± SD of three independent determinations ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Among the examined *WRKY* genes, only *ClWRKY23* showed a significant difference between the ungrafted and grafted groups due to drought treatment (at 60% and 50% treatment groups) (Figs. 3 and 4). Compared to the control grafted watermelon group and treated grafted watermelon group most *ClWRKY* gene responses were significantly increased (Fig. 6). Compared to the control ungrafted group and the treated ungrafted watermelon group *ClWRKY* showed a significant increase (Fig. 7).

Salt Stress

During 14-day treatment, the plants treated with 25 mmol/L NaCl solution in the three groups showed a normal rate of development compared to the control groups. As stress increased, we observed a delay in development in ungrafted watermelon plants (fewer new leaves, color changes, and even necrosis). This change was noticeable at the grafted watermelon in a later stage of treatment, and even later in a bottle ground (Fig. 8).

As a result of salt treatment, when comparing the grafted and ungrafted plant groups, the gene expression of the grafted watermelon was higher for all tested genes. Genes with significant differences were found in each treatment group. The grafted plant showed a stronger response in their gene expression to the two lowest salt treatments than the ungrafted plants (25, 50 mmol/L) (Fig. 9). In the case of the lowest salt treatment (25 mM), only three genes in the grafted-ungrafted plant groups showed significant differences (*ClNAC69*, *ClNAC72*, *ClWRKY13*). As the concentration of treatment increased, the expression of the genes increased in both groups of plants. However, there was a significantly greater increase in gene expression in the grafted watermelon compared to the ungrafted watermelon. Therefore, a significant difference was found in four of the six tested genes (*ClNAC69*, *ClNAC72*, *ClWRKY13*, *ClWRKY23*) (Fig. 9).

As a result of salt treatment of 200 mmol/L, the gene expression of the ungrafted plants decreased drastically for all genes tested genes (Fig. 10). In the 200 mmol/L NaCl treatment, there was a significant difference in the expression of the six genes (*ClNAC2b*, *ClNAC69*, *ClNAC72*, *ClWRKY13*, *ClWRKY14*, *ClWRKY23*) between the grafted and ungrafted groups. In the 300 mmol/L NaCl treatment, there was a significant difference in the expression of five of the six genes (*ClNAC2b*, *ClNAC69*, *ClNAC72*, *ClWRKY14*, *ClWRKY23*) between the grafted and ungrafted groups (Fig. 10).

The influence of the different treatments on the expression patterns of the examined genes reveals a distinct trend. As a result of the three lowest treatment concentrations, the expression of most genes shows an increase in the examined groups. The plants sensed the presence of the stressor and reacted to it. As stress increases to 200 and 300 mM NaCl, the expression pattern of the genes in ungrafted plants shows a drastic decrease (Fig. 11). In grafted watermelon plants, the expression of genes is much more uniform (Fig. 12).

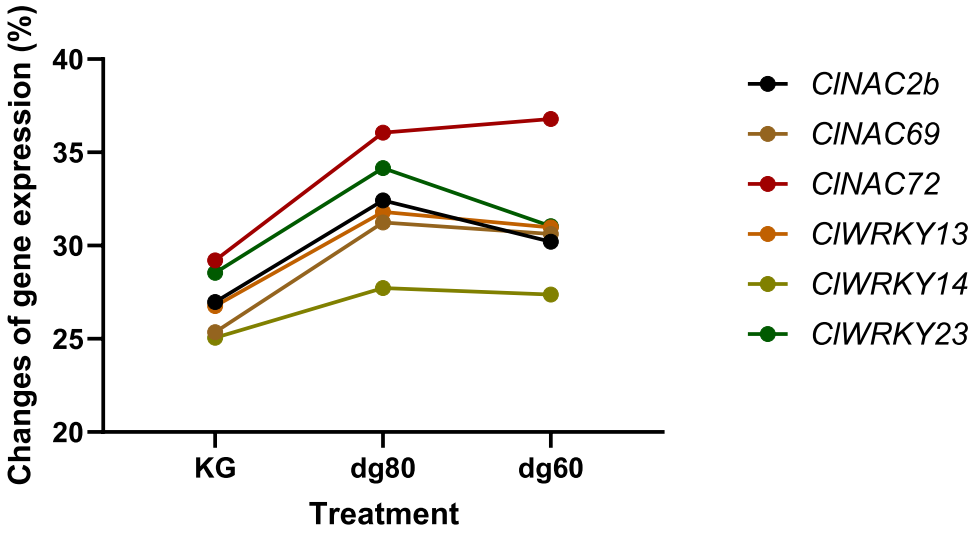


Fig. 6 The gene expression changes of grafted watermelon plants are KG (control group grafted), dg80 (drought treatment; grafted watermelon, 80% mass), and dg60 (drought treatment; grafted watermelon, 60% mass). Compared to the treatment group, the expression of the studied genes was significantly increased even with mild water deprivation

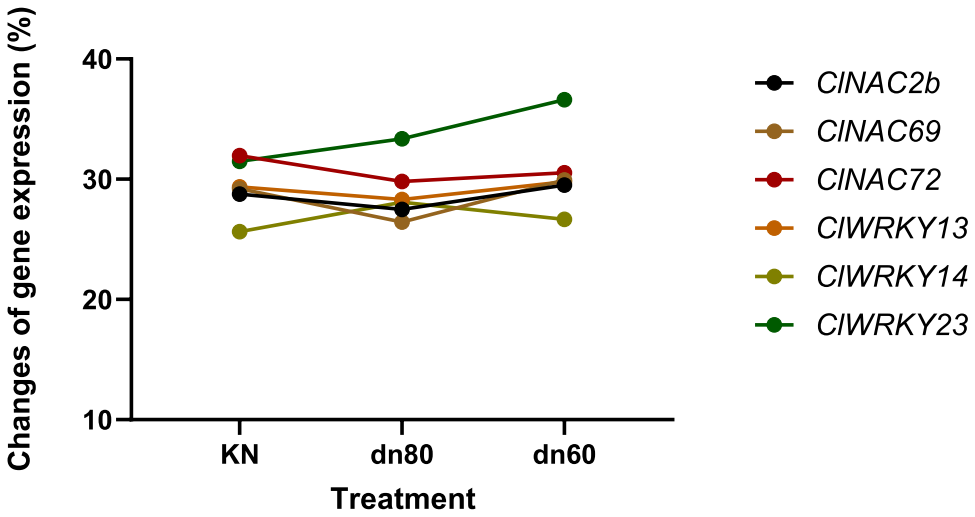


Fig. 7 The gene expression changes of ungrafted watermelon plants are KN (control group ungrafted), dn80 (drought treatment; ungrafted watermelon, 80% weight), and dn60 (drought treatment; ungrafted watermelon, 60% weight). Compared to the treatment group, the expression of the most tested genes showed a significant increase in the medium (60% weight retention) treatment group

Discussion

Drought Stress

Climate change has a profound impact on agricultural productivity, with droughts becoming more frequent and severe (Simelton et al., 2009). Watermelon, being a water-demanding crop, is particularly vulnerable to drought (Dietz et al. 2021). Certain environmental stresses, including drought and salinity and various biotic factors, significantly impair cucurbits growth by disrupting its physiological processes (Sharif et al. 2021; Zhang et al. 2022). Grafting offers an effective method to improve drought resistance in plants, though its molecular mechanisms remain largely unexplored.

Our study simulated drought conditions by creating four treatment groups with varying water retention levels (80%, 60%, 50%, and 40%). The grafted watermelon plants demonstrated a stronger response to low-intensity drought stress, particularly in genes such as *ClNAC69* and *ClNAC72*, which known for their sensitivity to water deficits. In the 80% weak treatment, three genes (*ClNAC2b*, *ClNAC69*, *ClNAC72*) showed a significant difference between the grafted and ungrafted groups.

In the study by Lv et al. (2016), the *ClNAC69* and *ClNAC72* genes in watermelon responded rapidly and positively to water deficit, aligning with our findings. These genes are orthologous to the *ANAC19*, *ANAC55*, and *ANAC72* genes in *Arabidopsis thaliana*, which are closely linked to stress responses triggered by drought, salinity, and plant hormones such as abscisic acid (ABA) and jasmonic acid (JA). Specifically, *ANAC72* and *ANAC19* positively regulate ABA signaling pathways (Fujita et al. 2004; Jensen et al. 2010), while *ANAC19* and *ANAC55* activate JA-mediated defense responses (Bu et al. 2008). Similarly,

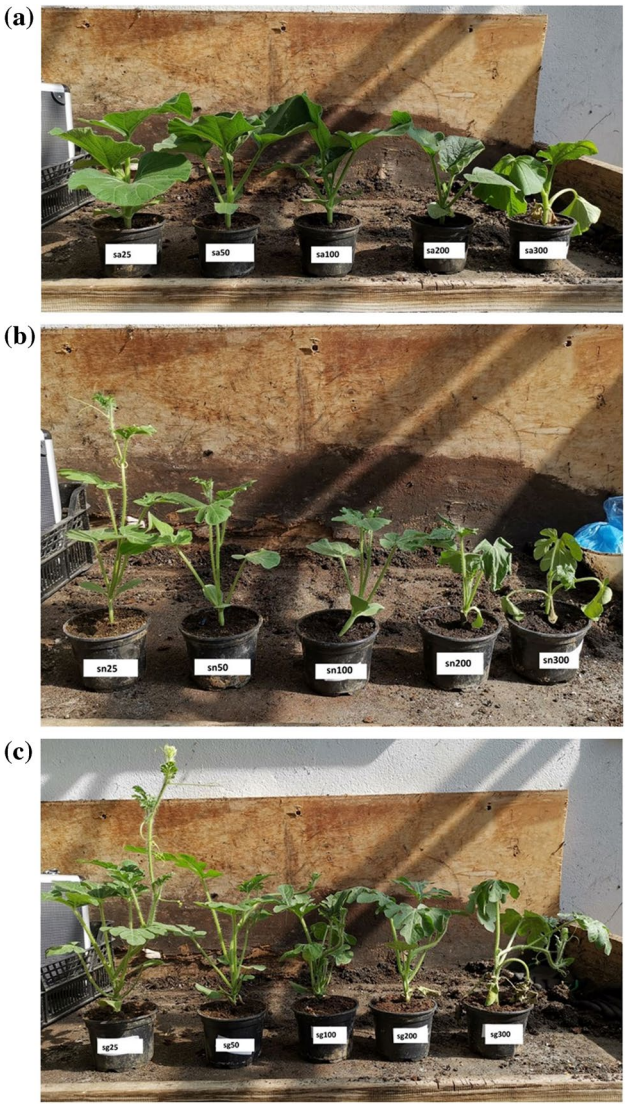


Fig. 8 Individuals of the different plant groups (bottle gourd (sa), grafted watermelon (sg), ungrafted (sn) watermelon), under different treatments (25 mmol/L, 50 mmol/L, 100 mmol/L, 200 mmol/L, 300 mmol/L NaCl). On the last day of treatment. In the case of bottle gourd, the least difference in the development of the groups can be perceived; in the case of the grafted plants, a decrease in size and a slight yellowing of the leaves can be observed as a result of the two strongest salt treatments (200 mmol/L and 300 mmol/L NaCl). In the case of ungrafted plants, the growth of individuals was significantly reduced, their stems became thinner, and their leaves were uneven or started to turn yellow

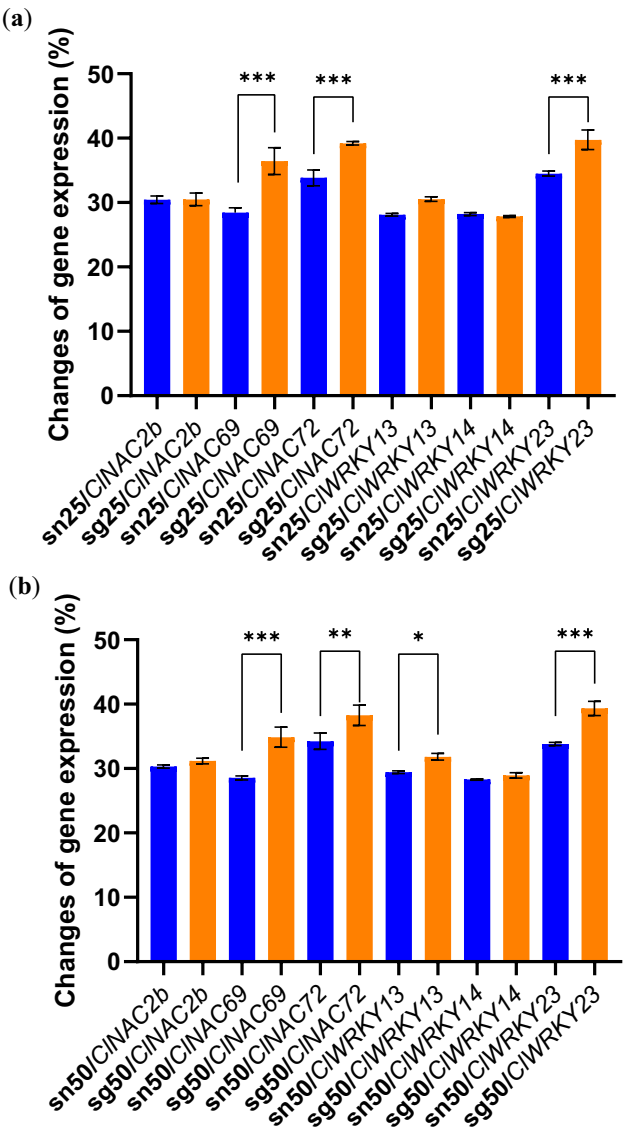


Fig. 9 Changes in gene expression between grafted and ungrafted plant types as a result of (**a**) 25 mmol/L and (**b**) 50 mmol/L NaCl salt treatment. At 25 mM NaCl, there was a significant difference in the expression of three genes (*ClNAC69*, *ClNAC72*, *ClWRKY23*) between the grafted and ungrafted groups. At 50 mM NaCl treatment, there was a significant difference in the expression of 4 out of 6 genes (*ClNAC69*, *ClNAC72*, *ClWRKY13*, *ClWRKY23*) between the grafted and ungrafted groups. Data are means ± SD of three independent determinations ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

ClNAC69 and *ClNAC72* in watermelon exhibit expression patterns analogous to their *Arabidopsis* orthologs under NaCl, ABA, and JA treatments (Tran et al. 2004). Notably, *ClNAC69* also plays a role in ABA-mediated stomatal closure (Du et al. 2014), a critical mechanism for minimizing water loss under drought stress.

Additionally, *ClNAC2b* expression was observed in multiple watermelon tissues, including shoots, leaves, flowers, and fruits, with particularly high levels in vascular tissues and their surrounding environments (Lv et al. 2016). Its ortholog, *ANAC002/ATAF1*, is known to modulate plant responses to abiotic stress (Wu et al. 2009). Elevated expression of *ClNAC2b* enhances drought sensitivity, allowing plants to tolerate prolonged water deficit through mechanisms such as stomatal closure and vascular system protection. Our findings corroborate these observations, indicating that grafted plants exhibit heightened gene expression

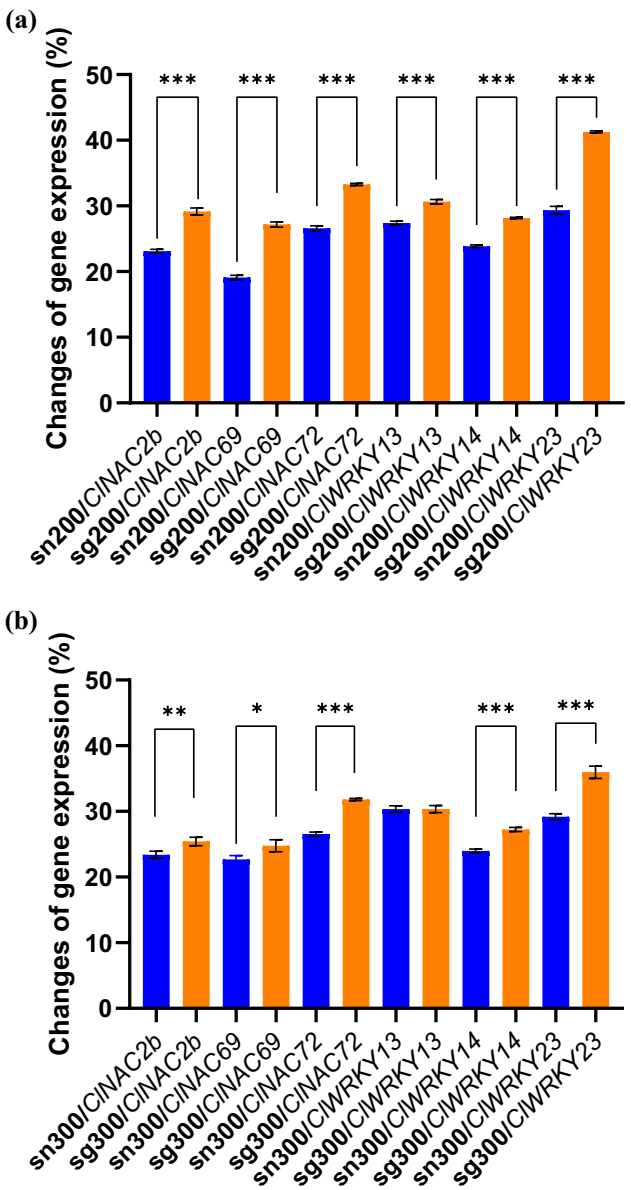


Fig. 10 Changes in gene expression between grafted and ungrafted plant types as a result of NaCl salt treatment of 200 mmol/L (**a**) and 300 mmol/L (**b**). Significant difference in gene expression between treatments of 200 mmol/L (ClNAC2b, *ClNAC69*, *ClNAC72*, *ClWRKY13*, *ClWRKY14*, *ClWRKY23*) and 300 mmol/L (*ClNAC2b*, *ClNAC69*, *ClNAC72*, *ClWRKY14*, *ClWRKY23)*. Data are means ± SD of three independent determinations ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

and stronger drought responses, contributing to improved homeostasis and drought resilience.

Among the analyzed *ClWRKY* genes, *ClWRKY23* was the only gene to show significant differences between grafted and ungrafted plants under drought stress. *ClWRKY23* is orthologous to *AtWRKY46*, which has a dual role in regulating plant responses to drought. It is rapidly activated by drought, salinity, and oxidative stress and also plays a role in light-dependent stomatal opening (Ding et al. 2014). Both *ClWRKY23* and *AtWRKY46* contribute to cellular osmotic responses and overall plant homeostasis. *ClWRKY23* is crucial for regulating not only stomatal closure but also the dynamic processes of stomatal opening and closing, emphasizing its role in maintaining homeostasis under stress conditions.

These findings underline the critical roles of *ClNAC* and *ClWRKY* genes in regulating physiological and molecular responses to drought. The elevated expression of these genes in grafted plants can supports their enhanced capacity to adapt to water deficits, thereby promoting sustained development and resilience under drought conditions. Our observations indicate that grafted plants were better equipped to adapt to water limitations by enhancing stomatal regulation and protecting vascular systems. This increased molecular responsiveness allowed grafted plants to maintain growth and homeostasis under prolonged drought conditions, outperforming their ungrafted counterparts.

Salt Stress

Salt stress poses a significant challenge for crop plants, originating from both natural processes and human activities such as irrigation. Salt stress disrupts various physiological, biochemical, and molecular processes (Raza et al. 2022). Using salt treatments of varying concentrations (25, 50, 100 mM/L NaCl), we observed substantial differences in gene expression between grafted and ungrafted watermelon plants.

The relationship between the level of salt stress and the response to it is crucial to its survival. Grafted plants rely on their rootstock part for their root system, which actively absorbs ions and water. This absorption is influenced by the length and density of the root (Krasilnikoff et al. 2003). The increased salt tolerance in grafted plants is due to their root system and how it responds to soil stress factors. Grafted plants exhibit more efficient water and ion uptake, resulting in higher concentrations of stress hormones (such as abscisic acid (ABA), and gibberellic acid (GA)) compared to ungrafted plants (Colla et al. 2010; Kumar et al. 2017). Our observations indicate that members of the grafted and bottle gourd groups were more resilient to increased salt concentrations than those in the ungrafted group.

The *NAC* and *WRKY* genes that were investigated play an important role in regulating the plant response to abiotic stresses such as salt stress (Nakashima et al. 2012; Chen et al. 2012). As the salt concentration increased, the ungrafted plants became less effective in defending themselves. They lost a significant amount of water, their biological activity slowed down, and the expression pattern of most genes showed a drastic decrease. A much milder decrease in gene expression was observed in grafted watermelon (Figs. 11 and 12).

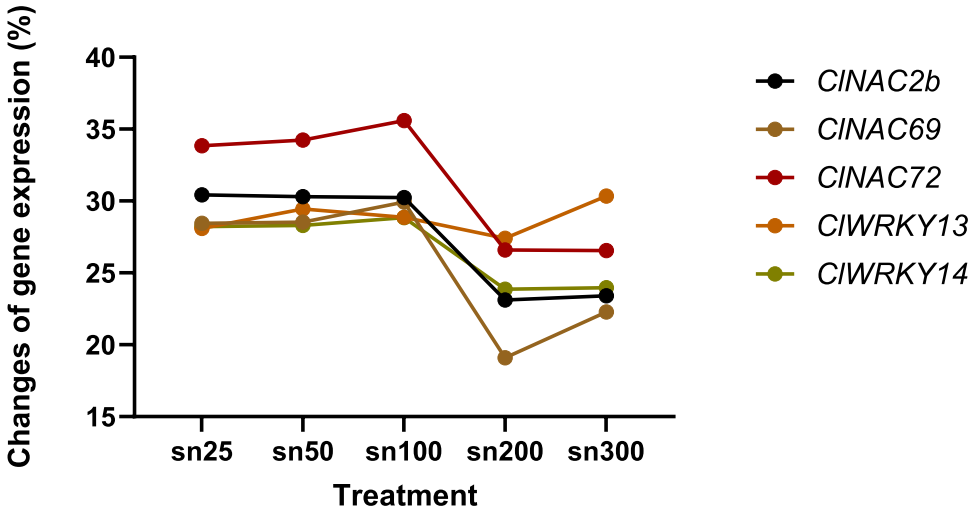


Fig. 11 Changes in gene expression pattern depending on treatment groups in ungrafted plants

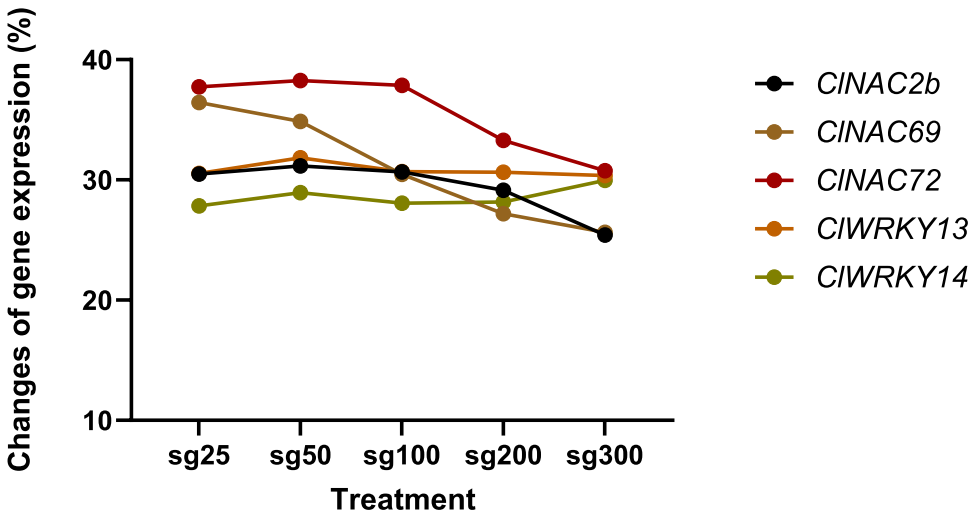


Fig. 12 Changes in the gene expression pattern depending on the treatment groups in the grafted plants

Grafted plants consistently showed higher expression of stress-responsive genes, such as *ClNAC72* and *ClWRKY23*, across all salt treatments. In contrast, ungrafted plants exhibited a sharp decline in gene expression under extreme salt stress (200–300 mM/L NaCl), accompanied by growth retardation and tissue damage. Grafted plants maintained their physiological functions more effectively because their enhanced root systems allowed for better water and ion absorption.

The *ClNAC72* gene is highly sensitive to salt stress and resembles its *Arabidopsis* orthologs (*ANAC19*, *ANAC55*, *ANAC72*), which regulate responses to salinity and play a role in ABA and JA signalling (Lv et al. 2016). *ClWRKY23* plays a dual role in regulating stomatal movements, promoting both closure to reduce water loss and opening to enhance gas exchange. This dynamic regulation is vital for maintaining plant homeostasis under stress (Ding et al. 2014). Other genes, such as *ClWRKY13* and *ClWRKY14*, contribute to vascular tissue formation and transport system integrity, further enhancing the plant's resilience to salt stress.

Our findings highlight that grafted plants are better equipped to manage salt stress due to their enhanced molecular and physiological responses. The consistently higher expression of *ClWRKY23* and *ClNAC72* in grafted plants underscores their critical role in stress adaptation. These genes not only activate early defence mechanisms but also ensure long-term stability, enabling grafted plants to survive and thrive under high salinity conditions.

Conclusion

Vegetables and fruits play a crucial role in human nutrition. As they are vulnerable to various biotic and abiotic stressors, droughts, extreme temperatures, or salinized soils further innovation is needed to ensure food crops

remain stable. Grafting is an environmentally friendly, fast and effective technique to protect plants.

During our tests, we successfully maintained different degrees of drought and salt stress for the plant groups. We used *Citrullus lanatus as* the scion and *Lagenaria siceraria* as the rootstock. During the 14-day treatment, individuals from the groups of grafted watermelon and bottle gourd plants were more resistant to strong and extreme treatments based on visual observations. They were able to maintain their water balance and their growth rate. In the group of individuals of the ungrafted plants, as the stress increased, growth retardation and signs of tissue damage were observed especially during salt treatment.

Based on the results of gene expression research, we can say that in the low-treatment groups, the expression of the salt and drought-sensitive genes in the grafted plants increased significantly compared to the ungraded group. They adapt more successfully to limitations (stoma closure, regulation of evaporation, etc.) that can play an essential role in their survival.

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Data Availability All tables and figures were created by the authors. All sources of information are adequately referenced. No copyright permissions are needed. The data can be provided by the first and corresponding authors upon request.

Declarations

Conflicts of Interest The authors declare no competing interests.

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