



## DROUGHT-TOLERANT *OSCIPK* GENES IN LOCAL AROMATIC RICE CULTIVARS

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### SUMMARY

The promising study transpired in the laboratory of the Department of Field Crops, College of Agriculture, University of Karbala, Karbala, Iraq. The six aromatic local rice cultivars, i.e., V1: Ebaa1, V2: Baraka, V3: Furat, V4: Diggla, V5: Yasmine, and V6: Amber-33, served as materials in the presented study. Ten genes of the CIPK family were indicators for drought-tolerant genes. Detecting these genes from the leaves after exposure to drought stress used the Real Time device PCR after RNA extraction and converted to cDNA. The results revealed that the aromatic rice cultivar Amber-33 contained almost all the genes except the gene, *OsCIPK04*. Cultivars Furat and Diggla were also superior for gene detection. The genes *OsCIPK06* and *OsCIPK07* appeared in three rice cultivars (Diggla, Yasmine, and Amber-33). The three genes, i.e., *OsCIPK08*, *OsCIPK09*, and *OsCIPK10*, were absent in all the aromatic rice cultivars, while the *OsCIPK05* gene appeared in all the studied rice cultivars under drought stress conditions. The technique used for detecting *OsCIPK* genes in the rice crop, matched with the results in the field experiments, resulted in the potential use of this method to screen the rice cultivars and determine their degree of drought tolerance.

**Keywords:** Aromatic rice, *CIPK* gene, gene expression, RNA, drought stress conditions

**Key finding:** In the presented molecular study, *CIPK* genes can assess the genetic variation generated by different rice cultivars under drought-stress conditions.

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### INTRODUCTION

The crop plants' exposure to abiotic stresses, such as, drought, high salinity, and temperature in different stages of growth, has affected their development and productivity.

The study of the mechanisms of plant responses to abiotic stresses is one of the basics sought by plant breeders; however, most of these studies focused on studying phenotypic variations and the degree of their differences among the genotypes of the same

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species. Recently, genetic engineering in plants began to study molecular and cellular changes linking them with physiological and phenotypic changes to respond and adapt to these stresses (Hatem *et al.*, 2022).

The cultivars vary in their ability to withstand water stress in many research studies. The data of Salleh *et al.* (2020) on screening rice genotypes for drought-stress tolerance showed that rice varieties varied in their degree of tolerance to water stress, as it revealed that reducing the amount of irrigation water by half affected the plant's vegetative growth. It indicated that genotype V7 gave a biological yield of 2.37 t ha<sup>-1</sup>, but when exposed to stress, the output decreased to 1.8 t ha<sup>-1</sup>. Meanwhile, genotype V5 yielded a harvest of 1.48 t ha<sup>-1</sup>, yet, with a lack of water, the yield increased to 1.6 t ha<sup>-1</sup>. These differences between cultivars are due to the genome content of stress-tolerant genes, which play a major role during plants' exposure to environmental stress. Likewise, the studies of Konate *et al.* (2021) on high-yield phenotyping of drought tolerance in rice showed that the Apo cultivar gave the highest grain yield of 5,589 kg ha<sup>-1</sup>; however, acquired severe damages when exposed to water stress, giving an output of 3,160 kg ha<sup>-1</sup>. Similarly, the cultivar B6144F\_M\_6 gave a produce of 3,139 kg ha<sup>-1</sup>, with its yield upon pressure at 2,993 kg ha<sup>-1</sup>. Notably, not all cultivars that provide high productivity in natural conditions can yield well under stress conditions because they have a high content of productivity genes lacking environmental stress genes.

The Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR), and Random Amplification of Polymorphic DNA (RAPD) have characteristics linked to unspecified random loci, which are unaffected much by the environment, and their influence does not determine the strength and weaknesses of plant strains (Sitaresmi *et al.*, 2019). However, studying the genes responsible for physiological processes to withstand environmental stress can provide more accurate readings in distinguishing the strains of the same species when exposed to environmental stress conditions. Nowadays, several identified kinase enzymes have served

as sensors for Ca to help in environmental stress responses. These enzymes included protein kinases, the most important of which are calcium-dependent protein kinases. There are 38 SnRKs, including the SnRK3 subfamily, also named CIPK (calcineurin B-like [CBL] protein interaction protein kinase) (Mahajan *et al.*, 2006).

Sequence analysis of the Arabidopsis genome revealed 10 AtCBL/SCaBP and 25 CIPK/PKS genes by having expression patterns of these CBL/SCaBP and CIPK/PKS genes suggest diverse functions in different signaling processes, such as, light, hormone, sugar, and stress responses (Luan *et al.*, 2002). Sequence analysis suggested 30 putative CIPK genes in the rice genome, and the predicted rice CIPK proteins have the same domain compositions as Arabidopsis CIPK proteins. However, the functions of almost all the rice CIPK proteins remain to be revealed except for very few reports on the expression of rice CIPK genes, including *OsCK1*, with the induction of diverse stimuli, such as, cold, light, salt, sugar, calcium, and cytokinin (Kim *et al.*, 2003).

The 30 *OsCIPK* genes identified in Japanese rice had different locations in the genome, as located on chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 11, and 12 (Ohba *et al.*, 2000). These genes determine their vital role in their mechanism of tolerance to salinity, drought, and cold stresses in various genotypes of Japanese rice. Their findings revealed that stress-tolerant rice genotypes always have a high content of these genes (Xiang *et al.*, 2007). Therefore, the presented study aimed to screen the local rice (Aromatic rice) cultivars grown in Iraq for various genes after exposure to water stress conditions.

## MATERIALS AND METHODS

### Genetic material and procedure

This study went on in the Laboratory of the Department of Field Crops, College of Agriculture, University of Karbala, Karbala, Iraq. The six aromatic rice were V1: Ebaa1, V2: Baraka, V3: Furat, V4: Diglla, V5: Yasmine, and V6: Amber-33, used in the

**Table 1.** Primers and symbol of *OsCIPK* genes (Xiang *et al.*, 2007).

Gene name	Gene symbol	Forward Primer (5'–3')	Reverse Primer (5'–3')
OsCIPK01	Ak065588	CATGAAAAATGGCAGGGGTT	CAGACGGGCAAGAAGAGAGC
OsCIPK02	AK072868	ATGGGCATTGATGCAGAGAT	GCAAAAACAGTAACATCCAGAAAC
OsCIPK03	AK111929	CAGGCACTGAATCTGGACAA	GCTACTCTACGGCGAACACC
OsCIPK04	Os12g41090	CGTTCGACATCATCTCCATGTC	TGTCGTGTTCGCCGAACA
OsCIPK05	AK065589	AAGAAGGGGAGGAAAGGGCAG	GAACAGGAGATCCATGTAGACAAA
OsCIPK06	Os08g34240	GATGCGGGTGACCAAGAG	ACCACCCAAACAGCGATACT
OsCIPK07	AK111510	ATGGAGATGTCGGAGGTGTC	CATTCCTACCAACAATTTAG
OsCIPK08	AK120431	TGAGGCATCCGAATGTGGTT	CGCCAGTGATGAACTCCAAGAT
OsCIPK09	OJ1015F07.8	CTCGACCGAACCATGTG	TCATTGTGGAAATCTCCCGTTT
OsCIPK10	AK066541	TGCTAGCGACGAGGAACACTCT	GCGAGTGGCTGTACACACAGA

research. Ten genes of the CIPK family were indicators for gene expression (Table 1). These genes aided in screening the rice cultivars since they were stress-tolerant genes, enhancing tolerance in rice.

All the rice cultivars' seeds, grown in pots with 3 kg of soil, had a rate of 10 plants in a can. After two weeks of germination, plants' exposure to water stress proceeded. Removing the vegetative parts from the leaves of the growing plants and keeping them refrigerated avoided RNA degradation. The *CIPK* family water-stress-tolerant genes' detection in plant leaves used the Real Time device PCR.

### RNA extraction

Ribonucleic acid (RNA) extraction was the first step to study gene expression. Different types of RNA molecules include the messenger RNA (mRNA), ribosomal RNA (rRNA), and small interfering RNA (siRNA). The mixture of total RNA generally showed in the rice leaves and shoots samples. The TRIzol® protocol used for

RNA extraction from the leaf had the general RNA extraction from three sources: tissues, adherent cells, and suspension cells. The protocol proceeded according to TRIzol® RT FDMix Kit (Wizbiosolution, Seongnam, South Korea).

### Reverse transcriptase

Conversion of the total single-stranded RNA into complementary DNA (cDNA) progressed according to the protocol of WizScript RT FDMix Kit (Wizbiosolution, Seongnam, South Korea). The standard Kit application was for a single reaction, adding only water and placing the RNA on a template to the RT FDMix (Hexamer) tube. The reaction components appear in Table 2. Carrying out the thermocycler program followed the procedure of the FDMix Kit (Table 3). Immediately using the synthesized cDNA as a product continued with the product prepared for PCR electrophoresis materials, according to Xiang *et al.* (2007).

**Table 2.** Reaction components for converting single-strand RNA to complementary DNA (cDNA).

No.	Reagent	Volume
1	RT_FDMix (Hexamer)	1 Tube contained all the components for the reaction
2	Primers	1 µL in all primer
3	Template RNA	< 5 µL
4	RNase free water	Up to 20 µL

**Table 3.** Thermal cycling program for conversion of RNA into cDNA.

No. of cycles	Temperature °C	Time (min)
1	25	10
2	42	30
3	85	5
4	4	holding

## RESULTS AND DISCUSSION

The results revealed that the genes *OsCIPK08*, *OsCIPK09*, and *OsCIPK10* were absent in all the aromatic rice cultivars included in the study. The lack of these genes also proves a distinctive feature of the local aromatic type of rice cultivars, as other rice cultivars were distinguishable with these genes, especially those cultivated in other sites (Asano *et al.*, 2005; Chen *et al.*, 2011). Therefore, aromatic rice cultivars can be notable from different cultivars by detecting these genes after exposure to drought-stress conditions. It is possible to use some of the missing genes in the local genotypes to distinguish and compare with the imported rice cultivars, determining the degree of their drought tolerance efficiency (Zhu *et al.*, 2002).

The *OsCIPK05* gene appeared in all the rice cultivars and showed a comprehensive characteristic as a basic gene for stress tolerance (Figures 1-7). Therefore, this gene was a basic rule in drought-stress tolerance in different cultivars of various plant species because it is valuable in many physiological processes related to stress conditions (Yang *et al.*, 2022; Tran *et al.*, 1999). However, the *OsCIPK06* and *OsCIPK07* genes appeared in three rice cultivars found most tolerant to drought compared with other rice cultivars because of their continuous growth for a longer period and delayed yellowing of the leaves, making such genes as high-stress-tolerant (Kanwar *et al.*, 2014; Zhu *et al.*, 2016).

A survey of stress-induced expression patterns can often provide clues for speculating the putative functions of the genes, which may be especially true for a functionally diversified gene family, such as the CIPK family (Kolukisaoglu *et al.*, 2004). To prove that some of these stress-responsive *OsCIPK* genes may

be potentially useful for enhancing stress tolerance in rice, choosing 10 *CIPK* genes (*OsCIPK01*, *OsCIPK10*, responsive to drought) served as examples and over-expressed in six Iraqi aromatic types of rice cultivars. The study results indicated that rice plants expressing all genes can significantly improve the rice genotype's tolerance to drought-stress conditions. The study further implied the significant role of *CIPK* genes in processing accumulating dry matter and increasing the cell content of sugars and amino acids, especially proline, which plays an important role in drought tolerance. It also appears that rice cultivars with a high content of *CIPK* genes can grow closer to normal through plant branching, transpiration rate, and photosynthesis reflected in chlorophyll content in leaves, dry matter production, and economic yield (Hashimoto *et al.*, 2012; Tsai *et al.*, 2012).

Rice cultivars showed variations in gene detection relating to stress. A high discrepancy emerged among the rice cultivars for their gene expression under drought tolerance with high superiority in rice cultivars, i.e., Digglā and Yasmine, by having seven genes of *OsCIPK*, compared with other rice cultivars (Figures 1-7). The two cultivars were late in showing signs of drought in the vegetative growth of seedlings, indicating the efficiency of gene expression in two rice cultivars with enhanced adaptability (Agbeleye *et al.*, 2019; Sujariya *et al.*, 2019). Kadim's *et al.* (2018) findings further revealed in the study of eight Iraqi rice cultivars that the cultivar Jasmine and Digglā gave the highest economic grain yield compared with different rice cultivars under natural and drought-stress conditions because the genome contained high drought-tolerant genes.



**Figure 1.** The *OsCIPK01* gene appeared in rice cultivars (V3, V4, V5, and V6) and the gene size was 414 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK1* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.



**Figure 2.** The *OsCIPK02* gene appeared in rice cultivars (V3, V4, V5, and V6) and the gene size was 887 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK2* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.



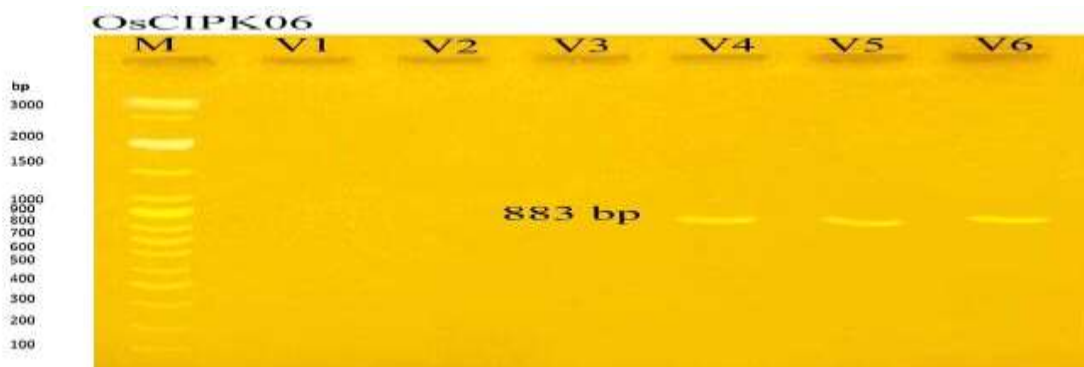
**Figure 3.** The *OsCIPK03* gene appeared in rice cultivars (V2, V3, V4, V5, and V6) and the gene size was 1199 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK3* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.



**Figure 4.** The *OsCIPK04* gene appeared in rice cultivars (V2, V3, V4, and V5) and the gene size was 1161 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK4* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.



**Figure 5.** The *OsCIPK05* gene appeared in all rice cultivars and the gene size was 684 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK5* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.



**Figure 6.** The *OsCIPK06* gene appeared in rice cultivars (V4, V5, and V6) and the gene size was 883 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK6* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.



**Figure 7.** The *OsCIPK07* gene appeared in rice cultivars (V4, V5, and V6) and the gene size was 1124 bp. Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *OsCIPK7* in *Oryza sativa* L. detected under UV light, cDNA strain was used, first line: 3000 bp DNA ladder.

The genome of the rice cultivar Aromatic rice-33 contained all the genes that appeared for the studied cultivars except the *OsCIPK04* gene (Figures 1-7). Hence, the said cultivar came second in its tolerance to water stress; however, it revealed as one of the cultivars tolerant to water stress conditions compared with other cultivars, as it showed better growth under drought-stress conditions (Rasheed et al., 2017). The said cultivar exhibited perfect development after exposure to drought stress and was also late in yellowing compared with the rest of the rice cultivars (Awasthi and Lal, 2014).

The aromatic rice cultivar Furat contained five genes, but its genome did not have the genes *OsCIPK06* and *OsCIPK07*, with the cultivar considered a medium drought-stress tolerant cultivar (Figures 1-7). These results were consistent with the findings of Al-Azzawi et al. (2020), who determined the level of drought tolerance for the said rice cultivar under drought stress conditions. The rice cultivar Baraka revealed three genes, i.e., *OsCIPK03*, *OsCIPK04*, and *OsCIPK05*, and such cultivar appeared as a low drought-tolerant cultivar like rice cultivar Ebaa, which also showed weak growth and greater yellowness of the plants (Al-Azzawi et al., 2020).

## CONCLUSIONS

The detection of *OsCIPK* genes in the rice cultivars and matching the results with field experiments resulted in the potential use of this method to screen the rice genotypes and determine their degree of tolerance to drought-stress conditions in a record period not exceeding two weeks compared with the field experiments, which need around six months to establish the water-stress tolerance.

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