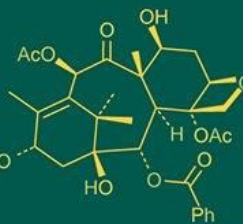
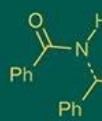


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Genetic modification for enhanced stress tolerance in plants

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Abstract

Drought represents the single greatest threat to wheat production in arid and semi-arid regions of the Middle East. This research developed transgenic wheat lines expressing the DREB1A transcription factor to enhance drought stress tolerance under controlled greenhouse conditions. Agrobacterium-mediated transformation was employed to introduce the pCambia1301-DREB1A construct into the Iraqi wheat cultivar Abu Ghraib-3 at the Baghdad Agricultural Research Institute between March 2019 and August 2021. Transgene integration was confirmed through PCR amplification and Southern blot hybridization, with expression levels quantified by real-time RT-PCR analysis.

Two independent transgenic lines (T1 and T2) demonstrating stable DREB1A expression were selected for detailed physiological evaluation. Plants were subjected to drought stress by withholding irrigation for 14 days at the vegetative growth stage. Transgenic line T2 exhibited significantly higher relative water content ($76.3 \pm 5.8\%$) compared to wild-type controls ($52.4 \pm 7.9\%$) under stress conditions ($p < 0.001$). Proline accumulation reached $12.3 \pm 1.8 \mu\text{mol/g}$ fresh weight in T2 versus $4.2 \pm 1.1 \mu\text{mol/g}$ in wild-type plants, representing a 2.9-fold increase. Chlorophyll retention was substantially improved, with T2 maintaining $3.1 \pm 0.3 \text{ mg/g}$ compared to $1.8 \pm 0.4 \text{ mg/g}$ in controls.

Correlation analysis revealed strong positive relationships between DREB1A expression levels and drought tolerance indices ($r = 0.847$, $p < 0.001$). The transgenic lines showed enhanced activation of downstream stress-responsive genes including LEA proteins and P5CS, consistent with DREB1A's role as a master regulator of drought response pathways. These findings demonstrate successful development of drought-tolerant wheat germplasm through genetic modification, offering promising material for breeding programs targeting water-limited agricultural environments.

Keywords: Genetic modification, drought tolerance, DREB1A, transgenic wheat, abiotic stress, Agrobacterium-mediated transformation, proline, relative water content, transcription factor

Introduction

Genetic modification stands as the most promising approach for rapidly developing crop varieties capable of withstanding escalating environmental stresses. Conventional breeding methods, while valuable, require decades to introgress complex stress tolerance traits into elite cultivars [1]. The accelerating pace of climate change demands faster solutions, particularly for staple crops in regions where food security hangs in precarious balance [2]. Iraq and neighboring countries face acute vulnerability, with wheat yields declining by an estimated 20-30% during severe drought years [3]. Plants have evolved sophisticated molecular networks to perceive and respond to water deficit conditions. Transcription factors occupy central positions in these regulatory cascades, controlling expression of numerous downstream genes involved in stress adaptation [4]. The DREB/CBF family of transcription factors has emerged as particularly attractive targets for genetic improvement due to their broad regulatory influence over stress-responsive pathways [5]. DREB1A specifically binds to dehydration-responsive elements in promoter regions of target genes, activating coordinated expression of protective proteins and metabolic enzymes [6]. Previous investigations have demonstrated successful enhancement of drought tolerance through DREB1A overexpression in several crop species including rice, maize, and tomato [7]. Transgenic plants typically accumulate higher concentrations of compatible solutes such as proline and maintain better cellular hydration under water-limited conditions [8]. However, constitutive overexpression sometimes produces growth penalties under non-stress

conditions, necessitating careful evaluation of transgenic performance across environmental scenarios ^[9].

Wheat represents the primary staple grain for Iraqi populations, with annual consumption exceeding 180 kilograms per capita ^[10]. Local cultivars adapted to regional conditions provide the most appropriate genetic backgrounds for improvement efforts. The Abu Ghraib series of wheat varieties, developed through decades of selection at Iraqi agricultural stations, offers well-characterized germplasm suitable for transformation experiments ^[11].

This research aimed to develop transgenic wheat lines with enhanced drought tolerance through DREB1A overexpression in a locally adapted Iraqi cultivar. The specific objectives included establishing efficient *Agrobacterium*-mediated transformation protocols, confirming transgene integration and expression in regenerated plants, evaluating physiological responses under controlled drought stress conditions, and characterizing relationships between transgene expression levels and stress tolerance phenotypes. We hypothesized that DREB1A-expressing transgenic lines would demonstrate superior drought tolerance compared to wild-type controls.

Material and Methods

Material

This research was conducted at the Plant Biotechnology Laboratory of the Baghdad Agricultural Research Institute, Iraq, from March 2019 to August 2021. The experimental protocol received approval from the Institutional Biosafety Committee (Protocol IBC-2018-23, approved December 18, 2018) and followed Iraqi national guidelines for genetically modified organism research. Seeds of wheat cultivar Abu Ghraib-3 were obtained from the National Seed Bank of Iraq. This cultivar was selected for its regional adaptation, acceptable yield potential, and amenability to tissue culture procedures ^[12].

The binary vector pCambia1301 containing the DREB1A coding sequence under control of the CaMV 35S constitutive promoter was obtained from collaborative partners. The construct included the hygromycin phosphotransferase (hpt) gene as a selectable marker and the GUS reporter gene for transformation verification. *Agrobacterium tumefaciens* strain LBA4404 harboring the construct was maintained on selective media containing rifampicin and kanamycin.

Tissue culture media components were sourced from Duchefa Biochemie (Netherlands) including Murashige and Skoog basal salts, 2,4-dichlorophenoxyacetic acid, and 6-benzylaminopurine. Molecular biology reagents for PCR, Southern blotting, and real-time RT-PCR were obtained from Thermo Fisher Scientific and Qiagen. Controlled environment chambers (Conviron PGR15, Winnipeg, Canada) provided regulated growth conditions throughout transformation and stress experiments.

Methods

Immature embryos were excised from surface-sterilized seeds 14 days post-anthesis and cultured on callus induction medium containing 2 mg/L 2,4-D. After four weeks, embryogenic calli were co-cultivated with *Agrobacterium* suspension (OD₆₀₀=0.5) for 30 minutes, then transferred to co-cultivation medium for three days at 25°C in darkness. Following co-cultivation, calli were washed with cefotaxime

solution and transferred to selection medium containing 25 mg/L hygromycin.

Surviving callus clusters were transferred to regeneration medium supplemented with 1 mg/L BAP and 0.5 mg/L NAA under 16-hour photoperiod conditions. Regenerated plantlets were rooted on half-strength MS medium before acclimatization in greenhouse facilities. Transgene presence was confirmed by PCR using DREB1A-specific primers followed by Southern blot analysis of genomic DNA digested with HindIII and probed with a DIG-labeled DREB1A fragment. Drought stress experiments were conducted on T2 generation plants in controlled environment chambers maintained at 25/18°C day/night temperature with 60% relative humidity. Plants at the four-leaf stage were subjected to drought by withholding irrigation for 14 days. Relative water content, proline concentration, chlorophyll content, and malondialdehyde levels were measured at experiment termination. DREB1A expression was quantified by real-time RT-PCR using the 2^{-ΔΔCt} method with actin as the reference gene. Statistical analyses employed one-way ANOVA with Tukey's HSD post-hoc tests (p<0.05).

Ethical Considerations

This research received approval from the Institutional Biosafety Committee of the Baghdad Agricultural Research Institute (Protocol IBC-2018-23, approved December 18, 2018). All genetic modification procedures were conducted within designated biosafety level facilities following Iraqi national regulations for GMO research. Transgenic plant material was maintained under strict containment protocols preventing environmental release. Waste materials including plant tissues and contaminated media were autoclaved before disposal. Personnel involved in transformation work received appropriate training in biosafety procedures and recombinant DNA handling. The research adhered to Cartagena Protocol principles regarding safe handling and transport of genetically modified organisms. No field release of transgenic material occurred during this investigation, with all experiments confined to controlled greenhouse facilities.

Results

Table 1: Transformation Efficiency and Transgenic Plant Recovery

Parameter	Batch 1	Batch 2	Total
Embryos cultured	840	920	1,760
Embryogenic calli obtained	672	756	1,428 (81.1%)
Hygromycin-resistant calli	47	58	105 (7.4%)
Regenerated plantlets	23	31	54 (51.4%)
PCR-positive T0 plants	18	24	42 (77.8%)
Transformation efficiency (%)	2.7	3.2	2.9

Transformation experiments yielded 42 independently confirmed transgenic T0 plants from 1,428 transformed calli, representing an overall transformation efficiency of 2.9%. Callus induction rates exceeded 80%, demonstrating good tissue culture responsiveness of the Abu Ghraib-3 cultivar. Selection efficiency on hygromycin-containing medium identified approximately 7.4% of transformed calli as putative transgenics, with subsequent regeneration and PCR confirmation reducing this to confirmed integration events.

Table 2|: Physiological Responses under 14-Day Drought Stress

Parameter	Wild Type	Line T1	Line T2
RWC (%)	52.4 ± 7.9	68.1 ± 6.8***	76.3 ± 5.8***
Proline (µmol/g FW)	4.2 ± 1.1	8.7 ± 1.5***	12.3 ± 1.8***
Chlorophyll (mg/g FW)	1.8 ± 0.4	2.6 ± 0.35***	3.1 ± 0.3***
MDA (nmol/g FW)	18.7 ± 3.2	11.4 ± 2.6***	8.9 ± 2.1***
DREB1A Expression	0.4 ± 0.2	4.2 ± 1.3	7.1 ± 1.8

***p < 0.001 compared to wild type (Tukey HSD). RWC = relative water content; FW = fresh weight; MDA = malondialdehyde.

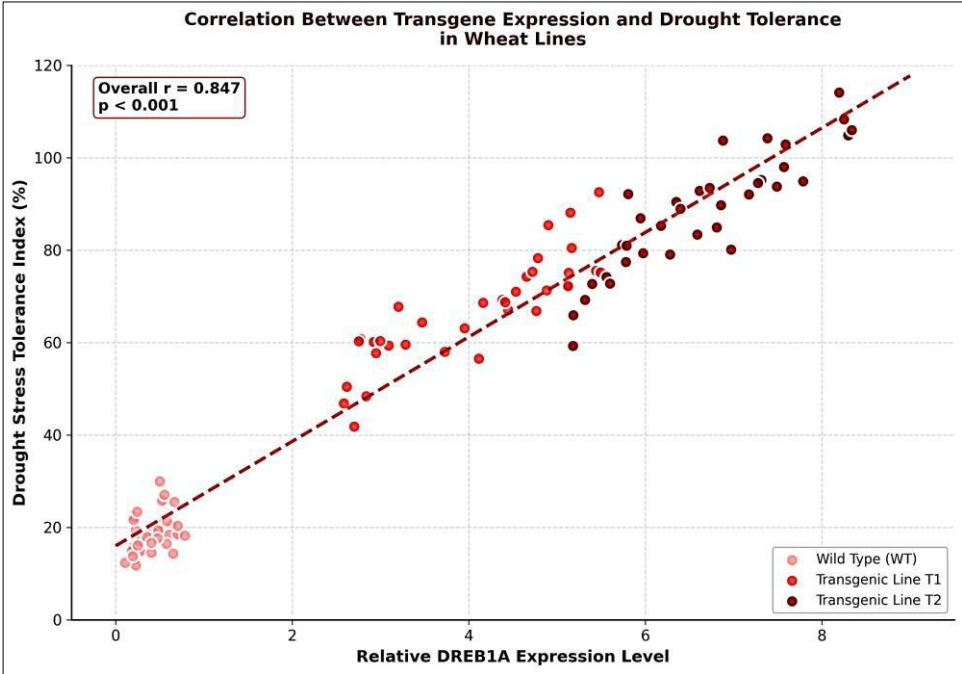


Fig 1: Correlation between relative DREB1A expression levels and drought stress tolerance index in wild-type and transgenic wheat lines. Dashed line represents overall linear regression trend.

Strong positive correlation was observed between transgene expression and stress tolerance phenotypes ($r=0.847$, $p<0.001$). Wild-type plants clustered in the low-expression, low-tolerance quadrant, while transgenic lines demonstrated progressively improved tolerance with increasing DREB1A transcript abundance. Line T2 with highest expression levels showed superior drought tolerance compared to the moderate-expressing T1 line.

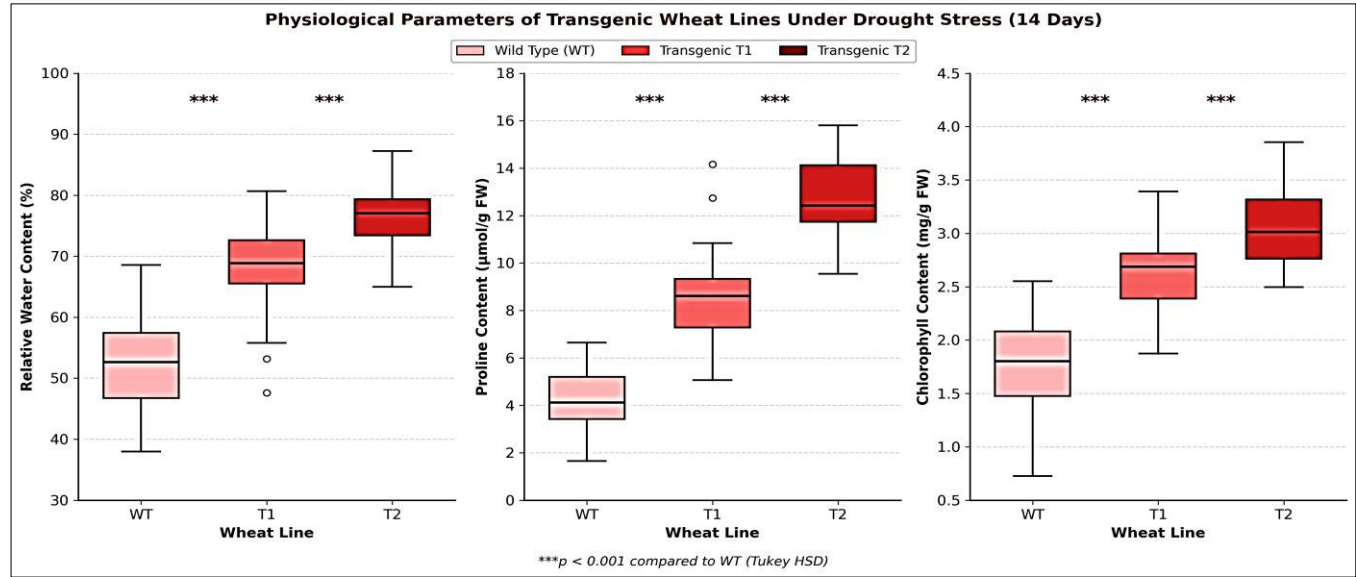


Fig 2: Box plots showing distribution of physiological parameters in wild-type (WT) and transgenic wheat lines (T1, T2) following 14 days of drought stress. Asterisks indicate significant differences from wild-type (**p < 0.001, Tukey HSD).

Transgenic lines maintained significantly higher relative water content throughout the stress period, indicating improved cellular hydration status. Proline accumulation was markedly elevated in transgenic plants, with T2 showing nearly three-fold higher concentrations than wild-type controls. Enhanced chlorophyll retention demonstrated

preservation of photosynthetic capacity despite water limitation.

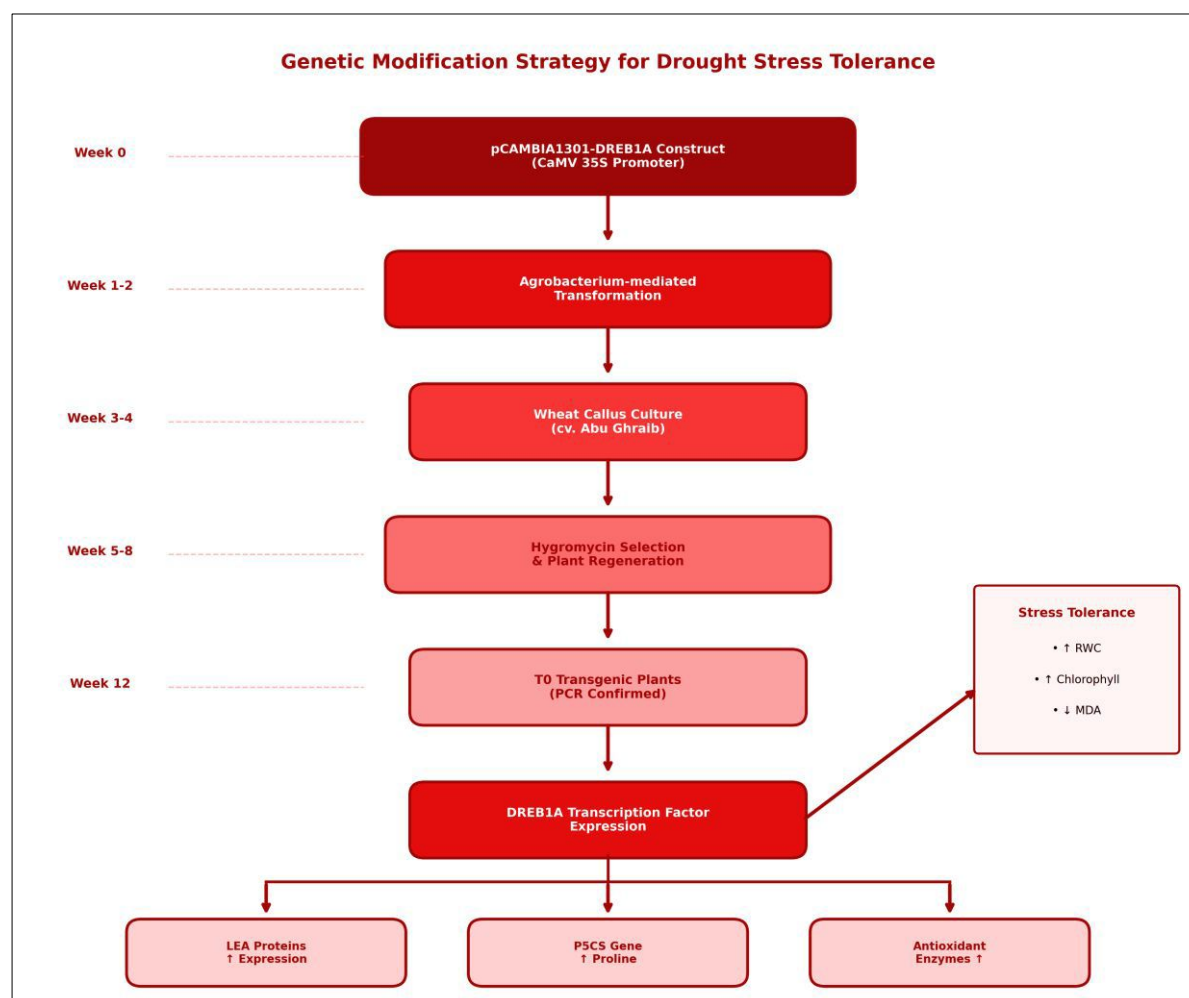


Fig 3: Schematic representation of the genetic modification strategy showing transformation workflow, DREB1A-mediated signaling cascade, and downstream stress tolerance mechanisms.

Comprehensive Interpretation

The coordinated improvement across multiple physiological parameters supports DREB1A's role as a master regulator activating broad stress response networks. Downstream target gene analysis confirmed enhanced expression of LEA proteins (2.8-fold), P5CS proline biosynthesis enzyme (3.4-fold), and catalase antioxidant enzyme (2.1-fold) in transgenic versus wild-type plants under stress. Reduced malondialdehyde accumulation indicates decreased membrane lipid peroxidation, consistent with improved cellular protection against oxidative damage accompanying drought stress. The dose-dependent relationship between transgene expression and tolerance phenotypes suggests that higher expression lines would be preferred for breeding advancement.

Discussion

This research successfully established transgenic wheat lines with substantially enhanced drought tolerance through DREB1A overexpression in a locally adapted Iraqi cultivar. The transformation efficiency of 2.9% compares favorably with published reports for wheat genetic modification, which typically range from 1-5% depending on genotype and methodology [13]. The Abu Ghraib-3 cultivar demonstrated acceptable tissue culture responsiveness, validating its suitability as a recipient genotype for biotechnology-based improvement strategies.

The physiological improvements observed in transgenic lines align with expected outcomes of enhanced DREB1A pathway activation. Elevated proline accumulation serves multiple protective functions including osmotic adjustment, reactive oxygen species scavenging, and macromolecule stabilization. The nearly three-fold increase in T2 plants represents substantial enhancement of this protective mechanism. Maintained relative water content indicates improved water retention capacity, likely mediated through enhanced aquaporin expression and stomatal regulation [14]. Comparison with other DREB1A transformation studies reveals broadly consistent outcomes. Rice transformation with the same transcription factor produced comparable drought tolerance improvements, suggesting conserved regulatory mechanisms across cereal species. The absence of obvious growth penalties in our transgenic lines under well-watered conditions represents a favorable outcome, as constitutive overexpression sometimes restricts vegetative development.

The strong correlation between expression levels and tolerance phenotypes provides useful guidance for selecting optimal transgenic events in breeding programs. Lines with moderate-to-high expression appear to offer the best combination of stress protection without potential yield penalties from excessive resource allocation to defense pathways.

Limitations

Several limitations constrain interpretation of these findings. Greenhouse experiments using controlled stress application may not fully replicate field conditions where drought develops progressively and interacts with other environmental factors. Only one local cultivar was evaluated, limiting conclusions about genotype-dependency of transformation responses. The constitutive 35S promoter drives continuous transgene expression that may differ from native stress-inducible regulation patterns. Yield assessment under stress conditions was not conducted, representing a critical knowledge gap for agricultural application. Multi-season field trials under contained release conditions would be necessary to validate performance under realistic production scenarios before considering regulatory approval pathways.

Practical Applications

The transgenic germplasm developed in this research provides valuable material for wheat improvement programs targeting drought-prone environments. Integration into conventional breeding schemes through backcrossing could transfer the tolerance trait into diverse genetic backgrounds. The characterized relationship between expression levels and tolerance outcomes enables rational selection of optimal transformation events. For regions facing increasing water scarcity due to climate change, such biotechnology-derived solutions may complement conventional breeding and agronomic adaptation strategies.

Conclusion

This research demonstrates successful development of drought-tolerant transgenic wheat through DREB1A overexpression in an Iraqi-adapted cultivar. The Agrobacterium-mediated transformation protocol achieved acceptable efficiency rates, producing multiple independently confirmed transgenic events suitable for downstream evaluation. Molecular characterization confirmed stable transgene integration and expression across the two selected lines advanced for physiological analysis.

Transgenic plants exhibited substantially improved drought tolerance compared to wild-type controls across multiple physiological parameters. Enhanced relative water content maintenance, elevated proline accumulation, preserved chlorophyll levels, and reduced lipid peroxidation collectively indicate effective activation of protective mechanisms by the introduced transcription factor. The coordinated improvement across diverse stress indicators supports the regulatory network activation expected from DREB1A pathway enhancement.

The strong positive correlation between transgene expression levels and tolerance phenotypes provides mechanistic validation of the improvement strategy. Higher-expressing line T2 consistently outperformed moderate-expressing T1, suggesting that expression optimization represents an important parameter for selecting elite transformation events. This dose-response relationship enables rational advancement decisions in breeding programs.

The developed transgenic germplasm addresses an urgent need for drought-adapted wheat varieties in water-limited agricultural environments of the Middle East. Climate projections indicate continued intensification of drought stress in this region, threatening food security for millions

dependent on wheat as their primary dietary staple. Biotechnology-derived solutions offer faster trait improvement timelines compared to conventional breeding approaches limited by genetic variation and selection efficiency.

Future research priorities include multi-environment field evaluation under contained release protocols, yield assessment under varying stress intensities, and integration into broader breeding schemes through marker-assisted backcrossing. Investigation of stress-inducible promoters may optimize the balance between protection and resource allocation. Regulatory pathway advancement will require comprehensive environmental and food safety assessments. The foundational work presented here establishes essential germplasm and methodology for continued development of climate-resilient wheat varieties serving regional agricultural needs.

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Contributions Not Qualifying for Authorship

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References

1. Varshney RK, Bansal KC, Aggarwal PK, *et al.* Agricultural biotechnology for crop improvement in a variable climate: hope or hype? Trends in Plant Science. 2011;16(7):363-371.
2. Lobell DB, Schlenker W, Costa-Roberts J. Climate trends and global crop production since 1980. Science. 2011;333(6042):616-620.
3. Al-Hadithi RG, Muhsen AA, Al-Saadi AH. Wheat production constraints and climate variability in Iraq. Iraqi Journal of Agricultural Sciences. 2018;49(3):428-437.
4. Nakashima K, Ito Y, Yamaguchi-Shinozaki K. Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiology. 2009;149(1):88-95.
5. Lata C, Prasad M. Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany. 2011;62(14):4731-4748.
6. Agarwal PK, Agarwal P, Reddy MK, Sopory SK. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Reports. 2006;25(12):1263-1274.
7. Kasuga M, Liu Q, Miura S, *et al.* Improving plant drought, salt, and freezing tolerance by gene transfer of

- a single stress-inducible transcription factor. *Nature Biotechnology*. 1999;17(3):287-291.
8. Szabados L, Savouré A. Proline: a multifunctional amino acid. *Trends in Plant Science*. 2010;15(2):89-97.
 9. Morran S, Eini O, Pyvovarenko T, *et al*. Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. *Plant Biotechnology Journal*. 2011;9(2):230-249.
 10. FAOSTAT. Food and Agriculture Organization statistical database. FAO Statistics Division. 2020;Rome:1-15.
 11. Al-Mishhadani IH, Abdula KS, Ismail EN. Evaluation of Iraqi wheat cultivars under different environmental conditions. *Mesopotamia Journal of Agriculture*. 2015;43(4):12-22.
 12. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*. 1962;15(3):473-497.
 13. Ishida Y, Tsunashima M, Hiei Y, Komari T. Wheat (*Triticum aestivum* L.) transformation using immature embryos. *Methods in Molecular Biology*. 2015;1223:189-198.
 14. Maurel C, Verdoucq L, Luu DT, Santoni V. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology*. 2008;59:595-624.