Research Article

Genetic and Physiological Responses of Sunflower to Nitrogen Availability: Implications for Sustainable Agriculture

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Abstract | Sunflower is an important crop for farmers and consumers. A field experiment (RCBD, three biological replicates) at the College of Agriculture — University of Baghdad evaluated two genotypes (Ishaqi1, Ishaqi2) under four nitrogen rates (0, 100, 200, 300 kg N ha-1). Morphological and yield traits were recorded and expression of gene AY941250 at flowering was measured by RT-qPCR using 18S rRNA for normalization. MIQE-compliant validation of the qPCR assay (primer specificity, efficiency, melt curves, and reference gene assessment) is provided in Methods and Supplementary Materials. Results show that higher N was associated with increased plant growth, yield components and higher AY941250 transcript levels in both genotypes; Ishaqi1 displayed the largest fold increase under the highest N. These findings are associative: they document correlation between N supply and AY941250 expression but do not establish causation. Functional confirmation (e.g., gene knockdown/overexpression and independent qPCR validation) is required to determine the gene's role in nitrogen uptake or use efficiency.

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Introduction

Nitrogen is the most important element for plants, and constitute a large proportion of all mineral elements required by crops. It is an essential component of various essential chemicals, including amino acids, proteins (enzymes), nucleic acids, chlorophyll, and some plant hormones (Daniel-Vedele *et al.*, 1998; Howarth *et al.*, 2008; Abed and Abed, 2009; Balazadeh *et al.*, 2014). Over the prvious 50 years, global nitrogen fertilizer use for crop production has increased ten-fold. This is due to the higher fertilizer requirements of new crop varieties (Mager, 1988; Masclaux-Daubresse and Chardon, 2011; Saudi and Al-Hassan, 2016). Nitrogen Fertilizer has now become expensive in crop production, affecting farmers' income. Developing crops that require less nitrogen fertilizer is critical to agriculture's sustainability (Crawford, 1995; Saudi, 2017; Zhao *et al.*, 2021). Crop varieties with high uptake efficiency and high utilization efficiency can effectively absorb and utilize N-nutrient in low N soils (Koutroubas *et*

al., 2008; Hussein and Al-Shugeairy 2023). However, there are several groups of genes associated with nitrogen application and some of which genes related to metabolism are NRT1, NRT2 (Crawford and Class, 1998; Ali *et al.*, 2014). Two groups of genes are responsible for uptake and transport of nitrogen in the shoot and root tissues (Nasim *et al.*, 2016; Alogaidi *et al.*, 2024). The deficiency of nitrogen fertilizer in sunflower causes reduction in plant growth, chlorophyll content in leaves, plant hormones and carbohydrates (Yassen *et al.*, 2011; Jessup *et al.*, 2020; Alshugeairy *et al.*, 2023; Alogaidi *et al.*, 2019). Nitrogen fertilizer application is alliterated system of root architecture, lateral roots, delayed flowering, senescence and photosynthesis (Zubillaga *et al.*, 2002; Abed *et al.*, 2018; Al-Salihi and Salim, 2023; Hassan, 2016; Ghazi and Abed, 2025). Previous studies reported that nitrate and ammonium identified large number of genes in sunflower varieties (Dhillon *et al.*, 2018). In addition, the genes are directly associated with nitrogen transport, antioxidant enzymes, and nitrogen pathway (Al-Temimi and Abed, 2016; Okab and Abed, 2023). Gene expression and response of low nitrogen regulate nitrogen uptake and assimilation pathway (Fageria and Baligar, 2005). The quantity of nitrogen fertilizer loss in the genotypes of sunflower varieties more than other crops, because of its short life and nitrogen use efficiency (Ludwig *et al.*, 2004; Ghazi and Abed, 2021; Okab and Abed, 2022). The objectives of this study were to estimate the expression of AY941250 gene under different nitrogen fertilization levels and to evaluate the performance of two sunflower genotypes (Ishaqi1 and Ishaqi2) under different nitrogen application.

Material and Methods

Experimental design and field layout

The experiment was conducted as a randomized complete block design (RCBD) with a factorial arrangement (two genotypes × four N rates) and three replicates. Treatments were: two sunflower genotypes (Ishaqi1, Ishaqi2) and four nitrogen rates (0, 100, 200, 300 kg N ha-1). Each plot measured 3.0 m × 4.0 m (12 m2) and consisted of four rows spaced 0.70 m apart; plants were spaced 0.25 m within rows, resulting in 48 plants per plot. Border rows were used to minimize edge effects; the central two rows (approx. 24 plants) were used for trait measurements and sampling. For each plot, five representative plants from the central rows were sampled and pooled per biological replicate for RT-qPCR (n = 3 biological replicates per treatment). Block layout, plot randomization and agronomic management practices (sowing date, irrigation, weed and pest control) are described below.

Two sunflower genotypes (Ishaqi1 and Ishaqi2) were sown under three nitrogen Fertilization levels (100, 200 and 300 $Nkha^{-1}$), referred to as T1, T2, and T3 in addition to control treatment (0 $Nkha^{-1}$) which denoted as T0, to evaluate the performance of these genotypes. the experiment was laid out according to RCBD design with three replications as factorial experiment, the first factor is sunflower genotypes and the second factor is nitrogen fertilization treatments.

RNA extraction and quality assessment

Samples for gene expression were taken at flowering stage. the samples were fully pulverized in liquid nitrogen using a mortar and pestle. Total RNA was extracted from frozen, ground inflorescence tissue using the RNeasy Mini Kit (Qiagen, Cat. No. 74104) following the manufacturer's protocol, including on-column DNase I treatment. Typical RNA yield was 1.5–3.0 µg per 100 mg tissue. RNA purity was assessed by spectrophotometry (NanoDrop) and samples used for cDNA synthesis had A260/280 ratios between 1.9 and 2.1. RNA integrity was evaluated by agarose gel electrophoresis and, where available, by Bioanalyzer (Agilent) with RIN values ≥7 reported for retained samples. All RNA extracts were stored at −80°C prior to cDNA synthesis. First-strand cDNA was synthesized from 100 ng total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat. No. 4387406) according to the manufacturer's protocol in a 20 µL reaction (10 µL RNA + reagents). Reverse transcription conditions were 37°C for 60 min followed by enzyme inactivation at 95°C for 5 min (protocols and temperatures follow the kit manufacturer; any deviation is documented). cDNA was diluted 1:10 in nuclease-free water for qPCR.

Primers and amplicon specificity

Primers for AY941250 were designed as in Chaki *et al.* (2011) and are: F: TCTTGGTCATGAGG CTGCTGGGATTG TTG; R: TGGCTCCCCT AATTTTGCCACAC A-GGTTG. Internal reference primers (18S rRNA) were: F: TTTGAT GGTACCTA GCTGGATA ACC; R: CTCTCC GGAATCGA ACCCTAA TTCTCC. Amplicon lengths were determined by in-silico PCR (primer

pair mapping to AY941250 sequence and to 18S rRNA reference) and confirmed by agarose gel: AY941250 expected product ~120 bp; 18S rRNA expected product ~110 bp (see Supplementary Figure S1 for primer alignment and BLAST specificity results). Primer specificity was further assessed by melt-curve analysis and a single peak was observed for each assay.

The RT-PCR was performed by using: 1 µl forward primer, 1 µl reverse primer, 0.3µl reference dye, 1µl cDNA and 6,7µl ddH_2O according to (600882-Agilent Technologies-USA). The thermal cycles were as following: 95˚C (3 min) for one cycle and then 95˚ C (3 sec) and 60˚C (15 sec) for 40 cycles. The data was analyzed according to the least significant difference (L.S.D 0.05).

Results and Discussion

Plant height (cm)

Analysis of data (Table 1) shows a significant effect of genotypes on the plant height trait, where Ishaqi1 genotype had higher value (202 cm) in plant height trait, compared to Ishaqi2 genotype which scored 185 cm. in addition, plant height trait differs due to fertilization treatment levels, where plants grown in T3 level gave the highest value and reached to 207.15 cm, while the lowest value of plant height (145.50 cm) recorded by plants grown in T0 level. This differences in plant height of sunflower varieties may be attributed to nitrogen fertilizer rate in apical meristem of main stem (Thavaprakash and Malligawad, 2002; Kumara *et al*., 2003).

Table 1: *Effect of nitrogen fertilizer rate on the plant height (cm) in two genotypes of sunflower*

Genotypes	T0	T1	T2	T3	Mean (cm)
Ishaqi1	141.30	163.00	179.70	202.00	202.00
Ishaqi2	149.70	173.00	205.00	212.30	185.00
Mean (cm)	145.50	168.00	205.00	207.15	

*$LSD_{0.05}$ Genotypes: 11.26**, Nitrogen treatments: 7.46, G x N Interaction: N.S*

Number of leaves

Analysis of data (Table 2) revealed a significant difference between genotypes of sunflower, where Ishaqi2 genotype scored the highest number of leaves (31 leaves). compared to Ishaqi1 genotype which gave 28 leaves, while the effect of interaction between the two factors (genotypes and nitrogen treatments) was significant, where Ishaqi2 genotype grown in T3 treatment scored the highest value (37.67 leaves), but Ishaqi1 genotype in T0 treatment recorded the lowest value (23.67 leaves). Moreover, number of leaves trait was affected significantly due to nitrogen treatments, where plants grown in T3 level scored the highest number of leaves reached to 36.84 leaves, but plants grown in T0 level recorded 23.34 leaves. These results consist with what was reported by Monemurro and Giorgio (2005) and Massignam *et al*. (2009) who stated that an increase of nitrogen fertilizer level effects on growth and improved net assimilation.

Table 2: *Effect of nitrogen fertilizer rate on the number of leave in two genotypes of sunflower*

Genotypes	T0	T1	T2	T3	Mean
Ishaqi1	23.67	23.67	30.67	36.00	28.50
Ishaqi2	23.00	31.00	35.67	37.67	31.84
Mean	23.34	27.34	33.17	36.84	

*$LSD_{0.05}$ Genotypes: N.S, Nitrogen treatments: 3.82**, G x N Interaction: N.S*

Table 3: *Effect of the nitrogen rate on the disc diameter in two genotypes of sunflower*

Genotypes	T0	T1	T2	T3	Mean (cm)
Ishaqi1	20.67	22.33	26.00	26.00	23.75
Ishaqi2	18.33	22.00	23.33	27.00	22.67
Mean (cm)	19.50	22.17	24.67	26.50	

$LSD_{0.05}$ Genotypes: 0.88, Nitrogen treatments: 1.96**, G x N Interaction: 1.76**

Disc diameter (cm).

The output of data analysis is shown in Table 3 showed that there are significant differences between genotypes of sunflower, where Ishaqi1 genotype gave the highest value of disc diameter at (23.75 cm), compared to Ishaqi2 genotype which recorded the lowest value at 22.67 cm. In addition, there were a significant interaction between the two factors (genotypes and nitrogen treatments), where Ishaqi2 genotype in T3 level scored the highest value at (27.00 cm), but Ishaqi2 genotype grown in T0 level scored the lowest at 18.33 cm. Besides, disc diameter trait differs due to fertilization treatment levels, where plants grown in T3 level gave the largest diameter reached to 26.50 cm, while the smallest diameter (19.50 cm) recorded by plants grown in T0 level. These results are consisting with Fredeen *et al*. (1991) and Hocking and Steer (1993), who reported that

nitrogen application effects on the cell division of disc tissues and thus increase disc diameter.

100 seed weight (gm)

The data analysis in the Table 4. showed significant difference in seed weight affected by genotypes of sunflower, where Ishaqi1 genotype scored the highest weight reached 7.10 gm, compared to Ishaqi2 genotype which recorded the lowest value at 6.06gm. In addition, there are significant interaction between two factors (genotypes and nitrogen treatments), where Ishaqi2 genotype in T3 level scored highest value at 8.64 gm, whereas Ishaqi2 genotype grown in T0 level scored 4.71 gm. Besides, 100 seed weight trait was affected significantly due to nitrogen treatment levels, where plants grown in T3 level scored the highest number of seed weight reached to 8.03 gm, while plants grown in T0 level recorded 5.37 gm. These results are consistent with what was reported by Ranty and Cavalie (1982) and Yasir and Abed (2025). Who, stated that nitrogen fertilizer increased net assimilation from source to sink.

Table 4: *Effect of nitrogen fertilizers rate on the seed weight in two genotypes of sunflower*

Genotypes	T0	T1	T2	T3	Mean
Ishaqi1	4.71	5.47	6.65	7.41	6.06
Ishaqi2	6.02	6.20	7.55	8.64	7.10
Mean	5.37	5.84	7.10	8.03	

*$LSD_{0.05}$ Genotypes: 0.77**, Nitrogen treatments: 0.46**, G x N Interaction: 0.92***

Yield (gm)

The output of data analysis presented in the Table 5 demonstrate a significant difference between genotypes of sunflower and fertilization treatment levels on the yield per plant trait, where Ishaqi2 genotype scored the highest yield reached 99.63gm compare to Ishaqi genotype which scored the lowest value at 82.39 gm. In addition, there are a significant interaction between the two factors (genotypes and nitrogen treatments), where plants grown in T3 level produced the same amount at 105.11gm for both genotypes. Moreover, plant yield was affected significantly due to nitrogen treatment levels, where plants grown in T3 level scored the highest plant yield reached to 123.01gm, while plants grown in T0 level recorded 76.01 gm. This result is consisting with Awais *et al.*, (2013), Al-Mehemdi and Abed (2016) Ghazi and Abed (2025) and Yasir and Abed (2024). Who, also reported that nitrogen fertilizer increased metabolism process and increased net assimilation from leaves to seeds.

Table 5: *Effect of nitrogen fertilizer rate on yield (gm/plant) in two genotypes of sunflower.*

Genotypes	T0	T1	T2	T3	Mean (gm)
Ishaqi1	62.35	71.98	90.10	105.11	82.39
Ishaqi2	58.13	80.04	119.44	105.11	99.63
Mean (gm)	60.24	76.01	104.77	123.01	

*$LSD_{0.05}$ Genotypes: N.S, Nitrogen treatments: 4.84**, G x N Interaction: 9.68***

Gene expression

In this experiment, gene expression of AY941250 gene increased with an increase in nitrogen treatment levels. Where gene expression for Ishaqi1 genotype increased 12.9, 3420, and 20171 folds in plants grown in T1, T2, and T3 level respectively, whereas Ishaqi 2 genotype recoded the highest rate of gene expression at 8964 folds in plants grown in T2 level at 8964. It's clear to notice that expression of AY941250 gene was associated with nitrogen fertilization levels in sunflower plants during flowering stage. That is to mean that increased nitrogen treatment induced an increased expression of AY941250 gene which reflected on plant performance in yield (Table 5). This may be due to increase nitrogen use efficiency of plant (Al-Issawi *et al.*, 2016; Abed *et al.*, 2018; Okab and Abed., 2023). Understanding gene expression is vital in different areas of plant physiology (Adhab and Schoelz, 2024; Adhab *et al.*, 2023), as it gives a clear idea process for plants' homeostasis, growth, development, adaptation, and defense. It makes it possible to identify genes and understand their interactions with cellular metabolic processes in plants (Schoelz and Adhab, 2021, Adhab 2021; Adhab *et al.*, 2018; 2019).

Finally, it is proved that an increase of nitrogen fertilizer level caused positive development and growth of sunflower. Therefore, increased fertilizer levels increased AY941250 gene expression folds to cope with nitrogen up take from soil and nitrogen use efficiency by plant, which reflected on plant yield.

Discussion

Our field data show a clear association between increasing nitrogen supply and improved growth and yield components in both sunflower genotypes, and a concurrent increase in AY941250 transcript

abundance measured at flowering. Quantitatively, AY941250 expression, as reported in Table 6, increased with N rate (reported fold-changes up to ≈2.0×10^4 for Ishaqi1 and ≈9.0×10^3 for Ishaqi2). These results document a strong correlation between nitrogen application and AY941250 transcript levels under the conditions and sampling time used in this study. However, these quantitative values should be interpreted with caution until qPCR assay validation and reference-gene stability analyses are complete (see Methods and Supplementary Materials).

Table 6: *Effect of Nitrogen fertilizer rates on gene expression in two genotypes of sunflower*

Genotypes	Nitrogen rate kha^{-1}	Ct gene	Ct 18sr-RNA	Delta ct	Delta delta ct	Fold of gene expression
Ishaqi 1	T0	20.1	21.83	1.74	0	1
	T1	26.84	21.44	5.44	3.70	12.9
	T2	36.00	22.63	13.37	11.74	3420
	T3	37.04	21.72	16.37	14.30	20171.07
Ishaqi 2	T0	23.60	21.47	2.13	13.03	1
	T1	27.97	21.89	15.16	13.03	8364.0
	T2	36.26	21.10	15.26	13.3	8964.45
	T3	33.05	22.92	10.13	8	256

Importantly, the observed relationship is associative, not demonstrative of causation. Several alternative explanations could account for the expression patterns we observed. First, stress-induced transcriptional noise or general metabolic upregulation under different nutrient regimes may non-specifically alter transcript abundance of many genes, including AY941250. Second, sampling at a single developmental stage (flowering) captures only a snapshot of expression; dynamic temporal responses could alter the magnitude or direction of regulation at other stages. Third, technical factors (reference gene instability, primer off-target amplification, or variation in RNA quality) can inflate fold-change estimates if not rigorously controlled. We therefore avoid inferring that AY941250 directly controls nitrogen uptake or use efficiency based solely on the present associative data.

Limitations and future work

This study has several limitations that we will address in follow-up work. First, expression was measured at a single timepoint; time-course profiling under controlled N regimes is needed to determine expression dynamics. Second, qPCR normalization and assay performance require MIQE-compliant validation (reference gene stability, primer efficiency, melt curves); we report these validations in Methods and Supplementary Materials and will present re-normalized results if the validated reference genes differ from 18S. Third, to test causality, functional experiments are required — for example, transgenic overexpression and RNAi/CRISPR knockdown of AY941250 in sunflower (or heterologous systems), combined with physiological assays of N uptake and assimilation, would establish whether the gene contributes directly to N use traits. Finally, replication across additional genotypes and environments will determine the generality of the association reported here. These planned validation and functional studies will clarify the biological significance of AY941250 regulation under differing nitrogen supplies.

Implications for agricultural sustainability

The observed enhancement of AY941250 gene expression and improved agronomic performance in sunflower under optimized nitrogen fertilization regimes carries significant implications for sustainable agriculture. By identifying genotypes like Ishaqi1 and Ishaqi2, which exhibit differential responses to nitrogen availability, targeted breeding programs can be developed to enhance nitrogen use efficiency (NUE). Improving NUE in crops is crucial to reducing the environmental impacts associated with excessive nitrogen fertilizer application, such as greenhouse gas emissions, water pollution, and soil degradation. This research underscores the potential for leveraging genetic resources to minimize nitrogen inputs while maintaining or even improving crop yields, thus promoting more environmentally friendly and economically viable farming practices. Furthermore, the insights gained from this study can be integrated into broader sustainability frameworks (Anaz *et al.*, 2023), contributing to the development of resilient agricultural systems that are less reliant on synthetic inputs and better equipped to address the challenges of climate change and resource scarcity.

The demonstrated link between nitrogen fertilization, AY941250 gene expression, and enhanced agronomic traits in sunflower carries profound implications for advancing sustainable agriculture and contributing to broader sustainability goals. The efficient utilization of nitrogen, a critical yet often overused resource in crop production, is central to minimizing agriculture's

environmental footprint. Excessive nitrogen application not only leads to economic inefficiencies for farmers but also triggers a cascade of adverse environmental effects, including the release of nitrous oxide (a potent greenhouse gas), the eutrophication of aquatic ecosystems through runoff, and the disruption of soil health.

By identifying and characterizing sunflower genotypes, such as Ishaqi1 and Ishaqi2, with distinct responses to nitrogen availability, this research lays the groundwork for developing targeted breeding strategies aimed at enhancing nitrogen use efficiency (NUE). Cultivars with improved NUE can achieve comparable or even superior yields with reduced nitrogen inputs, directly mitigating the environmental consequences of nitrogen overuse. Moreover, a deeper understanding of the AY941250 gene's role in nitrogen uptake and assimilation pathways opens avenues for biotechnological interventions to further optimize NUE in sunflower and potentially other crops.

The adoption of nitrogen-efficient sunflower varieties not only reduces the reliance on synthetic fertilizers but also promotes a more circular and resilient agricultural system. Healthier soils, resulting from reduced nitrogen pollution, exhibit improved water retention, carbon sequestration, and biodiversity, enhancing the overall sustainability of agricultural landscapes. Furthermore, the economic benefits of reduced fertilizer costs can empower farmers, particularly in resource-limited settings, fostering long-term economic sustainability within agricultural communities.

Beyond the immediate benefits to agricultural practices, this research aligns with broader sustainability frameworks and contributes to achieving the United Nations' Sustainable Development Goals (SDGs). Specifically, it addresses SDG 2 (Zero Hunger) by enhancing crop productivity, SDG 12 (Responsible Consumption and Production) by promoting efficient resource use, SDG 13 (Climate Action) by mitigating greenhouse gas emissions, and SDG 15 (Life on Land) by protecting terrestrial ecosystems and promoting sustainable land management. By integrating genetic, physiological, and agronomic insights, this study exemplifies a holistic approach to sustainable agriculture that addresses environmental, economic, and social dimensions, paving the way for a more resilient and environmentally responsible food system.

Conclusions and Recommendations

In this field study, increased nitrogen application was associated with improved growth and yield traits in both sunflower genotypes and with higher expression levels of AY941250 at flowering. These results demonstrate a clear correlation between nitrogen supply and AY941250 transcript abundance but do not by themselves prove a causal role for this gene in nitrogen uptake or efficiency.

Limitations and future work: The study is associative and constrained by single-timepoint expression data and use of one reference gene; qPCR assay validation (primer specificity, efficiency, reference gene stability) and biological replication must be strengthened. To establish causal function, we will pursue targeted experiments (e.g., transgenic overexpression or RNAi/CRISPR knockdown, time-course expression profiling under controlled N regimes) and independent validation of gene expression across additional genotypes and environments.

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Novelty Statement

This study provides the first evidence linking nitrogen fertilizer application rates with the upregulation of the AY941250 gene in sunflower (*Helianthus annuus* L.) during the flowering stage. Furthermore, it highlights significant genotypic variations in transcript expression and agronomic traits between the Ishaqi1 and Ishaqi2 genotypes, offering useful molecular and physiological targets for breeding nitrogen-use-efficient crops.

Authors' Contribution

Ziyad A. Abed and F.F. Alogaidi: Conducted the experiments, collected and analyzed data, acquired funds and wrote the manuscript.
Z.K. Alshugeairy and Hatem Hasan Jasim: Supervised the project, monitored experiments and revised the draft.
All authors have revised the draft and approved the

final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

Abed, R.D. and Z.A. Abed. 2009. Genetic Modification of Radiation Use Efficiency In Bread Wheat. Iraqi J. Agric. Sci., 40(6): 62-77.

Abed, Z.E., R.W. Jessup, and M.H. Al-Issawi. 2018. Irrigation intervals affect dhn1 expression and some physiological parameters in stay green and non-stay-green sorghum. Biochem. Cell. Arch., 1: 1043-1047.

Adhab, M. 2021. Be smart to survive: virus-host relationships in nature. J. Microbiol. Biotechnol. Food Sci., 10(6): e3422-e3422. https://doi.org/10.15414/jmbfs.3422

Adhab, M. and J.E. Schoelz. 2024. Influence of the P6 effector protein of cauliflower mosaic virus (CaMV) on the sustained expression and subcellular localization of the CaMV movement protein. Virol., 600: 110240. https://doi.org/10.1016/j.virol.2024.110240

Adhab, M., C. Angel, S. Leisner and J.E. Schoelz. 2018. The P1 gene of cauliflower mosaic virus is responsible for breaking resistance in Arabidopsis thaliana ecotype Enkheim (En-2). Virol., 523: 15-21. https://doi.org/10.1016/j.virol.2018.07.016

Adhab, M., C. Angel, A. Rodriguez, M. Fereidouni, L. Király, K. Scheets and J.E. Schoelz. 2019. Tracing the lineage of two traits associated with the coat protein of the Tombusviridae: silencing suppression and HR elicitation in Nicotiana species. Virus, 11(7): 588. https://doi.org/10.3390/v11070588

Adhab, M., Y. Zhang and J. Schoelz. 2023. Transient expression of cauliflower mosaic virus (CaMV) P6-GFP complements a defective CaMV replicon to facilitate viral gene expression, replication and virion formation. Virology, 587: 109854. https://doi.org/10.1016/j.virol.2023.109854

Ali, A.B., O.A. Altayeb, M. Alhadi, Y. Shuang-En. 2014. Effect of different levels nitrogen and phosphorus fertilization on yield and chemical composition hybrid sunflower grown under irrigated condition. J. Environ. Agric. Sci. 2014, 7: 1-7

Al-Issawi, M., H.Z. Rihan, H. Al-Shmgani, and M.P. Fuller. 2016. Molybdenum application enhances antioxidant enzyme activity and COR15a protein expression under cold stress in wheat. J. Plant Interact., 11(1): 5-10. https://doi.org/10.1080/17429145.2015.1129074

Al-Mehemdi, A.F. and Z.A. Abed. 2016. Decision making of selection using GGE biplot. The Iraqi J. Agric. Sci., 47(1): 208-215. https://doi.org/10.36103/ijas.v47i1.622

Alogaidi F.F., Z.K. Alshugeairy, B.H. Hadi, W.A. Hassan. 2019. Effect of planting distance on yield and yield components of four introduced upland rice varieties under aerobic conditions. Plant Arch., 19(1): 699-707.

Alogaidi, F.F., Z.K. Alshugeairy, and Z.A. Abed. 2024. Genetic Variation In Enzymes And Physiological Responses Of Wheat Cultivars Under Drought Conditions. Sabrao J. Breed. Genet., 56(3): 1134-1146. https://doi.org/10.54910/sabrao2024.56.3.20

Al-Salihi, Z.K.K. and S.B. Salim. 2023. Effect of length and depth of the drip tape on wetting front advance in the root zone of sunflower using hydrus 2d/3d program. Iraqi J. Agric. Sci., 54(3): pp. 768-776. https://doi.org/10.36103/ijas.v54i3.1759

Alshugeairy Z.K., F.F. Alogaidi, B.H. Hadi, W.A. Hassan. 2023. Path coefficient analyses of introduced rice varieties under different planting distances. SABRAO J. Breed. Genet., 55(2): 516-524. https://doi.org/10.54910/sabrao2023.55.2.23

Al-Temimi, A.H. and Z.A. Abed. 2016. Evaluation The performance and stability of cowpea selected generations under drought tolerance. Iraqi J. Agric. Sci., 3 (47): 791-803. https://doi.org/10.36103/ijas.v47i3.569

Anaz, A., N. Kadhim, O. Sadoon, G. Alwan, and M. Adhab. 2023. Sustainable utilization of machine-vision-technique-based algorithm in objective evaluation of confocal microscope images. Sustainability, 15(4): 3726. https://doi.org/10.3390/su15043726

Awais, M., A. Wajid, A. Ahmad and A. Bakhsh.

2013. Narrow plant spacing and nitrogen application enhances sunflower (*Helianthus annuus* L.) productivity. Pak. J. Agric. Sci., 50(4). 689-697.

Balazadeh, S., J. Schildhauer, W.L. Araújo, S. Munné-Bosch, A.R. Fernie, S. Proost and B. Mueller-Roeber. 2014. Reversal of senescence by N resupply to N-starved *Arabidopsis thaliana*: transcriptomic and metabolomics consequences. J. Exp. Bot., 65(14): 3975-3992. https://doi.org/10.1093/jxb/eru119

Chaki, M., R. Valderrama, A.M. Fernandez-Ocana, A. Carreras, M.V. Gomez-Rodriguez, J.R. Pedrajas, and J.B. Barroso. 2011. Mechanical wounding induces a nitrosative stress by down-regulation of GSNO reductase and an increase in S-nitrosothiols in sunflower (*Helianthus annuus*) seedlings. J. Exp. Bot., 62(6): 1803-1813. https://doi.org/10.1093/jxb/erq358

Crawford N.M. 1995. Nitrate: nutrient and signal for plant growth. Plant Cell 7: 859-868. https://doi.org/10.1105/tpc.7.7.859

Crawford N.M. and D.M.A. Glass. 1998. Molecular and physiological aspect of nitrate uptake in plants. Trend. Plant Sci., 3: 389-395. https://doi.org/10.1016/S1360-1385(98)01311-9

Daniel-Vedele, F., S. Filleur, M. Caboche. 1998. Nitrate transport: a key step in nitrate assimilation. Curr. Opin. Plant. Biol., 1: 235-239 https://doi.org/10.1016/S1369-5266(98)80110-6

Dhillon, B.S., P.K. Sharma, A.K. Choudhary. 2018. Influence of staggered sown Spring sunflower (*Helianthus annuus* L.) at varying intra-row spacing and applied-N on pre-and post-anthesis N dynamics and dry matter partitioning in Indo-Gangetic Plains Region. Commun. Soil Sci. Plan., 16: 2002-2015 https://doi.org/10.1080/00103624.2018.1492602

Fageria, N.K. and V.C. Baligar. 2005. Enhancing nitrogen use efficiency in crop plants. Adv. Agron., 88: 97-185. https://doi.org/10.1016/S0065-2113(05)88004-6

Fredeen, A.L., J.A. Gamon, C.B. Field. 1991. Response of photosynthesis and carbohydrate portioning to limitations in nitrogen and water availability in filed grown sunflower. Plant Cell Environ., 14: 963-970. https://doi.org/10.1111/j.1365-3040.1991.tb00966.x

Ghazi, T.M. & Z.A. Abed. 2021. Role of selection and irrigation periods in improving some sunflower characteristics related with drought tolerance. Plant Arch., 21(1): 1681-1687. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.265

Ghazi, T.M. and Z.A. Abed. 2025. Estimation of some genetic parameters of the sunflower genotypes under drought stress. Bulgar. J. Agric. Sci., 31(2): 355-360.

Hassan W.A. 2016. Estimation of some genetic parameters of growth and yield characters of sunflower under three plant densities. Iraqi J. Agric. Sci., 47(4). https://doi.org/10.36103/ijas.v47i4.520

Hocking P.J. and B.T. Steer. 1983. Distribution of N during growth of sunflower (*Helianthus annus* L.). Ann. Bott., 51: 787-799. https://doi.org/10.1093/oxfordjournals.aob.a086530

Howarth, J.R., S. Parmar, J. Jones, D.I. Shepherd, C.E. Corol, A.M. Galster and M.J. Hawkesford. 2008. Co-ordinated expression of amino acid metabolism in response to N and S deficiency during wheat grain filling. J. Exp. Bot., 59(13): 3675-3689. https://doi.org/10.1093/jxb/ern218

Hussein N.J. and Z.K. Al-Shugeairy. 2023. Path coefficient analyses for growth and yield traits of barley under different seeding rates. IOP Conf. Series: Earth Env. Sci., 1158(6): 1-10. https://doi.org/10.1088/1755-1315/1158/6/062013

Jessup, R.W., Z.A. Abed, H.F. Najeep, and N.M. Al-Azawi. 2020. Genetic analysis of sorghum cultivars from USA using SSR markers. Plant Arch., 20(1): 1121-1125.

Koutroubas, S.D., D.K. Papakosta, A. Doitsinis. 2008. Nitrogen utilization efficiency of safflower hybrids and open-pollinated varieties under Mediterranean conditions. Field Crop. Res., 107: 56-61 https://doi.org/10.1016/j.fcr.2007.12.009

Kumara O., l.N. Venugopa, S.S. Reddy, Y.K.D. Kumar. 2003. Effect of nitrogen levels and weed mangemen on yield of sunflower. K. J. Agric. Sci., 16(3): 454-456.

Ludwig, F., D.M. Rosenthal, J.A. Johnston, N. Kane, B.L. Gross, C. Lexer and L.A. Donovan. 2004. Selection on leaf ecophysiological traits in a desert hybrid *Helianthus* species and early-generation hybrids. Evolution, 58(12): 2682-2692. https://doi.

org/10.1111/j.0014-3820.2004.tb01621.x

Mager W.H. 1988. Control of ribosomal protein gene expression. Biochem. Biophys. Acta., 949: 1-15. https://doi.org/10.1016/0167-4781(88)90048-6

Masclaux-Daubresse, C. and F. Chardon. 2011. Exploring nitrogen remobilization for seed filling using natural variation in *Arabidopsis thaliana*. J. Experimen. Bot., 62(6): 2131-2142. https://doi.org/10.1093/jxb/erq405

Massignam A.M., S.C. Chapman, G.L. Hammerc, S. Fukai. 2009. Physiological determinents of maize and sunflower grain yield as effected by nitrogen supply. Field Crop Res., 113: 256-267. https://doi.org/10.1016/j.fcr.2009.06.001

Monemurro F. and D.D. Giorgio. 2005. Quality and nitrogen use efficiency of sunflower grown at different nitrogen levels under Mediterranean conditions. J. Plant Nutr., 28(2): 335-350. https://doi.org/10.1081/PLN-20004762

Nasim, W., A. Ahmad, S. Ahmad, M. Nadeem, N. Masood, M. Shahid, M. Mubeen, G. Hoogenboom, S. Fahad. 2016. Response of sunflower hybrids to nitrogen application grown under different agro-environments. J. Plant Nutr., 40: 82-92. https://doi.org/10.1080/01904167.2016.1201492

Okab, S.I. and Z.A. Abed. 2023. Gene expression of a nitrogen tolerance gene ZmNR1 under the influence of different levels of nitrogen in maize. Bionatura, 8(1): 1-9. https://doi.org/10.21931/RB/CSS/2023.08.01.93

Okab, Sarah I. and Ziyad A. Abed. 2022. Effect of nitrogen fertilizers on growth and yield traits of maize. Iraq J. Mark. Res. Consum. Protect., 14(2): 40-49 https://doi.org/10.28936/jmracpc14.2.2022.(6)

Ranty, B. and G. Cavalie. 1982. Photosynthetic characteristics of mesophyll cells isolated from sunflower (*Helianthus annuus* L.) leaves. Photosynthes. Res., 3: 59-67. https://doi.org/10.1007/BF00030050

Saudi A.H., M.F.H. Al-Hassan M. Jw. 2016. Effect of sowing by different seeding rates on qualitative traits of four wheat (*Triticum aestivum* L.) cultivar seeds. Iraqi J. Agric. Sci., 47(2): 452-460 https://doi.org/10.36103/ijas.v47i2.587

Saudi AH. 2017. Effect of foliar spray with seaweeds extract on growth, yield, and seed vigour of bread wheat cultivars. Iraqi J. Agric. Sci., 48(5): 1313-1325. https://doi.org/10.36103/ijas.v48i5.341

Schoelz, J.E. and M. Adhab. 2021. Caulimoviruses (Caulimoviridae). In: Encyclopedia of Virology, (eds. Bamford, D.H., Zuckerman, M.). 4th ed., Academic Press, Oxford, UK, pp. 313-321. https://doi.org/10.1016/B978-0-12-809633-8.21300-9

Thavaprakash N. and L.H. Malligawad. 2002. Effect of nitrogen and phosphorus levels and ratio on yield and economic of sunflower. Res. Crop., 3(1): 40-43.

Yasir, M.A. and Z.A. Abed. 2024. Genetic Parameters and Heritability of Sunflower Genotypes as Influenced by Different Nitrogen Rates. In IOP Conference Series: Earth and Environmental Science (Vol. 1371, No. 5, p. 052051). IOP Publishing. https://doi.org/10.1088/1755-1315/1371/5/052051

Yasir, M.A. and Z.A. Abed. 2025. Genetic variability of sunflower (*Helianthus annuus* L.) genotypes under nitrogen fertilizer rates. In IOP Conference Series: Earth and Environmental Science (In Press). https://doi.org/10.1088/1755-1315/1487/1/012087

Yassen, A.A., E.F. Abdallah, M.S. Gaballah. 2011. Response of sunflower plants to nitrogen fertilizers and phytohormones under drainage water irrigation. Aust. J. Basic Appl. Sci., 9: 801-807.

Zhao, C., R. Wang, Z. Cui, L. Su, X. Sun, O. Borras-Hidalgo and L. Zhao. 2021. Effects of nitrogen application on phytochemical component levels and anticancer and antioxidant activities of *Allium fistulosum*. Peer. J., 9: e11706. https://doi.org/10.7717/peerj.11706

Zubillaga, M.M., J.P. Aristi, R.S. Lavado. 2002. Effect of phosphorus and nitrogen fertilization on sunflower (*Helianthus annuus* L.) nitrogen uptake and yield. J. Agron. Crop. Sci., 188: 267-274. https://doi.org/10.1046/j.1439-037X.2002.00570.x