Cicer arietinum abscisic acid receptor PYL1 and glycine-rich RNA-binding, abscisic acid-inducible protein-like differential expression under induced draught conditions

Heorhii Slishchuk[1*], *Nataliia* Volkova[1, 2], and *Kholmurodova* Dilafruz[3]

[1]Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, Odesa, Ukraine, 24 Maiakska doroha str., Khlibodarske, Odesa Region, 67667, Ukraine
[2]Western Caspian University, Baku, AZ1001, Azerbaijan
[3]Samarkand State Medical University, Samarkand, Uzbekistan

Abstract. Chickpea (*Cicer arietinum* L.) is a key legume crop with significant economic and nutritional importance, often cultivated in drought-prone regions. The development of drought-tolerant varieties is crucial for maintaining food security amidst the challenges posed by climate change. This study focuses on the differential expression of two key drought-responsive genes: abscisic acid receptor PYL1 and glycine-rich RNA-binding abscisic acid-inducible protein-like in two chickpea genotypes, Desi PI598080 (non-drought-tolerant) and Kabuli Flip07 318C (drought-tolerant), under both control and simulated drought stress conditions. Results showed a significant interaction between genotype and condition for abscisic acid receptor PYL1, where expression increased in the drought-tolerant genotype under stress and decreased in the non-tolerant genotype. This suggests the gene's potential role in conferring drought tolerance, making it a promising target for future molecular breeding efforts. On the other hand, no significant differences in glycine-rich RNA-binding abscisic acid-inducible protein-like gene expression were detected between genotypes or conditions, indicating its limited role in drought response. These results highlight the importance of the abscisic acid receptor PYL1 in adaptation to drought stress and provide insight into the molecular response of chickpea to drought, laying the foundation for further genetic improvement of drought tolerance in this crop.

1 Introduction

Rising temperatures and altered precipitation patterns negatively impact crop yields by increasing crop respiration rates, evapotranspiration, and pest infestations, while reducing crop duration and soil microbial activity [1-4]. Extreme weather events such as droughts and heat waves lead to significant yield anomalies and harvest failures, particularly in critical regions like North America, Asia, and Europe [5,6]. Effective adaptation requires a

* Corresponding author: geoncrfqg@gmail.com

combination of technological advancements, improved agricultural practices, and proactive policy measures to ensure sustainable food production in the face of ongoing climate change.

Chickpea (*Cicer arietinum* L.) is a vital legume crop known for its high protein content and economic importance. It is cultivated globally, often in regions prone to drought and other abiotic stresses. Given the increasing frequency of extreme weather events due to climate change, developing drought-resilient chickpea varieties is crucial for ensuring food security and sustainable agriculture. Genetic variability, genomic selection, molecular markers, and biotechnological tools have been utilized to develop drought-tolerant chickpea cultivars [7-10]. Chickpea exhibits various physiological and biochemical responses to drought, such as increased proline, sugar, glycine betaine, and antioxidant enzyme activities, which help in mitigating stress effects [8,11]. Abscisic acid (ABA) is a crucial plant hormone that plays a significant role in mediating plant responses to drought stress. It is involved in various physiological and molecular processes that enhance drought tolerance in plants. ABA acts as a signaling mediator for various stress responses, including stomatal closure, root system modulation, and activation of stress-responsive genes [12, 13]. ABA and cytokinin signalling pathways interact antagonistically to mediate drought stress responses. ABA signalling components, such as SnRK2 kinases, phosphorylate and stabilize ARR5, a negative regulator of cytokinin signalling, enhancing drought tolerance [14]. That's why this work is devoted to the problems of chickpea abscisic acid receptor PYL1 and glycine-rich RNA-binding, abscisic acid-inducible protein-like differential expression under induced draught conditions.

2 Materials and methods

For the differential expression analysis, we utilized twelve transcriptomes from two chickpea genotypes [15]: Desi PI598080, which is non-drought-tolerant, and Kabuli Flip07 318C, which is drought tolerant. Both tolerant and non-tolerant genotypes were analysed under non-treated control conditions and simulated drought conditions. Transcriptomes are shown in table 1.

XM_004512472.3 PREDICTED: *Cicer arietinum* abscisic acid receptor PYL1 (LOC101500611), mRNA and M_004507394.3 PREDICTED: *Cicer arietinum* glycine-rich RNA-binding, abscisic acid-inducible protein-like (LOC101507444), mRNA mRNAs were used as the targets for differential expression analysis, and NM_001365163.1 Cicer arietinum elongation factor 1-alpha (EF1A), mRNA was used as reference gene for counts normalisation. Bowtie2 software was used to construct mRNA indices and to align sequencing reads with the reference genome [16]. Two-way Analysis of Variance (ANOVA) was performed using R programming language to determine if there were statistically significant differences in mRNA expression levels between different genotypes and conditions for two selected targets. An ANOVA test was conducted to evaluate the main effects of genotype and condition, as well as their interaction, with a p-value below 0.05 deemed statistically significant. Following the two-way ANOVA, a post-hoc analysis was conducted to identify specific group differences using Tukey's Honest Significant Difference (HSD) test. The comparisons were reported with adjusted p-values to account for the potential inflation of Type I error due to multiple comparisons. Significant differences were determined at a threshold of $p < 0.05$. Confidence intervals that did not cross zero were interpreted as significant. All statistical analyses were performed in the R software environment (version 4.3.1) [17]. The packages used included *stats* for ANOVA and *TukeyHSD()* for post-hoc analysis.

Table 1. Transcriptomes, used to perform analysis.

Transcriptome ID	Genotype	Conditions
ERR11526165	Desi PI598080	Control
ERR11526166	Desi PI598080	Control
ERR11526167	Desi PI598080	Control
ERR11526168	Desi PI598080	Drought
ERR11526169	Desi PI598080	Drought
ERR11526170	Desi PI598080	Drought
ERR11526171	Kabuli Flip07 318C	Control
ERR11526172	Kabuli Flip07 318C	Control
ERR11526173	Kabuli Flip07 318C	Control
ERR11526174	Kabuli Flip07 318C	Drought
ERR11526175	Kabuli Flip07 318C	Drought
ERR11526176	Kabuli Flip07 318C	Drought

3 Results and Discussions

Raw count data, as well as normalised values are shown in table 2. Both genotypes exhibited a difference in abscisic acid receptor PYL1 and glycine-rich RNA-binding, abscisic acid-inducible protein-like mRNA expression levels.

For Abscisic acid receptor PYL1 mRNA differential expression, such key findings were observed (figure 1):

Genotype ($p = 0.1687$):

The p-value for the Genotype effect (0.1687) is above 0.05, so there is no statistically significant difference between the two genotypes under the combined conditions.

Condition ($p = 0.7821$):

The p-value for Condition (0.7821) is also above 0.05, indicating no significant difference between the control and stress conditions across both genotypes.

Interaction ($p = 0.0206$):

Table 2. Differential expression data.

Genotype/ Condition	EF1A, raw count	Abscisic acid receptor PYL1, raw count	Glycine-rich RNA-binding, abscisic acid-inducible protein-like, raw count	Abscisic acid receptor PYL1, normalised	Glycine-rich RNA-binding, abscisic acid-inducible protein-like, normalised
Desi PI598080 Control	70530	583	11326	0.0083	0.1606
	70793	460	6238	0.0065	0.0881
	74862	445	14257	0.0059	0.1904
Desi PI598080 Stress	63320	398	10907	0.0063	0.1723
	65516	196	9681	0.0030	0.1478
	46327	218	6743	0.0047	0.1456
Kabuli Flip07 318C Control	45256	155	4152	0.0034	0.0917
	50796	223	10863	0.0044	0.2139
	38723	58	4834	0.0015	0.1248
Kabuli Flip07 318C Stress	34869	253	5721	0.0073	0.1641
	72622	450	23359	0.0062	0.3217
	63888	260	10349	0.0041	0.1620

The interaction between Genotype and Condition has a p-value of 0.0206, which is below the 0.05 threshold. This means that the effect of Condition depends on the Genotype, indicating a statistically significant interaction. In other words, the difference between control and stress conditions differs between the Desi PI598080 and Kabuli Flip07 318C genotypes.

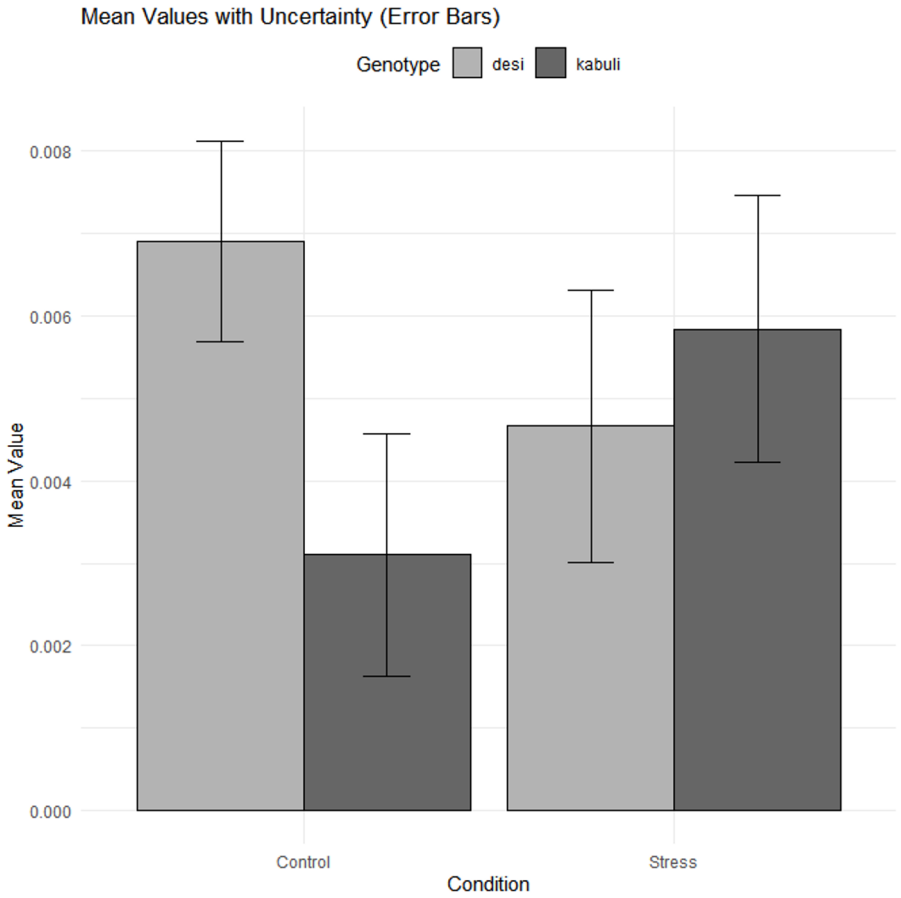

Fig. 1. Differential expression levels of Abscisic acid receptor PYL1 mRNA.

The Tukey HSD test helps to identify where the specific differences lie. For Genotype Comparison: kabuli - desi: The difference between kabuli and desi is -0.0013, but with a p-value of 0.1687, this difference is not significant.

Condition Comparison: Stress - Control: The difference between Stress and Control conditions is very small (0.0002), and the p-value (0.7821) indicates no significant difference between these conditions.

Interaction Comparison: The most notable comparison is kabuli:Control - desi:Control, which has a p-value of 0.0574, close to the 0.05 significance threshold. This suggests a borderline significant interaction where kabuli under control conditions differs from desi under control conditions, but it's not quite statistically significant at the 5% level.

The interaction plot (Figure 2) shows the nature of this interaction. The lines intersect, indicating a significant interaction. This suggests that the effect of the condition (control or stress) on the response variable differs between genotypes.

For Desi PI598080 (solid line), the value decreases from Control to Stress, meaning that expression decreases under stress.

For Kabuli Flip07 318C (dashed line), the value increases from Control to Stress, suggesting that specific mRNA expression increases under stress.

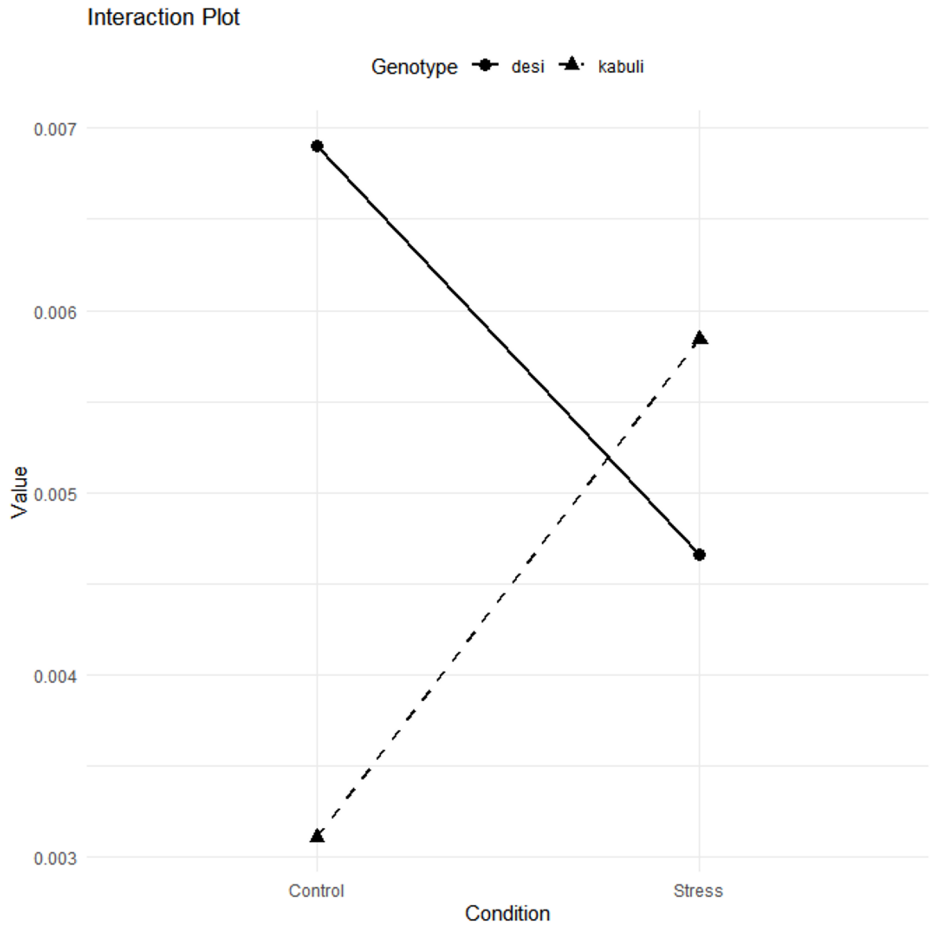

Fig. 2. Interaction plot for Abscisic acid receptor PYL1 mRNA.

For Glycine-rich RNA-binding, abscisic acid-inducible protein-like no statistically significant changes in specific mRNA expression was found for Genotype, Condition, as well as for interaction (figure 3). Genotype ($p = 0.443$): the p-value is 0.443, which is greater than the significance threshold of 0.05. This means there is no statistically significant difference between the two genotypes. Condition ($p = 0.289$): the p-value is 0.289, which is also greater than 0.05. This suggests that there is no statistically significant difference between the control and stress conditions. Genotype Interaction ($p = 0.400$): the p-value for the interaction effect between Genotype and Condition is 0.400, meaning that the combined effect of genotype and condition is not significant.

Interpretation of Tukey HSD Test:

Genotype: diff = 0.0289: The difference in means between kabuli and desi is 0.0289, but the p-value (0.443) is not significant.

The confidence interval (-0.0536, 0.1114) includes zero, reinforcing that there is no significant difference between the two genotypes.

Condition: diff = 0.0406: The difference between Stress and Control conditions is 0.0406, with a p-value of 0.289, which is not significant.

The confidence interval (-0.0419, 0.1231) includes zero, indicating no significant difference between the two conditions. Interaction (Genotype): none of the pairwise comparisons for the Genotype interaction are significant.

All the confidence intervals for the interaction comparisons include zero, further confirming the lack of significant interaction effects.

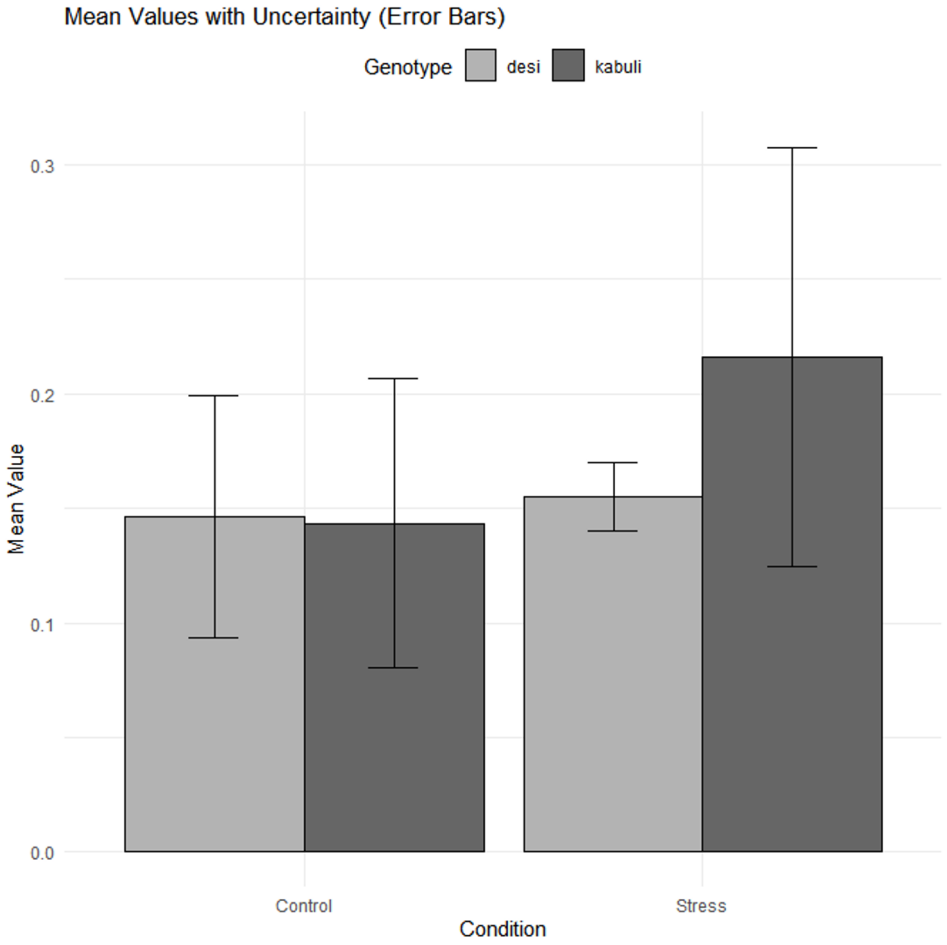

Fig. 3. Differential expression levels of Glycine-rich RNA-binding, abscisic acid-inducible protein-like mRNA.

Concerning interaction plot (figure 4), the lines are not parallel, which suggests there is an interaction between Genotype and Condition. However, the ANOVA results show this interaction was not statistically significant ($p = 0.400$). This means the interaction we see in the plot, while visually apparent, is not strong enough to be statistically meaningful.

Genotype-Specific Trends: Kabuli Flip07: Shows a stronger response to stress, with a sharp increase in the Value.

Desi PI598080: Has a relatively flat line, meaning the stress condition does not significantly impact its performance.

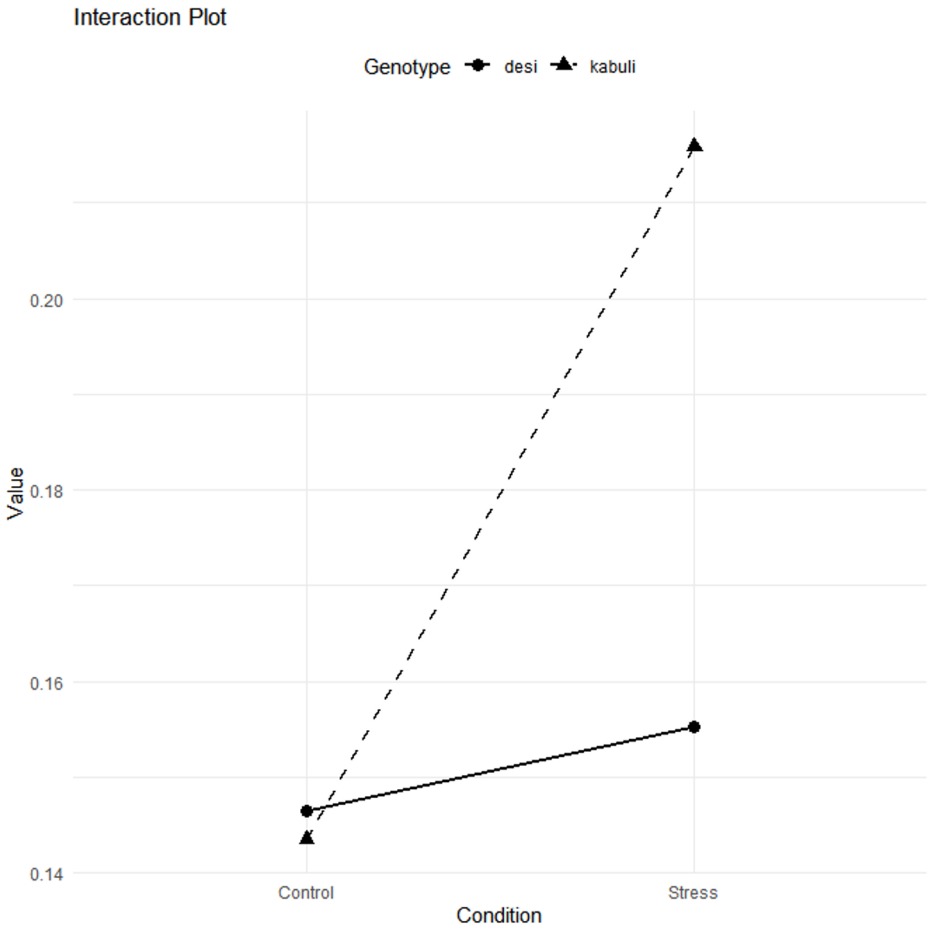

Fig. 4. Interaction plot for Glycine-rich RNA-binding, abscisic acid-inducible protein-like mRNA.

Differential expression analysis showed a key difference between abscisic acid receptor PYL1 mRNA and glycine-rich RNA-binding, abscisic acid-inducible protein-like mRNA regulation under the simulated drought stress in both susceptible and drought-tolerant genotypes. For abscisic acid receptor PYL1 mRNA, there is no statistically significant difference between the genotypes overall. Control and stress conditions also have no significant effect.

Interaction: There is a significant interaction, indicating that the effect of the condition (control or stress) on the measured value depends on the genotype. This interaction suggests that Desi PI598080 and Kabuli Flip07 318C respond differently to stress and control conditions, even though the individual effects of genotype and condition are not significant. The interaction analysis shows that Kabuli Flip07 318C, a tolerant genotype, exhibits an increase in the mRNA of the abscisic acid receptor PYL1 under drought stress conditions. In contrast, Desi PI598080, which is susceptible, shows a decrease in the expression level of PYL1 mRNA, highlighting the role of PYL1 in drought tolerance. The increased mRNA expression of the abscisic acid receptor PYL1 may be a key factor enhancing drought tolerance, suggesting this gene as a potentially important target for further research in the search for molecular markers associated with drought tolerance in chickpeas. As for glycine-rich RNA-binding, abscisic acid-inducible protein-like mRNA differential expression, no

significant differences were found between the genotypes, between the conditions, or in their interaction. The data does not provide strong evidence that either genotype performs differently under stress vs. control conditions or that one genotype outperforms the other across conditions. Although interaction analysis showed differences between Genotypes/Conditions (slight increase in glycine-rich RNA-binding, abscisic acid-inducible protein-like mRNA expression level in Kabuli Flip07 318C and almost no reaction in Desi PI598080), since none of these effects were statistically significant, the observed trends may be due to natural variation in the data rather than true differences in how the genotypes respond to the conditions.

4 Conclusions

In our study, we investigated the differential expression of abscisic acid receptor PYL1 and glycine-rich RNA-binding genes like inducible abscisic acid protein in two chickpea genotypes, Desi PI598080 (drought-sensitive) and Kabuli Flip07 318C (drought-resistant), under controlled and induced drought stress conditions. The results showed that the mRNA expression of abscisic acid receptor PYL1 demonstrated a significant genotype × condition interaction. In particular, while the expression of this gene was increased under drought stress conditions in the drought-tolerant Kabuli Flip07 318C genotype, it was decreased in the susceptible Desi PI598080 genotype, indicating its role in enhancing drought tolerance in chickpea. This indicates that the abscisic acid receptor PYL1 is a potential target for the development of drought-tolerant chickpea varieties.

In contrast, no statistically significant differences in the expression of the gene induced by abscisic acid-binding protein-like glycine-rich RNA were observed between genotypes or conditions. Although small differences in expression trends were observed, these differences were not significant enough to draw definitive conclusions.

Overall, this study highlights the importance of abscisic acid receptor PYL1 in drought response mechanisms in chickpea, providing a basis for further study of molecular markers for breeding drought-tolerant chickpea varieties. Differential expression analysis revealed a key difference between abscisic acid receptor PYL1 mRNA and abscisic acid-inducible glycine-rich RNA-binding protein-like mRNA regulation under simulated drought stress in both drought-susceptible and drought-tolerant genotypes.

References

1. G. Malhi, M. Kaur, P. Kaushik, Impact of climate change on agriculture and its mitigation strategies: A review. Sustainability **13**, 131318 (2021). https://doi.org/10.3390/SU13031318
2. J. Hatfield, K. Boote, B. Kimball, L. Ziska, R. Izaurralde, D. Ort, A. Thomson, D. Wolfe, Climate impacts on agriculture: Implications for crop production. Agron. J. **103**, 351-370 (2011). https://doi.org/10.2134/AGRONJ2010.0303
3. S. Chen, B. Gong, Response and adaptation of agriculture to climate change: Evidence from China. J. Dev. Econ. **148**, 102557 (2021). https://doi.org/10.1016/j.jdeveco.2020.102557.
4. M. Agovino, M. Casaccia, M. Ciommi, M. Ferrara, K. Marchesano, Agriculture, climate change and sustainability: The case of EU-28. Ecol. Indic. **84**, 64-71 (2019). https://doi.org/10.1016/J.ECOLIND.2018.04.064

5. E. Vogel, M. Donat, L. Alexander, M. Meinshausen, D. Ray, D. Karoly, N. Meinshausen, K. Frieler, The effects of climate extremes on global agricultural yields. Environ. Res. Lett. **14**, 083002 (2019). https://doi.org/10.1088/1748-9326/ab154b
6. J. Olesen, M. Trnka, K. Kersebaum, A. Skjelvåg, B. Séguin, P. Peltonen-Sainio, F. Rossi, J. Kozyra, F. Micale, Impacts and adaptation of European crop production systems to climate change. Eur. J. Agron. **34**, 96-112 (2011). https://doi.org/10.1016/J.EJA.2010.11.003
7. A. Rani, P. Devi, U. Jha, K. Sharma, K. Siddique, H. Nayyar, Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Front. Plant Sci. **10**, 01759 (2020). https://doi.org/10.3389/fpls.2019.01759
8. E. Karalija, C. Vergata, M. Basso, M. Negussu, M. Zaccai, M. Grossi-de-Sá, F. Martinelli, Chickpeas' tolerance of drought and heat: Current knowledge and next steps. Agronomy **12**, 2248 (2022). https://doi.org/10.3390/agronomy12102248
9. R. Asati, M. Tripathi, S. Tiwari, R. Yadav, N. Tripathi, Molecular breeding and drought tolerance in chickpea. Life **12**, 11846 (2022). https://doi.org/10.3390/life12111846
10. O. Arriagada, F. Cacciuttolo, R. Cabeza, B. Carrasco, A. Schwember, A comprehensive review on chickpea (*Cicer arietinum* L.) breeding for abiotic stress tolerance and climate change resilience. Int. J. Mol. Sci. **23**, 26794 (2022). https://doi.org/10.3390/ijms23126794
11. Y. Sree, N. Lakra, K. Manorama, Y. Ahlawat, A. Zaid, H. Elansary, S. Sayed, M. Rashwan, E. Mahmoud, Drought-induced morpho-physiological, biochemical, metabolite responses and protein profiling of chickpea (*Cicer arietinum* L.). Agronomy **13**, 71814 (2023). https://doi.org/10.3390/agronomy13071814
12. S. Ali, K. Hayat, A. Iqbal, L. Xie, Implications of abscisic acid in the drought stress tolerance of plants. Agronomy **10**, 91323 (2020). https://doi.org/10.3390/agronomy10091323
13. M. Aslam, M. Waseem, B. Jakada, E. Okal, Z. Lei, H. Saqib, W. Yuan, W. Xu, Q. Zhang, Mechanisms of abscisic acid-mediated drought stress responses in plants. Int. J. Mol. Sci. **23**, 31084 (2022). https://doi.org/10.3390/ijms23031084
14. X. Huang, L. Hou, J. Meng, H. You, Z. Li, Z. Gong, S. Yang, Y. Shi, The antagonistic action of abscisic acid and cytokinin signaling mediates drought stress response in *Arabidopsis*. Mol. Plant **11**, 970-982 (2018). https://doi.org/10.1016/j.molp.2018.05.001
15. M. Negussu, E. Karalija, C. Vergata, et al., Drought tolerance mechanisms in chickpea (*Cicer arietinum* L.) investigated by physiological and transcriptomic analysis. Sci. Direct. https://www.sciencedirect.com/science/article/abs/pii/S0098847223002836
16. B. Langmead, S. L. Salzberg, Fast gapped-read alignment with Bowtie 2. Nat. Methods **9**, 357-359 (2012). https://doi.org/10.1038/nmeth.1923
17. R Core Team, R: A language and environment for statistical computing, (R Foundation for Statistical Computing, Vienna). URL: https://www.R-project.org