J. Agric. Res. Technol., 50 (2) : 170-177 (2025) https://doi.org/10.56228/JART.2025.50207

Expression Analysis of Candidate Genes in Response to PEG Induced Osmotic Stress in Soybean [*Glycine max* (L.) Merrill]

S. S. Nikam[1*], V. P. Chimote[1], A. A. Kale[1], A. R. Aher[1] and M. P. Deshmukh[2]
[1]State Level Biotechnology Centre, Mahatma Phule Krishi Vidhypeeth, Rahuri - 413 722 (India)
[2]Agricultural Research Station, Kasbe Digraj, Sangli - 416 305 (India)
*Corresponding author E-mail : nikambiotech@gmail.com
(Received : 05.08.2024 Accepted : 24.02.2025)

Abstract

Drought stress poses a significant challenge to soybean production, necessitating the understanding of molecular mechanisms underlying stress tolerance for breeding resilient varieties. This study evaluated the expression of three drought-responsive candidate genes-*GmLEA2-1*, *GmXTH1*, and *DREB*-in seven soybean genotypes with varying tolerance levels under mild (0.5% PEG 6000) and severe (1% PEG 6000) osmotic stress, induced by immersing 3-week-old seedlings in PEG 6000-modified 1/10 strength MS nutrient solution for 18 hours. Semi-quantitative RT-PCR and spot density analysis revealed that tolerant genotypes, such as EC 538828 and EC 602288, exhibited significant upregulation of *GmLEA2-1* and *GmXTH1*, emphasizing their roles in cellular stabilization and water uptake. DREB expression varied, with tolerant genotypes maintaining consistent or enhanced expression, while sensitive genotypes, such as NRC 37, showed downregulation under stress. Notably, MACS 330, a sensitive genotype, exhibited a delayed but significant response under severe stress. The findings underscore the utility of PEG 6000 as an effective osmotic agent for simulating drought stress, while highlighting the potential of these genes as molecular markers for breeding drought-resistant soybean varieties, offering insights into improving crop resilience in water-limited environments.

Key words : Soybean, Drought, Osmotic Stress, Polyethylene glycol (PEG) 6000.

Soybean [*Glycine max* (L.) Merrill] is the world's leading oilseed crop. Soybeans are an exceptional source of complete plant-based protein, comprising 36-40% protein content and providing all essential amino acids. Soybean provides an inexpensive source of protein and fats and natural nitrogen for the soil (Foyer *et al*., 2016). Interestingly, the economic benefits derived from soybean cultivation are not just limited to the food supply; it is also an important industrial crop utilised in producing edible oils, wax, paints, dyes, and fibre (Rezaei *et al*., 2002; Raghuvanshi and Bisht, 2010). In India, the area under soybean crops for the past five decades has increased appreciably. During the kharif season of 2023, the crop was cultivated on a 118.547 lakh ha area with a productivity of 1002 kg ha^{-1}, and the production was 118.747 lakh metric tons. Madhya Pradesh, Maharashtra, and Rajasthan are the leading states in soybean cultivation (SOPA, 2023). In Maharashtra it is mainly cultivated as a rain-fed crop from June to November. Latur, Buldhana, Nanded, Osmanabad, Beed, Washim, Yeotmal, Parbhani, etc. are major soybean cultivating districts of Maharashtra (SOPA, 2023).

Soybean is a water-sensitive crop, requiring substantial water to grow and reproduce (Bhardwaj, 1986). Consequently, rising global temperatures and changing precipitation patterns pose a significant threat to soybean production, especially in under-irrigated or rainfed areas (Jin *et al*., 2017; Cotrim *et al*., 2021). Drought is the most significant abiotic threat to the agricultural productivity of soybeans throughout the world and can reduce yield by more than 40% (Specht *et al*., 1999; Purcell and Specht, 2016). The essential factor to improve drought tolerance in crops, including

soybeans, is to use available water more effectively through the growing season so that the plant's physiological activity is sustained through to maturity (Turner *et al*., 2001; Blum, 2009; Sinclair, 2018; Ye *et al*., 2018). Drought impacts soybean cultivars differently, as some cultivars are more susceptible than others (Oya *et al*., 2004; Maleki *et al*., 2013; Du *et al*., 2020; Dayoub *et al*., 2021). Also, the timing of drought stress, whether at the vegetative or the reproductive phase, is important in determining yield loss. Drought stress at vegetative stages led to reduced plant height and a decline in seed number in the early reproductive stages and reduced seed weight in late reproductive stages (Desclaux *et al*., 2000). Improving the drought resistance of soybeans and selecting new drought-resistant soybean varieties is an important way to ensure high and stable yields in soybean production (Manavalan *et al*., 2009).

To develop drought-resilient soybean lines either through genetic engineering or molecular breeding, it is necessary to identify genes whose expression is associated with drought resistance about which little is known. There is significant natural variation in soybeans' ability to withstand drought. The availability of cultivars that are susceptible and drought-tolerant at various stages of development (seedling, pre-flowering, and post-flowering) makes it easier to analyse gene expression globally and find genes that are only regulated by drought in tolerant genotypes. In order to find genes linked to drought tolerance during the seedling stage, this study aims to take advantage of the inherent heterogeneity in drought tolerance among genotypes. To accomplish this, we conducted a transcriptome analysis of gene expression in aqueous cultures of four drought-resistant, two drought-sensitive, and one moderately drought-sensitive soybean genotypes in response to drought stress generated by polyethylene glycol (PEG) (Kulkarni *et al*., 2014; Michel and Kaufmann, 1973). Our study uncovered the differential gene expression between drought-tolerant and drought-sensitive genotypes.

Materials and methods

Experimental materials : The present investigation focused on seven soybean genotypes, which include tolerant (TGX 709-50E, EC 333901, EC 538828, EC 602288), sensitive (NRC 37, MACS 330), and moderately sensitive genotypes (JS 335). All the genotypes used in the present study were collected from the Soybean Breeder, Agriculture Research Station, Kasbe Digraj, Sangli. The clean, round, and normal-sized seeds of each of the 10 genotypes were planted in plastic pots in a greenhouse for inducing osmotic stress.

Osmotic stress induction : To study the expression patterns of candidate genes, soybean genotypes were subjected to treatments simulating mild and severe osmotic stress using PEG 6000 solutions, details of which are given in Table 1. Different concentrations of PEG 6000 dissolved in a 1/10 strength modified MS nutrient solution (as given in Table 2) were tested to induce varying levels of osmotic stress, with the responses of various genotypes observed at 6, 12, 18, and 24 hours. Based on these observations, a PEG 6000 concentration of 0.5% was selected for inducing mild osmotic stress, and 1% was chosen for inducing severe osmotic stress, both applied for a duration of 18 hours overnight. To induce osmotic stress, healthy 3-week-old soybean seedlings were carefully removed from the soil, their roots washed clean, and then attached to a cardboard support before being placed in a tray containing 2 litres of 1/10 strength modified MS nutrient solution. For the control group, seedlings were submerged in this nutrient solution for 18 hours. For mild osmotic stress, a 0.5% PEG 6000 solution was prepared by dissolving 5 g of PEG 6000 in 1 litre of the nutrient solution, and

seedlings were similarly submerged for 18 hours. For severe osmotic stress, a 1% PEG 6000 solution was prepared by dissolving 10 g of PEG 6000 in 1 litre of the nutrient solution, and seedlings were treated in the same manner. After 18 hours, drought symptoms in the bottom axial leaves were observed, and these leaves were collected for RNA extraction across all treatments.

RNA isolation and cDNA synthesis : The total RNA extraction from leaf samples of control and stressed soybean plants was carried out by the Trizol method, described by Sah *et al*. (2014). The concentration and quality of RNA were checked by using a Nanodrop ND-1000 Spectrophotometer (Thermofisher Scientific, USA) and by running the product on a 1% formaldehyde agarose gel. After RNA extraction, first-strand cDNA synthesis was performed using an RTIII RT-PCR kit (GeNeiTM, Bangalore), with Oligo(dT)18 primers to ensure the transcription of polyadenylated mRNA into cDNA. The resulting cDNA served as the template for quantitative PCR (qPCR) analysis, targeting specific genes associated with osmotic stress tolerance.

Table 1. Details of PEG mediated stress treatments given to different genotypes

Treatment		
Control	**Mild Stress**	**Severe Stress**
TGX 709-50E	TGX 709-50E	TGX 709-50E
EC 333901	EC 333901	EC 333901
EC 538828	EC 538828	EC 538828
EC 602288	EC 602288	EC 602288
NRC 37	NRC 37	NRC 37
MACS 330	MACS 330	MACS 330
JS 335	-	JS 335

Table 2. Constitution of modified MS nutrient solution

Constituents of nutrient solution	Concentration (mg l^{-1})
NH_4NO_3	1650.000
KNO_3	1900.000
H_3BO_3	6.200
KH_2PO_4	170.000
KI	0.830
$Na_2MoO_4.2H_2O$	0.250
$CoCl_2.2H_2O$	0.025
$CaCl_2.2H_2O$	440.000
$MgSO_4.7H_2O$	370.000
$MnSO_4.7H_2O$	22.300
$ZnSO_4.7H_2O$	8.600
CuSO4.5H2O	0.025
Na_2EDTA	37.250
$FeSO_4.7H_2O$	27.850

Candidate genes and primer designing: Candidate genes responsible for drought stress tolerance were identified through literature search, details of which are given in Table 3. The nucleotide sequences (FASTA sequences) of CDS of selected candidate genes responsible for drought stress tolerance were retrieved from the Glycine max Wm82 genome assembly version 4 (glyma.Wm82.gnm4) at SoyBase. The primers were designed using the primer-BLAST tool at NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) with 16 to 25 base lengths. All the gene-specific primers designed and used in the present study were synthesised by GeNeiTM, Bangalore.

Semi-quantitative RT-PCR : Semi-quantitative RT-PCR was performed using a 10-fold diluted cDNA product in a PCR reaction mixture (12 µl) that contained 2 µl template cDNA (30 ng μl^{-1}), Taq buffer B (1 µl), $MgCl_2$ (0.8 µl), 1 µl of dNTP (2.5 mM), Taq DNA polymerase (0.2 µl), 0.5 µl of forward and reverse primer (25 picomoles μl^{-1}), and sterile distilled water (6 µl). Amplification was performed in 0.2 ml (each tube) thin-walled PCR tubes in an Eppendorf thermal cycler. The PCR regime comprised an initial incubation at 94.0°C for 5 min and was then subjected to 40 cycles: 94.0°C for 30 sec, 45.5-60°C for 45 sec, and 72.0°C for 1 min, and a final extension was

carried out at 72.0°C for 7 min. The PCR amplification was carried out using 3 primers, and its products were evaluated on 3% agarose gel stained with ethidium bromide and visualised under a UV transilluminator.

Analysis of gene expression by spot density measurement method : The transcript abundance was measured by 'GelQuant.NET' software version 1.8.2. by BioChemLab Solutions. The gel-captured image was subjected to spot density analysis, and each band was quantified in the form of integrated density value (IDV). The transcript abundance of a target gene in response to induced osmotic stress was compared with the expression of that gene in control.

Results

***RNA* isolation :** Total RNA was extracted from the leaves of soybean plants subjected to control, mild, and severe osmotic stress treatments. The RNA extraction was performed using Trizol reagent, a widely used method that efficiently isolates high-quality RNA. The purity and integrity of the RNA samples were assessed using a Nanodrop spectrophotometer, which showed high-quality, non-degraded RNA, suitable for downstream applications like quantitative PCR (qPCR).

Further validation of RNA integrity was carried out by running the samples on a 1% formaldehyde agarose gel, which revealed two distinct bands corresponding to the 18S and 28S rRNA. These bands were sharp and free of degradation, confirming the integrity of the RNA and its suitability for gene expression studies. The yield of total RNA varied across genotypes, ranging from 850 ng μl^{-1} to 1500 ng μl^{-1}, which is typical for high-quality RNA extraction from soybean leaves.

Gene expression analysis : The expression of three key drought-responsive genes-*GmLEA2-1*, *GmXTH1*, and *DREB* was measured across control, mild, and severe osmotic stress treatments. The primers for each gene were designed to amplify the respective genes, ensuring accurate and specific amplification. The expression levels were quantified relative to the control condition (no stress), and fold changes in gene expression were calculated to determine the impact of osmotic stress.

The GmLEA2-1 gene, which encodes a late embryogenesis abundant protein, is known to play a critical role in enhancing plant tolerance to water deficit by stabilising cellular structures and accumulating osmoprotectants like proline. Gene expression analysis revealed significant changes in the expression levels of GmLEA2-1 under osmotic stress. For example, in the drought-tolerant genotype EC 538828, the expression increased by 1.93-fold under mild drought and 1.55-fold under severe drought, indicating its robust response to stress. Similarly, EC 602288, another tolerant genotype, exhibited a 1.09-fold increase under mild drought and a 1.48-fold increase under severe drought. Conversely, the sensitive genotype NRC 37 showed a substantial reduction in GmLEA2-1 expression under mild drought (0.44-fold decrease), though a slight recovery was observed under severe drought (1.12-fold increase), suggesting that it could adapt to extreme stress but with a less effective early response. MACS 330, another sensitive genotype, displayed a similar trend, with a modest reduction in expression under mild drought (0.89-fold decrease) but a pronounced increase (1.75-fold) under severe drought, indicating a delayed but significant response under extreme drought conditions (Fig. 1).

The *GmXTH1* gene, which encodes a xyloglucan endotransglycosylase/hydrolase involved in cell wall loosening and water uptake,

showed significant variability in its expression across different genotypes under drought conditions. The drought-tolerant genotype EC 602288 exhibited a dramatic increase in expression, with a 3.16-fold increase under mild drought and an even more pronounced 5.48-fold increase under severe drought. This indicates that *GmXTH1* plays a key role in the drought tolerance mechanisms of this genotype by enhancing root growth and water absorption under stress. In contrast, the sensitive genotype NRC 37 showed a marked reduction in expression under mild drought (0.42-fold decrease) and a slight recovery under severe drought (0.70-fold increase), indicating that this genotype struggles to adapt to osmotic stress at the transcriptomic level. The genotype MACS 330, while showing reduced expression under mild drought (0.75-fold decrease), demonstrated a recovery in expression under severe drought (1.21-fold increase), highlighting a delayed but effective response mechanism to severe stress conditions (Fig. 2).

The DREB gene family, known for its role in binding dehydration-responsive elements in the promoters of drought-responsive genes, was also assessed. TGX 709-50E, a drought-tolerant genotype, showed a significant upregulation of DREB expression with a 2.26-fold increase under mild drought, though a slight reduction (0.98-fold) was observed under severe drought, suggesting a strong initial response to osmotic stress that weakens under more extreme conditions. The genotype EC 333901 exhibited a steady upregulation of DREB, with a 1.22-fold increase under mild drought and a 1.25-fold increase under severe drought, indicating stable drought tolerance and a sustained response to stress. Interestingly, EC 602288 showed a 0.86-fold decrease in DREB expression under mild drought but a significant 2.59-fold increase under severe drought, reflecting its adaptive response mechanism that becomes more pronounced as drought conditions intensify. On the other hand, the sensitive genotype NRC 37 showed substantial reductions in DREB expression under both mild (0.58-fold decrease) and severe drought (0.35-fold decrease), confirming its sensitivity to osmotic stress at the gene expression level (Fig. 3).

Interpretation of gene expression patterns : The differential expression of these drought-responsive genes provides valuable insights into how various genotypes cope with water deficit. Tolerant genotypes like EC 602288, EC 538828, and TGX 709-50E generally exhibited upregulation of GmLEA2-1, GmXTH1, and DREB under both mild and severe osmotic stress, highlighting their ability to enhance cellular stability, improve water uptake, and activate stress-responsive pathways. These genotypes also demonstrated more stable and consistent expression profiles, suggesting their suitability for breeding drought-resistant varieties. In contrast, sensitive genotypes such as NRC 37 exhibited reduced expression of these key genes, particularly under mild osmotic stress, suggesting their inability to effectively manage water deficit in early stages. However, some sensitive genotypes, like MACS 330, showed recovery and increased expression under severe drought, indicating that while they are less equipped for early drought responses, they can still mount a significant stress response at later stages.

Table 3. List of genes studied in the present study

Candidate gene	Gene id	References
Dehydration responsive element binding protein gene (DREB)	Glyma.13g304300	Zhou *et al.*, (2020)
Endo-xyloglucan transferase 1 gene (XTH1)	Glyma.16g045000	Zhang *et al.*, (2021)
Late-embryogenesis abundant protein 1 gene 2 (LEA-2)	Glyma.20g044800	Wang *et al.*, (2018)

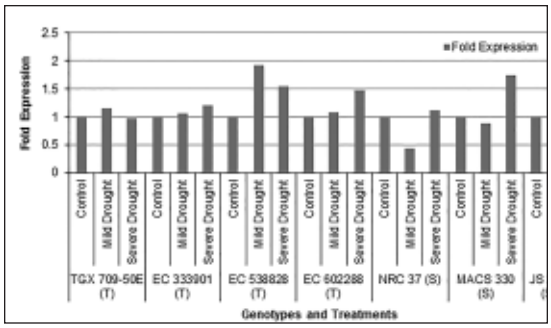

Fig. 1. Fold Expression of *GmLEA2-1* over control

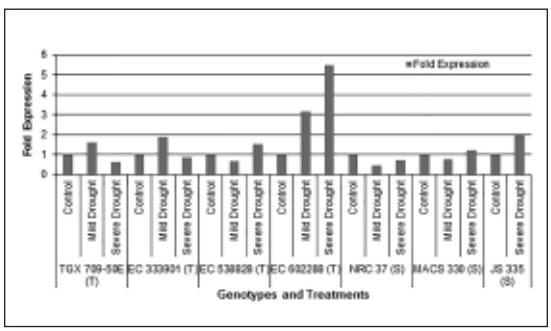

Fig. 2. Fold Expression of *GmXTH1* over control

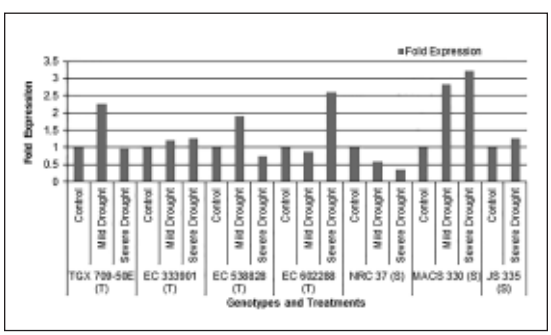

Fig. 3. Fold Expression of *DREB* over control

Conclusions

In conclusion, this study highlights the critical role of genes like *GmLEA2-1*, *GmXTH1*, and *DREB* in the osmotic stress tolerance of soybean. These genes are critical in regulating cellular responses, including water retention, cell wall modification, and the activation of stress-related pathways. The findings underscore the potential of these genes as biomarkers for drought resistance, offering a valuable foundation for future breeding programs aimed at improving drought resilience in crops. The study also demonstrated that PEG induced osmotic stress is a reliable and reproducible method for simulating drought conditions in soybean, providing a robust experimental platform for evaluating drought responses in various genotypes.

Future Research Directions : While this study lays the groundwork for understanding the genetic basis of drought tolerance in soybean, further research is needed to explore the functional validation of the identified genes. Future studies should focus on transgenic approaches, where genes like *GmLEA2-1*, *GmXTH1*, and *DREB* can be overexpressed or silenced to confirm their direct role in improving drought resistance. Additionally, studies examining the synergistic effects of multiple drought-responsive genes in transgenic plants could provide a more comprehensive understanding of how these genes interact to enhance drought tolerance. Additionally, genetic mapping and genome-wide association studies (GWAS) could be employed to identify other potential candidate genes associated with drought resistance in soybean. These approaches will help to further unravel the complex genetic networks governing drought tolerance and allow breeders to incorporate a broader range of genes into their selection criteria.

Finally, given the complexity of drought tolerance, integrating these genetic findings with phenotypic data on yield, water-use efficiency, and plant growth under field conditions will be essential for developing soybean varieties that are both drought-resistant and high-yielding. This integration will ensure that the genetic improvements made in controlled environments translate into real-world benefits for farmers.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

We are very thankful to the Post Graduate Institute and State Level Biotechnology Centre, MPKV, Rahuri, for providing all facilities and research grants to carry out this research work and to the authors cited in references for providing necessary literature.

References

Bhardwaj, S. F. 1986. Consumptive use and water requirement of soybeans. Journal of Irrigation and Drainage Engineering,112(2), 157-163.

Blum, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Research, 112(2-3), 119-123.

Cotrim, M. F., Gava, R., Campos, C. N. S., De David, C. H. O., De Assis Reis, I., Teodoro, L. P. R., and Teodoro, P. E. 2020. Physiological performance of soybean genotypes grown under irrigated and rainfed conditions. Journal of Agronomy and Crop Science, 207(1), 34-43.

Dayoub, E., Lamichhane, J. R., Schoving, C., Debaeke, P. and Maury, P. 2021. Early-stage phenotyping of root traits provides insights into the drought tolerance level of soybean cultivars. Agronomy 11(1), 188.

Desclaux, D., Huynh, T. T., and Roumet, P. 2000. Identification of soybean plant characteristics that indicate the timing of drought stress. Crop Science, 40(3), 716–722.

Du, Y., Zhao, Q., Chen, L., Yao, X., and Xie, F. 2020. Effect of drought stress at reproductive stages on growth and nitrogen metabolism in soybean. Agronomy 10(2), 302.

Foyer, C. H., Lam, H.-M., Nguyen, H. T., Siddique, K. H., Varshney, R. K., Colmer, T. D., *et al.* 2016. Neglecting legumes has compromised human health and sustainable food production. Nature plants. 2(8).

Jin, Z., Zhuang, Q., Wang, J., Archontoulis, S. V., Zobel, Z. and Kotamarthi, V. R. 2017. The combined and separate impacts of climate extremes on the current and future US rainfed maize and soybean production under elevated CO_2. Global Change Biology, 23(7), 2687-2704.

Kulkarni, S., Hongal, S., and Shoba, N. 2014. Standardization of optimal concentration of PEG 6000 for induction of drought and screening of coriander (*Coriandrum sativum* L.) genotypes. The Asian Journal of Horticulture, 9(1), 100-105.

Maleki, A., Naderi, A., Naseri, R., Fathi, A., Bahamin, S. and Maleki, R. 2013. Physiological performance of soybean cultivars under drought stress. Bulletin of Environment, Pharmacology and. Life Sciences,2(6), 38-44.

Manavalan, L. P., Guttikonda, S. K., Tran, L. S. and Nguyen, H. T. 2009. Physiological and molecular approaches to improve drought resistance in soybean. Plant and Cell Physiology, 50(7), 1260-1276.

Michel, B. E. and Kaufmann, M. R. 1973. The osmotic potential of polyethylene glycol 6000. Plant physiology, 51(5), 914-916.

Oya, T., Nepomuceno, A. L., Neumaier, N., Farias, J. R. B., Tobita, S. and Ito, O. 2004. Drought tolerance characteristics of Brazilian soybean cultivars- Evaluation and characterization of drought tolerance of various Brazilian soybean cultivars in the field. Plant. Prod. Sci. 7(2), 129-137.

Purcell, L. C. and Specht, J. E. 2016. Physiological traits for ameliorating drought stress. In Agronomy monograph/Agronomy (pp. 569-620).

Raghuvanshi, R. S. and Bisht, K. 2010. Uses of soybean: products and preparation. In CABI eBooks (pp. 404-426).

Rezaei, K., Wang, T., and Johnson, L. A. 2002. Hydrogenated vegetable oils as candle wax. Journal of the American. Oil Chemists Society, 79(12), 1241-1247.

Sah, S. K., Kaur, G. and Kaur, A. 2014. Rapid and Reliable Method of High-Quality RNA Extraction from Diverse Plants. American Journal of Plant Sciences, 5(21), 3129-3139.

Sinclair, T. R. 2018. Effective water use required for improving crop growth rather than transpiration efficiency. Frontiers in Plant Science 9, 1442.

SOPA (The Soybean Processors Association of India) 2023. Soybean hectares planted SOPA. https://www.sopa.org/statistics/soybean-hectares-planted/.

Specht, J. E., Hume, D. J. and Kumudini, S. V. 1999 Soybean yield potential-A genetic and Physiological perspective. Crop Science, 39(6), 1560-1570.

Turner, N. C., Wright, G. C. and Siddique, K. 2001. Adaptation of grain legumes (pulses) to water-limited environments, In Advances in Agronomy (pp. 193-231).

Wang, Z., Yang, Q., Shao, Y., Zhang, B., Feng, A., Meng, F. and Li, W. 2018. *GmLEA2-1*, a late embryogenesis abundant protein gene isolated from soybean (*Glycine max* (L.) Merr.), confers tolerance to abiotic stress. Acta Biologica Hungarica, 69(3), 270-282.

Ye, H., Roorkiwal, M., Valliyodan, B., Zhou, L., Chen, P., Varshney, R. K., and Nguyen, H. T. 2018. Genetic diversity of root system architecture in response to drought stress in grain legumes, Journal of Experimental Botany, 69(13), 3267-3277.

Zhang, Y., Zhang, H., Fu, J., Du, Y., Qu, J., Song, Y., & Wang, P. 2021. The *GmXTH1* gene improves drought stress resistance of soybean seedlings. Molecular Breeding, 42(1).

Zhou, Y., Zhou, W., Liu, H., Liu, P. and Li, Z. 2020. Genome wide analysis of the soybean DREB gene family: Identification, genomic organization and expression profiles in response to drought stress. Plant Breeding, 139(6), 1158-1167.