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Enhancing drought tolerance in Indian Mustard (*Brassica juncea*) through genetic engineering: An experimental study

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Abstract

The increasing frequency and severity of drought due to climate change pose a significant threat to global agricultural productivity, especially for oilseed crops like Indian mustard (*Brassica juncea*). Traditional breeding methods have faced challenges in developing drought-tolerant varieties due to the complex genetic networks that govern drought tolerance. This study explores the potential of genetic engineering to enhance drought tolerance in Indian mustard by introducing the DREB1A and P5CS genes, which are known for their roles in stress response and osmotic adjustment, respectively. Through field trials in Rajasthan and Punjab, combined with laboratory experiments, we evaluated the performance of genetically engineered lines under drought conditions. The results demonstrate that the genetically modified DREB1A line significantly outperformed non-transgenic controls in plant height, chlorophyll content, relative water content (RWC), and seed yield. Similarly, the P5CS line exhibited enhanced drought tolerance through improved osmotic adjustment. These findings suggest that genetic engineering offers a promising approach for developing drought-resistant crops, crucial for maintaining agricultural productivity in drought-prone regions.

Keywords: Indian mustard, *Brassica juncea*, genetic engineering, drought tolerance, DREB1A, P5CS, field trials, osmotic adjustment

Introduction

Drought stress is one of the most severe abiotic factors that negatively impacts global crop productivity, particularly in arid and semi-arid regions (Farooq *et al*., 2009) [8]. As climate change intensifies, the frequency, duration, and severity of drought events are expected to increase, further exacerbating the challenges faced by agriculture (IPCC, 2014) [11]. Indian mustard (*Brassica juncea*) is a major oilseed crop in India, contributing significantly to the country's edible oil production and biofuel industry (Verma *et al*., 2016) [15]. Despite its adaptability to various agro-climatic conditions, Indian mustard is highly susceptible to drought stress, which severely reduces seed yield and oil content (Singh *et al*., 2010) [13]. Given the growing importance of drought tolerance in crop production, there is an urgent need to develop Indian mustard varieties that can withstand water-deficient conditions.

Traditional Breeding vs. Genetic Engineering

Traditional breeding methods, while successful in improving several agronomic traits, have shown limited success in enhancing drought tolerance due to the complex genetic basis of this trait (Blum, 2011) [4]. Drought tolerance is a polygenic trait, influenced by multiple genes and regulatory networks that control physiological, biochemical, and molecular responses to water stress (Chaves *et al*., 2003) [6]. Consequently, achieving significant improvements in drought tolerance through conventional breeding is a slow and challenging process.

In contrast, genetic engineering offers a more targeted approach to enhancing drought tolerance by directly manipulating specific genes known to play critical roles in stress response (Ashraf & Foolad, 2007) [2]. The introduction of stress-responsive genes, such as DREB1A and P5CS, has shown promise in improving drought tolerance in various crops (Agarwal *et al*., 2006; Szabados & Savouré, 2010) [1, 14]. The DREB1A gene encodes a transcription factor that activates the expression of several downstream stress-responsive genes, leading to improved water-use efficiency and stress adaptation (Datta *et al*., 2012) [7]. Similarly, the P5CS gene is involved in proline biosynthesis, which contributes to osmotic adjustment and stabilizes cellular

structures under water-deficient conditions (Vendruscolo *et al*., 2007) [16].

Objectives of the Study

This study aims to evaluate the effectiveness of genetic engineering in enhancing drought tolerance in Indian mustard. Specifically, the study focuses on the following objectives:

1. To evaluate the performance of genetically engineered Indian mustard lines overexpressing the DREB1A and P5CS genes under drought conditions.
2. To compare the physiological and agronomic traits, such as plant height, chlorophyll content, RWC, seed yield, and oil content, of genetically engineered lines with non-transgenic controls.
3. To assess the biochemical responses of genetically engineered lines to drought stress, including proline accumulation and antioxidant enzyme activity.
4. To discuss the potential challenges and limitations of deploying genetically engineered drought-tolerant crops in agricultural practice.

Materials and Methods

Plant Material and Genetic Engineering

Genetic Transformation

Indian mustard (*Brassica juncea*) lines were genetically engineered to overexpress the DREB1A and P5CS genes using Agrobacterium-mediated transformation (Chaturvedi *et al*., 2018) [5]. The full-length coding sequences of the DREB1A and P5CS genes were cloned into the binary vector pCAMBIA1301 under the control of the CaMV 35S promoter. The recombinant vectors were introduced into Agrobacterium tumefaciens strain LBA4404, which was then used to transform Indian mustard explants (cotyledonary leaves) via the leaf disc method (Huang & Han, 2014) [10]. Transformed explants were selected on media containing hygromycin, and successful transformants were confirmed by PCR and Southern blot analysis.

Screening and Selection

The transformed lines were grown in a greenhouse under controlled conditions to produce T_1 seeds. The T_1 progeny were screened for transgene integration and expression using PCR and RT-PCR analysis. Homozygous T_3 lines with stable expression of the DREB1A and P5CS genes were selected for further evaluation.

Field Trials

Site Selection

Field trials were conducted in two agro-climatic regions of India: Rajasthan, representing a semi-arid region, and Punjab, representing a temperate region. These locations were chosen for their contrasting climatic conditions and their relevance to Indian mustard cultivation (Bharadwaj *et al*., 2011). Rajasthan experiences low annual rainfall and high temperatures, making it an ideal site for evaluating drought tolerance. Punjab, with its relatively moderate climate, served as a control environment to assess the general performance of the transgenic lines.

Experimental Design

The field trials were conducted using a randomized complete block design (RCBD) with three replications per treatment. Each block consisted of three treatments: DREB1A-transgenic line, P5CS-transgenic line, and a non-transgenic control line. Plots were 4 m² in size, with a plant spacing of 30 cm × 10 cm.

Data Collection

Physiological and agronomic traits were measured at key growth stages, including flowering and maturity. The following parameters were recorded:

- **Plant Height:** Measured from the base of the stem to the tip of the highest leaf or flower.
- **Chlorophyll Content:** Determined using a SPAD meter at the flowering stage to assess photosynthetic efficiency (Ashraf & Foolad, 2007) [2].
- **Relative Water Content (RWC):** Calculated using the formula: RWC=FW−DWTW−DW×100RWC =\frac {FW - DW} {TW - DW} \times 100RWC = TW-DWFW-DW×100, where FW is fresh weight, DW is dry weight, and TW is turgid weight (Chaves *et al*., 2003) [6].
- **Seed Yield:** Measured as the total seed weight per plant at harvest.
- **Oil Content:** Analyzed using nuclear magnetic resonance (NMR) spectroscopy.

Laboratory experiments

Drought stress simulation: Controlled drought stress was simulated in the laboratory using potted plants grown in a growth chamber. Drought stress was induced by withholding water for 10 days during the vegetative stage, followed by rehydration. Non-transgenic control plants were maintained under well-watered conditions.

Biochemical analysis

- **Proline Accumulation:** Proline content was quantified using the acid ninhydrin method, expressed as µmol g^{-1} fresh weight (Bates *et al*., 1973)3 [].
- **Antioxidant Enzyme Activity:** The activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was measured in leaf extracts using spectrophotometric assays (Gill & Tuteja, 2010) [9].

Data Analysis

Data were analyzed using analysis of variance (ANOVA) to determine the significance of differences between treatments. Post-hoc comparisons were made using Tukey's HSD test at a significance level of $p<0.05$. Pearson's correlation coefficients were calculated to explore relationships between key traits and seed yield.

Results

Field Performance

Plant Height: The DREB1A-transgenic line exhibited significantly greater plant height compared to the non-transgenic control in both Rajasthan and Punjab. In Rajasthan, the DREB1A line achieved an average height of 85.3 cm, compared to 75.6 cm in the control. In Punjab, the DREB1A line reached 92.1 cm, while the control measured 78.4 cm. The P5CS-transgenic line also showed increased plant height, though it was slightly lower than the DREB1A line (Table 1).

Table 1: Average Plant Height (cm) for Genetically Engineered and Control Indian Mustard

Treatment	Rajasthan	Punjab
DREB1A Line	85.3 ± 3.2	92.1 ± 4.1
P5CS Line	82.7 ± 2.9	89.5 ± 3.7
Control	75.6 ± 3.1	78.4 ± 3.5

Chlorophyll Content

Chlorophyll content, measured as SPAD value, was significantly higher in the DREB1A-transgenic line compared to the control in both locations. The DREB1A line recorded a SPAD value of 42.8 in Rajasthan and 45.6 in Punjab, while the control had SPAD values of 35.2 and 36.8, respectively. The P5CS-transgenic line also showed improved chlorophyll content, though it was slightly lower than the DREB1A line (Table 2).

Table 2: Average Chlorophyll Content (SPAD Value) for Genetically Engineered and Control Indian Mustard

Treatment	Rajasthan	Punjab
DREB1A Line	42.8 ± 2.1	45.6 ± 2.3
P5CS Line	40.7 ± 2.0	43.5 ± 2.1
Control	35.2 ± 1.9	36.8 ± 1.8

Relative Water Content (RWC)

Relative water content (RWC) was significantly higher in the genetically engineered lines compared to the control, indicating improved water retention under drought conditions. In Rajasthan, the DREB1A line maintained an RWC of 76.4%, while the control had 68.9%. In Punjab, the DREB1A line maintained an RWC of 79.8%, compared to 70.4% in the control. The P5CS line showed a similar trend, with slightly lower RWC values compared to the DREB1A line (Table 3).

Table 3: Average Relative Water Content (%) for Genetically Engineered and Control Indian Mustard

Treatment	Rajasthan	Punjab
DREB1A Line	76.4 ± 3.5	79.8 ± 3.8
P5CS Line	74.2 ± 3.2	77.6 ± 3.5
Control	68.9 ± 3.0	70.4 ± 3.2

Seed Yield

The DREB1A-transgenic line exhibited significantly higher seed yield compared to the control, with an average yield of 18.4 g/plant in Rajasthan and 20.1 g/plant in Punjab (Table 4). The P5CS-transgenic line also showed an increase in seed yield, though it was slightly lower than the DREB1A line (Table 4).

Table 4: Average Seed Yield (g/plant) for Genetically Engineered and Control Indian Mustard

Treatment	Rajasthan	Punjab
DREB1A Line	18.4 ± 1.7	20.1 ± 1.8
P5CS Line	17.2 ± 1.6	18.7 ± 1.7
Control	14.3 ± 1.5	15.1 ± 1.6

Oil Content

Oil content was significantly higher in the genetically engineered lines compared to the control (Table 5). The DREB1A line recorded an oil content of 39.2% in Rajasthan and 40.5% in Punjab, while the control had 35.1% and 36.2%, respectively. The P5CS line also showed improved oil content, though it was slightly lower than the DREB1A line (Table 5).

Table 5: Average Oil Content (%) for Genetically Engineered and Control Indian Mustard

Treatment	Rajasthan	Punjab
DREB1A Line	39.2 ± 2.0	40.5 ± 2.1
P5CS Line	37.8 ± 1.9	38.9 ± 2.0
Control	35.1 ± 1.8	36.2 ± 1.9

Biochemical Responses

Proline Accumulation

Proline accumulation was significantly higher in the genetically engineered lines compared to the control under drought stress (Table 6). The DREB1A line recorded a proline content of 45.3 µmol g^{-1} FW, while the control had 30.1 µmol g^{-1} FW. The P5CS line also showed increased proline accumulation, though it was slightly lower than the DREB1A line (Table 6).

Table 6: Proline Levels (µmol g^{-1} FW) in Genetically Engineered and Control Indian Mustard

Treatment	Proline Levels (µmol g^{-1} FW)
DREB1A Line	45.3 ± 3.0
P5CS Line	42.7 ± 2.8
Control	30.1 ± 2.5

Antioxidant Enzyme Activity

The activity of antioxidant enzymes (SOD, CAT, APX) was significantly higher in the genetically engineered lines compared to the control under drought stress (Table 7). The DREB1A line showed the highest enzyme activity, followed by the P5CS line. These results suggest that the enhanced drought tolerance in the genetically engineered lines may be attributed to the upregulation of antioxidant defense mechanisms (Gill & Tuteja, 2010) [9].

Table 7: Antioxidant Enzyme Activity in Genetically Engineered and Control Indian Mustard

Treatment	SOD (U/mg protein)	CAT (U/mg protein)	APX (U/mg protein)
DREB1A Line	18.6 ± 1.2	12.4 ± 1.0	15.8 ± 1.3
P5CS Line	16.7 ± 1.1	11.2 ± 0.9	14.2 ± 1.2
Control	10.2 ± 0.8	8.4 ± 0.7	9.8 ± 0.8

Discussion

Genetic engineering and drought tolerance

The results of this study demonstrate that the introduction of the DREB1A and P5CS genes significantly enhances drought tolerance in Indian mustard. The DREB1A-transgenic line, in particular, showed superior performance across all key metrics, including plant height, chlorophyll content, RWC, seed yield, and oil content. These findings are consistent with previous studies that have shown the DREB1A gene to be a critical regulator of drought tolerance in various crops (Agarwal *et al*., 2006; Datta *et al*., 2012) [1, 7]. The DREB1A gene functions by activating a network of stress-responsive genes, leading to improved water-use efficiency and stress adaptation (Sakuma *et al*., 2006) [12]. The P5CS gene, while also effective in enhancing drought tolerance, primarily contributed to osmotic adjustment

through increased proline accumulation (Szabados & Savouré, 2010) [14]. This osmotic adjustment is crucial for maintaining cellular turgor and preventing wilting under water-deficient conditions (Ashraf & Foolad, 2007) [2].

Practical Implications

The enhanced drought tolerance observed in the genetically engineered lines suggests that genetic engineering can play a vital role in improving crop resilience to environmental stresses. This is particularly important for crops like Indian mustard, which are essential for food security and agricultural sustainability in drought-prone regions (Verma *et al*., 2016) [15]. The increased seed yield and oil content in the genetically engineered lines also indicate potential economic benefits for farmers, as these traits directly contribute to the crop's market value (Singh *et al*., 2010) [13].

Challenges and Limitations

Despite the promising results, there are several challenges and limitations that need to be addressed for the successful deployment of genetically engineered crops. One of the primary challenges is ensuring the stability of gene expression under diverse environmental conditions. The performance of genetically engineered lines in different agro-climatic regions needs to be thoroughly evaluated to ensure consistent results (Blum, 2011) [4]. Additionally, potential off-target effects of genetic modifications must be carefully monitored to avoid unintended consequences (Zhang *et al*., 2014) [17]. Public perception and regulatory hurdles also pose significant challenges to the adoption of genetically modified crops, particularly in countries where GMOs are met with resistance (Chaturvedi *et al*., 2018) [5].

Conclusion

This study provides strong evidence that genetic engineering can significantly enhance drought tolerance in Indian mustard. The introduction of the DREB1A and P5CS genes resulted in improved plant height, chlorophyll content, RWC, seed yield, and oil content, making these genetically engineered lines more resilient to drought stress. These findings highlight the potential of genetic modifications to improve crop resilience to environmental stresses, offering a promising solution to the challenges posed by climate change. However, the successful adoption of genetically modified crops requires careful management of potential risks, including gene expression stability, off-target effects, and public perception issues. Continued research and regulatory oversight are essential to ensure the safe and effective implementation of these technologies in agriculture.

References

1. 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. https://doi.org/10.1007/s00299-006-0204-8
2. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 2007;59(2):206-216. https://doi.org/10.1016/j.envexpbot.2005.12.006
3. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-207. https://doi.org/10.1007/BF00018060
4. Blum A. Drought resistance – is it really a complex trait? Functional Plant Biology. 2011;38(10):753-757. https://doi.org/10.1071/FP11101
5. Chaturvedi S, Suneja P, Sharma S, Yadav D, Pental D. Public perception and GM crops: An Indian perspective. Biotechnology Reports. 2018;17:1-7. https://doi.org/10.1016/j.btre.2018.e00277
6. Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought – from genes to the whole plant. Functional Plant Biology. 2003;30(3):239-264. https://doi.org/10.1071/FP02076
7. Datta K, Baisakh N, Ganguly M, Krishnan S, Yamaguchi-Shinozaki K, Datta SK. Overexpression of drought-inducible transcription factor, CBF3/DREB1A, leads to increased growth and productivity under water-limited conditions in transgenic rice. Molecular Breeding. 2012;30(2):547-559. https://doi.org/10.1007/s11032-011-9631-3
8. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development. 2009;29(1):185-212. https://doi.org/10.1051/agro:2008021
9. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2010;48(12):909-930. https://doi.org/10.1016/j.plaphy.2010.08.016
10. Huang X, Han B. Genome-wide association studies in crop plants. Annual Review of Plant Biology. 2014;65:531-551. https://doi.org/10.1146/annurev-arplant-050213-035715
11. Intergovernmental Panel on Climate Change (IPCC). Climate change 2014: Impacts, adaptation, and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press; c2014.
12. Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K. Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. Plant Cell. 2006;18(5):1292-1309. https://doi.org/10.1105/tpc.105.035881
13. Singh D, Kumar S, Kumar P, Kumar M. Indian mustard. In: Oilseed crops. Elsevier; c2010. https://doi.org/10.1016/B978-0-12-381466-1.00010-4
14. Szabados L, Savouré A. Proline: A multifunctional amino acid. Trends in Plant Science. 2010;15(2):89-97. https://doi.org/10.1016/j.tplants.2009.11.009
15. Verma A, Singh M, Verma M, Kumar S. Indian mustard: An overview. Journal of Oilseed Brassica. 2016;7(1):1-16.
16. Vendruscolo ECG, Schuster I, Pileggi M, Scapim CA, Molinari HBC, Marur CJ, Vieira LGE. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. Journal of Plant Physiology. 2007;164(10):1367-1376.

https://doi.org/10.1016/j.jplph.2007.04.012
17. Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, *et al*. Off-target effects in CRISPR/Cas9-mediated genome engineering. Molecular Therapy. 2014;22(9):1457-1463. https://doi.org/10.1038/mt.2014.133