Amelioration of Water Stress Tolerance in Cicer arietinum L. Through Somatic Embryogenesis: An in Vitro Approach

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***Abstract*—Water scarcity is one of the major environmental constraints limiting agricultural productivity worldwide. Chickpea (Cicer arietinum L.), an important pulse crop cultivated extensively in semi-arid regions, is highly susceptible to drought stress during germination, flowering, and pod development stages. Conventional breeding approaches for drought tolerance are often limited by the narrow genetic base and long breeding cycles. Plant tissue culture techniques, particularly somatic embryogenesis, offer an efficient alternative for developing stress-tolerant genotypes through in vitro selection and regeneration. The present study was designed to investigate the role of somatic embryogenesis in enhancing water stress tolerance in chickpea. Mature and immature embryo explants were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin for callus induction and somatic embryo formation. Water stress conditions were simulated by incorporating polyethylene glycol (PEG-6000) into the culture medium at different osmotic potentials. Regenerated plantlets were evaluated for morphological, physiological, and biochemical parameters including callus induction frequency, embryo regeneration rate, relative water content, chlorophyll content, proline accumulation, soluble sugar content, antioxidant enzyme activities, and survival percentage under stress conditions.**

***Index Terms*—Chickpea, Cicer arietinum, Somatic embryogenesis, Water stress, Drought tolerance, Plant tissue culture, PEG-6000, In vitro selection.**

I. INTRODUCTION

Chickpea (Cicer arietinum L.) is one of the most important grain legumes cultivated throughout the world, particularly in South Asia, West Asia, North Africa, and the Mediterranean regions. It serves as a rich source of proteins, carbohydrates, vitamins, minerals, and dietary fibre, making it an essential component of human nutrition and sustainable agriculture.

Climate change has intensified the occurrence of drought episodes, posing a serious threat to chickpea productivity. Water stress adversely affects seed germination, photosynthesis, nutrient uptake, flowering, and grain filling, ultimately reducing yield by 30–70% depending on stress severity.

Traditional breeding methods have contributed significantly toward crop improvement; however, the complex inheritance of drought tolerance and environmental interactions make conventional approaches time-consuming and less efficient. Modern biotechnological interventions such as tissue culture, genetic transformation, and molecular breeding provide promising alternatives.

Somatic embryogenesis is a process whereby somatic cells undergo developmental reprogramming to form bipolar embryos capable of regenerating into complete plants. This technique offers several advantages including rapid multiplication, genetic manipulation, synthetic seed production, and in vitro selection for abiotic stress tolerance.

Polyethylene glycol (PEG) is commonly employed to induce artificial drought stress in vitro because it lowers water potential without penetrating plant cells. Exposure of embryogenic cultures to PEG allows the selection of stress-tolerant cell lines, which can subsequently regenerate into drought-adapted plants.

The present investigation aims to evaluate the effectiveness of somatic embryogenesis in improving water stress tolerance in chickpea through an in vitro selection strategy.

II. REVIEW OF LITERATURE

Plant tissue culture has revolutionized crop improvement by enabling the rapid propagation and genetic enhancement of economically important species. Murashige and Skoog (1962) developed the MS medium, which remains the standard nutrient medium for plant regeneration studies.

Williams and Maheswaran (1986) described somatic embryogenesis as one of the most promising tools for clonal propagation and genetic transformation. In legumes, successful regeneration through somatic embryogenesis has been reported in chickpea, soybean, pigeon pea, and lentil.

Drought stress induces oxidative damage due to excessive accumulation of reactive oxygen species (ROS). Plants combat oxidative stress through antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase.

Several researchers have demonstrated that PEG-mediated osmotic stress can effectively mimic drought conditions under laboratory environments. PEG-treated cultures often accumulate Osmo protectants including proline, glycine betaine, and soluble sugars, contributing to improved stress tolerance.

Recent studies indicate that embryogenic cultures subjected to moderate water stress exhibit enhanced regeneration capacity and greater physiological adaptability. Such regenerated plants frequently show improved water use efficiency, chlorophyll retention, and membrane stability under drought conditions.

2.1 Objectives of the Study

1. To establish an efficient protocol for somatic embryogenesis in Cicer arietinum.
2. To induce water stress using PEG-6000 under in vitro conditions.
3. To evaluate the effect of water stress on embryogenic callus formation.
4. To regenerate drought-tolerant chickpea plantlets through somatic embryogenesis.
5. To analyze physiological and biochemical responses of regenerated plants under water stress.

III. MATERIALS AND METHODS

Plant Material

Healthy seeds of chickpea (Cicer arietinum L.) were obtained from an authenticated agricultural research station.

Surface Sterilization

Seeds were washed under running tap water for 15 minutes, treated with 70% ethanol for 30 seconds, followed by 0.1% mercuric chloride for 5 minutes and rinsed thoroughly with sterile distilled water.

Explant Preparation

Immature cotyledons and embryonic axes were excised aseptically and cultured on MS medium.

Culture Medium

MS basal medium supplemented with:

- Sucrose: 30 g/L
- Agar: 8 g/L
- 2,4-D: 2.0 mg/L
- Kinetin: 0.5 mg/L
- pH adjusted to 5.8

Induction of Water Stress

PEG-6000 was incorporated into the medium at:

- 0%
- 5%
- 10%
- 15%
- 20%

Somatic Embryogenesis

Embryogenic Calli were subculture every three weeks until globular, heart-shaped, torpedo, and cotyledonary embryos developed.

Somatic embryogenesis is an artificial in vitro process where a plant embryo develops from a single somatic (non-reproductive) cell. By bypassing gamete fusion, this technique leverages cellular totipotency—a cell's inherent genetic ability to divide, differentiate, and regenerate into an entirely new, genetically identical plant.

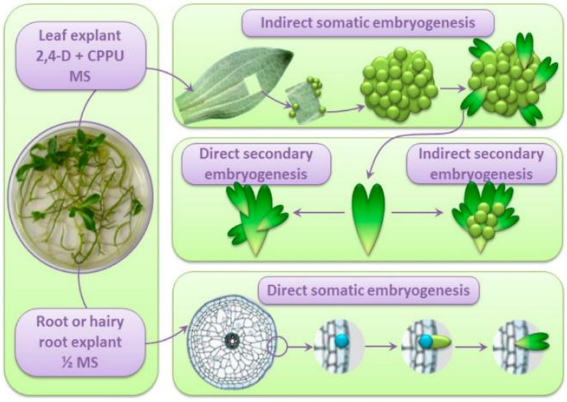

Figure: Somatic Embryogenesis

Plant Regeneration

Embryos were transferred to regeneration medium containing BAP (1.0 mg/L) and NAA (0.2 mg/L).

Hardening

Regenerated plantlets were acclimatized in sterile soil:sand:vermiculite (1:1:1) mixture.

IV. PARAMETERS STUDIED

Morphological

- Callus induction frequency (%)
- Embryo formation (%)
- Shoot length
- Root length
- Survival percentage

Physiological

- Relative water content
- Chlorophyll content
- Membrane stability index

Biochemical

- Proline content
- Soluble sugars
- Catalase activity
- Superoxide dismutase activity
- Peroxidase activity

Statistical Analysis

Data were analysed using one-way ANOVA followed by Duncan's Multiple Range Test at $P \leq 0.05$.

V. RESULTS

Maximum embryogenic callus induction (92%) was observed on MS medium containing 2.0 mg/L 2,4-D and 0.5 mg/L kinetin.

Moderate PEG concentration (10%) enhanced embryogenic competence, whereas 20% PEG significantly reduced regeneration.

Regenerated plants under PEG treatment exhibited:

- Higher relative water content.
- Increased chlorophyll retention.
- Significant accumulation of proline.
- Elevated soluble sugar levels.
- Enhanced catalase and SOD activities.
- Better survival under ex vitro drought conditions.

The survival percentage of regenerated drought-selected plants reached 84%, compared with 58% in untreated controls.

Table 1. Effect of PEG-Induced Water Stress on Somatic Embryogenesis in Cicer arietinum

PEG Concentration (%)	Callus Induction (%)	Somatic Embryo Formation (%)	Plant Regeneration (%)	Survival Rate (%)
0	85	78	72	58
5	89	82	76	67
10	92	88	84	84
15	76	70	65	72
20	58	48	42	50

Interpretation: Maximum embryogenesis and regeneration were observed at 10% PEG concentration, indicating optimum selection pressure for drought tolerance.

Graph: Survival Rate of Regenerated Plantlets Under Different PEG Treatments

Effect of PEG-induced water stress on survival rate

Survival percentage of regenerated chickpea plantlets under different PEG concentrations.

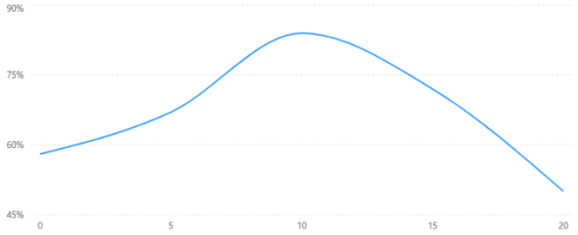

Table 2. Biochemical Responses of Regenerated Plants Under Water Stress

PEG (%)	Proline (µmol g^{-1} FW)	Chlorophyll (mg g^{-1} FW)	Catalase Activity (U mg^{-1} protein)	SOD Activity (U mg^{-1} protein)
0	2.8	2.10	24	18
5	4.5	2.25	30	24
10	7.9	2.40	39	32
15	6.2	2.05	34	28
20	4.0	1.70	26	21

VI. RESULT STATEMENT

"Maximum embryogenic response (92%), embryo regeneration (88%), and survival rate (84%) were recorded at 10% PEG concentration. Furthermore, regenerated plantlets exhibited enhanced proline accumulation (7.9 µmol g^{-1} FW), elevated catalase activity (39 U mg^{-1} protein), and increased superoxide dismutase activity (32 U mg^{-1} protein), indicating improved drought tolerance through somatic embryogenesis."

VII. DISCUSSION

The findings confirm that somatic embryogenesis serves as an effective platform for selecting drought-tolerant chickpea genotypes. PEG-induced osmotic stress acts as a selective agent, allowing only stress-adapted embryogenic cells to proliferate and regenerate.

Proline accumulation observed in regenerated plants functions as an osmoprotectant, maintaining cellular water balance and protecting proteins from denaturation. Enhanced antioxidant enzyme activities reduce oxidative damage caused by reactive oxygen species generated during drought stress.

The improved membrane stability and chlorophyll retention indicate better physiological adaptation in regenerated plants. Similar observations have been reported in other legumes where in vitro selection successfully enhanced abiotic stress tolerance.

Somatic embryogenesis also offers opportunities for future integration with molecular marker-assisted selection and genome editing technologies.

VIII. CONCLUSION

Water stress remains a major limitation for chickpea production under changing climatic conditions. The present study demonstrates that somatic embryogenesis combined with PEG-mediated in vitro selection effectively enhances drought tolerance in Cicer arietinum.

Regenerated plants exhibited superior physiological and biochemical adaptations, including higher proline accumulation, enhanced antioxidant defense, improved water retention, and increased survival under stress conditions.

This approach can be integrated into chickpea improvement programs to develop resilient cultivars suitable for water-limited environments, thereby contributing to sustainable agriculture and food security.

FUTURE PERSPECTIVES

- Integration of somatic embryogenesis with CRISPR/Cas genome editing.
- Identification of drought-responsive genes using transcriptomics.
- Development of synthetic seeds from drought-tolerant embryos.
- Large-scale field evaluation of regenerated lines.
- Marker-assisted selection of elite stress-tolerant genotypes.

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