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Article

Genetic Modification of Tomatoes for Increased Stress Tolerance via HSP101 Gene Integration

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Abstract

Climate change poses a serious threat to global food security, as increasing environmental stresses adversely affect plant growth and crop yield. Key abiotic stresses impacting agricultural productivity include heat stress, drought stress, salinity, and oxidative stress, all of which disrupt cellular homeostasis and protein stability in plants. In this study, we propose a novel genetic engineering approach to enhance stress tolerance in *Solanum lycopersicum* (tomato) by introducing the HSP101 (Heat Shock Protein 101) coding sequence. HSP101 is an AAA+ molecular chaperone essential for both basal and acquired thermotolerance in plants. It prevents protein misfolding and aggregation, providing cross-protection against multiple abiotic stresses. Here we describe a genetic modification strategy to introduce the HSP101 gene into tomato using *Agrobacterium*-mediated transformation. This work aims to generate transgenic tomato lines with improved resilience to high temperatures and other environmental stresses. Subsequent phases will include molecular confirmation of gene integration and evaluation of transgenic plants under controlled stress conditions. The expected outcome is the development of tomato lines with improved resilience to high temperature, drought, and oxidative stress. This ongoing work aims to contribute to the creation of climate-resilient crops and to support sustainable agricultural productivity in arid and semi-arid regions.

Keywords: HSP101; *Solanum lycopersicum*; heat stress; drought stress; agrobacterium-mediated transformation; Ti-plasmid; abiotic stress tolerance; climate-resilient crops

1. Introduction

Climate change is one of the most pressing challenges of our time, profoundly impacting ecosystems, economies, and human livelihoods. Among its many consequences, the disruption of agriculture stands out as a critical issue, with rising temperatures and extreme weather events threatening the stability of global food production. Crops that are vital to both nutrition and economic development, such as tomatoes, are particularly vulnerable to heat stress, leading to reduced yields, lower quality, and increased susceptibility to pests and diseases. In arid regions like the UAE, where agriculture already faces challenges due to limited water resources and harsh climatic conditions, the need for innovative solutions is more urgent than ever.

In response to this pressing issue, this preprint focuses on exploring the potential of genetically modifying tomato plants to increase their tolerance to abiotic stress by introducing the **HSP101 gene**, a Heat Shock Protein (HSP) known for its ability to help plants endure high temperatures. The **HSP101 gene** plays a critical role in enhancing plant thermotolerance, enabling them to withstand environmental stress such as extreme heat, drought, and other abiotic factors. By incorporating this gene into tomato plants, this project aims to develop a more resilient crop capable of thriving in the harsh climate conditions prevalent in arid regions such as the UAE.

The primary objective of this research is to genetically enhance tomato plants to withstand the increasing heat and environmental stress brought on by climate change.

The project is structured into several key stages, including the selection of the **HSP101 gene**, the design of a suitable vector for gene insertion, the use of **Agrobacterium-mediated transformation** for

gene transfer, and the subsequent testing of the modified plants for their resilience and performance under heat stress. The research methodology involves both PCR (Polymerase Chain Reaction) testing to confirm the successful insertion of the gene and long-term testing to assess the impact of the gene on plant growth, yield, and stress tolerance.

2. Materials and Methods

Since this project is still in progress, the methodology is presented as **planned experimental approaches** to guide the research workflow and provide transparency about the procedures.

2.1. Plant Material

- *Solanum lycopersicum* (tomato) seedlings will serve as explants for transformation.
- Cotyledons from 7–10-day-old sterile seedlings will serve as the primary tissue for *Agrobacterium*-mediated gene transfer.

2.2. Gene Selection and Vector Construction

- The **HSP101** coding sequence (*Arabidopsis thaliana* ath_HSP101, NM_106091.4) was selected due to its role in enhancing thermotolerance and cross-protection against other abiotic stresses such as drought, salinity, and oxidative stress.
- The gene was synthesized at vectorBuilider inc. and cloned into a **Ti-plasmid binary vector** (pPBV[Exp]-Bar-(CaMV 35S Promoter)>ath_HSP101), under the **CaMV 35S promoter** to allow constitutive expression in tomato plants.
- The vector includes the **Bar gene** for herbicide selection and **Kanamycin resistance** for bacterial selection.
- The **HSP101-containing vector was constructed and successfully introduced into *Agrobacterium tumefaciens* LBA4404** by VectorBuilder (company), prior to the start of plant transformation experiments.

2.3. Plasmid Details

Table 1. Plasmid detials.

FEATURE	DETAILS
VECTOR NAME	pPBV[Exp]-Bar-(CaMV 35S Promoter)>ath_HSP101
SIZE	11,586 bp
TYPE	Agrobacterium Binary Vector
INSERTED PROMOTER	CaMV 35S Promoter
INSERTED ORF	ath_HSP101 [NM_106091.4]
SELECTABLE MARKER	Bar (herbicide resistance)
ANTIBIOTIC RESISTANCE	Kanamycin
CLONING HOST	VB UltraStable1

2.4. Protocol and Procedures

- *LB Broth and Agar Preparation*
 - **Materials (per 1 L):**
 - Tryptone: 10 g
 - Yeast Extract: 5 g
 - NaCl: 10 g
 - Agar (for solid medium): 15 g
 - **Steps:**
 1. Dissolve components in 1 L distilled water and adjust pH to 7.0 using HCl or NaOH.

2. Autoclave at 121°C for 15–20 minutes.
 3. For LB agar, cool to 55°C before adding antibiotics.
- Kanamycin Stock Solution
 - **Materials:**
 - Kanamycin 500 mg, sterile water 10 mL
 - **Steps:**
 1. Dissolve Kanamycin in sterile water and filter sterilize using a 0.22 µm syringe filter.
 2. Store at 50 mg/mL; for LB agar, add 1 mL per 1 L medium.
 - Culturing *Agrobacterium tumefaciens* LBA4404
 - Preparation of Culture Plates
 - Add Kanamycin (final concentration: 50 µg/mL) to LB agar once cooled to 55°C.
 - Pour the agar into sterile Petri dishes and let it solidify.
 - Streaking *Agrobacterium*
 - Scrape a small amount of frozen glycerol stock and streak it on an LB-Kanamycin plate.
 - Incubate plates upside down at 28°C for 2-3 days until colonies form.
 - Preparing Liquid Culture of the LBA4404 strain
 - Pick a single colony and inoculate it into 5–10 mL LB broth containing Kanamycin.
 - Incubate at, 28 °C, 200–250 rpm, for 16–24 hours (until turbid).
 - Transfer 1.5 mL of the culture into a 50 mL Erlenmeyer flask containing 50 mL LB broth with Kanamycin.
 - Incubate at the same conditions.
 - Preparation of Transformation Suspension
 - Centrifuge the bacterial culture at 4000–5000 rpm (5000g) for 10 minutes.
 - Discard the supernatant and resuspend the pellet in 5% sucrose and 0.05% Silwet-L77 solution:
 - Use a vortex mixer and pipette to ensure the bacterial pellet is fully resuspended.
 - *Agrobacterium*- Mediated Transformation of Tomato Cotyledons
 - *Material*
 - 7–10-day-old sterile tomato seedlings
 - Infection suspension (prepared *Agrobacterium* culture)
 - Co-cultivation medium (MS medium + acetosyringone 100 µM)
 - Selection medium (MS + 50 mg/L kanamycin + 250 mg/L cefotaxime + 3% sucrose + 0.8% agar)
 - Regeneration medium (MS + 1 mg/L BAP + 0.1 mg/L NAA + 3% sucrose + agar)
 - Rooting medium (½ MS + 0.5 mg/L IAA + 3% sucrose + agar)

2.5. Evaluation of Stress Tolerance and Growth Performance

To assess the functional impact of *HSP101* gene integration on tomato stress tolerance and growth, a series of controlled greenhouse and field experiments will be conducted. The goal is to determine how *HSP101* expression influences plant growth, flowering, and fruit yield under various abiotic stress conditions.

Testing Environments

Experiments will be performed under both **greenhouse** and **open-field** conditions to simulate realistic environmental stress exposure. Environmental parameters (temperature, light intensity, humidity) will be recorded using digital sensors to ensure consistency between replicates.

Data Collection

Data will be collected at **periodic intervals** specific to each type of stress, enabling detailed temporal tracking of physiological and morphological changes. Traits assessed include plant height, leaf area, chlorophyll content, photosynthetic efficiency, survival rate, and recovery time.

Table 2. High Temperature Stress Test.

PARAMETER	DETAILS
EXPOSURE CONDITIONS	35–40 °C for 5 days
TESTING FREQUENCY	Every 3 days during exposure and post-stress recovery
OBJECTIVE	Evaluate thermotolerance and recovery capacity
MEASUREMENTS (WILD-TYPE)	Plant height (cm), leaf area (cm ²), photosynthesis rate (CO ₂ assimilation), leaf damage (scorching/wilting), survival rate (%)
MEASUREMENTS (HSP101 TRANSGENIC)	same parameters for comparison, including recovery time (days)

Table 3. UV-B Radiation Stress Test.

PARAMETER	DETAILS
EXPOSURE CONDITIONS	3 hours/day of UV-B for 7 days
TESTING FREQUENCY	Measurements before, during, and after exposure
OBJECTIVE	Assess the protective effects of <i>HSP101</i> on photosynthesis and chlorophyll maintenance
MEASUREMENTS (WILD-TYPE)	Chlorophyll content (SPAD values), leaf necrosis/browning, photosynthesis rate, plant height, growth rate (cm/day)
MEASUREMENTS (HSP101 TRANSGENIC)	Same parameters with emphasis on chlorophyll retention and reduced tissue damage

Table 4. Drought Stress Test.

PARAMETER	DETAILS
EXPOSURE CONDITIONS	Water withheld for 10–14 days
TESTING FREQUENCY	Before stress, during stress (days 3, 7, 10), and during recovery
OBJECTIVE	Determine plant resilience to limited water availability
MEASUREMENTS (WILD-TYPE)	leaf wilting index (0–5 scale), survival rate (%), water loss rate (by weight), plant height, photosynthesis rate
MEASUREMENTS (HSP101 TRANSGENIC)	Same parameters including recovery time (days) and water retention efficiency

Table 5. Heavy Metal Exposure Test.

PARAMETER	DETAILS
EXPOSURE CONDITIONS	100 mM and 200 mM NaCl, or Cd/Pb solutions for 7–14 days
TESTING FREQUENCY	Every 3 days during exposure and recovery
OBJECTIVE	Evaluate metal tolerance, photosynthesis stability, and leaf health
MEASUREMENTS (WILD-TYPE)	Metal accumulation (mg/kg in roots, stems, leaves), leaf necrosis/chlorosis (%), root length (cm), photosynthesis rate, survival rate (%)

MEASUREMENTS (HSP101 TRANSGENIC)	Same parameters with expected lower metal accumulation and higher survival under identical stress levels
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The outcomes from these experiments will collectively provide insight into the **functional role of HSP101** in enhancing tolerance to multiple abiotic stresses. Comparative data between wild-type and transgenic lines will determine whether the introduction of *HSP101* confers statistically significant improvements in plant vigor, photosynthetic efficiency, and yield stability under challenging environmental conditions.

3. Excepted Results

It is anticipated that transgenic tomato plants expressing HSP101 will:

- Exhibit enhanced thermotolerance compared to wild-type controls.
- Maintain higher chlorophyll content and lower MDA accumulation under heat or drought stress.
- Show reduced cellular damage and improved recovery post-stress exposure.
- Demonstrate stable gene integration and expression across subsequent generations (T₁ and T₂ lines).

4. Discussion

The integration of the *HSP101* gene into *Solanum lycopersicum* represents a strategic approach toward improving crop resilience under climate-induced stress conditions. Heat shock proteins (HSPs) are well known for their protective role in maintaining protein stability, refolding denatured proteins, and preventing aggregation during cellular stress (Wang et al., 2004; Queitsch et al., 2000). Among them, *HSP101* functions as a molecular disaggregase, facilitating the reactivation of aggregated proteins and thereby enhancing cellular survival under extreme environmental conditions (Hong & Vierling, 2001).

This study’s approach differs from conventional breeding or stress-specific transgenes by targeting a broad-spectrum stress regulator. Constitutive expression of *HSP101* under the CaMV 35S promoter is expected to provide multi-stress protection, not only against heat but also secondary stresses such as drought, salinity, and oxidative damage, all of which share common pathways of protein denaturation and metabolic disruption.

5. Conclusions

This Research-in-Progress project outlines a genetic engineering strategy to enhance abiotic stress tolerance in tomato through *HSP101* gene integration. By combining *Agrobacterium*-mediated transformation with a well-characterized promoter and selectable markers, the proposed workflow establishes a replicable framework for producing climate-resilient crops. The expected enhancement in heat, drought, and oxidative stress resistance could substantially benefit agricultural productivity in hot, arid regions.

The broader implication of this work extends beyond tomatoes, as similar *HSP101*-based strategies could be applied to other horticultural and staple crops to safeguard food security under global climate change.

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