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Proceeding paper Genetic and Microbial Insights into Drought Stress Alleviation in Tomato (Solanum lycopersicum L.)

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Abstract: Drought is a significant environmental stress that severely affects various key crops de-9 velopment, productivity, and overall quality. Tomato production is globally significant due to its 10 economic importance and is considered the second horticultural crop produced in terms of yield 11 and consumption worldwide, and yet, it is facing challenges posed by drought in agriculture. 12 Drought stress negatively affects various characteristics of tomato plants, including physiological, 13 genetic, biochemical, and morphological traits, leading to reduced seed production and fruit quality 14 and, also poses threat towards significant yield loss. In response to the need to mitigate the impacts 15 of drought stress on tomato plants, it is focused on the assessment of the delicate interplay between 16 genetic variables and microbial interactions. Some key genes, such as ABA-responsive genes, tran-17 scription factor genes, aquaporin genes, ROS-related genes, etc., and their function in drought tol-18 erance in tomato plants have been discovered and analyzed to understand their role in stress adap-19 tation. Additionally, Microbial interactions, notably with plant growth-promoting rhizobacteria 20 (PGPR), mycorrhizal fungi, and Pseudomonas, have emerged as key components in the context of 21 drought stress alleviation. Mycorrhizal fungi form symbiotic relationships with plant roots, expand-22 ing the root system's reach and improving water and nutrient availability. On the other hand, certain 23 microorganisms, like Bacillus subtilis, produce antioxidants such as catalase and superoxide dis-24 mutase, which scavenge reactive oxygen species (ROS), protect plant cells, and enhance resistance 25 to oxidative damage during drought. Overall, this study emphasizes existing information on mo-26 lecular principles underpinning stress tolerance and underscores the relevance of microbial-assisted 27 stress amelioration and the interplay between genetic variables and microbial populations in reliev-28 ing drought in tomato. 29

Keywords: Drought; Resistance; Sustainability; Drought tolerance; Genetic variables; Microbial insights 31

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1. Introduction

Drought stress poses a significant challenge to global tomato (Solanum lycopersicum 34 L.) production, potentially resulting in up to 50% yield losses due to factors such as inad-35 equate rainfall, elevated temperatures, and low soil moisture. Genetic and microbial fac-36 tors collectively influence drought tolerance in tomatoes. Genetic determinants include 37 the expression of drought-responsive genes, accumulation of compatible solutes, and wa-38 ter transport regulation. Key genes enhancing drought tolerance include DREB1A, a tran-39 scription factor activating drought-responsive genes, PAD4, which produces protective 40 proline, LEA genes for protein stabilization, and P5CS for proline synthesis. 41

Additionally, genes like MdEPF2, cwInv, AtGAMT1, SIADL1, ATHB-7, SIPIP2;1, 42 SIPIP2;7, SIPIP2;5, osmotin, TAS14, SIMAPK3, and SIJUB1 contribute to drought resistance in tomatoes. Plant growth-promoting bacteria (PGPB) stimulate growth and aid 44

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drought tolerance by hormone production, inhibition of harmful microbes, nutrient enhancement, and improved water relations. Biofertilizers, housing beneficial microorganisms, enrich soil quality and nutrient availability, further enhancing drought resistance.

Understanding the interplay of these genetic and microbial elements is crucial for developing drought-resistant tomato cultivars, ultimately promoting sustainable agriculture by mitigating drought's adverse effects on crop yields.

2. Genetic basis of drought tolerance in tomatoes

2.1. ABA-responsive genes

ABA is an important plant hormone that plays an important role in drought tolerance 9 and stress response. When plants experience drought stress, ABA levels increase, which 10 activates a signaling cascade that leads to physiological changes and the expression of 11 drought-responsive genes [1-3]. The effects of ABA are complex and multifaceted. It reg-12 ulates stomatal closure to reduce water loss, enables intercellular signaling of plant water 13 stress status, stimulates antioxidant production to protect against drought damage, and 14 activates transcription factors that activate drought-responsive genes [1-5]. ABA regulates 15 two types of drought responsive genes: those directly related to drought tolerance, such 16 as aquaporins and antioxidants, and genes related to stress signaling, such as DREB and 17 RD29 [6-8]. DREB genes activate other drought responsive genes in response to ABA and 18 stress signals [6]. RD29 genes produce proteins that are important for ABA synthesis [6]. 19 LEA genes encode proteins that protect cells against dehydration damage [6]. ABA activ-20 ity is mediated by ABA-responsive transcription factors that bind to ABREs, cis-acting 21 DNA sequences in the promoters of ABA-responsive genes [1-4, 8]. When ABA levels in-22 crease due to drought stress, ABA-responsive transcription factors are activated and bind 23 to ABREs, which activates ABA-responsive genes [1-4]. In summary, ABA is essential for 24 drought tolerance and activation of drought responsive genes. It stimulates physiological 25 changes, antioxidant production and transcription factors that bind to ABREs and activate 26 genes involved in drought tolerance and stress signaling. ABA-responsive genes produce 27 proteins that reduce water loss, protect cells and facilitate the transmission of stress sig-28 nals. 29

2.2. Aquaporin genes

Aquaporins are water channel proteins that facilitate the passage of water through 31 cell membranes and are important in drought tolerance of plants [9]. Reuscher and his 32 team identified 47 aquaporin genes in the tomato genome and found that they are ex-33 pressed in a tissue-specific manner. Some aquaporin genes are upregulated in response to 34 drought, suggesting a role in drought tolerance [10]. Wang (2017) found that overexpres-35 sion of the apple aquaporin gene MdPIP1;3 in tomato increased fruit size, improved 36 drought tolerance and reduced water loss. Transgenic tomatoes had slower water loss, 37 more drought-sensitive stomatal feathers, larger fruit cells and larger fruits [11]. This in-38 dicates that MdPIP1;3 increases drought tolerance by increasing water transport and reg-39 ulating stomata. Ouziad found that colonization of tomato roots with arbuscular mycor-40 rhizal fungi (AMF) resulted in silencing of two aquaporyngenes and increased drought-41 induced downregulation of three aquaporyngenes. This suggests that AMF regulates aq-42 uaporin expression and water flow in tomato during drought [12]. This indicates that 43 AMF directly ameliorate plant drought through aquaporins. Gong identified many 44 drought-responsive genes in drought-tolerant tomato, including aquaporins, transcrip-45 tion factors, signaling proteins, and genes related to growth, development, energy pro-46 duction, and oxidative stress. Drought-tolerant lines had unique expression of ~400 47 drought-responsive genes, suggesting that these genes, especially aquaporins and tran-48scription factors, likely contain drought-tolerant QTLs [13]. Transgenic plants showed im-49 proved chlorophyll fluorescence parameters during drought, indicating that chlorophyll 50 fluorescence can inform drought tolerance and can be used for high-throughput screening 51

[14]. Zupin found that two varieties of beans with different drought tolerance had differ-1 ent expression of four aquaporin genes in response to drought and liquefaction. The more 2 drought-tolerant variety had a greater downregulation of the two aquaporins during 3 drought and a higher relative water content, suggesting that aquaporin regulation is im-4 portant for drought tolerance [15]. In summary, aquaporins and their regulation are im-5 portant genetic components in drought tolerance in tomatoes and other plants. Overex-6 pression of certain aquaporins or transcription factors can improve drought tolerance. 7 AMF also directly improves drought tolerance by regulating aquaporin expression. Chlo-8 rophyll fluorescence parameters can be used as a proxy for drought tolerance screening. 9 The genetic basis of drought tolerance is complex, but understanding aquaporins and 10 their regulators is key. 11

2.3. ROS genes are related

Drought stress induces an influx of reactive oxygen species (ROS) in tomato plants, 13 which triggers a series of reactions to neutralize their harmful effects while exploiting their 14 signaling potential [16]. Dehydration accelerates ROS generation due to increased respi-15 ration and oxygenation in dehydrated cells [17]. Because antioxidants are limited during 16 drought, ROS accumulate rapidly [17]. However, ROS have a dual role, as they mediate 17 defense genes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate perox-18 idase (APX) to produce antioxidants, as well as promote the synthesis of osmolytes (eg, 19 proline, glycine, betaine) for defense. [18]. Stress response genes such as heat shock pro-20 teins (HSP) and transcription factors also help plants adapt [19]. The ROS signaling path-21 way activates various target genes involved in antioxidant defense, stress signaling and 22 cell death, removing damaged cells and preventing further damage [20]. In fact, drought-23 tolerant tomatoes use a complex ROS-related network in which ROS simultaneously trig-24 ger defense responses that reduce damage and increase resistance to adversity [21]. ROS 25 are key players in stress signaling involved in growth, development and biotic/abiotic re-26 sponses [22]. Recent findings indicate that stress responses depend on ROS signals that 27 interact with other signals using stress-specific chemicals, compounds and hormones [22]. 28 In summary, drought stress in tomatoes increases ROS production through different cel-29 lular mechanisms [16-17]. Although ROS accumulate and cause damage without suffi-30 cient antioxidants [17], they also signal defense genes and osmolytes for protection [18-31 19]. Stress response genes further aid adaptation [19]. The ROS signaling pathway acti-32 vates target genes of antioxidant defense, stress signaling and cell death, mitigating dam-33 age and increasing tolerance [20-21]. ROS are crucial in stress signaling and response 34 [22]. Stress responses depend on ROS signaling, which interacts with other signals [22]. 35

Table 1. List of other genes that plays crucial role against drought stress in Tomato.

Gene/Origin	Function	Expression/ Regulation	Results
BEL1-like genes	Transcription factors, part of the TALE superfamily, regulate various plant biological processes	Exhibited tissue-specific expressions and responded to heat, cold, and drought stress	Plant growth and abiotic stress response [23]
AtCDF3, AtDREB1a, AtJUB1, CcHRD	Increases abiotic stress tolerance of tomatoes, including cold, salt, and drought stress	Overexpression	Stress tolerance [23]
SIHB2, SIAGO4A, SIMBP8	Tolerance to salt, drought stress	Overexpression, Gene silencing	Tolerance to salt, drought stress [23]
SlbZIP1	Salt and drought stress tolerance	Expression	Salt and drought stress Tolerance [23]

3. Metabolic pathways

Drought tolerance in tomato plants is orchestrated through a sophisticated network 2 of metabolic pathways, strategically designed to uphold essential physiological processes 3 and safeguard cellular integrity in the face of water scarcity. The photosynthetic pathway, 4 challenged by drought-induced stomatal closure limiting carbon dioxide availability, em-5 ploys adaptive strategies to elevate leaf carbon dioxide levels and transition to a less wa-6 ter-sensitive form of photosynthesis, thus ensuring sustained energy production. Concur-7 rently, increased respiratory rates, a response to drought, furnish the requisite energy for 8 growth, reproduction, and defense, albeit accompanied by the generation of reactive ox-9 ygen species (ROS). To counteract ROS-induced cellular damage, the plant activates de-10 fense mechanisms, including the production of antioxidants. Moreover, drought-induced 11 stress leads to the augmented production of specific amino acids, like proline, serving as 12 osmolytes for cellular protection, although amino acids may be catabolized to meet energy 13 demands. Complementary metabolic pathways, encompassing carbohydrate synthesis 14 and degradation, lipid metabolism for membrane stability, and a sophisticated stress sig-15 naling pathway, collectively underpin the plant's adaptation to water deficit, thereby en-16 hancing its drought resilience. 17

4. Microbial interactions and drought tolerance in tomatoes

Drought significantly impacts tomato plant growth, development, and productivity, but microbial interactions offer a versatile strategy to enhance drought tolerance.

4.1. Beneficial Microbes

Microbes like plant growth-promoting rhizobacteria (PGPR) stimulate drought tol-22 erance by producing plant growth hormones (e.g., auxins, gibberellins) that foster root 23 growth and water absorption. Additionally, mycorrhizal fungi form symbiotic relation-24 ships, expanding the root surface area for improved water absorption and nutrient provision [24].

4.2. Antioxidant Production

Microbes like *Bacillus subtilis* produce antioxidants that shield plants from reactive oxygen species (ROS) generated during drought stress, mitigating damage [25].

4.3. Genetic Modulation

Microbes can influence drought tolerance-related gene expression, aiding tomato 31 plants in adapting more efficiently to dry conditions [26].

Examples of microbial effects on tomato drought tolerance encompass PGPR pro-33 moting root growth, enhancing water absorption, and ROS protection. Mycorrhizal fungi 34 improve both water absorption and nutrient supply. Pseudomonas bacteria produce anti-35 microbial compounds to fend off pathogens. These diverse microbial mechanisms collec-36 tively empower tomato plants to better withstand drought stress. 37

5. Molecular mechanisms of microbial-mediated drought tolerance in tomatoes

The molecular mechanisms underlying microbe-mediated drought tolerance in to-39 mato plants are complex and multifaceted, involving several important processes. To-40 gether, these processes allow tomato plants to better withstand the challenges of water 41 scarcity. 42

First, hormonal regulation plays a central role in this microbial interaction. Beneficial 43 microorganisms produce plant hormones, including auxins and gibberellins. Hormones 44 introduced by microbes stimulate root growth and increase water absorption. In addition, 45 these microorganisms can modulate the expression of genes related to stress signaling 46 pathways. This modulation improves the adaptation of the plant to dry conditions and 47 contributes to drought tolerance. [24] 48

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Secondly, Stomatal regulation is another critical aspect of microbe-mediated drought 1 tolerance. Microbes can affect stomatal conductance, gatekeepers control water loss 2 through evaporation. Some microorganisms have the ability to fine-tune stomata, which 3 reduces water loss during drought. This reduction in evaporation helps the plant conserve 4 water and maintain optimal hydration. [27] 5

Thirdly, Osmotic regulation is another mechanism by which microbial partners improve drought tolerance in tomato plants. Drought-tolerant microorganisms contribute to osmotic adaptation, a process that involves the accumulation of solutes such as proline and sugars in plant cells. This accumulation reduces the water potential of the cells, allowing them to retain water and maintain turgor pressure even in the absence of water. [28]

Fourthly, Antioxidant defense mechanisms play an important role in protecting to-12mato plants against the harmful effects of reactive oxygen species (ROS) generated during13drought stress. Certain microorganisms such as *Bacillus subtilis* produce antioxidants such14as catalase and superoxide dismutase. These antioxidants scavenge ROS, protect plant15cells from oxidative damage and strengthen overall resistance. [25]16

Fifthly, Nutrient uptake and homeostasis are also influenced by microbial interactions, particularly those associated with mycorrhizal fungi. These fungi form symbiotic 18 relationships with plant roots, effectively expanding the reach of the root system. This 19 extension improves both water and nutrient availability, which critically contributes to 20 plant survival during periods of water scarcity. In addition, mycorrhizal fungi increase 21 the assimilation of essential elements such as phosphorus, which are often limited during 22 drought stress. [26] 23

Finally, cell wall modification is another layer of protection provided by certain microorganisms. These microbes can change the composition and structure of a plant's cell wall, strengthening it against drought challenges. These changes improve the plant's ability to retain water, maintain cell turgidity and resist cell damage, increasing overall dryness.

Taken together, the complex molecular mechanisms of microbial-mediated drought 29 tolerance in tomato plants include hormonal regulation, stomatal control, osmotic regula-30 tion, antioxidant defense, nutrient uptake, induced systemic resistance, gene expression 31 regulation and cell wall modification. These processes work together to allow tomato 32 plants to thrive under water-scarce conditions. Understanding these mechanisms offers 33 enormous potential for developing innovative strategies to improve drought tolerance not 34 only in tomatoes but also in other crops, thus promoting more sustainable and sustainable 35 agricultural practices in a changing climate. 36

6. Conclusion:

Using microorganisms to enhance drought tolerance in tomato plants is a promising 38 area of research. Understanding how microorganisms mediate drought tolerance can help 39 devise new strategies for tomato plant adaptation. The study of microbial mediated 40drought tolerance in tomatoes is a rapidly developing field. Understanding how microor-41 ganisms help plants manage arid conditions can lead to effective strategies for increasing 42 yields and food security in the face of climate change. Prospects for the molecular mecha-43 nisms of microbial mediated drought tolerance in tomatoes are promising. Advancements 44 in technology and research have improved scientists' comprehension of microorganism-45 plant interactions, allowing for the development of new strategies to increase drought 46 tolerance in tomato plants. Gene editing techniques, such as CRISPR-Cas9, can be used to 47 introduce drought-resistant plant genes into tomato plants, enhancing their ability to cope 48with adversity. Sustained research can further develop strategies to increase the arid tol-49 erance of tomato plants and ensure food security in the face of climate change. 50

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