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Poliamine come agenti di priming e biostimolanti contro stress abiotici e biotici nelle piante

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Polyamines as priming agents and biostimulants against plant abiotic and biotic stress

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Table of contents

ABSTRACT

By 2050, global food demand is projected to increase by approximately 100–110 %, posing an unprecedented challenge to agricultural systems and requiring a substantial enhancement of crop productivity. Concurrently, climate change projections predict a global temperature rise of about 2 °C, which is expected to exacerbate a wide range of abiotic and biotic stresses, including drought, soil salinization, and increased pressure from pests and pathogens. Traditionally, modern agriculture has relied heavily on genetic improvement, chemical fertilizers, and pesticides to sustain crop yields. However, the growing urgency to reconcile food security with environmental sustainability has accelerated the development and adoption of eco-friendly and sustainable agronomic strategies. In this context, biostimulants (BSs) have emerged as promising tools to enhance plant resilience and mitigate yield losses under adverse environmental conditions. BSs improve plant performance by promoting nutrient uptake, modulating stress-responsive pathways, and supporting growth and development. Among the most promising BSs, polyamines (PAs), including putrescine (Put), spermidine (Spd), and spermine (Spm), play multifaceted roles in plant stress tolerance. These compounds function as osmoprotectants, scavengers of reactive oxygen species (ROS), regulators of gene expression, and signalling molecules. Moreover, PAs serve as precursors of hydrogen peroxide (H_2O_2), a key signalling component in plant stress-response networks.

Cucumber (*Cucumis sativus* L.), one of the oldest domesticated vegetable crops, was selected as the model species for this study due to its economic relevance and marked sensitivity to soil salinity. Salinity was chosen as the primary abiotic stress factor, as it represents a major and increasingly restrictive constraint for cucumber cultivation worldwide. In addition to abiotic stress, cucumber production is severely limited by biotic challenges. The whitefly *Bemisia tabaci* (Bemisia) is a major phloem-feeding pest that reduces photosynthate availability, compromises plant vigor, and promotes the development of sooty mold through honeydew deposition, ultimately impairing photosynthetic efficiency. Furthermore, powdery mildew, predominantly caused by obligate biotrophic fungi such as *Golovinomyces cichoracearum*, constitutes another critical threat, particularly in protected cultivation systems where environmental conditions favor rapid pathogen proliferation and disease spread.

Despite extensive knowledge of PAs as multifunctional biomolecules, their relative effectiveness as priming agents under agriculturally relevant abiotic and biotic stress remains poorly understood. In this study, the molecular, cellular, and biochemical mechanisms underlying plant responses to salinity stress, insect herbivory, and fungal infection following exogenous PA application were investigated, using *A. thaliana* as a model species and *C. sativus* as a crop plant.

Experimental activities were conducted on seeds, seedlings, and adult plants across three research sites: Roma Tre University (Rome, Italy), C.R.O. Agrigeos S.r.L. (Acireale, Italy), and the School of Biosciences at Cardiff University (Cardiff, UK). This multi-site experimental framework enabled the integration of complementary expertise and infrastructures, thereby enhancing the robustness, reproducibility, and translational relevance of the results.

The first objective of this study was to evaluate the contribution of PA seed priming to to stress tolerance during germination and early seedling development under salinity conditions. In Arabidopsis, seed priming with 1 mM Put consistently improved the germination rate (GR) and germination index (GI) under both optimal growth conditions and severe salinity stress (200 mM NaCl). Notably, the beneficial effects of 1 mM Put were maintained under saline conditions, resulting in enhanced germination kinetics during the early phases following seed imbibition.

To further investigate the molecular mechanisms underlying the protective effect of 1 mM Put during germination and based on the optimal efficacy observed for the 1 mM Put treatment in the previous experiments, germination parameters in loss-of-function *Atcuao* mutant lines were analysed. These mutants carry loss-of-function mutations in genes encoding copper amine oxidases (CuAOs), key enzymes involved in PA oxidation, specifically in Put catabolism. This genetic impairment results in altered Put turnover, thereby allowing the assessment of how altered Put oxidation influences salt-stress tolerance during early developmental stages.

Specifically, loss-of-function mutants of *AtCuAOβ* (*Atcuaoβ.2*), *AtCuAOγ1* (*Atcuaoγ1.1*), and *AtCuAOγ2* (*Atcuaoγ2.1*) were examined. Data suggested that CuAO-dependent Put catabolism is required for the proper regulation of germination under both optimal and saline conditions. Under control conditions, *Atcuaoγ1* and *Atcuaoγ2* displayed altered germination dynamics and reduced germination efficiency compared to WT. Under salinity stress, these mutants exhibited increased sensitivity, characterized by delayed and incomplete germination. Notably, seed priming with 1 mM Put failed to restore germination performance in the *Atcuao* mutants and instead further exacerbated stress sensitivity. These findings indicate that unaltered Put oxidation is essential for the beneficial priming effect observed in WT plants, highlighting a critical role for CuAO-mediated Put catabolism in the regulation of germination under saline conditions.

Experiments were then conducted on cucumber seeds, to evaluate the agricultural relevance of PA priming. Seed priming with 1 mM Put significantly mitigated the detrimental effects of severe salinity (200 mM NaCl), resulting in increased GR and final GI compared to unprimed stressed seeds. In contrast, priming with Spd and Spm, although less effective or even inhibitory of Put during germination, selectively promoted early root growth in Arabidopsis seedlings, more than Put priming. This observation highlights a developmental stage– and response-specific role of distinct PAs**.**

Collectively, these findings identified Put as the most effective priming agent during germination, thereby providing a strong rationale for its further evaluation in adult plants.

The second objective of this study focused on the effects of Put priming on salinity tolerance in adult cucumber plants. Foliar application of 0.5 mM Put was selected based on preliminary dose-response analysis and significantly mitigated salt-induced reductions in root growth, plant height, and total biomass, while also limiting salt-induced foliar damage. Physiological analysis revealed a recovery of photosynthetic efficiency, improved plant water status, maintenance of chlorophyll content, and enhanced stomatal regulation. Furthermore, biochemical and molecular analysis indicated reduced oxidative damage, increased antioxidant capacity, and modulation of stress-responsive gene expression. Importantly, long-term agronomic assessments demonstrated significant improvements in fruit length and weight under salinity stress.

The third objective of this study investigated PA-mediated resistance to Bemisia. Pre-infestation treatments revealed a strong and persistent protective effect of foliar spray with 5 mM Put , which markedly reduced whitefly population density, delayed insect development, and increased mortality. further confirmed that Put retained protective activity even when applied after pest establishment. These effects were associated with an improved accessory photosynthetic pigment content in Put-treated plants and a marked alteration of their volatilome, as revealed by TD–GC×GC–TOF–MS analysis. Indeed, Put priming induced distinct volatile organic compound (VOC) emission patterns, enhancing specific secondary metabolites involved in pest-defence signalling. Surprisingly, VOC-mediated effects were also observed in neighbouring Put-untreated plants, demonstrating that treatment of a single plant with Put is sufficient to confer indirect protection against pest infestation to adjacent, untreated plants.

The fourth objective of this study investigated PA-mediated defense against powdery mildew pathogen *G. cichoracearum*. Among the tested PAs, 1 mM Spd emerged as the most effective priming agent, significantly reducing fungal incidence and colony expansion, achieving protection levels like the fungicide azoxystrobin. These findings confirm that PA-induced resistance is stress-specific and that different PAs preferentially activate distinct defence pathways.

Overall, this research systematically evaluated targeted PA–stress combinations to identify optimal priming strategies for major agricultural constraints, including soil salinity, insect herbivory, and fungal disease. The results establish Put, at optimized concentrations, as a robust and versatile BS capable of enhancing stress tolerance, physiological performance, and potentially crop yield. By integrating molecular, physiological, and agronomic analysis, this work provides a solid scientific foundation for the development of sustainable PA-based strategies aimed at improving crop resilience and supporting global food security.

ABSTRACT ITALIANO

Entro il 2050, si prevede che la domanda alimentare globale aumenterà di circa il 100–110 %, ponendo una sfida senza precedenti ai sistemi agricoli e richiedendo un sostanziale incremento della produttività delle colture. Parallelamente, le proiezioni sui cambiamenti climatici indicano un aumento della temperatura globale di circa 2 °C, che dovrebbe aggravare un'ampia gamma di stress abiotici e biotici, tra cui siccità, salinizzazione dei suoli e una maggiore pressione da parte di parassiti e patogeni. Tradizionalmente, l'agricoltura moderna ha fatto ampio affidamento sul miglioramento genetico, sui fertilizzanti chimici e sui pesticidi per sostenere le rese colturali. Tuttavia, la crescente urgenza di conciliare la sicurezza alimentare con la sostenibilità ambientale ha accelerato lo sviluppo e l'adozione di strategie agronomiche ecocompatibili e sostenibili. In questo contesto, i biostimolanti (BS) sono emersi come strumenti promettenti per aumentare la resilienza delle piante e mitigare le perdite di resa in condizioni ambientali avverse. I BS migliorano le prestazioni delle piante promuovendo l'assorbimento dei nutrienti, modulando le vie di risposta allo stress e sostenendo la crescita e lo sviluppo. Tra i BS più promettenti, le poliammine (PA), tra cui putrescina (Put), spermidina (Spd) e spermina (Spm), svolgono ruoli multifunzionali nella tolleranza delle piante agli stress. Questi composti agiscono come osmoprotettori, *scavenger* delle specie reattive dell'ossigeno (ROS), regolatori dell'espressione genica e molecole di segnalazione. Inoltre, le PA fungono da precursori del perossido di idrogeno (H_2O_2), un componente chiave delle reti di segnalazione coinvolte nella risposta allo stress nelle piante.

Il cetriolo (*Cucumis sativus* L.), una delle più antiche colture orticole addomesticate, è stato scelto come specie modello per questo studio in virtù della sua rilevanza economica e della marcata sensibilità alla salinità del suolo. La salinità è stata selezionata come principale fattore di stress abiotico in quanto rappresenta un vincolo rilevante e in costante aumento per la coltivazione del cetriolo a livello mondiale. Oltre allo stress abiotico, la produzione di cetriolo è fortemente limitata anche da sfide biotiche. La mosca bianca *Bemisia tabaci* (Bemisia) è un importante fitofago succhiatore di floema che riduce la disponibilità di fotosintati, compromette il vigore delle piante e favorisce lo sviluppo della fumaggine attraverso la deposizione di melata, con un conseguente peggioramento dell'efficienza fotosintetica. Inoltre, l'oidio, causato prevalentemente da funghi biotrofi obbligati come *Golovinomyces cichoracearum*, costituisce un'ulteriore minaccia critica, in particolare nei sistemi di coltivazione protetta, dove le condizioni ambientali favoriscono una rapida proliferazione del patogeno e la diffusione della malattia.

Nonostante le ampie conoscenze sulle PA come biomolecole multifunzionali, la loro efficacia relativa come agenti di *priming* in condizioni di stress abiotici e biotici rilevanti dal punto di vista agricolo rimane ancora poco compresa. In questo studio sono stati analizzati i meccanismi

molecolari, cellulari e biochimici alla base delle risposte delle piante allo stress salino, all'erbivoria da insetti e all'infezione fungina in seguito all'applicazione esogena di PA, utilizzando *Arabidopsis thaliana* come specie modello e *C. sativus* come pianta coltivata.

Le attività sperimentali sono state condotte su semi, plantule e piante adulte presso tre sedi di ricerca: l'Università Roma Tre (Roma, Italia), il C.R.O. Agrigeos S.r.L. (Acireale, Italia) e la *School of Biosciences* della *Cardiff University* (Cardiff, Regno Unito). Questo approccio sperimentale multi-sito ha consentito l'integrazione di competenze e infrastrutture complementari, aumentando così la robustezza, la riproducibilità e la rilevanza applicativa dei risultati.Il primo obiettivo dello studio è stato valutare il contributo del *priming* dei semi con PA alla tolleranza allo stress durante la germinazione e le prime fasi di sviluppo delle plantule in condizioni di salinità. In Arabidopsis, il *priming* dei semi con 1 mM Put ha migliorato in modo consistente la percentuale di germinazione (GR) e l'indice di germinazione (GI) sia in condizioni di crescita ottimali sia in presenza di severo stress salino (200 mM NaCl). In particolare, gli effetti benefici della 1 mM Put si sono mantenuti anche in condizioni saline, determinando un miglioramento della cinetica di germinazione nelle fasi iniziali successive all'imbibizione dei semi.

Per approfondire i meccanismi molecolari alla base dell'effetto protettivo della 1 mM Put durante la germinazione e sulla base dell'efficacia ottimale osservata per questo trattamento nei precedenti esperimenti, sono stati analizzati i parametri di germinazione in linee mutanti loss-of-function di *Atcuao*. Questi mutanti presentano mutazioni loss-of-function nei geni che codificano per le ammino ossidasi rame-dipendenti (CuAO), enzimi chiave coinvolti nell'ossidazione delle PA, in particolare nel catabolismo della Put. Tale compromissione genetica determina un'alterazione del turnover della Put, consentendo di valutare come la modificazione della sua ossidazione influenzi la tolleranza allo stress salino nelle prime fasi dello sviluppo. In particolare, sono stati esaminati i mutanti *loss-of-function* di *AtCuAOβ* (*Atcuaoβ.2*), *AtCuAOγ1* (*Atcuaoγ1.1*) e *AtCuAOγ2* (*Atcuaoγ2.1*).

I dati hanno suggerito che il catabolismo della Put dipendente dalle CuAO è necessario per una corretta regolazione della germinazione sia in condizioni ottimali sia saline. In condizioni di controllo, *Atcuaoγ1* e *Atcuaoγ2* hanno mostrato dinamiche di germinazione alterate e una ridotta efficienza di germinazione rispetto al WT. In condizioni di stress salino, questi mutanti hanno manifestato una maggiore sensibilità, caratterizzata da una germinazione ritardata e incompleta. È degno di nota che il *priming* dei semi con 1 mM Put non sia stato in grado di ripristinare le prestazioni germinative nei mutanti *Atcuao* e, al contrario, abbia ulteriormente accentuato la sensibilità allo stress. Questi risultati indicano che un'ossidazione della Put non alterata è essenziale per l'effetto

benefico del *priming* osservato nelle piante WT, evidenziando un ruolo cruciale del catabolismo della Put mediato dalle CuAO nella regolazione della germinazione in condizioni saline.

Successivamente, sono stati condotti esperimenti su semi di cetriolo al fine di valutare la rilevanza agronomica del *priming* con PA. Il *priming* dei semi con 1 mM Put ha mitigato in modo significativo gli effetti negativi di una severa salinità (200 mM NaCl), determinando un aumento della GR e del GI finale rispetto ai semi non trattati e sottoposti a stress. Al contrario, il *priming* con Spd e Spm, sebbene meno efficace o addirittura inibitorio rispetto alla Put durante la germinazione, ha promosso selettivamente la crescita precoce delle radici nelle plantule di Arabidopsis, in misura maggiore rispetto al *priming* con Put. Questa osservazione evidenzia un ruolo specifico delle diverse PA in funzione della fase di sviluppo e del tipo di risposta. Complessivamente, questi risultati hanno identificato la Put come l'agente di *priming* più efficace durante la germinazione, fornendo una solida base per la sua ulteriore valutazione nelle piante adulte.

Il secondo obiettivo dello studio si è concentrato sugli effetti del *priming* con Put sulla tolleranza alla salinità nelle piante adulte di cetriolo. L'applicazione fogliare di 0,5 mM Put, selezionata sulla base di analisi preliminari dose-risposta, ha mitigato in modo significativo le riduzioni indotte dal sale nella crescita radicale, nell'altezza delle piante e nella biomassa totale, limitando al contempo i danni fogliari causati dalla salinità. Le analisi fisiologiche hanno evidenziato un recupero dell'efficienza fotosintetica, un miglioramento dello stato idrico della pianta, il mantenimento del contenuto di clorofilla e una regolazione stomatica più efficiente. Inoltre, le analisi biochimiche e molecolari hanno indicato una riduzione del danno ossidativo, un aumento della capacità antiossidante e una modulazione dell'espressione dei geni responsivi allo stress. È importante sottolineare che le valutazioni agronomiche a lungo termine hanno mostrato un miglioramento significativo della lunghezza e del peso dei frutti in condizioni di stress salino.

Il terzo obiettivo dello studio ha indagato la resistenza mediata dalle PA nei confronti di *Bemisia tabaci*. I trattamenti effettuati prima dell'infestazione hanno rivelato un forte e persistente effetto protettivo della nebulizzazione fogliare con 5 mM Put, che ha ridotto marcatamente la densità della popolazione di mosca bianca, ritardato lo sviluppo degli insetti e aumentato la mortalità. Ulteriori analisi hanno confermato che la Put mantiene la sua attività protettiva anche quando applicata dopo l'insediamento del fitofago. Tali effetti sono stati associati a un miglioramento del contenuto di pigmenti fotosintetici accessori nelle piante trattate con Put e a una marcata alterazione del loro volatiloma, come evidenziato dall'analisi TD–GC×GC–TOF–MS. In particolare, il *priming* con Put ha indotto specifici profili di emissione di composti organici volatili (VOC), potenziando metaboliti secondari coinvolti nella segnalazione di difesa contro i parassiti. Sorprendentemente, effetti mediati dai VOC sono stati osservati anche nelle piante vicine non trattate con Put, dimostrando

che il trattamento di una singola pianta è sufficiente a conferire una protezione indiretta contro l'infestazione anche alle piante adiacenti non trattate.

Il quarto obiettivo dello studio ha riguardato la difesa mediata dalle PA contro il patogeno dell'oidio *G. cichoracearum*. Tra le PA testate, la 1 mM Spd è risultata l'agente di *priming* più efficace, riducendo significativamente l'incidenza dell'infezione fungina e l'espansione delle colonie, con livelli di protezione paragonabili a quelli del fungicida azoxystrobin. Questi risultati confermano che la resistenza indotta dalle PA è specifica per il tipo di stress e che differenti PA attivano preferenzialmente vie di difesa distinte.

Nel complesso, questa ricerca ha valutato in modo sistematico combinazioni mirate PA–stress per identificare strategie di *priming* ottimali nei confronti di importanti limitazioni agricole, tra cui la salinità del suolo, l'erbivoria da insetti e le malattie fungine. I risultati identificano la Put, a concentrazioni ottimizzate, come un BS robusto e versatile, in grado di migliorare la tolleranza allo stress, le prestazioni fisiologiche e potenzialmente la resa delle colture. Integrando analisi molecolari, fisiologiche e agronomiche, questo lavoro fornisce una solida base scientifica per lo sviluppo di strategie sostenibili basate sulle PA, finalizzate a migliorare la resilienza delle colture e a sostenere la sicurezza alimentare globale.

1. Introduction

1.1 Global food security: current challenges and perspectives

The challenge of meeting the growing global demand for food, which is expected to increase by 50 to 100 % by 2050, remains one of the most urgent and important issues for the international community (Tilman *et al*., 2011; Alexandratos & Bruinsma, 2012; Rockström *et al*., 2017).

Since agriculture is crucial for achieving the UN Sustainable Development Goals of eradicating hunger, reaching the projected demand requires a 60–110 % boost in food production by 2050 (Mba *et al*., 2012; Pardey *et al*., 2014; Rockström *et al*., 2017). The Integrated Pollution Prevention and Control (IPPC) of 2018 states that a major obstacle to reaching this goal will be the rise of 2 °C of global temperature, which is likely to result in more extreme weather events and more frequent crop failures (Hoegh-Guldberg *et al*., 2018).

The resulting scenario is further complicated, as climate change amplifies numerous abiotic and biotic stresses, which already constrain plant growth and limit agricultural output. Understanding and mitigating these stresses are therefore fundamental steps toward achieving resilient and sustainable food production systems.

1.2 Abiotic and biotic stresses as major constraints to crop productivity

Plants are sessile organisms, constantly interacting with a wide range of environmental factors that can adversely affect their growth, development, and productivity These adverse conditions are generally classified into abiotic stresses, such as salinity, drought, extreme temperatures, or nutrient imbalances, and biotic stresses, caused by organisms including insects, fungi, bacteria and viruses. Both categories of stress can significantly compromise plant performance, leading to reductions in yield and overall crop quality (Rejeb *et al*., 2014; Zhang *et al*., 2022).

Abiotic and biotic stresses act through distinct mechanisms, triggering specific physiological and molecular responses in plants. Abiotic stresses often disrupt fundamental processes such as water balance, ion homeostasis, and photosynthesis, whereas biotic stresses typically activate immune pathways aimed at recognizing and combating invading organisms. Despite these differences, plants must continuously coordinate these responses to maintain growth and survival. The study of plant-environment interactions, especially regarding plant responses to stress, holds significant economic and agricultural importance (Cramer *et al*., 2011; Rejeb *et al*., 2014).

1.2.1 Abiotic stress in plants: salt stress

Abiotic stresses are the most significant constraint on global agricultural productivity, with estimates indicating that it can reduce average yields of major crops by more than 50 % (Lamaoui *et*

al., 2018; Godoy *et al*., 2021). These stresses arise from different environmental factors, including drought, extreme temperatures (both heat and cold), salinity, heavy metal toxicity, flooding, and nutrient imbalances. Exposure to such suboptimal conditions forces plants to activate a variety of physiological and molecular defence mechanisms. The challenge posed by abiotic stresses is being further intensified by climate change, which increases the frequency and severity of extreme weather events, thereby amplifying their detrimental effects on agricultural systems worldwide. Among different abiotic stresses, soil salinity is considered one of the most pervasive and damaging, affecting extensive areas of arable land on a global scale (Chele *et al*., 2021). Its profound impact on plant health and crop productivity underscores the need for a deeper understanding of its mechanisms and the development of effective strategies to mitigate its effects (Munns & Tester, 2008a).

The Soil Science Society of America defines saline soil as "*non-sodic soil containing sufficient amount of soluble salt which could adversely influence most crop plants*". (Hassani *et al*., 2021). Soil salinization is a growing global concern, further intensified by climate change, which accelerates processes such as seawater intrusion, increased evaporation, and reduced freshwater availability. These phenomena contribute to the degradation of agricultural lands, resulting in lower crop productivity and desertification. Globally, salt-affected soils cover approximately 17 million km² (Negacz *et al*., 2022), and some of the world's most populated countries, including the United States, China and Russia, show extensive salinity-affected regions (Ivushkin *et al*., 2019).

Although Earth's land surface is totals 13.2×10^9 ha, only 53 % is arable, and merely 11.4 % is currently cultivated (Nachtergaele *et al*., 2006). Of these cultivated areas, about 23 % are saline and 37 % are sodic, highlighting the global scale of the problem.

Salinity compromises plant growth and yield primarily through osmotic, ionic and oxidative stresses. High concentrations of sodium (Na^+) interfere with nutrient homeostasis, particularly by reducing potassium (K^+) uptake and increasing its exclusion from cells, leading to K^+ deficiency (Ma *et al*., 2016). The soil accumulation of soluble salts also lowers the water potential at the root–soil interface, limiting water availability and causing physiological drought even when soil moisture is present (Hasegawa *et al*., 2000). Once absorbed, salts are transported through the transpiration stream, accumulating predominantly in leaves where their toxic effects are most pronounced (Munns & Tester, 2008; Taha *et al*., 2020).

These osmotic and ionic disturbances frequently trigger secondary stresses, including altered nutrient balance, impaired metabolic functions, and the buildup of ROS such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), hydroxyl radicals (•OH), singlet oxygen ($^1O_2$; Yang & Guo, 2018). Excessive ROS accumulation can lead to oxidative damage to lipids, proteins, and nucleic acids, further exacerbating the detrimental impact of salinity on plant performance.

1.2.2 Biotic stress in plants: *Bemisia tabaci* and Powdery mildew as common agricultural pests

Biotic stresses arise from interactions between plants and living organisms such as insects, fungi, bacteria, viruses, and nematodes, which can significantly compromise plant health and agricultural productivity. In recent decades, climate change has markedly influenced biotic stress patterns, altering both pest dynamics and plant/pest interactions. These effects occur through a combination of direct and indirect mechanisms. Direct impacts involve changes in pest development, reproduction, and survival, while indirect effects include modifications in the ecological relationships among pests, their host plants, and associated organisms such as natural enemies, competitors, vectors, and mutualists (Prakash *et al*., 2014). Among the climatic variables, temperature is one of the most critical determinants of insect biology. Insects are ectothermic organisms, and their physiological processes, particularly metabolism, are susceptible to temperature fluctuations. On average, metabolic rates can double with a 10 °C increase (Dukes *et al*., 2009). Therefore, rising temperatures can reshape pest population dynamics by affecting fecundity, survival, development time, the number of generations per year, population size and the geographical distribution of species (Bale *et al*., 2002). Temperature also plays a central role in determining insect mobility, metamorphosis, and host availability, all of which contribute to shifts in pest prevalence and the severity of infestations (Shrestha, 2019).

The whitefly *Bemisia tabaci* (Gennadius, Hemiptera: Aleyrodidae; Bemisia) is a globally distributed phloem-feeding pest originating from India and capable of infesting more than 600 plant species in both open-field and greenhouse conditions (Li *et al*., 2011). Its extensive host range includes numerous economically important vegetables, fiber, and ornamental crops. Because of its rapid population growth and adaptability, Bemisia often leads to intensive insecticide application, which contributes to the development of resistance. This pest causes damage through multiple mechanisms: direct feeding on phloem sap weakens plant vigor, honeydew accumulation promotes the growth of sooty mold that interferes with photosynthesis, and Bemisia serves as a highly efficient vector of several plant viruses, particularly those belonging to the genus *Begomovirus* (Geminiviridae; Mound *et al*., 1978; Secker *et al*., 1998; Fauquet *et al*., 2005; Polston *et al*., 2014). These combined effects can severely compromise crop productivity and quality across a wide range of cultivated species (Oliveira *et al*., 2001).

Powdery mildew is among the most widespread and destructive fungal diseases globally, affecting more than 10,000 plant species across 200 countries and causing estimated annual agricultural losses of 6.3 billion USD (Glawe, 2008; Sotiropoulos *et al*., 2022; Bradshaw *et al*., 2024; Gan *et al*., 2025). Powdery mildew fungi are obligatory biotrophic Ascomycetes (order *Erysiphales*) that depend on living host tissue to complete their life cycle. During infection, they manipulate host

cellular structures and functions to support their growth, disrupting key physiological processes at the plant–fungus interface. Infection typically begins when conidiospores land on the leaf surface, germinate, and form appressoria that attempt to penetrate the epidermis. Once inside, the pathogen develops haustoria, specialized feeding structures that enable nutrient uptake from host cells. Powdery mildew fungi may also secrete epicuticular alkanes or very-long-chain aldehydes, which can contribute to adhesion and the initiation of infection structures (Noir *et al*., 2009; Hückelhoven & Panstruga, 2011). Following penetration, powdery mildew fungi releases effector molecules that suppress host immune responses and inhibit host cell death (Bhat *et al*., 2005). As colonization progresses, the pathogen establishes a strong nutrient sink, altering local tissue physiology and enabling sustained parasitism.

1.3 *Cucumis sativus*: agronomic relevance and sensitivity to salt stress

The cucumber, *Cucumis sativus* L., is a member of the Cucurbitaceae, a family comprising 90 genera and 750 species (Sitterly, 1972). It is one of the oldest cultivated vegetable crops, domesticated over 5000 years ago in India and now grown throughout temperate regions worldwide. Cucumber represents a major horticultural commodity, with global production reaching approximately 87.8 million tons in 2021 (Whitaker, 1962; Wehner & Robinson, 1991; Wang, 2021). Its economic relevance, combined with a notable susceptibility to diverse environmental constraints, makes the cucumber an important system for studying plant stress responses.

C. sativus is generally classified as a glycophyte and is recognized as a salt-sensitive crop (Zhu *et al*., 2004). Even moderate salinity strongly limits its overall performance, with electrical conductivity values above 2.5 dS m^{-1} in the root zone typically leading to measurable yield reductions (Al-Momany & Abu-Romman, 2023). The early phases of development are particularly vulnerable: salinity rapidly decreases germination rates and severely constrains seedling establishment, largely due to osmotic effects that reduce water uptake (Munns & Tester, 2008).

Beyond osmotic constraints, salt stress imposes substantial ionic and oxidative burdens on cucumber plants. Excess Na^+ disrupts nutrient homeostasis, while the associated physiological imbalance promotes the overproduction of ROS. Under salinity, impaired electron transport in chloroplasts and mitochondria further amplifies ROS generation. Accumulation of ROS leads to lipid peroxidation and damage to photosynthetic pigments, proteins, nucleic acids, and membrane structures. Consequently, the plant's antioxidant machinery becomes essential: numerous studies indicate that salt tolerance in cucumber is closely associated with a more efficient antioxidant system capable of maintaining redox balance under stress (Naliwajski *et al*., 2021). Interestingly, several studies have provided important insights into how exogenous ~~Put~~ PA, especially Put, modulate~~s~~ plant

stress responses, particularly in cucumber under salinity, demonstrating that salt stress impairs photosynthesis by damaging PSII and reducing Fv/Fm, while Put mitigates these effects by preserving thylakoid structure and restoring PSII efficiency (Shu *et al*., 2012). Similarly, in cucumber, it has been reported that Put enhances photoprotection under salt stress by promoting regulated energy dissipation, reducing uncontrolled heat loss, and stimulating xanthophyll cycle de-epoxidation, thereby improving the capacity to dissipate excess excitation energy (Yuan *et al*., 2014). Put also influences root development and cellular resilience, alleviating salt-induced inhibition of root growth by modulating proteins involved in energy metabolism, amino acid pathways, defence, and protein turnover, supporting structural and metabolic stability of the root system (Yuan *et al*., 2016). It has also been shown that Put supports ionic homeostasis by limiting Na^+ buildup, preserving K^+ levels, and modulating SOS1, NHX, and HKT1 transporters, with PA-derived H_2O_2 acting as a signalling mediator in this regulation (Yuan *et al*., 2019).

1.3.1 Interaction between cucumber and Bemisia

Cucumber is highly susceptible to pest attacks like Bemisia, and infestations impair plant health through various mechanisms. Direct feeding on phloem sap reduces photosynthates and weakens plant vigor, while honeydew deposits encourage the growth of sooty mold that blocks light and hampers photosynthesis (Oliveira *et al*., 2001). Feeding also disrupts host physiology at the cellular level, leading to decreases in chlorophyll content, changes in photosynthetic efficiency, shifts in carbohydrate and amino acid profiles, and increased production of ROS (He *et al*., 2022; Morin *et al*., 2024).

The interaction also involves complex immune responses. Like other piercing–sucking insects, *B. tabaci* saliva suppresses jasmonic acid (JA)-mediated defences while activating salicylic acid (SA)-dependent pathways, allowing prolonged feeding and aiding colonization of cucumber tissues (Lin *et al*., 2019; Zhao *et al*., 2025). These immune responses are further amplified when the whitefly carries plant viruses (Novaes *et al*., 2020). In fact, Bemisia is an extremely effective vector of several begomoviruses, including Tomato Yellow Leaf Curl Virus (TYLCV), which can drastically reduce cucumber growth and yield. Viral infection influences the plant–insect relationship: TYLCV not only manipulates host defences but can also improve the fitness, fecundity, and survival of Bemisia, creating a mutually reinforcing cycle that worsens disease severity (Luan *et al*., 2014). As a result, virus–vector–host interactions pose a major threat in cucurbit production.

The impact of Bemisia on cucumbers can be significant. Infestations can cause severe yield losses during severe outbreaks, along with reductions in fruit size, weight, and quality for market sales (Dong *et al*., 2014). Effective management is further complicated by the insect's high

reproductive rate and its rapid development of resistance to multiple insecticide classes (Fernández *et al*., 2009; Erdogan *et al*., 2024). Overall, Bemisia remains one of the most critical pests in cucumber farming and a primary focus of integrated pest management strategies.

1.3.2 Powdery Mildew infection in cucumber

Like many members of the Cucurbitaceae, cucumber is highly susceptible to powdery mildew, a disease that remains one of the most persistent constraints in cucumber cultivation worldwide (Xu *et al*., 2024). Powdery mildew in cucumber is primarily associated with two obligate biotrophic fungi, *G. cichoracearum* and *Podosphaera xanthii*, with *G. cichoracearum* representing one of the main causal agents in many production regions (Block & Reitsma, 2005; Corneo *et al*., 2021).

Under conducive conditions, especially in protected environments such as greenhouses, the pathogen rapidly colonizes leaf surfaces, forming extensive mycelial growth that drastically reduces the functional photosynthetic area. This leads to premature leaf senescence, impaired carbon assimilation, reduced plant vigor, and ultimately significant declines in fruit set, fruit quality, and overall yield (Gordon & Duniway, 1982; Tian *et al*., 2024).

1.4 Salt stress and pest infestation impact on crop yield and management strategies

To contrast abiotic stress, such as salinity, and biotic stress, such as pest and pathogen infestation, modern agriculture requires continuous development of new methodologies, procedures, and protective compounds. Management strategies vary widely depending on the type of stress. For fungal diseases like powdery mildew, particularly those caused by *G. cichoracearum* and *Podosphaera xanthii*, control in cucumber traditionally relies on frequent fungicide applications (McGrath, 2001). However, resistance to several fungicide classes has increasingly been reported (Lebeda *et al*., 2010), reducing the long-term effectiveness of chemical control.

At present, despite progress in environmentally friendly pest management approaches, chemical control remains one of the most widely used methods due to its cost-effectiveness and ease of implementation (Choudhary *et al*., 2021). In the case of salinity, management requires context-specific strategies ranging from agro-hydrotechnical interventions to chemical amendments, depending on soil type, salt composition, and severity of the stress (Shahid *et al*., 2018).

There is increasing interest in new compounds able to mitigate abiotic and biotic stress in plants, such as BSs and physio-activators, which are among the most promising alternatives today for coping with yield losses caused by plant stresses intensified by climate change (Bulgari *et al*., 2019; Ondrasek *et al*., 2022).

1.5 Biostimulants as a sustainable solution

BSs are an emerging class of agricultural inputs that play a crucial role in sustainable crop production by enhancing plant growth, development, and stress tolerance without exerting direct pesticidal or fertilizing effects. Unlike traditional agrochemicals, BSs mainly work by modulating plant physiological processes, such as nutrient uptake, photosynthesis, and defence responses, thereby improving crop resilience to adverse environmental conditions. Over the past decade, increasing scientific interest and regulatory developments have led to a clearer definition of BSs. According to the European Union Regulation 2019/1009, as reported by the European Biostimulants Industry Council (EBIC), a plant BS is defined as "A product that stimulates plant nutrition processes independently of the product's nutrient content, with the primary goal of improving one or more of the following characteristics of the plant or the plant rhizosphere: (*i*) nutrient use efficiency; (*ii*) tolerance to abiotic stress; (*iii*) quality traits; or (*iv*) availability of confined nutrients in the soil or rhizosphere." (García-García *et al*., 2020). This regulatory recognition has promoted increased research and commercial development of BSs, especially in the context of climate-resilient agriculture, where the demand for tools that can improve crop performance under abiotic and biotic stress conditions is rapidly rising.

BSs encompass a diverse array of natural or biologically derived substances and microorganisms, and their classification has been the subject of several studies. Among the most widely adopted systems, a classification based on the origin of active compounds was proposed, grouping BSs into seven main categories: (*i*) humic and fulvic acids, organic substances derived from the decomposition of plant material; (*ii*) algae and botanical extracts, rich in phytohormones, polysaccharides, and antioxidants; (*iii*) hydrolyzed proteins and nitrogen-containing compounds, precursors for metabolic pathways and signalling molecules; (*iv*) chitosan and other biopolymers, derived from chitin, these substances are recognized for their elicitor activity in plant defence mechanisms; (*v*) inorganic compounds, including certain silicon and selenium formulations, which can strengthen plant tissues and enhance resistance; (*vi*) fungi, notably arbuscular mycorrhizal fungi (AMF), which facilitate nutrient exchange and water absorption, (*vii*) beneficial bacteria, such as plant growth-promoting rhizobacteria, which contribute to root health and nutrient (du Jardin, 2015; Bulgari *et al*., 2019). The multifaceted nature of BSs allows them to interact with both plant metabolism and the surrounding rhizosphere microbiome (du Jardin, 2015; Rouphael & Colla, 2020), often leading to synergistic effects that go beyond the simple addition of nutrients or protective agents. As such, BSs represent a promising strategy for improving crop performance under non-optimal growth conditions, such as high salinity, drought, or pathogen pressure, and rising interest in these compounds reflects a broader shift toward eco-compatible agriculture, driven by the need to reduce

the environmental footprint of traditional inputs and meet increasingly strict regulatory and sustainability goals globally.

Research in the field of plant BS has mainly focused on complex mixtures like plant extracts and recycled waste products, an approach that supports the principles of a circular economy and can lead to more effective and synergistic effects of the different compounds in the mixture (Bulgari *et al*., 2019). However, using such mixtures presents challenges, especially regarding batch-to-batch consistency, which can vary depending on factors as the source material's development stage, environmental conditions, and time of year (Yakhin *et al*., 2017). While some companies attempt to control production and analyse the final product, establishing a fully standardized protocol remains difficult, particularly when active principles are unknown or scarce. This has spurred increased efforts to develop BS based on pure active compounds, which offer advantages in understanding physiological effects, modes of action, and simplifying certification and registration processes (Yakhin *et al*., 2017; García-García *et al*., 2020). Examples of these pure compounds against abiotic and biotic stress include melatonin, GABA, and PAs (Yong *et al*., 2017; Arnao & Hernández-Ruiz, 2019).

1.5.1 Polyamines and their potential role in plant stress tolerance

PAs are polycationic compounds with two or more amino groups ($-NH_3^+$). They are present in all living organisms and are involved in many different defence and development processes in plants, from seed germination (Lenis *et al*., 2017; Blázquez, 2024) to abiotic and biotic stress response. The most abundant PAs in plants are Put (di-ammine), Spd (tri-ammine), and Spm (tetra-ammine; Killiny & Nehela, 2020; **Figure 1**).

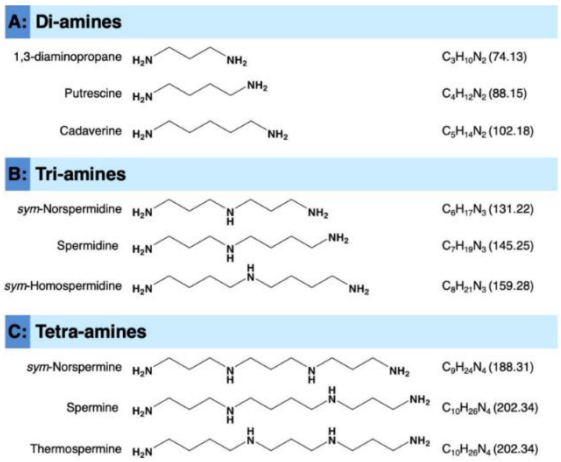


Figure 1: PA chemical structures. Chemical structures of the PAs and other nitrogen-containing molecules are discussed throughout this review. (**A**) Diamines, (**B**) Tri-amines, (**C**) Tetra-amines. Molecular weight/molar mass (g·mol−1) is mentioned between parentheses beside the chemical formula of each compound (Modified by Killiny & Nehela, 2020).

Put is the precursor compound for the longest PAs, Spd and Spm. In plants, there are two pathways for Put biosynthesis: the ornithine decarboxylase (ODC, EC 4.1.1.17) pathway and the arginine decarboxylase (ADC, EC 4.1.1.19) pathway, both involving several intermediates from arginine. In the ornithine pathway, the process begins with the amino acid arginine, which is converted into ornithine by the enzyme arginase. Ornithine is subsequently decarboxylated by ODC to obtain Put. In the alternative ADC pathway, arginine is decarboxylated by ADC to form agmatine. Agmatine is subsequently converted into *N*-carbamoyl Put, which is hydrolysed to generate Put. The synthesis of Spd proceeds through the action of Put aminopropyl transferase (PAPT), which catalyses the transfer of an aminopropyl group to Put. This aminopropyl group is donated by decarboxylated S-adenosylmethionine (dcSAM), the universal donor of aminopropyl moieties in PA biosynthesis. Spd can be further converted into Spm via spermidine-aminopropyl-transferase (SAPT), which facilitates the addition of another aminopropyl group (Kuznetsov *et al*., 2006; **Figure 2**).

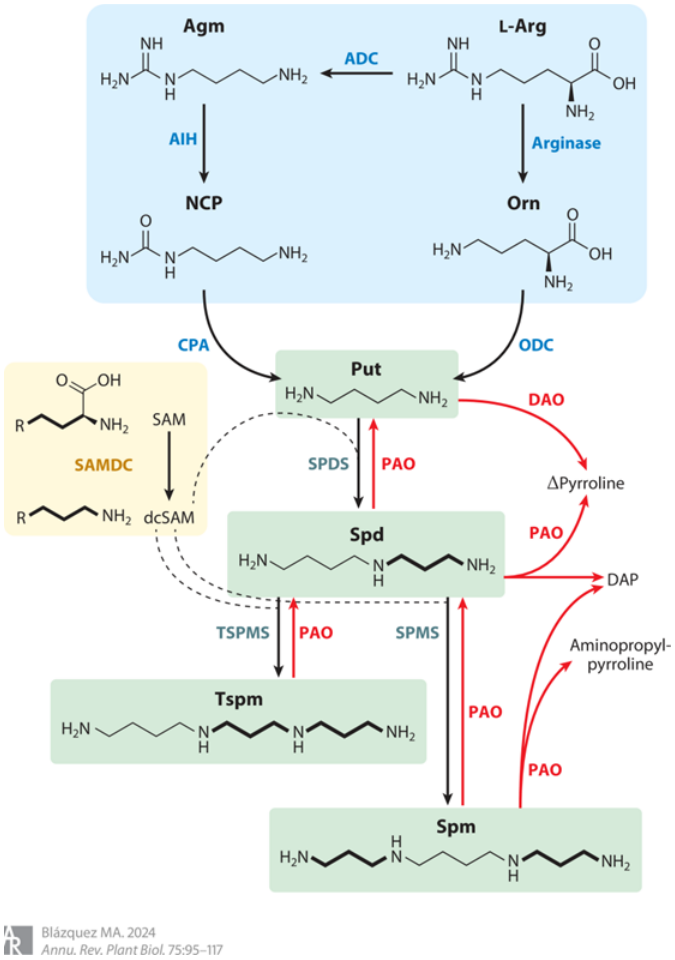


Figure 2: Polyamines (PAs) biosynthesis pathways in plants. The two pathways leading to Put biosynthesis are depicted in blue, the four major PAs in plants are shown in green, and the production of dcSAM as the aminopropyl donor for the synthesis of tri and tetra amines is marked in yellow. Reactions involved in PA catabolism and back conversion are shown in red (Blázquez, 2024).

The formation of Put from arginine involving ADC is typically associated with plant responses to stress. ADC's higher activity in light compared to darkness explains its localization in chloroplasts (Borrell *et al*., 1995). Molecular biology analysis has shown that ADC is localized in nearly all organs, from flowers to roots (Bortolotti *et al*., 2004; Paschalidis & Roubelakis-Angelakis, 2005). Although polyamine biosynthesis via the arginine and ornithine pathways has been extensively studied in plants, the coordinated regulation and functional interaction of these pathways under physiological and stress conditions are not yet fully understood (Tiburcio *et al*., 2014). Data indicate that Put accumulation and ADC activation occur under unfavorable conditions such as drought, salinity, hyperthermia, potassium, and sulfur deficits (Bouchereau *et al*., 1999). Typically, in sensitive plant species, stress leads to a selective accumulation of Put (Basu *et al*., 1988). The two pathways of Put biosynthesis are spatially separated, tissue-specific, and developmentally regulated. Early studies reported a nuclear localization of plant ODC (as summarized by Slocum & Flores, 2024). Other subsequent biochemical and molecular evidence indicate that ODC is predominantly localized in the cytosol and mitochondria and is tightly associated with chromatin (Bagni & Tassoni, 2001; Takahashi & Kakehi, 2010). Conversely, ADC is found in the thylakoid membranes of chloroplasts (Borrell *et al*., 1995; Tiburcio *et al*., 1997).

Beyond their synthesis and localization, PA homeostasis is also maintained through catabolic pathways, primarily mediated by Amine oxidases (AOs).

AOs are a diverse group of enzymes that include copper amine oxidases (CuAOs) and flavin-containing PA oxidases (PAOs). They are part of the complex enzyme network responsible for the oxidative deamination of PAs into amino aldehydes. During AO oxidation reactions, an amine group, ammonium, and biologically active H_2O_2 are produced as co-products. Plant PAOs oxidize PAs at the secondary amino groups through either terminal catabolism or interconversion pathways, with the reaction products depending on both the catalytic mechanism and specific substrates. Conversely, CuAOs are involved in the terminal breakdown of PAs, oxidizing PAs and monoamines at the terminal amine group and producing ammonium, the corresponding aminoaldehyde from the substrate, and H_2O_2 (Fraudentali *et al*., 2021).

CuAOs are homodimeric enzymes composed of 70–90 kDa subunits, each containing a copper ion and a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor, which is formed through a post-translational, autocatalytic modification of an active site tyrosine residue CuAOs have been identified and characterized in various plant species (Cona *et al*., 2006; Tavladoraki *et al*., 2016; Wang *et al*., 2019). The most comprehensive analysis of a CuAO gene family has been performed in *Arabidopsis thaliana* (Arabidopsis), where 10 different CuAO-encoding genes have been identified. Among them, eight are predicted to encode functional enzymes: *AtCuAOα1* (At1g31670), *AtCuAOα2*

(At1g31690), *AtCuAOα3* (At1g31710), *AtCuAOβ* (At4g14940), *AtCuAOγ1* (At1g62810), *AtCuAOγ2* (At3g43670), *AtCuAOδ* (At4g12290), and *AtCuAOζ* (At2g42490). The remaining two genes, *AtCuAOε1* (At4g12270) and *AtCuAOε2* (At4g12280), are located upstream of *AtCuAOδ* on chromosome 4 and are thought to have originated from a duplication event of *AtCuAOδ*, followed by insertion of the transposable element At4g12275 (Tavladoraki *et al*., 2016; Groß *et al*., 2017; Fraudentali *et al*., 2021).

Previous studies have linked the activity of *AtCuAOβ*, *AtCuAOγ1*, and *AtCuAOγ2* to the regulation of abiotic stress and developmental processes (Fraudentali *et al*., 2020), making them targets to investigate the role of PA catabolism in abiotic stress resistance.

Regarding the external application of PAs as BSs in crop species directly in the field, treated plants exhibit greater stress tolerance, whereas inhibiting PA biosynthesis results in increased stress damage. External application of Put has been shown to enhance growth, photosynthetic pigment content, yield, and quality in onion (*Allium cepa*). Similarly, foliar application of Put has been reported to improve growth and essential oil yield under stress conditions in thyme (*Thymus vulgaris* L.; Abd Elbar *et al*., 2019), to enhance drought tolerance in wheat and lettuce (Zhu *et al*., 2019), and to increase salt tolerance in yellow guava seedlings (*Psidium guajava* L.; Esfandiari Ghalati *et al*., 2020). Exogenous PAs may enhance stress tolerance through processes such as: (*i*) functioning as osmoprotectors; (*ii*) scavenging ROS; (*iii*) regulating gene expression; (*iv*) acting as signalling molecules or sources of bioactive molecules like H_2O_2; (*v*) modulating ion channels; and (*vi*) inducing programmed cell death (Minocha *et al*., 2014). Indeed, H_2O_2, originating from the oxidation of PAs catalysed by AOs, can act as a stress signalling molecule at low levels (Wang *et al*., 2019), but at high levels, it can be toxic, damaging lipids, proteins, and nucleic acids (Miller *et al*., 2009). This dual role suggests that the exogenous application of PAs as BSs may operate by finely modulating H_2O_2 concentration to promote hormesis and activate stress-responsive pathways without causing lethal oxidative damage. Many studies demonstrate that ROS mediates systemic propagation of signals triggered by abiotic stress through a process linked to Ca^{2+} signalling, called the "ROS wave" (Miller *et al*., 2009; Mittler *et al*., 2011; Gilroy *et al*., 2014; Gilroy *et al*., 2016).

Another key role of PAs in stress regulation is enhancing plant defence responses through the modulation of signalling pathways. Several studies have shown that exogenous application of PAs, particularly Spm, can activate JA biosynthesis and promote the accumulation of ROS, both of which are critical in signalling pathways leading to the induction of defensive volatile organic compounds (VOCs; Ozawa *et al*., 2009; Seifi *et al*., 2019). Specifically, treatment of *Phaseolus lunatus* leaves with Spm significantly increased the production of volatile terpenoids typically associated with responses to infestation by *Tetranychus urticae*, a phytophagous mite (Ozawa *et al*.,

2009). Moreover, when Spm was applied together with JA, VOC production was enhanced compared to JA alone, positively affecting the attraction of natural enemies of herbivores (Arimura *et al*., 2005). Finally, in a recent study, exogenously applied Spm also slowed down the development of *T. urticae* (Shahtousi & Talaee, 2023). These results suggest that PAs can act as co-activators of defence signalling pathways, influencing the emission of Herbivore-Induced Plant Volatiles (HIPVs) and thereby facilitating an indirect response to biotic stress (Pérez-Hedo *et al*., 2021). Additionally, their ability to modulate redox metabolism and intracellular Ca^{2+} flux may contribute to the activation of specific volatile profiles, making them promising tools for sustainable plant defence improvement (Li *et al*., 2019).

Furthermore, the effectiveness of Spm and Spd has been demonstrated against necrotrophic fungi, where low concentrations of Spm were found to be effective in 'priming' resistance against *Botrytis cinerea* in Arabidopsis by modulating ROS dynamics and related metabolic pathways (Janse van Rensburg *et al*., 2021).

Therefore, using PAs as BSs can improve tolerance to environmental stresses while promoting the production of functional VOCs, offering benefits both at the agronomic level, resistance to pathogens and insects, and post-harvest, like product quality and shelf life. In conclusion, PAs are not only essential endogenous regulators of plant growth and stress responses but also represent a promising class of BSs for enhancing crop resilience and productivity.

2. Aim of the thesis

In recent years, the application of BSs has gained increasing relevance as part of sustainable agricultural strategies aimed at enhancing crop yield and resilience, thereby contributing to global food security (Seifi *et al*., 2019). BS based approaches offer a promising alternative to conventional methods such as genetic crop improvement, which is often constrained by regulatory limitations and infrastructural requirements, and the widespread use of chemical fertilizers and pesticides.

This study aims to investigate the molecular, cellular, and biochemical mechanisms underlying plant responses to abiotic and biotic stresses following exogenous BS treatment, such as PAs, using both seeds, seedlings, and adult plants of the model organism Arabidopsis and the crop species *C. sativus*. Specifically, the research was carried out across three locations to integrate diverse scientific expertise and infrastructure: *Roma Tre* University (Rome, Italy), C.R.O. Agrigeos S.r.L. (Contract Research Organization; Acireale, Italy), and the School of Biosciences, Cardiff University (Cardiff, UK). This approach ensured the integration of detailed molecular analyses, focused on the variations in biochemical stress markers and the expression of stress-responsive genes, with the assessment of commercially valuable traits, as the analyses of plant morphological development and

fruit production, through large-scale greenhouse and field trials conducted under relevant agricultural stress conditions.

C. sativus is one of the world's major vegetable crops, with steadily rising production and a high global market value (Wang, 2021). However, its cultivation remains particularly vulnerable to both abiotic and biotic stresses, which reduce the extent of arable land suitable for its growth, lower its yield, and negatively affect its commercial potential (Zhu *et al*., 2004; Block & Reitsma, 2005; Novaes *et al*., 2020; He *et al*., 2022). Despite accumulating evidence supporting the beneficial effects of PAs on plant stress tolerance (Abd Elbar *et al*., 2019; Zhu *et al*., 2019; Arslan *et al*., 2019; Esfandiari Ghalati *et al*., 2020), the precise molecular and cellular mechanisms through which they act remain largely unknown. Indeed, limited information is available regarding how PAs modulate the interplay among signalling pathways activated by different stressors, the direct mechanisms through which they attenuate stress, and the most effective PA-based strategies for specific abiotic and biotic challenges.

Therefore, this research aims to investigate diverse PA–stress combinations to identify optimal treatment strategies for major agricultural constraints, particularly soil salinity and pest infestation to establish a robust scientific basis for developing sustainable, effective PA-based solutions aimed at improving crop resilience and supporting global food security.

This research aimed to investigate the following aspects:

- the effect of seed priming with PA on germination parameters of Arabidopsis and cucumber seeds, both in optimal and salt stress conditions
- the effect of PA-foliar spray on morphological, physiological, biochemical, and agronomic parameters of cucumber adult plants, both in optimal and salt stress conditions
- the effect of PA priming of cucumber adult plants against Bemisia infestation and VOC emission
- the effect of PA priming of cucumber adult plants against powdery mildew infection

3 Materials and Methods

3.1 Plant materials, chemical reagents, growth conditions, and stress

3.1.1 Plant material and chemicals

Arabidopsis thaliana experiments were performed using the Columbia-0 (Col-0) wild-type (WT) ecotype. T-DNA insertion lines impaired in copper amine oxidase (*CuAO*) genes were included: *Atcuaoβ.2* (SALK_077391; TAIR locus At4g14940), *Atcuaoγ1.1* (SALK_019030; TAIR locus At1g62810), and *Atcuaoγ2.1* (SALK_099684; TAIR locus At3g43670). These lines were used for *in vitro* germination and seedling root growth assays.

Experiments on crop species were conducted using *Cucumis sativus* L. (cucumber), cultivar "Marketmore". Seeds were supplied by Fratelli Ingegnoli S.p.A. (Milan, Italy).

The PAs used for all priming treatments were putrescine dihydrochloride (Put), spermidine trihydrochloride (Spd), and spermine tetrahydrochloride (Spm) (Sigma-Aldrich). For Bemisia resistance assays, acetamiprid (2.1 mM; EPIK® SL, Sipcam Italia S.p.A.) was included as a chemical control. For *G. cichoracearum* resistance assays, azoxystrobin (1.2 mM; ORTIVA®, Syngenta) was used as a chemical control.

3.1.2 Growth conditions and sterilization

All plants were grown in controlled-environment growth chambers under a 16 h light/8 h dark photoperiod. Arabidopsis and *C. sativus* cultures were maintained at 23 °C with 55 % relative humidity.

Before all *in vitro* assays, Arabidopsis and *C. sativus* seeds were surface sterilized as described by (Valvekens *et al*., 1988). After sterilization, Arabidopsis seeds were cold stratified by soaking in the dark at 4 °C for 2 days. Seeds were then sown on solid half-strength Murashige and Skoog (MS ½) medium (pH 5.7), supplemented with 0.5 % (w/v) sucrose and 0.8 % (w/v) agar. *C. sativus* seeds were stratified by soaking in darkness for 2 days at room temperature and subsequently placed on moist filter paper for germination.

For adult plant experiments, *C. sativus* seedlings were transplanted into commercial potting soil for morphological and biochemical analysis or cultivated in a recirculating hydroponic system for physiological and gene expression assays. Hydroponic cultivation was performed on 12 cm × 12 cm rockwool slabs (ROCKWOOL Italia S.p.A., Milan, Italy). The basal nutrient solution (control, C) consisted of water supplemented with Canna Hydro Vega (CANNA, London, UK; A + B) at the manufacturer-recommended dilution (1:250; 400 mL of solution A and 400 mL of solution B per 100 L of H_2O). Electrical conductivity (EC) was maintained at 3.0 dS m^{-1}, and solution temperature was kept at 25 °C. The nutrient solution was replaced daily to ensure a stable chemical composition. Plants were used for experiments once they reached BBCH 1.5 (*Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie*; Fifth true leaf fully expanded; Meier, 2018).

3.1.3 Seed-soaking priming (*in vitro* assays)

During the 2-day stratification period, seeds were primed by soaking in PA solutions. Preliminary dose–response assays were performed using putrescine (Put), spermidine (Spd), and spermine (Spm) at concentrations of 1 mM, 100 µM, and 10 µM. Based on the results of these assays,

the concentrations selected for subsequent treatments of WT seeds were 1 mM Put, 100 µM Spd, and 10 µM Spm.

For experiments involving Arabidopsis mutant lines, both WT and mutant seeds were treated exclusively with the optimal Put concentration (1 mM) or with distilled water (dH_2O), which served as the control. In all experiments, control seeds were primed only with dH_2O or grown on non-amended medium.

3.1.4 Foliar priming (*in vivo* assays)

PAs were applied to *Cucumis sativus* plants by foliar spraying until complete leaf wetting. Different priming regimes were adopted depending on the experimental assay: (*i*) for morphological, physiological, biochemical, and gene expression analysis, plants were treated with an aqueous solution of 0.5 mM Put. Foliar applications were performed four times per week for two consecutive weeks, using 10 mL per plant, starting at the BBCH 1.5 stage; (*ii*) For *Bemisia tabaci* infestation assays, Put, spermidine (Spd), and spermine (Spm) were tested at concentrations of 5 mM, 1 mM, and 0.5 mM. Treatments consisted of a single foliar spray (10 mL per plant) applied at the BBCH 1.5 stage; (*iii*) For *Golovinomyces cichoracearum* infection assays, Put, Spd, and Spm were applied at a single concentration of 1 mM. Foliar applications were performed four times per week for two weeks (10 mL per plant), starting at the BBCH 1.5 stage.

3.1.5 Abiotic/ biotic stress induction

Salinity stress was imposed using sodium chloride (NaCl). For *in vitro* assays, salt stress was applied by supplementing MS ½ medium or filter paper with 200 mM NaCl. 150 mM NaCl was used for Arabidopsis mutant germination assays. For *in vivo* experiments in soil-grown plants, salt stress was induced by irrigating plants with 200 mM NaCl to ensure a strong stress response despite the buffering capacity of the commercial substrate. In hydroponic experiments, salt stress was applied by adding 100 mM NaCl to the basal nutrient solution, resulting in a total EC of 11 dS/m. Salt treatments were initiated when plants reached the BBCH 1.2 stage.

For Bemisia infestation assays, plants at the BBCH 1.5 stage were uniformly infested with 10 adult *B. tabaci* per leaf. Adults were manually removed after 2 days to allow monitoring of egg deposition and nymphal development. In pre-infestation experiments, PA priming with Put, Spd, or Spm at concentrations of 5, 1, and 0.5 mM was performed before insect infestation. In post-infestation assays, foliar treatment with Put (5, 1, or 0.5 mM) was initiated 5 days after adult removal, targeting developing nymphal stages to evaluate curative efficacy.

For powdery mildew assays, plants at the BBCH 1.5 stage were inoculated with *G. cichoracearum* by spraying a water-based conidial suspension onto the leaves. The inoculum concentration was adjusted to 1×10^6 conidia mL^{-1} using a haemocytometer. Inoculation was performed under controlled conditions of 80 % humidity to optimize spore germination and infection success. Following inoculation, plants were maintained in growth chamber at 22 °C under the 16 h light/ 8 h dark photoperiod to allow disease progression before severity assessment.

3.1.6 Physiological, morphological, biochemical, and gene expression analysis

For the assessment of physiological**,** morphological, and biochemical parameters and subsequent gene expression analysis by real-time- quantitative – PCR (RT-qPCR), *C. sativus* plants that were grown in the hydroponic system (as described in 3.1.3), were divided into four distinct groups: C, control; P, Put-primed; S, salt-stressed; P+S, Put-primed and salt-stressed. Samples for RT-qPCR were harvested, frozen in liquid nitrogen, and stored at -80 °C until RNA extraction.

3.1.7 VOC profile analysis

The quantitative analysis of VOCs emitted by *C. sativus* (BBCH 1.5) was performed using Thermal Desorption coupled with two-dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (TD-GCxGC-TOF-MS), after treatment with 5 mM Put, which is the most effective treatment obtained in the previous experiment. VOC collection was conducted 21 days after treatment to evaluate the long-term volatile signature resulting from the priming and infestation effects. At this time point, four experimental groups were analysed: control non-primed non-infested (C), primed non-infested (Put), infested non-primed (I), and primed infested (Put + I). For infested treatments (I and Put + I), plants were infested with 10 adult Bemisia individuals per leaf.

3.1.8 *C. sativus* VOC priming against Bemisia infestation

A separate set of bioassays was conducted to evaluate the efficacy of volatile-mediated defence signalling against *B. tabaci* infestation, using a controlled system that allowed airborne communication between plants while preventing direct contact. The *C. sativus* plants at BBCH 1.5 were divided into five experimental groups to distinguish direct and indirect effects: (*i*) C: control, non-primed, non-infested, (*ii*) 5 mM Put: plants directly primed with 5 mM Put, non-infested, (*iii*) Bemisia + 5 mM Put: plants directly primed with Put, infested, (*iv*) Bemisia near 5 mM Put: plants infested that were placed adjacent to non-infested plants directly treated with Put (exposure to indirect VOC signal), (*v*) Bemisia near Bemisia + 5 mM Put: plants infested that were placed adjacent to plants both directly treated with Put and infested with Bemisia (exposure to indirect VOC and plant

damage signal). For all infested groups (Bemisia + 5 mM Put, Bemisia near 5 mM Put, and Bemisia near Bemisia + 5 mM Put), 10 adult Bemisia per leaf were used for the infestation.

3.2 Analysis of Arabidopsis and *C. sativus* germination parameters

Following treatment with PAs, the seeds placed on the respective growth media (Section 3.1.1 and 3.1.2) were observed using an optical microscope for 7 days in Arabidopsis, and 9 days in *C. sativus*. The extended observation period for cucumber seeds was based on the Fratelli Ingegnoli S.p.A. illustration schedule. The number of germinated seeds was counted daily. A seed was defined as germinated once its radicle had completely emerged. Based on the formulas derived from Kader, 2005, the following parameters were calculated for each experimental condition.

The Germination Rate (GR), calculated as:

$$\mathrm{GR} = \left(\frac{Number\ of\ geminated\ seeds\ in\ a\ seed\ lot}{Total\ number\ of\ seeds\ in\ a\ seed\ lot}\right) \times 100$$

and the Germination Index (GI), calculated as:

$$\mathrm{GI} = \left(\frac{\sum\left(\frac{Gt}{Dt}\right)}{Total\ number\ of\ seeds\ in\ a\ seed\ lot}\right)$$

where Gt is the number of germinated seeds on day t and Dt is the time corresponding to Gt in days. The GI was also calculated following the general methodology of (Kader, 2005), but with a specific modification to ensure normalization. Unlike the original formula, which calculates the cumulative index, our adaptation divides the final cumulative value by the total number of seeds sown.

3.3 Analysis of Arabidopsis and *C. sativus* seedlings' roots

Following the germination observation phase (Section 3.2), the morphological development of both Arabidopsis and *C. sativus* seedling roots was quantified using *ImageJ* software. Seedling roots were photographed directly within their growth substrate, with a scale reference (a ruler in cm) included in each image to ensure the conversion from pixels to physical units.

3.4 Analysis of BS activity of PAs in *C. sativus*: measurement of morphological, biochemical, and physiological parameters

For plants grown in the commercial soil mix (200 mM NaCl), morphological parameters were assessed on the entire shoot and roots of each plant: shoot height and root length were measured in cm from the soil surface to the apical meristem, and biomass was determined by measuring the fresh weight (FW) of the shoot and roots. Shoots were subsequently dried in a forced-air oven at 65 °C for 72 h to a constant weight to determine the dry weight (DW).

Relative Water Content (RWC) was calculated using the DW and FW values, along with the turgid weight (TW), obtained by deepening the leaves in dH_2O for 24 h, following the formula (González & González-Vilar, 2003):

$$\text{RWC} = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Biochemical assays were also conducted on these soil-grown plants, with the first, the second and the third true leaves harvested, immediately frozen in liquid nitrogen, and stored at -80 °C until analysis for stress markers: FRAP assay, photosynthetic pigments, and MDA quantification respectively. Specific protocols for the MDA, FRAP assay, and Chl + Car spectrophotometric quantification are detailed in Sections 3.6, 3.7, and 3.8, respectively.

Conversely, physiological parameters were assessed in plants grown in rockwool substrate (100 mM NaCl) immediately before sampling for gene expression. SPAD values, correlated with Chl content, were measured using a portable SPAD meter, SPAD-502 Plus (Konica Minolta Sensing) at three different points on the third fully expanded leaf. Photosynthetic efficiency, expressed as Fv/Fm ratio, was measured using a Photosynthetic Yield analyser mini-PAM portable chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich Germany). A SC-1 Leaf Porometer (METER, Müchen) was used to measure stomatal combined CO_2 and H_2O vapour flux (conductance).

3.5 Analysis of PA priming against Bemisia infestation (pre-infestation treatment)

PA priming against Bemisia was monitored at 7 and 15 days after treatment (T 7 and T 15, respectively). For plants treated with Put, observations were also conducted at 21 days (T 21) to evaluate the long-term effects of the priming agent.

Observations were conducted on leaf disks punched from infested cucumber leaves. For each disk, the total number of individuals was recorded, including all developmental stages: eggs, small nymphs (L2–L3), and large nymphs (L4–L5). Data were expressed as the mean number of total individuals per leaf disk and as the number of each developmental stage out of the total pest population counted.

The effectiveness of the PA pre-treatment against Bemisia infestation was quantified by calculating the percentage mortality (or reduction in infestation) using Abbott's formula (Abbott, 1925). This formula corrects the observed treatment effect for the natural mortality or infestation rate observed in the untreated control group. The efficacy was calculated as follows:

$$\text{Efficiency, (\% mortality)} = \frac{(Icontrol - Itreatment)}{Icontrol} \times 100$$

Where: *Icontrol* is the measure of infestation (number of individuals) in the untreated control group, *Itreatment* is the measure of infestation (number of individuals) in the PA treated group. This

standardized calculation ensures that the resulting efficacy value accurately reflects the direct effect of the applied PA treatment.

To ensure the absence of phyto-pathological effects of the PA treatments in cucumber plants, chlorophyll (Chl) and carotenoid (Car) content were estimated as an easy-to-see fitness indicator. Chl content was estimated in SPAD value units using a SPAD−502Plus (Konica Minolta Sensing), and Car content was estimated in Photochemical Reflectance Index (PRI) value units using a PSI's PlantPen (M&J SENSORES SL; Córdoba; Spain).

3.6 Analysis of PA priming against Bemisia infestation (post-infestation treatment).

Following the post-infestation treatment described in Section 3.1.5, the efficacy of Put against established Bemisia population was evaluated at 5 and 10 days after treatment. Observations were conducted on leaf disks punched from infested cucumber leaves. For each disk, the total number of Bemisia individuals was recorded, including all developmental stages: eggs, small nymphs (L2–L3), and large nymphs (L4–L5). Data were expressed as the mean number of total individuals per leaf disk and as the number of each developmental stage out of the total pest population counted.

The efficacy of PA-post treatment against Bemisia infestation was measured by calculating the percentage mortality using Abbott's formula (Abbott, 1925), as detailed in Section 3.5.

3.7 Analysis of VOC priming against Bemisia infestation

Following the VOC priming experiment and subsequent infestation (as described in Section 3.1.8), the effects of indirect and direct PA treatments on Bemisia infestation were observed at 7 and 15 days after treatment.

Observations were conducted on leaf disks obtained from the infested cucumber leaves using a leaf puncher. For each disk, the total number of Bemisia individuals was recorded, including all developmental stages: eggs, small nymphs (L2–L3), and large nymphs (L4–L5). Data were presented as the mean number of total individuals per leaf disk. To ensure the absence of phyto-pathological effects of the PA treatments, Chl and Car content were estimated as a fitness indicator: Chl content was estimated in SPAD value units (SPAD-502 Plus), and Car content was estimated in Photochemical Reflectance Index (PRI) value units (PSI's PlantPen).

3.8 PAs against *G. cichoracearum* infection

Following the PA treatments and subsequent inoculation with *G. cichoracearum* (as detailed in Section 3.1.5), the results were observed at 7, 15, and 21 days (T7, T 15, and T 21, respectively)

after the initiation of the infection. Disease severity was assessed by evaluating the percentage of leaf area covered by fungal mycelium and the average size of fungal colonies on *C. sativus*.

Percentages were then averaged to obtain a mean disease severity score for each treatment group at each observation time point (T 7, T 15, and T 21 days).

3.9 *C. sativus* VOC profile analysis using TD-GC x GC-TOF-MS

At 2 h and 21 days after treatment, plants were placed into a Nalophan bag (46 × 56 cm; Lakeland Ltd., Windermere, UK) and sealed using a food storage clip. Following an equilibration period of 1 h, headspace VOCs were sampled by collecting 1 L of air through Tenax TA/Sulficarb inert-coated SafeLok TD tubes (Markes International, Llantrisant, Wales, UK) using a SpeediVac high-vacuum pump (Edwards High Vacuum Ltd., Crawley, UK), with tubes connected via a Q-Max Tube Holder (Supelco, Bellefonte, PA, USA). Air blanks were collected under identical conditions from empty bags in the laboratory, without the presence of *C. sativus* plants (Spadafora *et al*., 2019; Mylett's Master's thesis, 2025). Blanks of air were identically sampled from the laboratory, without the addition of *C. sativus* plants (Spadafora *et al*., 2019; Mylett's master's degree thesis, 2025).

3.9.1 Two-dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (TD - GC x GC – TOF – MS)

Tubes were dry purged for 2 min with a flow rate of 50 mL/min. Sample tubes were then desorbed using a two-stage desorption. Stage 1: 120 °C for 5 min trap flow, 40 mL/ min. Stage 2: 280 °C for 5 min trap flow 50 mL/min. Desorption from the trap purge occurred for 50 mL/min, 1 min at 25 °C. The trap heating rate was MAX (>40 °C/s increase). The trap high was 300 °C, held for 3 min, and the desorb split was 2 mL/min. The carrier gas used was helium, and the sample extraction and enrichment platform was Centri ®. The primary column of the GC flow rate operated at 0.5 mL/min. The secondary column operated at 20 mL/min. Both columns have a constant flow rate. The GC oven was operated with a single temperature ramp. The initial temperature was 40 °C, held for 2 min; the 20 temp was ramped 4 °C / min to 240 °C and held for 5 mins (Total run time 57 mins). The INSIGHT flow modulator (SepSolve Analytical, Peterborough, UK) mounted within an 8890 GC (Agilent Technologies, Santa Clara, CA, USA) was operated with a 2 s modulation period with a 120 ms flush time. The RI standards were run identically but with a 2-second solvent delay. The bench TOF 2-TI mass spectrometer (SepSolve Analytical, Peterborough, UK) was operated with a filament voltage of 1.6 V and a mass range of 35-300 mass units. The transfer line temperature was 240 °C, and the ion source temperature was 230 °C. Ionisation was performed at -70V.

3.9.2 Peak identification

VOC data processing was done utilising ChromSpace (SepSolve Analytical, Peterborough, UK). Chromatographs generated by the TD – GC x GC – TOF – MS were aligned, and then a 2-second peak width dynamic baseline correction was applied. Integration was done using a deconvolution algorithm. The NIST spectral library (NIST v. 3.0, 2023) was used to identify compounds in the RI standard to create a ladder using a semi-non-polar, complete search. Using the created ladder as the template, all identified peaks in 4 individual samples were compared to the NIST17 spectral library (NIST v. 2.3, 2017) to create a custom library. Compounds were included in the custom library if the match factor > 750. Once the custom library was built, all peaks in all samples were identified using the custom library created with a match factor >750.

3.9.3 VOC normalisation

The average standard deviation (SD) of retention time across all samples was calculated for each compound. If SD was greater than 1 or the retention time indicated a known contamination coumpounds were removed. The other compounds were identified by NIST library matches of the chromatogram. Data were then normalized to the total peak area, and square root transformation was applied to reduce the influence of large components. Compounds were retained only if their abundance in samples was at least five times higher than in blanks and if they were detected in at least two biological replicates. Peak areas of the remaining compounds were used for statistical analysis. VOC statistical analysis was performed using R Studio. A permutational multivariate analysis of variance (Permanova) was used to test for differences between treatment groups. A Canonical Analysis of Principal Coordinates (CAP) was conducted, and the generated plot shows the separation of samples in two dimensions with 95 % confidence intervals. These analysis used the vegan package (Oksanen *et al*., 2012). A Random Forest (RF) analysis was also performed. Plots were generated with a 95 % confidence interval. RF identified the most informative compounds (Breiman, 2001). The most informative VOCs were identified using Mean Decreased Accuracy from the RF model, where higher Mean Decreased Accuracy values indicated a greater contribution to classification accuracy. This information was used to remove compounds that created noise from the dataset.

3.10 Malondialdehyde (MDA) quantification

MDA content was quantified in the third true leaves (counting from the apex) of *C. sativus* plants. Samples were collected from the severe stress soil experiment described in Section 3.1.5. The leaves were weighed, ground into a powder using a mortar and pestle, and subsequently homogenized

with 0.1 % (w/v) trichloroacetic acid (TCA) in a 2:1 ratio relative to the sample's weight in grams. The resulting homogenate was transferred to a 2 mL tube and centrifuged at 12,000 rpm for 15 min. Following centrifugation, 1 mL of the supernatant was collected and transferred to a 15 mL tube, to which 2 mL of 20 % (w/v) TCA containing 0.5 % (w/v) thiobarbituric acid (TBA) were added. The mixture was incubated in a thermostatic water bath at 95 ° C for 30 min. After incubation, the samples were centrifuged again at 12000 rpm for 10 min. at 4 °C. Finally, 1 mL of the supernatant was transferred to a cuvette for spectrophotometric analysis. Absorbance was measured at 532 nm to detect the presence of MDA and at 600 nm for background noise, using (w/v) TCA containing (w/v) TBA as the blank. The absorbance measured at 600 nm was subtracted from the absorbance measured at 532 nm to correct for background noise, and the MDA concentration was calculated by applying the Lambert-Beer law using the formula:

$$\mathrm{Abs}_{532}\mathrm{nm} - \mathrm{Ab}_{600}\mathrm{nm} = \varepsilon l c$$

where ε represents the molar extinction coefficient, equal to 155 M^{-1} cm^{-1}, *l* is the optical path length of the cuvette (1 cm) and *c* is the MDA concentration expressed in molarity. The concentrations were finally multiplied by 1000 to express them in mM.

3.11 Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of the plant samples was quantified using the FRAP assay, performed on the first leaves, counting from the apex, of *C. sativus* plants, utilizing the FRAP Assay Kit (Sigma-Aldrich). Samples were weighed and ground into a powder using a mortar and pestle using liquid nitrogen, then homogenized in an acid-methanolic solution composed of pure methanol, ultrapure water, and hydrochloric acid in the proportions of 70, 29.5, and 0.5, respectively, using a 2:1 ratio between the solvent volume and the sample weight in grams. The homogenate was transferred to Eppendorf tubes and centrifuged at 12,000 rpm for 15 min. Subsequently, 10 µL of the supernatant were added to 190 µL of the reaction mix, prepared with the kit reagents, in a 96-well plate, resulting in a total volume of 200 µL per well. For the positive control conditions, 10 µL of a specific reagent were added, while for the negative control, 10 µL of the acid-methanolic solution used for extraction were utilized. Furthermore, a standard curve was developed using known concentrations of reduced ions, as a function of the absorbance measured at 594 nm. Absorbance values at 594 nm were obtained using a multimodal microplate reader (Tecan Spark 10M). Each value was compared with the standard curve and subsequently divided by 10 µL (representing the amount of sample per well) to obtain the molar equivalent of reduced for each plant sample**.**

3. 12 Spectrophotometric quantifications of Chl and Car

The quantification of Chl and Car was performed on the second leaves, counting from the apex, of *C. sativus* plants, following the method described by Lichtenthaler, 1987 Initially, the fresh weight (FW) of the plant samples was measured. Subsequently, the leaves were pulverized using a mortar and pestle and mixed with pure methanol (MeOH) in a volume (expressed in mL) equal to 10 % of the fresh weight in grams. The resulting volume was transferred to a 2 mL Eppendorf tube and incubated in the dark at 4 °C for 24 h. The following day, the sample was centrifuged at 13000 rpm for 5 min., and 1 mL of supernatant was collected from each sample, subsequently diluted 1:10 in MeOH and transferred to a spectrophotometer cuvette. Absorbance was measured at 665 nm for Chla, at 652 nm for Chlb, and at 470 nm for car, using pure MeOH as the blank.

The concentration of Chla, expressed in µg/mL of MeOH, was calculated using the formula:

$$16{,}72 \times \mathrm{Abs665} - 9{,}16 \times \mathrm{Abs652}$$

The concentration of Chlb, expressed in µg/mL of MeOH, was calculated using the formula:

$$34{,}09 \times Abs652 - 15{,}28 \times Abs665$$

The concentration of car, expressed in µg/mL of MeOH, was calculated using the formula:

$$\frac{(1000 \times Abs470 - 1{,}63 \times Chla - 104{,}96 \times Chlb)}{221}$$

Finally, to obtain the concentration of Chla, Chlb, or car in µg/g of fresh weight (FW), the following formula was used:

$$\frac{Chla, Chlb\ o\ car\ (\mu g/ml) \times MeOH\ volume\ used\ (ml)}{sample\ fresh\ weight\ \ (g)} \times 10\ (diluition\ factor)$$

3.13 RNA extraction, RT-qPCR, and morphological parameters

For gene expression quantification, the expanded third leaf was harvested, immediately frozen in liquid nitrogen, and stored at −80 °C. Total RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. RNA quantity was determined using a Tecan Infinite 200 spectrophotometer (Männedorf, Switzerland). To eliminate genomic DNA contamination, RNA samples were treated with RQ1 DNase (Promega). First-strand cDNA synthesis was carried out using the ImProm-II™ Reverse Transcriptase (Promega), following the supplier's protocol. RT-PCR was performed using gene-specific primers listed in **Table 1**. The number of PCR cycles was optimized for each primer pair and cDNA batch by testing a series of cDNA dilutions across a suitable cycle range, to ensure a linear relationship between template and product concentration. All RT-PCR reactions were conducted at least in duplicate, with appropriate standards included in each run. Amplified products were visualized by ethidium bromide staining, and

fluorescence intensity was quantified using the GeneGenius Bioimaging System and GeneTools software (Syngene Ltd., Cambridge, UK). RT-qPCR was performed in 10 µL reaction volumes containing 5 µL of SYBR® Green Mix (PCR Biosystems), 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM), and 4 µL of diluted cDNA. Reactions were prepared in 96-well plates (Starlab), each well sealed with cling film, vortexed gently, and centrifuged briefly. For each gene and condition, two technical replicates were performed for each of three biological replicates. A no-template control (NTC; water) and a positive control (genomic DNA) were included in every qPCR run. To normalize cDNA input across samples, concentrations were adjusted based on the quantification cycle (Cq) values of the reference gene *ACT3*, following the method described by (Bustin *et al*., 2009). Samples were diluted to equalize Cq values within ±2.5 cycles, ensuring comparable starting template amounts across reactions. Reactions were amplified using a LightCycler® 96 system (Roche) with the following thermal profile: initial denaturation at 95 °C for 2 min., followed by 35 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds. A melting curve analysis (60 °C to 90 °C) was performed at the end of each run to verify primer specificity. For relative quantification, the mean of the technical replicates was used, provided the range between replicates was <0.5 Cq. The ΔCt for each gene of interest was calculated by subtracting the *ACT3* Ct value of the corresponding sample. ΔΔCt values were then calculated by comparing each treatment group to the calibrator sample, and the average ΔΔCt was determined across biological replicates, according to the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Name of Primer	**Sequence of Primer**
ACT3 Fw	TTCTGGTGATGGTGTGAGTC
ACT3 3 Rv	GGCAGTGGTGGTGAACATG
CsvNAC001 Fv	GGGTTTTACCTGGAAAGGCTT
CsvNAC001 Rv	CGAGGAGATGATTTTATCCGTAC
CsvNAC014 Fv	TTTTGCCAACTCCTTATCCC
CsvNAC014 Rv	CTGAGTTGGAGTCACTTTCAGGC
P5CS Fw	AGCCTTGCCAACTCCATCC
P5CS Rv	GAGAAGACCATTTCCACTCCG
SOD2 Fw	GGTGATGATGGTACTGCTACTTTC
SOD2 Rv	CAATAACGCCACAGCCTATTCTC
SOS3 Fw	CAAGGAAGAGTGGCGAAACC

SOS3 Rv	TGGGAACGTGGTCGTGATATC
CsZHD10 Fw	CAAGAGACCATCCTGAAGCAACCC
CsZHD10 Rv	TTGGCAGCGGAGTCGGAGTAG

Table 1: Primers used for RT-qPCR expression analysis in *C. sativus* adult plants.

3.14 Fruit development analysis

Following the main BS activity assays, a separate follow-up experiment was conducted under the same growth and treatment conditions (Section 3.1.5) to evaluate the long-term effect of 0.5 mM Put on yield-related traits under both optimal and saline stress (100 mM NaCl) conditions. For all ripe fruits harvested from the sampled plants, total fruit number, fresh fruit weight, and fruit length were measured.

3.15 Statistics

All statistical analysis and graph generation were performed using GraphPad Prism version 8.4.2, except for the advanced VOC data analysis, which was conducted within the R Studio environment (Version 4.0.2).

Statistical significance was determined using the Student's t-test to compare two means, control versus treatment in experiments involving germination, morphological, physiological, and biochemical parameters. For experiments with multiple comparison groups, like RT-PCR, fruit development, Bemisia, powdery mildew, and the relative VOC abundance changes, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used. Permanova was used to test for differences between treatment groups in VOCs experiment. Non-significant results are noted, while significance is indicated by *, **, ***, and **** corresponding to $p < 0.05$, 0.01, 0.001, and 0.0001, respectively. All experiments followed a fully randomized design, with the statistical unit carefully defined for each assay type.

For germination parameters, all treatments were analysed using 3 independent biological replicates (n=3). Each replicate consisted of 100 seeds for Arabidopsis and 25 seeds for *C. sativus*.

For digital image analysis of seedling root growth, the root lengths were measured on 25 individual seedlings per condition, which served as the biological replicates for analysis.

Morphological parameters and biochemical were conducted across 3 independent biological replicates (n=3), utilizing 6 to 10 plants per condition (totaling 18 to 30 plants).

Pre-Infestation (10 experimental conditions, 30 plants in total) and post-Bemisia Infestation (4 experimental conditions, 12 plants in total) experiments utilized 3 independent biological replicates

(n=3), each consisting of a single adult *C. sativus* plant. The data for each replicate were derived from the average number of Bemisia counted on 5 sampled leaf disks per plant.

VOC-priming effects against Bemisia experiment used 3 independent biological replicates (n=3) were used for the 5 experimental conditions (totaling 15 plants). The statistical unit for all Bemisia assays was the mean number of Bemisia per replicate, calculated from the average number of insects counted on 5 sampled leaf disks per plant.

G. cichoracearum assays used 10 independent biological replicates (n=10) per condition (totaling = 70 plants), with one adult plant per replicate.

VOC collection experiment (GC-MS) utilized 3 biological replicates (n=3), each consisting of 2 pooled adult *C. sativus* plants (totalling 24 plants). The measured VOC concentration in the pooled sample was the biological unit. The measured concentration of VOCs in the pooled sample was considered the technical unit.

For the analysis of the PA treatment effect against *G. cichoracearum*, 10 independent biological replicates were used per condition (n=10), with one adult plant per replicate (totalling 60 plants across 6 conditions).

RT-qPCR analysis was performed using 3 independent biological replicates (n=3), with each utilizing material from one single plant per experimental condition (totalling 3 plants). Each biological replicate included 2 technical replicates.

Fruit development analysis was conducted using 5 independent biological replicates n=5, with the measured fruit parameters (number and weight) derived from each of the 5 individual plants considered the biological replica. For fruit length, the mean length calculated from the pooled fruits collected across all 5 replicates was used for statistical analysis.

4. Results

4.1 Put enhances GR and GI of Arabidopsis seeds under saline stress conditions

Experiments were carried out to identify the most effective PA, and their optimal concentration for use as a seed priming agents in Arabidopsis under optimal growth conditions (**Figure 3**). The GR and GI of Arabidopsis seeds were analysed over 7 days (**Figure 3A** and **3B,** respectively) after seed priming with Put, Spd, and Spm at three different concentrations (1 mM, 100 µM, and 10 µM).

The data indicated that 1 mM Put, 100 µM Spd, and 10 µM Spm were the most effective PA treatments for enhancing germination parameters. Analysis showed that priming with 1 mM Put resulted in a statistically significant increase in GR during the first two days after sowing. Specifically, GR of seed primed with 1 mM Put reached 88.68 % and 93.82 % on 1st and 2nd days, respectively, compared to the values showed by control seeds of 69.12 % and 85.75 % for the same days.

Priming with 1mM Put also led to a significant increase in the final GI, reaching 4.88 compared to 4.40 for non-primed control seeds. Priming with 100 µM Spd significantly increased GR, peaking at 96.36% by the 2nd day, compared to the control value of 85.73%, and resulted in a final GI of 5.05. Finally, priming with 10 µM Spm resulted in a significant increase in GR, reaching 93.53% by the second day, compared with 85.73% in the control, and yielded a final GI of 5.03.

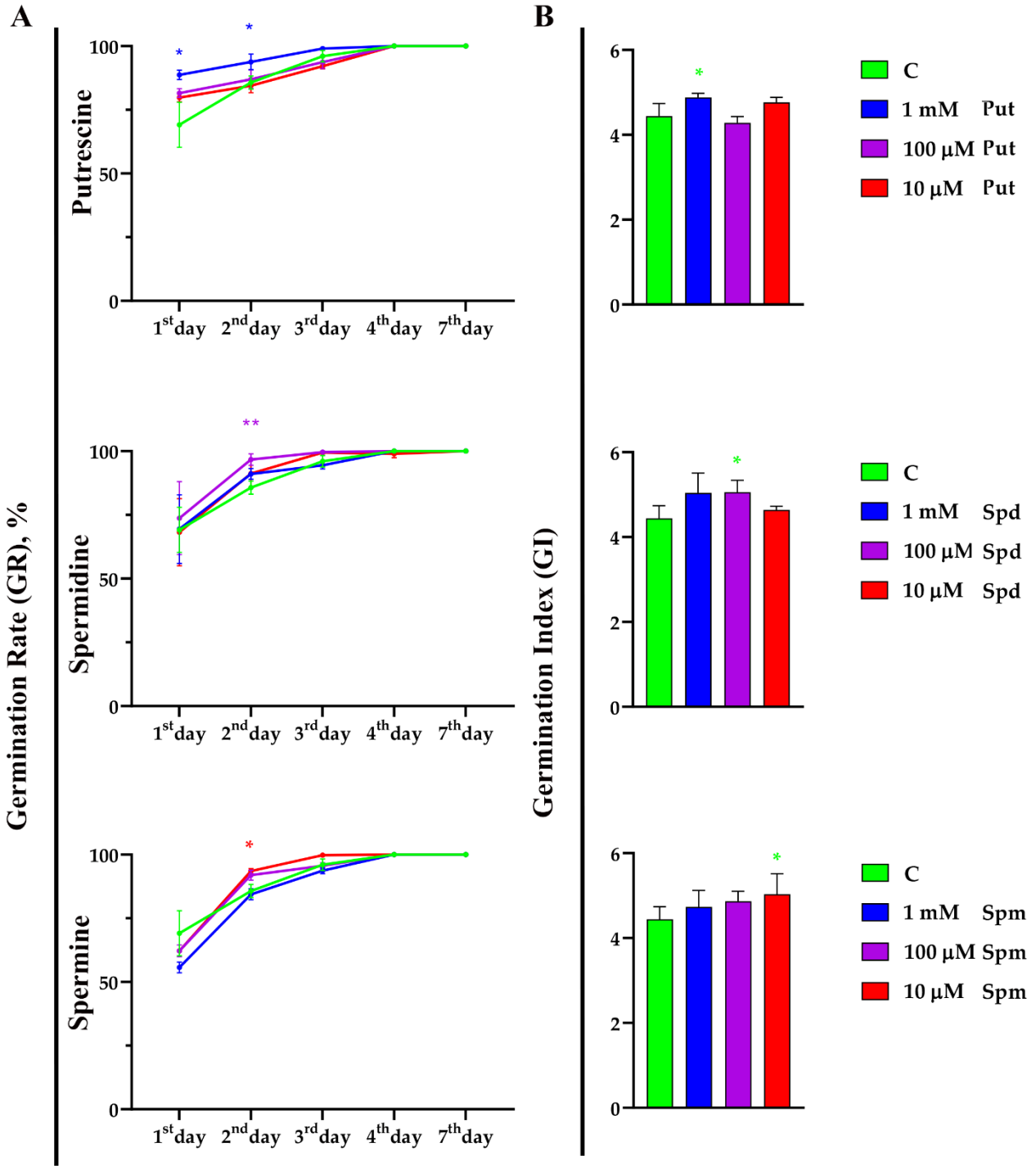


Figure 3. Effect of exogenous PA priming on Arabidopsis seed germination under optimal growth conditions. Time-course analysis from the 1st to the 7th day, measuring GR (**Figure 3A**) and GI (**Figure 3B**) following PA priming. C: non-primed control seeds, Put, Spd, and Spm at 1 mM, 100 µM, and 10 µM: PA primed seeds. Data are presented as mean ± SD (n = 3). Statistical significance between PA primed seeds and the respective control seeds was assessed using Student's t-test; $p > 0.05$ is considered not significant (not shown), and ** indicates $0.05 > p > 0.001$. Differences between each treatment and the control are highlighted in the corresponding treatment colour in **Figure 3A,** or in green in **Figure 3B**.

Following the preliminary assessment, experiments were conducted using the most effective treatments (1 mM Put, 100 µM Spd, and 10 µM Spm) to evaluate their potential in enhancing salt stress tolerance (200 mM NaCl; **Figure 4**). GR (**Figure 4A)** and GI (**Figure 4B)** of Put, Spd, and Spm primed seeds were analysed compared to unstressed unprimed control (C) and stressed unprimed control (S) seeds.

The results showed that 1 mM Put priming had a significant protective effect to counteract salt stress. Indeed, under optimal conditions, 1 mM Put sped up germination on the 1st day, achieving a GR of 86.66% compared to 67.21% in C seeds. Under salt stress, 1 mM Put primed seeds exhibited higher GR on the 3rd and 4th days, reaching 34.90% and 63.95%, respectively, both higher than the S values of 15.94% and 32.40%. 1 mM Put priming also notably increased the final GI, reaching 12 under optimal conditions compared to 9.07 for C seeds, and reaching 3.33 under salt stress compared to 2.47 in S seeds.

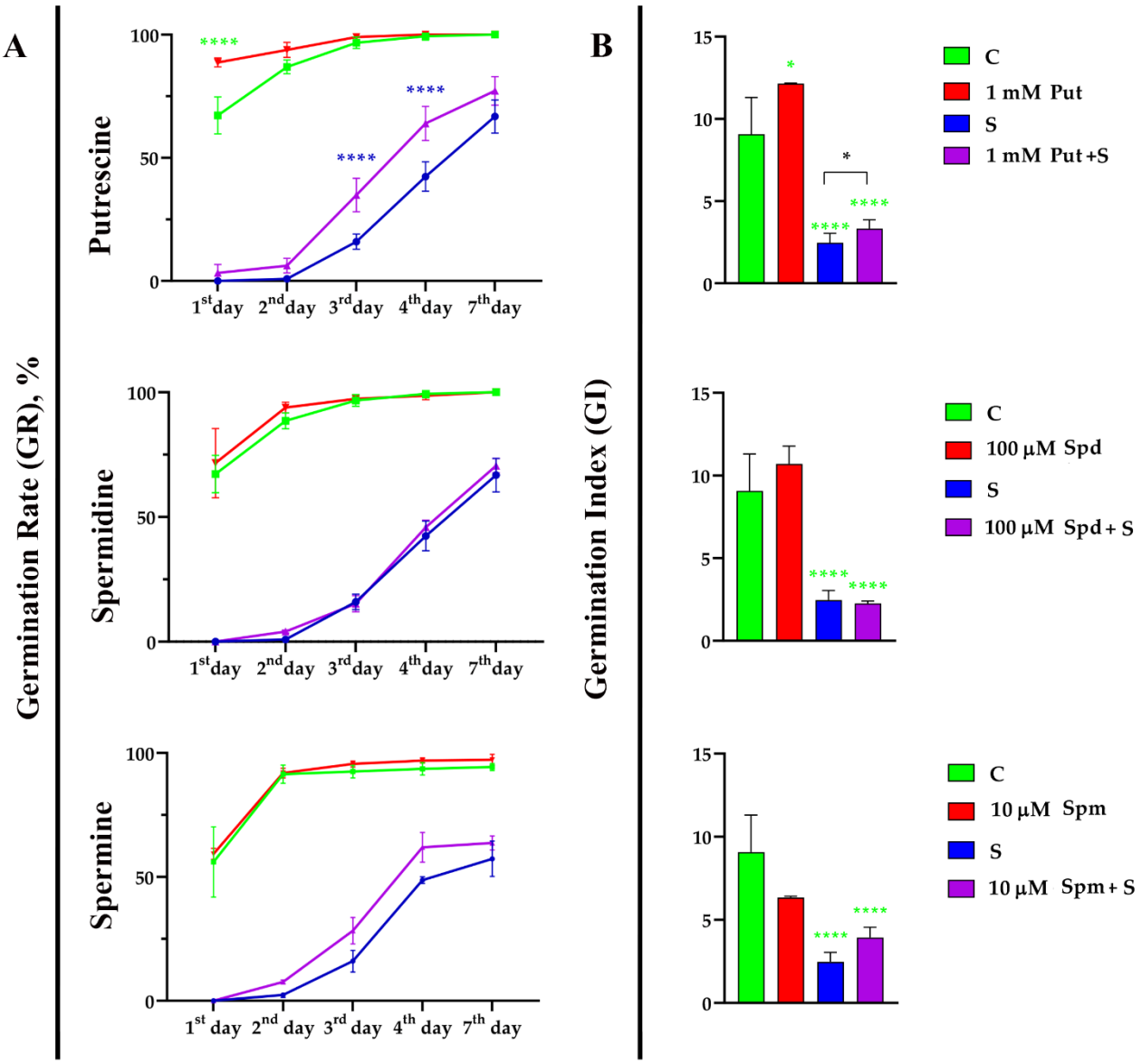


Figure 4. Effect of priming with 1 mM Put, 100 µM Spd, and 10 µM Spm on Arabidopsis seed germination under salt stress (200 mM NaCl). Time-course analysis from the 1st to the 7th day, measuring GR (**Figure 4A**) and GI (**Figure 4B**) following PA priming. C: non-primed, non-stressed control seeds, 1 mM Put, 100 µM Spd, and 10 µM Spm: PA-primed, non-stressed seeds, S: non-primed; salt-stressed seeds, 1 mM Put, 100 µM Spd, 10 µM Spm + S: PA-primed and salt-stressed seeds. Data are presented as mean ± SD (n = 3). Statistical significance between PA primed seeds and the respective control was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and **** indicates $p \leq 0.0001$. Differences between each treatment and C or S are highlighted in green or blue, respectively, other significant differences are highlighted in black.

4.2 Put enhances the GR of cucumber seeds under saline stress conditions

Following experiments conducted in Arabidopsis, additional tests were performed to identify the most effective PA and their optimal concentration for use as seed priming agents in cucumber seed priming under both optimal and salt-stressed growth conditions, to assess the effectiveness of PA priming in an agricultural system (**Figure 5**).

GR and GI of cucumber were analysed over 9 days (**Figure 5A** and **5B,** respectively) after seeds priming with Put, Spd, and Spm at three different concentrations (1 mM, 100 µM, and 10 µM), under both optimal and salt stress (200 mM NaCl) conditions.

The supply of 200 mM NaCl significantly reduced cucumber seed germination, both in GR and GI analysis (**Figure 5**). Data showed that only 1 mM Put significantly enhanced germination parameters under salt stress. GR of Put primed seeds reached 75.98 % at the 9^{th} day under salt stress, compared to 55.95 % of the stressed control seeds (S). In contrast, starting from the 7^{th} day, Spm priming led to a reduction in GR, most effectively at concentration of 1 mM, and this effect was evident under both optimal and salt-stress conditions (**Figure 5A**). Specifically, in optimal growth conditions, the GR of 1 mM Spm primed seeds reached 57.33 %, compared to 81.56 % recorded for C on the 7^{th} day. Similarly, under salt stress, the GR of 1 mM Spm primed seeds reached 34.66 %, compared to 55.95 % for stressed control (S). Furthermore, also the GI was also negatively affected by Spm treatments (**Figure 5B**).

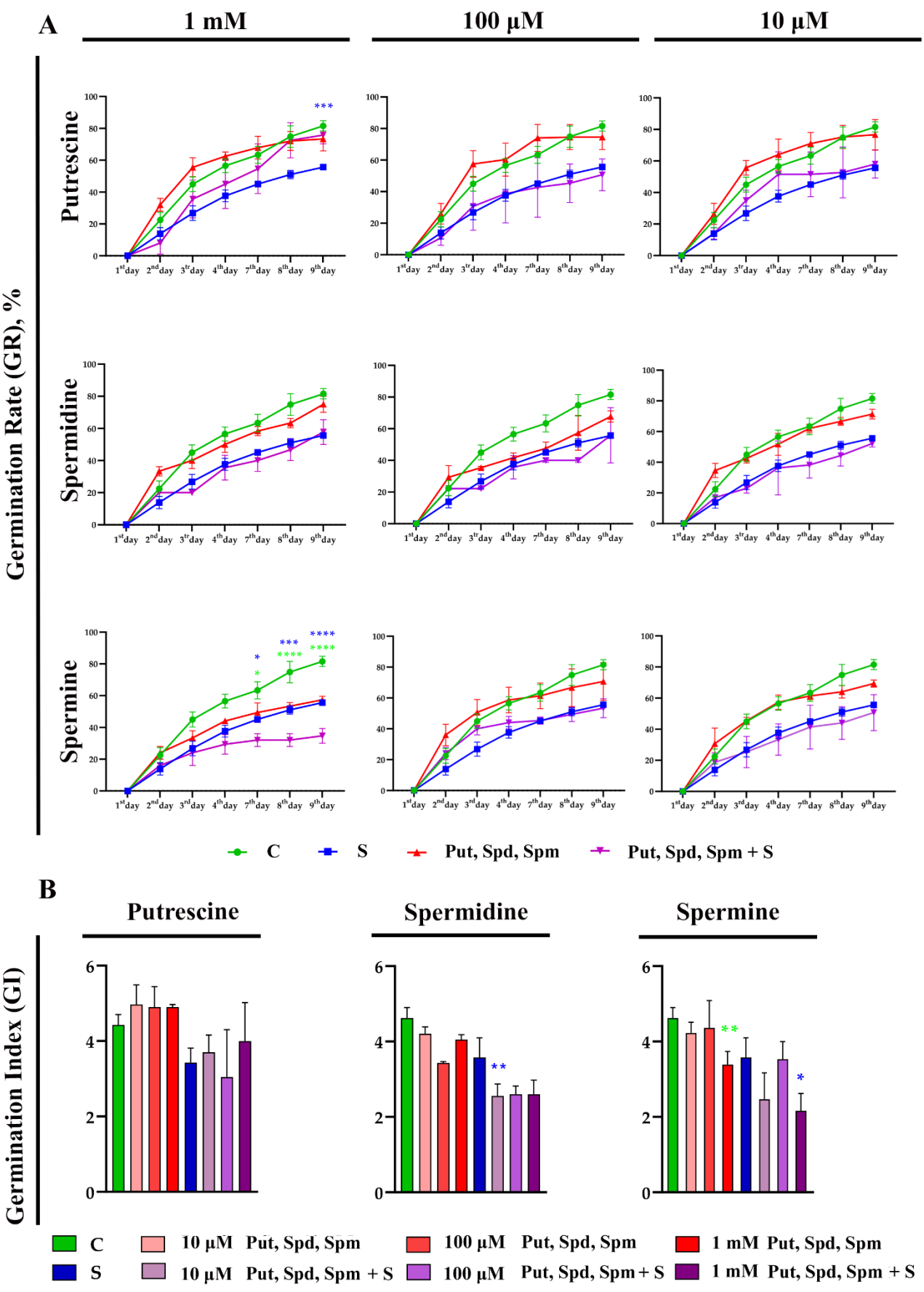

A
1 mM
100 µM
10 µM
Germination Rate (GR), %
Putrescine
Spermidine
Spermine
1st day 2nd day 3tr day 4th day 7th day 8th day 9th day
C
S
Put, Spd, Spm
Put, Spd, Spm + S
B
Germination Index (GI)
Putrescine
Spermidine
Spermine
C
S
10 µM Put, Spd, Spm
10 µM Put, Spd, Spm + S
100 µM Put, Spd, Spm
100 µM Put, Spd, Spm + S
1 mM Put, Spd, Spm
1 mM Put, Spd, Spm + S

Figure 5. Effect of Put, Spd, and Spm priming at different concentrations on germination parameters of cucumber seeds under optimal and salt stress conditions (200 mM NaCl). Time-course analysis from the 1st to the 9th day, measuring GR (**Figure 5A**) and GI (**Figure 5B**) following PA priming. C: non-primed, non-stressed control seeds, Put, Spd, and Spm at 1 mM, 100 µM, and 10 µM: PA-primed, non-stressed seeds, S: non-primed, salt-stressed seeds, Put, Spd, Spm at 1 mM, 100 µM, and 10 µM + S: PA-primed and salt-stressed seeds. Data are presented as mean ± SD (n = 3). Statistical significance between Cor S seeds and PA-primed seeds was assessed using Student's t-test; $p > 0.05$ is considered not significant (not shown), and **** indicates $p \leq 0.0001$. Differences between each treatment and C or S are highlighted in green or blue, respectively.

4.3 *AtCuAOγ1* and *AtCuAOγ2* are involved in Put priming effectiveness under salt stress

To further investigate the molecular mechanisms underlying the protective effect of Put during germination and based on the optimal efficacy observed for the 1 mM Put treatment in Sections 4.1 and 4.2, germination parameters in Arabidopsis loss-of-function *Atcuao* mutant lines were analysed (**Figure 6**). These mutants are impaired in CuAO enzymes, which oxidize PAs, specifically Put, allowing us to assess whether altered Put oxidation affects salt-stress tolerance **(Figure 6)**. Specifically, GR and GI of *Atcuaoβ.2*, *Atcuaoγ1.1*, and *Atcuaoγ2*.1. mutant lines were analysed over 7 days (**Figure 6A** and **6B,** respectively) after seeds were primed with 1 mM Put, under both optimal and salt stress (100 mM NaCl) conditions.

Under control condition (C), the *Atcuaoγ2.1* mutant exhibited an initial GR of 32 % on the 1st day, compared to 19 % in the WT. However, on the 2nd day, the GR of *Atcuaoγ2.1* reached only 65 %, significantly lower than the 84 % observed in the WT. Accordingly, the final GI of *Atcuaoγ2.1* under control condition was significantly reduced compared to the WT, with values of 4.85 and 6.21, respectively. Put priming of *Atcuaoγ1.1* and *Atcuaoγ2.1* resulted in a significantly lower GR on the 2nd day compared to the WT, with GI values of 4.90 and 5.09, respectively, versus 6.15 in the WT. When exposed to salt stress without priming (S), the *Atcuaoγ1.1* mutant displayed a significantly reduced GR on the 7th, day reaching 66.40 %, whereas the WT achieved complete germination (100 %). The combined treatment of Put priming and salt stress (P+S) led to a significant reduction in both GR and GI. Indeed, under this condition, both *Atcuaoγ1.1* and *Atcuaoγ2.1* showed a significantly lower final GR values on the 7th day (83.17 % and 83.87 %, respectively) compared to the WT (100 %). Furthermore, the final GI of *Atcuaoγ1.1* in the P+S condition was also significantly lower than that of the WT, with values of 3.17 and 3.99, respectively.

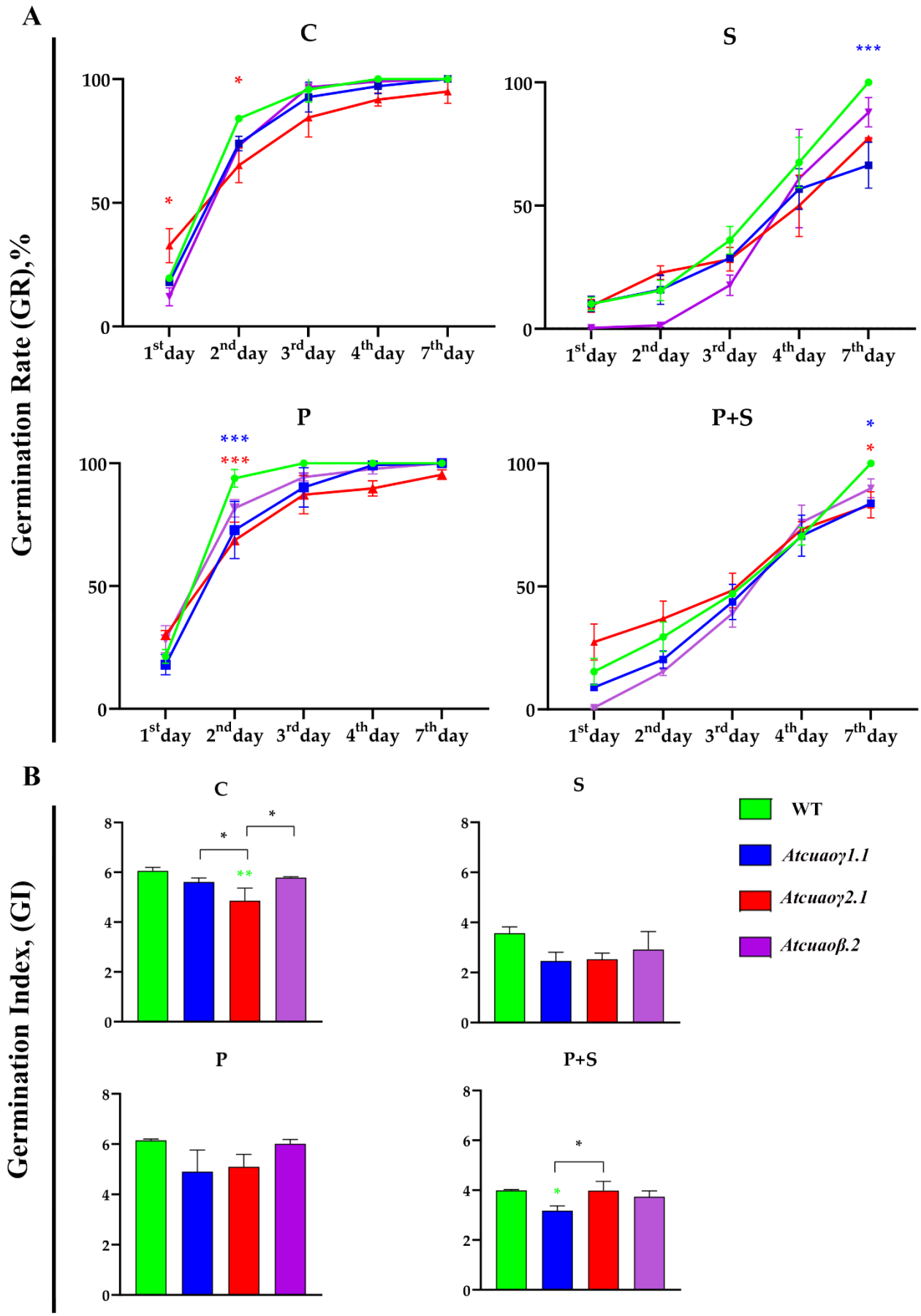

A
Germination Rate (GR),%
C
S
P
P+S
100
50
0
1st day
2nd day
3rd day
4th day
7th day
B
Germination Index, (GI)
WT
Atcuaoγ1.1
Atcuaoγ2.1
Atcuaoβ.2
8
6
4
2
0

Figure 6. Effect of 1 mM Put priming on germination parameters of Arabidopsis seeds of *Atcuao* mutant lines under both optimal and salt stress conditions (100 mM NaCl). Time-course analysis from the 1st to the 7th day, measuring GR **(Figure 6A)** and GI **(Figure 6B)** following Put priming. C: non-primed, non-stressed control seeds, P: Put primed, non-stressed seeds, S: non-primed; salt-stressed seeds, and P+S: Put primed and salt-stressed seeds. Data are presented as mean ± SD (n = 3). Statistical significance between *Atcuao* seeds and WT control was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between each mutant and WT in each condition are highlighted in green and with the colour of the treatments, other significant differences are highlighted in black.

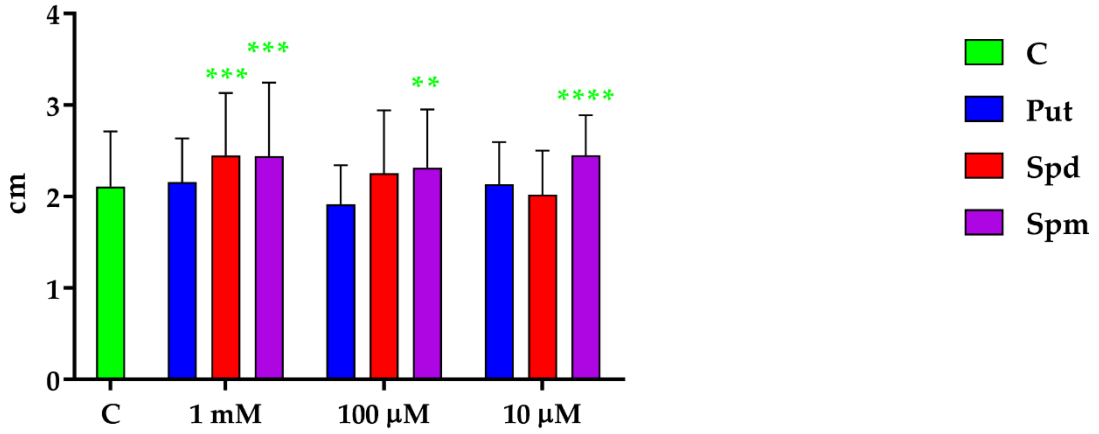
4.4 Spm priming promotes root elongation in Arabidopsis and highlights differential growth patterns among *Atcuao* mutant lines

To further investigate the effect of PA seed priming, the average of root length of Arabidopsis (WT and *Atcuao* mutant lines) and cucumber seedlings was analysed following the germination experiments (**Figure 7**).

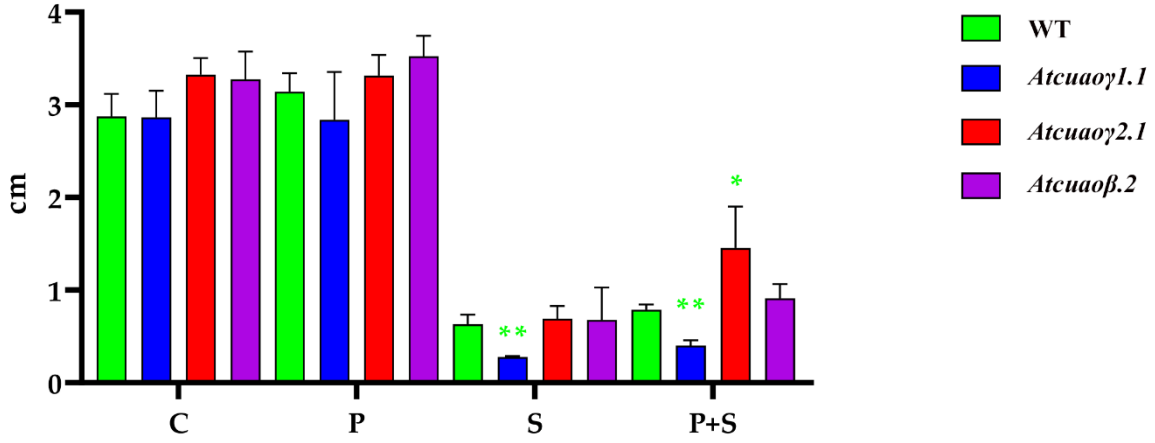
In Arabidopsis WT seedlings, Spm treatment significantly promoted root elongation at all tested concentrations. Indeed, 1 mM, 100 µM, and 10 µM Spm resulted in average root lengths of 2.44 cm, 2.33 cm, and 2.45 cm, respectively, compared to 2.18 cm in the untreated control (C). Spd induced a significant increase in root length only at the highest concentration tested (1 mM), reaching 2.44 cm, whereas Put did not produce any significant effect on WT root elongation (**Figure 7A**). Under salt stress (S) and combined priming plus stress (P+S) conditions, the *Atcuaoγ1.1* line showed significantly reduced root length compared to the WT, with values of 0.27 cm and 0.40 cm in respect to 0.63 cm and 0.78 cm in the WT, respectively. Conversely, the *Atcuaoγ2.1* line exhibited a significantly enhanced root elongation under P+S conditions, reaching 1.42 cm compared to 0.78 cm in the WT (**Figure 7B**).

In cucumber seedlings, a significant increase in root elongation was observed only under optimal growth conditions following 1 mM and 100 µM Spd treatments, resulting in average root lengths of 9.66 cm and 7.88 cm, respectively, compared with 5.08 cm in control plants. Under salt stress conditions, none of the tested PAs produced a statistically significant improvement in root length (**Figure 7C**).

A Average root length (*Arabidopsis thaliana*)



B Average root length (*Arabidopsis thaliana* mutants)



C Average root length (*Cucumis sativus*)

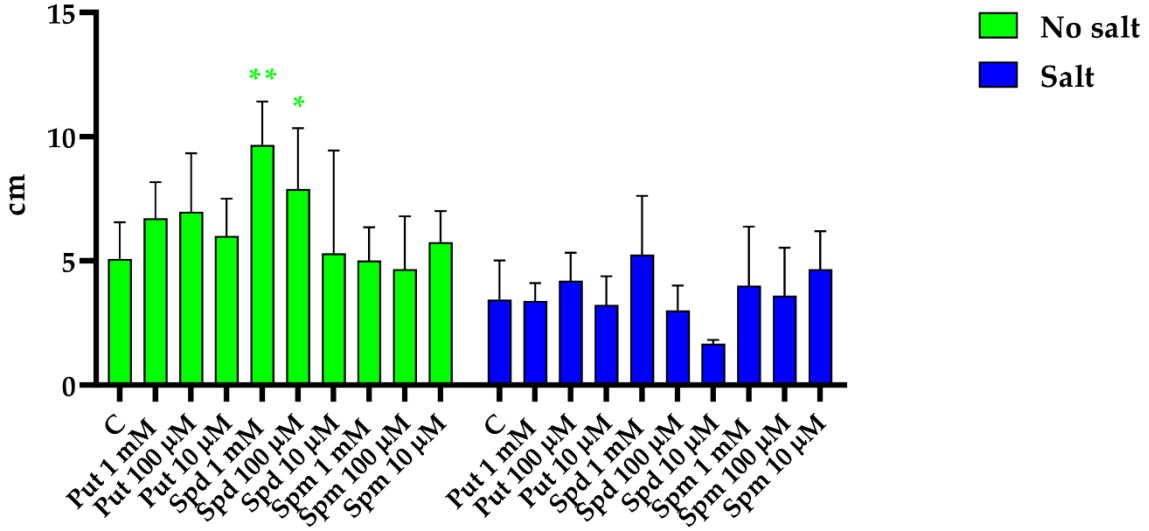


Figure 7. Effect of PA priming on root growth in WT Arabidopsis, *Atcuao* mutant lines, and cucumber seedlings. Average root length (cm) was measured to assess growth responses under the tested conditions. WT Arabidopsis (**Figure 7A**), *Atcuao* mutant line (**Figure 7B**), and cucumber under non-stressed and salt-stressed conditions (**Figure 7C**). Data are presented as mean ± SD (n = 50 for Arabidopsis, n = 25 for cucumber). Statistical significance between PA-primed and control seedling roots was assessed using Student's t-test; $p > 0.05$ is considered not significant (ns), and *** indicates $p \leq 0.001$. Differences between each treatment and C or S roots are highlighted in green or blue, respectively.

4.5 Put-foliar spray enhances morphological, growth, and damage-related parameters of cucumber adult plants, both in optimal and salt stress conditions

Experiments were carried out to analyse the BS effect of 0.5 mM Put applied as a foliar spray on cucumber plants at the BBCH 1.5 stage, both under optimal and salt-stressed growth conditions (200 mM NaCl), to evaluate morphological parameters **(Figure 8)**.

Average root length, average root weight, percentage of damaged leaf area, and average plant height was analysed in control (C), Put primed (P), salt stressed (S), and Put primed and salt stressed (P+S) plants.

Representative images of whole plants (aerial part and roots) under different treatments are shown in **Figure 8A**.

Regarding root growth, plants exposed to salt stress (S) showed a marked reduction in average root length. Roots of control plants reached 42.16 cm, whereas those of S plants reached only 30.43 cm. A similar trend was observed for root fresh weight: roots from the C condition weighed 8.19 g, compared with 3.70 g in the S condition. This detrimental effect was mitigated in the P+S treatment, which partially restored both root length (36.46 cm) and root fresh weight (6.15 g; **Figure 8B** and **8C**, respectively).

With respect to the percentage of leaf damaged area, P+S plants showed a pronounced reduction, decreasing from 26.19 % in S plants to only 1.74 % (**Figure 8D**). Analysis of average plant height further indicated that P+S plants were significantly taller, reaching 11.6 cm, whereas C and S plants reached 9.44 cm and 9.16 cm, respectively (**Figure 8E**).

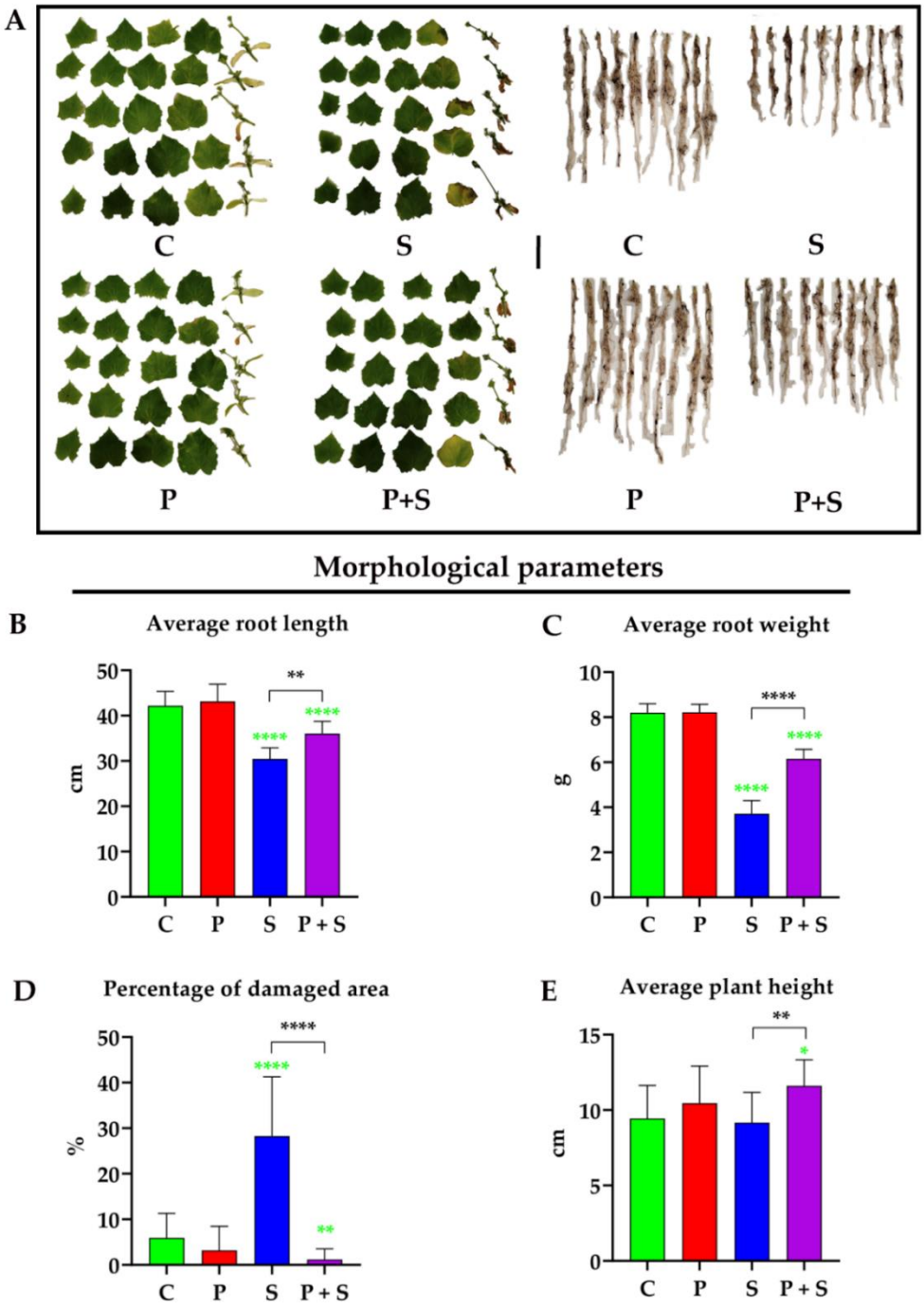


Figure 8. Effect of 0.5 mM Put foliar spray on morphological parameters in cucumber plants under optimal and salt stress conditions (200 mM NaCl). Representative images of whole plants, aerial parts, and roots under different treatments (**Figure 8A;** black bar = 9 cm), average root length (**Figure 8B**), average root fresh weight (**Figure 8C**), percentage of damaged leaf area (**Figure 8D**), average plant height (**Figure 8E**). C: non-primed, non-stressed control plants, P: Put-primed, non-stressed plants, S: salt-stressed, non-primed plants, and P+S: Put-primed under salt stress plants. Data are presented as mean ± SD (n = 18–30). Statistical significance was assessed using Student's t-test; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between each treatment and the control (C) are highlighted in green, other significant differences are highlighted in black.

4.6 Put foliar spray enhances the physiological parameters of cucumber adult plants, both in optimal and salt stress conditions

To further assess the effects of the 0.5 mM Put foliar spray, additional experiments were performed to evaluate its protective role in cucumber plants at the BBCH 1.5 stage exposed to 100 mM NaCl stress, with a focus on key physiological parameters. Photosynthetic efficiency (Fv/Fm), stomatal conductance, relative water content (RWC), and chlorophyll content (SPAD value) was analysed in control (C), Put primed (P), salt stressed (S), and Put-primed and salt stressed (P+S) plants **(Figure 9)**.Salt stress significantly reduced the photosynthetic efficiency (Fv/Fm), which decreased to 0.56 in S plants compared to 0.70 in C plants. The P+S combined priming effectively mitigated this reduction, restoring Fv/Fm to value statistically comparable to the C (0.68). In contrast, P plants showed a significantly lower Fv/Fm value (0.58) than C plants **(Figure 9A)**.

Stomatal conductance in S plants was 183.9 $mmol \cdot m^{-2} \cdot s^{-1}$, significantly higher than in P+S plants, which had a value of 142.87 $mmol \cdot m^{-2} \cdot s^{-1}$ (**Figure 9B**).

Leaf RWC declined from 79.97 % in C plants to 71.09 % in S plants. Put application alone resulted in a further reduction of RWC to 67.91 %. However, the combined P+S priming partially restored RWC to 73.86 %, a value statistically comparable to that of C plants (**Figure 9C**).

Chl content, expressed as SPAD values, was 35.3 in C, 33.2 in P, and 31.36 in S plants. Notably, the P+S treatment significantly increased SPAD values to 39.3, a level statistically comparable to the control and significantly higher than that observed under salt stress (**Figure 9D**).

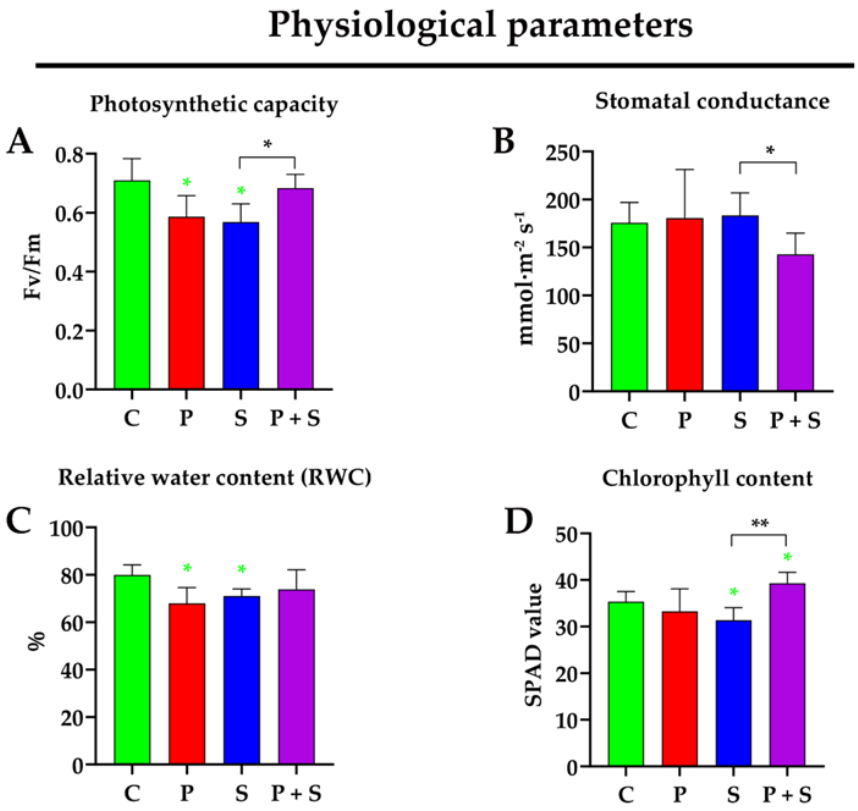


Figure 9. Effect of 0.5 mM Put foliar spray on physiological parameters in cucumber plant under optimal and salt stress conditions (100 mM NaCl). Photosynthetic efficiency (Fv/Fm; **Figure 9A**), stomatal conductance (mmol m^{-2} s^{-1}; **Figure 9B**), relative water content (RWC; **Figure 9C**), and Chl content (SPAD value; **Figure 9D**). C: non-primed, non-stressed control plants, P: Put-primed, non-stressed plants, S: salt-stressed, non-primed plants, and P+S: Put-primed under salt stress plants. Data are presented as mean ± SD (n = 5). Statistical significance was assessed using Student's t-test; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between each treatment and the control (C) are highlighted in green, other significant differences are highlighted in black.

4.7 Put foliar spray enhances biochemical parameters of cucumber adult plants, both in optimal and salt stress conditions

To further investigate the effects of 0.5 mM foliar Put application, biochemical analysis were conducted to evaluate its protective role in cucumber plants at the BBCH 1.5 stage subjected to 200 mM NaCl stress, focusing on key biochemical responses (**Figure 10**). Ferric reducing antioxidant power (FRAP), malondialdehyde (MDA) content, chlorophyll content (µg·mg^{-1} FW), and carotenoid content (µg·mg^{-1} FW) were analysed in control (C), Put primed (P), salt stressed (S), and Put-primed and salt stressed (P+S) plants.

FRAP increased markedly under salt stress, reaching 1.65 mmol Fe^{2+} compared with 1.11 mmol Fe^{2+} in control plants. The combined P+S treatment significantly reduced FRAP values to 1.26

mmol Fe^{2+}, restoring them to a level statistically comparable to the control, whereas P treatment alone resulted in FRAP values of 1.14 mmol Fe^{2+} (**Figure 10A**).

MDA content was also significantly elevated under salt stress, reaching 1.62 mM. The combined P+S treatment substantially decreased MDA levels to 1.14 mM, restoring values statistically equivalent to those observed in non-stressed control plants (**Figure 10B**).

Total chlorophyll content decreased under salt stress, dropping to 513 µg·mg^{-1} FW compared to 720 µg·mg^{-1} FW in control plants. The P+S treatment significantly increased Chl content to 633 µg·mg^{-1} FW, mitigating the impact of salt stress (**Figure 10C**).

Similarly, total car content was reduced by salt stress, decreasing to 72.84 µg·mg^{-1} FW compared with 105.94 µg·mg^{-1} FW in control plants. Under P+S conditions, Car content increased to 98 µg·mg^{-1} FW, reaching values statistically equivalent to the control (**Figure 10D**).

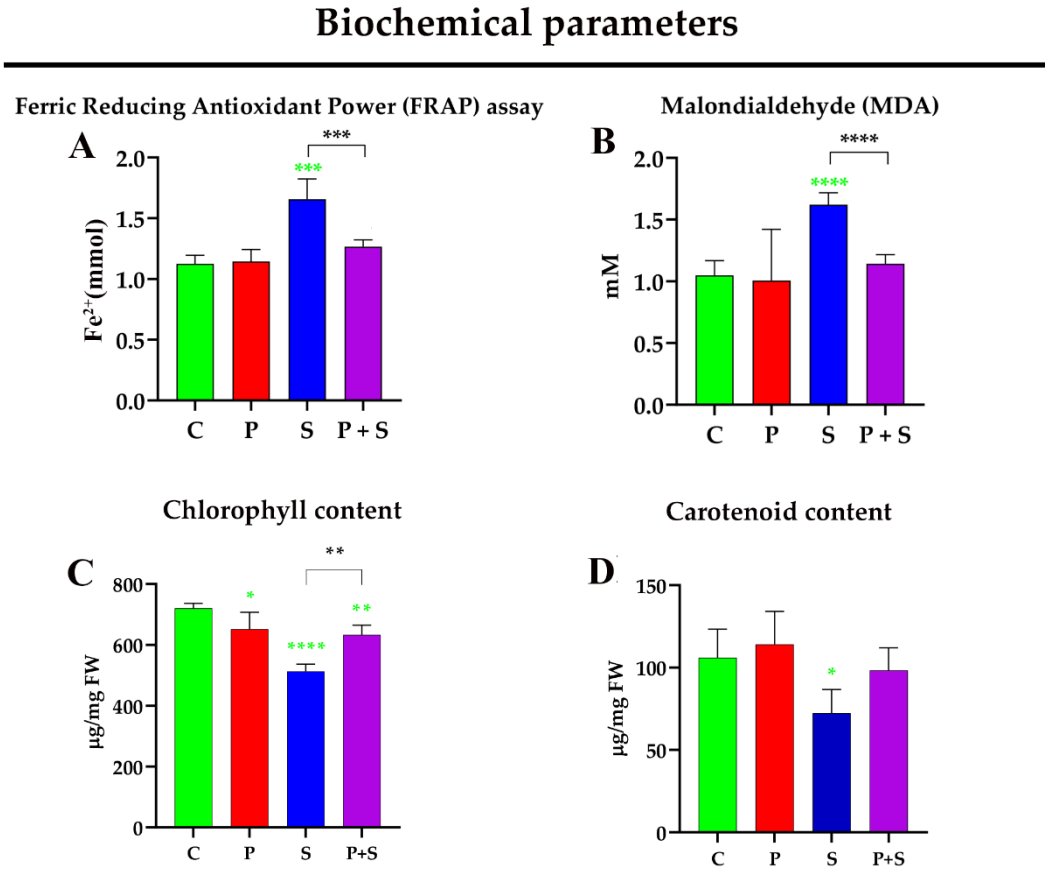


Figure 10. Effect of 0.5 mM Put foliar spray on biochemical parameters in cucumber plant under optimal and salt stress conditions (200 mM NaCl). Ferric reducing antioxidant power (FRAP) assay values (**Figure 10A**), MDA content **(Figure 10B)**, Chl content (µg/mg FW; **Figure 10C**), and Car content (µg/mg FW; **Figure 10D**). C: non-primed, non-stressed control plants, P: Put-primed, non-stressed plants, S: salt-stressed, non-primed plants, and P+S: Put-primed under salt stress plants. Data are presented as mean ± SD (n = 18–30). Statistical significance was assessed using Student's t-test; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between each treatment and the control (C) are highlighted in green¸other significant differences are highlighted in black.

4.8 Put foliar spray modulates the expression of osmotic- and oxidative-stress-related genes in cucumber

To further investigate the molecular responses associated with foliar application of 0.5 mM Put, the modulation of expression of key osmotic- and oxidative-stress-related genes was performed on cucumber plants at the BBCH 1.5 stage under 100 mM NaCl treatment (**Figure 11**). Parameters were assessed in control (C), Put-primed (P), salt-stressed (S); and Put-primed and salt-stressed (P+S) plants. Specifically, the expression levels of *SOS-3* (oxidative stress scavenger), *SOD-2* (oxidative stress scavenger), *P5CS* (osmo-protector), *ZDH-10* (osmo-protector), *NAC-14* (transcription factor), and *NAC-1* (transcription factor) were quantified and evaluated by RT-qPCR. Put priming alone induced significant changes in several genes compared with the non-stressed control. The ion homeostasis regulator *SOS-3* and the oxidative stress scavenger *SOD-2* were downregulated, with relative expression values of 0.66 and 0.76, respectively (**Figure 11A** and **11B**, respectively). Conversely, the osmo-protector *P5CS* and *ZDH-10* were upregulated, reaching 1.36- and 1.28-fold the control levels, respectively (**Figure 11C** and **11F,** respectively). The transcription factor *NAC-14* was downregulated to 0.78 relative to C (**Figure 11E**). Under combined Put priming and salt stress (P+S), gene expression patterns were significantly downregulated compared with salt stress alone (S). Notably, the transcription factor *NAC-1* showed the lowest expression in P+S plants, 0.48-fold that of C (**Figure 11D**), suggesting a Put-mediated adjustment of stress-responsive transcriptional activity

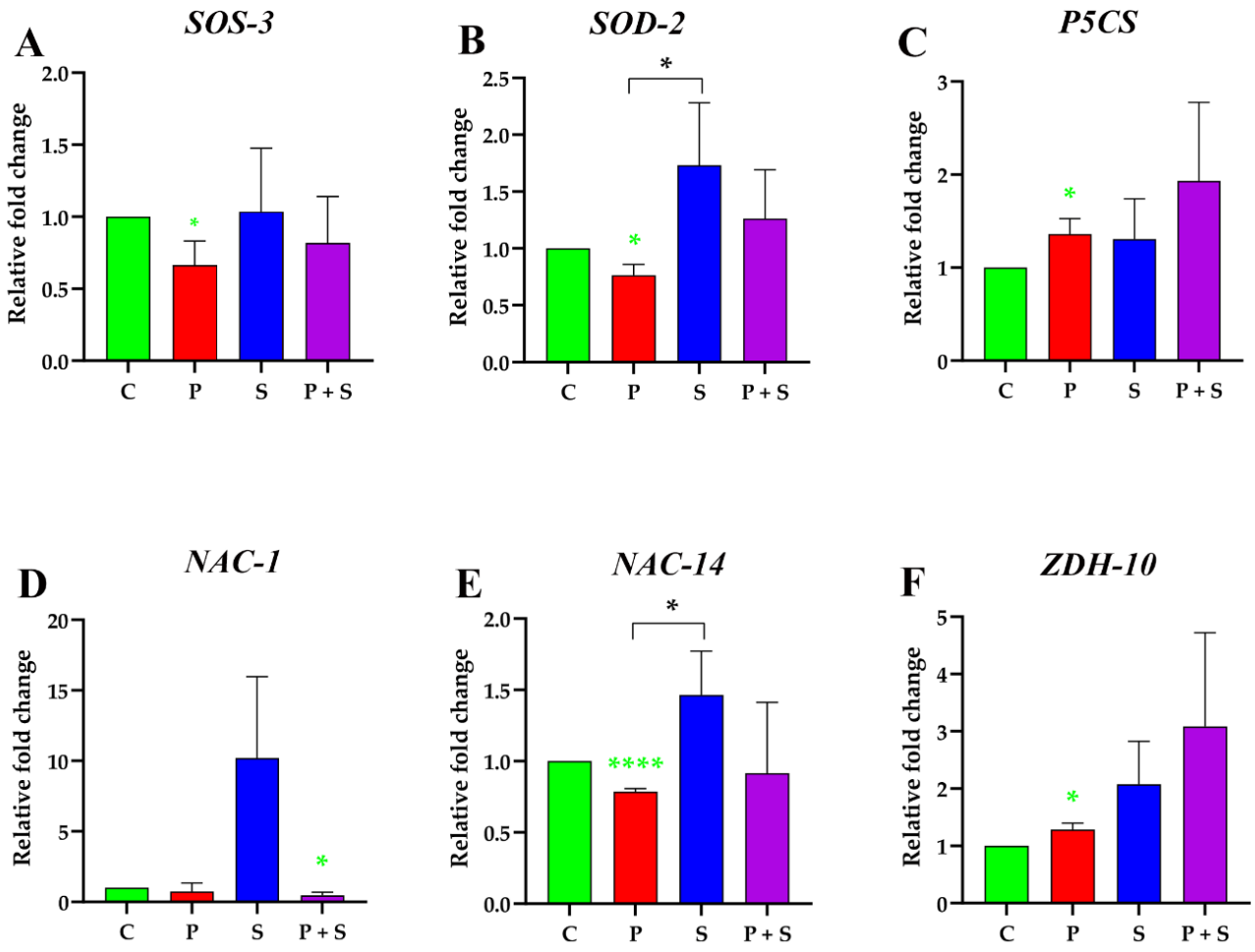


Figure 11. Effect of 0.5 mM Put foliar spray on expression levels of *SOS-3* (Figure 11A), *SOD-2* (Figure 11B), *P5CS* (Figure 11C), *NAC-1* (Figure 11D), *NAC-14* (Figure 11E), and *ZDH-10* (Figure 11F) genes in cucumber plant under optimal and salt stress conditions (100 mM NaCl). Gene expression was analysed in 1-month-old adult plants, untreated or treated with 0.5 mM Put, with and without salt stress. C: non-primed, non-stressed control plants, P: Put-primed, non-stressed plants, S: salt-stressed, non-primed plants, and P+S: Put-primed under salt stress plants. Values were normalized to C. Data are presented as mean ± SD (n = 3), each with two technical replicates. Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between each treatment and the control (C) are highlighted in green; other significant differences are highlighted in black.

4.9 Put foliar spray enhances cucumber fruit growth under salt stress

To further analyse the long-term agronomic effects of foliar 0.5 mM Put application, quantitative parameters of cucumber fruit, average fruit length, and average fruit weight, were assessed on adult plants at the BBCH 1.5 stage under both optimal and 100 mM NaCl stress conditions **(Figure 12)**. Parameters were assessed in Control (C), Put-primed (P), salt-stressed (S); and Put-primed and salt-stressed (P+S) plants. Representative images of fruits are presented in **Figure 12A.** Salt stress alone (S) did not cause a statistically significant reduction in either fruit length or fruit weight compared to the control (C). However, Put priming under stress (P+S) significantly enhanced both parameters, compared with salt stress treatment alone. Fruit length increased to 20.21 cm in P+S plants compared to 16.14 cm in S, while average fruit weight rose to 423.47 g per fruit, relative to 283.63 g in S (**Figure 12B** and **12C,** respectively).

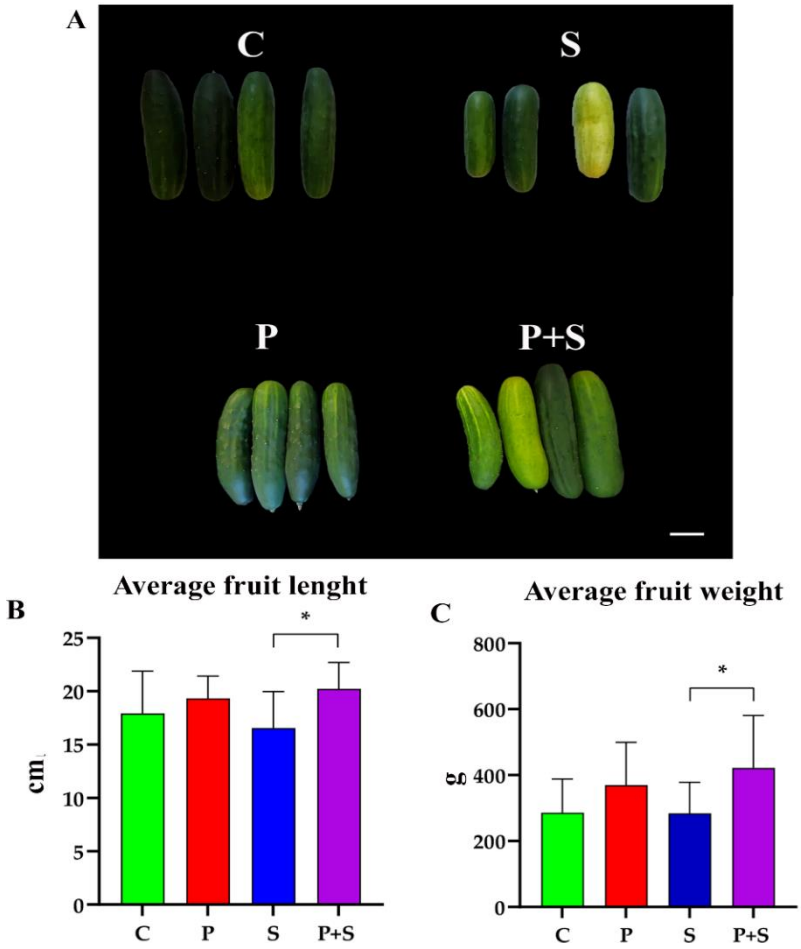


Figure 12. Effect of 0.5 mM Put foliar spray on fruit growth in cucumber plant under optimal and salt stress conditions (100 mM NaCl). Representative images of fruits (white bar = 3 cm; **Figure 12A**). C: non-primed, non-stressed control plants, P: Put-primed, non-stressed plants, S: salt-stressed, non-primed plants, and P+S: Put-primed under salt stress plants. Average fruit length (**Figure 12B**) and average fruit weight (**Figure 12C**). Data are presented as mean ± SD of five independent replicates (n = 5). Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between treated and control plants (C and S) are highlighted.

4.10 Put priming mitigates Bemisia infestation and alters VOC emission in cucumber

To investigate the protective effects of PA priming against Bemisia infestation, cucumber plants at the BBCH 1.5 stage were treated before pest exposure, and pest population dynamics, plant physiological responses, and VOC emission profiles were evaluated (**Figures 13, Figure 14,** and **Figure 15**, respectively). Bemisia population dynamics were assessed at T15 to evaluate the efficacy of PA treatment applied before infestation. Leaf disks were collected from treated and control plants, and the number of individuals per disk was recorded. The untreated infected control (C) harboured 42.1 individuals per leaf disk. Pre-infestation treatment with 5 mM Put reduced the population to 8.5 individuals, representing the most pronounced effect among all treatments. Dose-dependent reduction was observed, with 1 mM Put and 0.5 mM treatments reducing populations to 11.1 and 23.1 individuals, respectively 5 mM Spd and 5 mM Spm treatments decreased the population to 22.7 and 22.6 individuals, respectively (**Figure 13A).** Analysis of developmental stages indicated that 5 mM Put not only reduced the total population but also delayed development, resulting in a predominance of early instars at both T7 and T15 (**Figure 13B).** Treatment efficiency, quantified using Abbott's formula, confirmed the dose-dependent effect, with 5 mM Put achieving the highest mortality rate, exceeding 90 % (**Figure 13D).** At T21, persistence analysis revealed that the 5 mM Put treatment maintained the Bemisia population at 4.6 individuals, significantly lower than the 40.53 individuals recorded in C, indicating a sustained preventative effect (**Figure 13C**).

Physiological analysis were performed to assess plant health following PA treatments (**Figure 14).** At T7, SPAD values remained largely unchanged across treatments, while the photochemical reflectance index (PRI) was reduced by infestation; all PA treatments except 5 mM Put mitigated this reduction. At T15, 1 mM Put treatment increased SPAD values under infestation, whereas several Spd and Spm treatments, 1 mM and 0.5 mM, led to higher PRI compared to the infested control (**Figure 14A**). Representative images of leaves after 5 mM PA treatments are shown in **Figure 14B.**

VOC emissions were analysed at T21 to investigate potential indirect defence mechanisms **(Figure 15)**. A total of 20 VOCs were initially identified; however, following the removal of known contaminants and compounds with excessively SDs, 16 VOCs were retained and quantified (C1 to C16). Changes in VOC abundance were evaluated across four experimental conditions: infested control plants (I), Put primed plants (Put), non-infested control plants (C), and Put primed infested plants (Put + I). Relative quantitative profiling, summarized in a heat map, indicated that 5 mM Put treatment significantly altered the overall VOC profile, with Put + I plants exhibiting increased abundance of four compounds, including an unidentified Terpene 01, Styrene, and 3-Hexanol-2,4-dimethyl (**Figure 15A**). In the heat map, each compound is labelled with a colour-coded identifier

indicating its chemical class: green for terpenes, light blue for aromatic compounds, yellow for alcohols, pink for ketones, red for aldehydes, and orange for hydrocarbons. Statistical differences among experimental conditions were assessed using PERMANOVA. When all 16 compounds were included, no significant differences were detected in CAP analysis [Pr (>F) = 0.475; **Figure 15B**]. In contrast, PERMANOVA performed on six compounds identified by Random Forest analysis (C4, C5, C7, C10, C11, C14) revealed significant differences among treatments [Pr (>F) = 0.015; **Figure 15C**], without significant differences between I and Put plants.

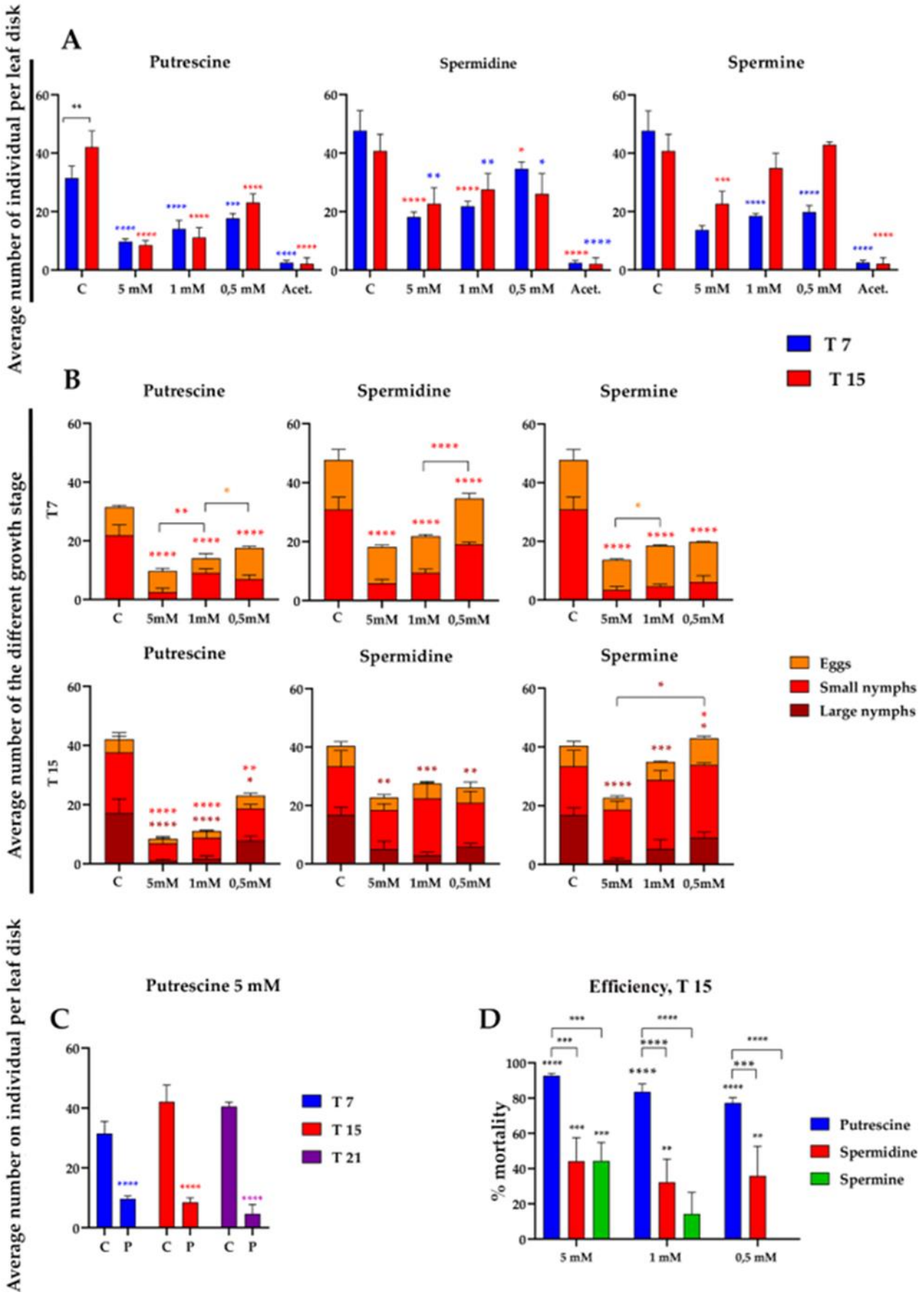

A
Putrescine
Spermidine
Spermine
Average number of individual per leaf disk
C
5 mM
1 mM
0,5 mM
Acet.
T 7
T 15
B
Putrescine
Spermidine
Spermine
T7
T 15
C
5mM
1mM
0,5mM
Eggs
Small nymphs
Large nymphs
Average number of the different growth stage
Putrescine 5 mM
Average number on individual per leaf disk
C
P
T 7
T 15
T 21
Efficiency, T 15
D
% mortality
Putrescine
Spermidine
Spermine
5 mM
1 mM
0,5 mM

Figure 13. Effect of PA foliar spray (pre-infestation) against Bemisia infestation in cucumber. Impact on Bemisia population dynamics. (**Figure 13A**) Average number of Bemisia individuals per leaf disk at T7 and T15, comparing three concentrations (5 mM, 1 mM, 0.5 mM) Put, Spd, and Spm against the non-treated control (C). 2.1 mM acetamiprid (Acet.) was used as a chemical control. Average number of eggs, small nymphs, and large nymphs at T7 and T15 (**Figure 13B**). Effects of Put treatment at T21 (**Figure 13C**). Treatment efficiency (% mortality) calculated using Abbott's formula for the treatments shown in **Figure 13A** at T15 (**Figure 13D**). Data are presented as mean ± SD of three independent replicates per concentration/PA (n = 3). For each replicate, data were derived from 5 sampled leaf disks per plant. Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between each PA treated plants and the control (C) are highlighted in blue (T7) or red (T15; **Figure 13A**); in significant differences in each developmental stage compared to C are indicated in orange (eggs), red (small nymphs), and dark red (large nymphs); in (C; **Figure 13B**), in differences between each Put treatment and C are highlighted in blue (T7), red (T15), and purple (T21; **Figure 13C**). Efficiency comparisons relative to C are reported in (**Figure 13D**). Other significant differences are highlighted in black.

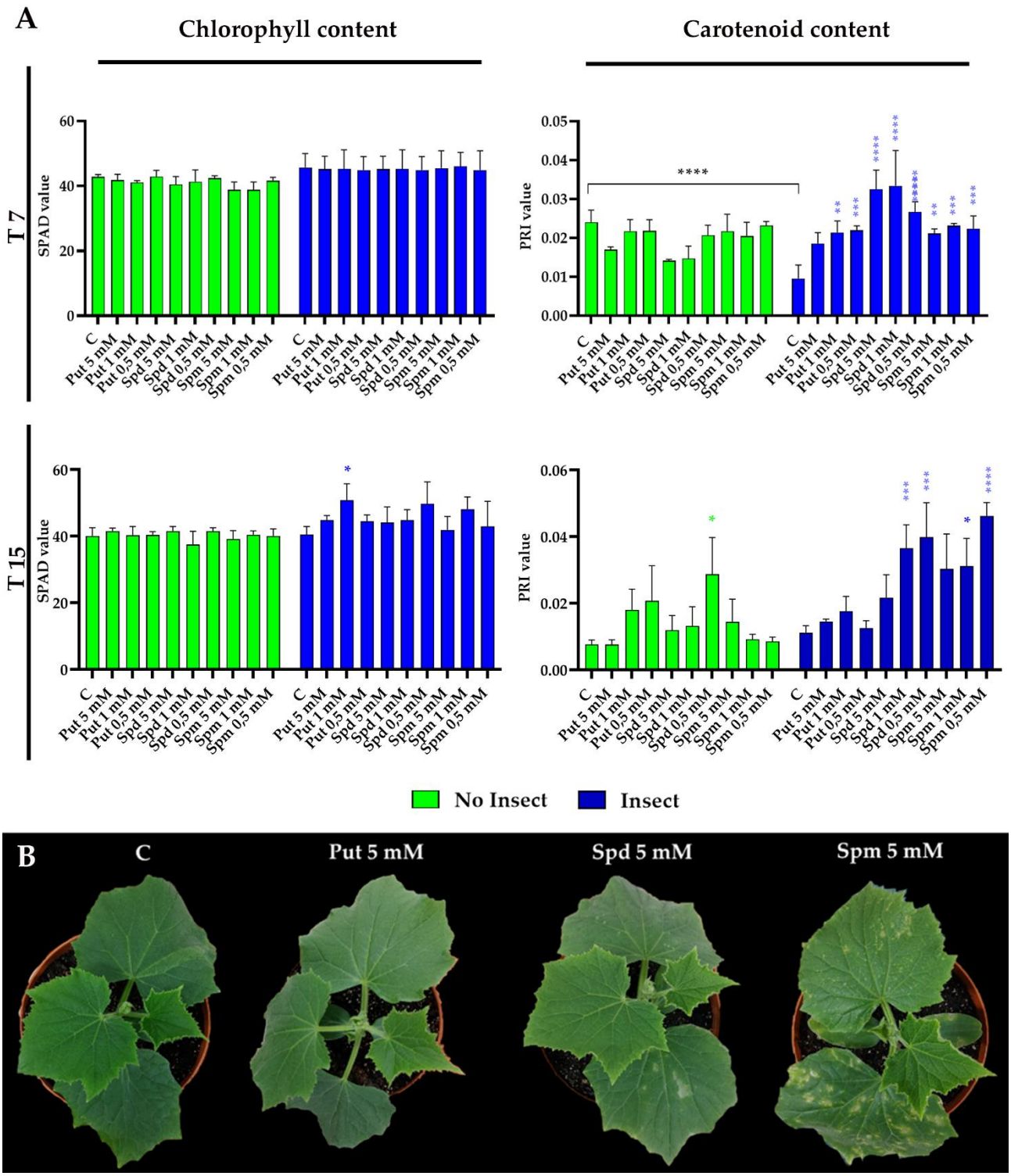
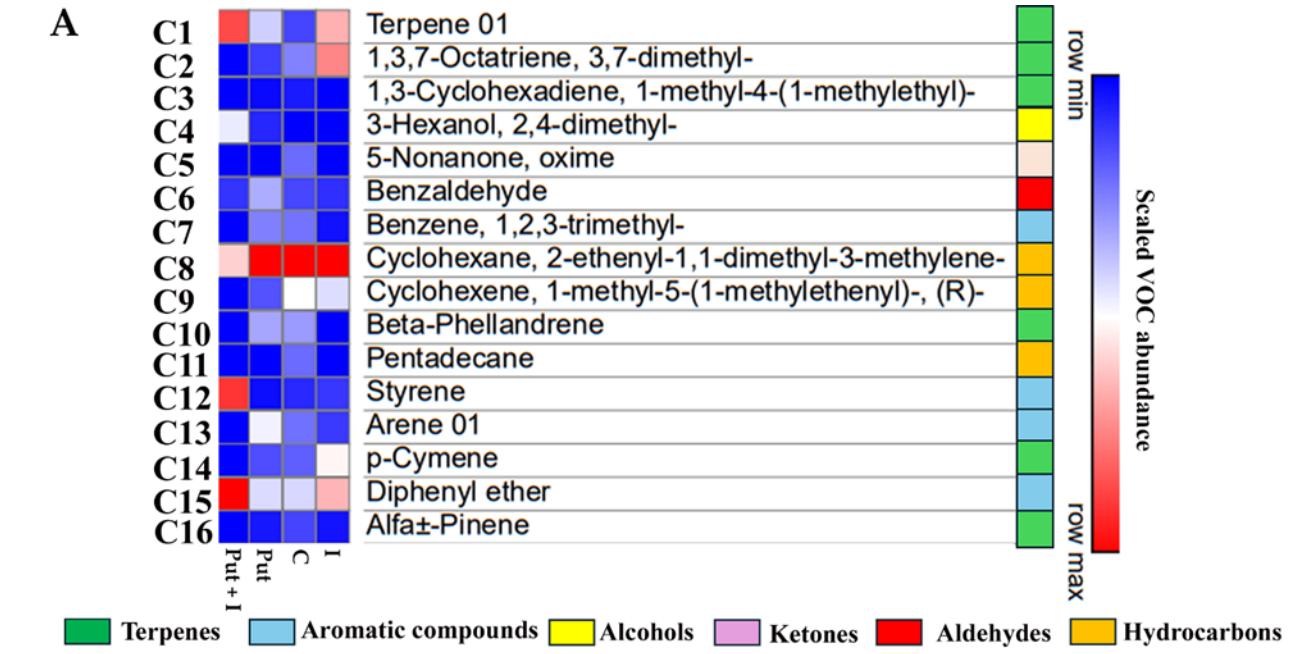
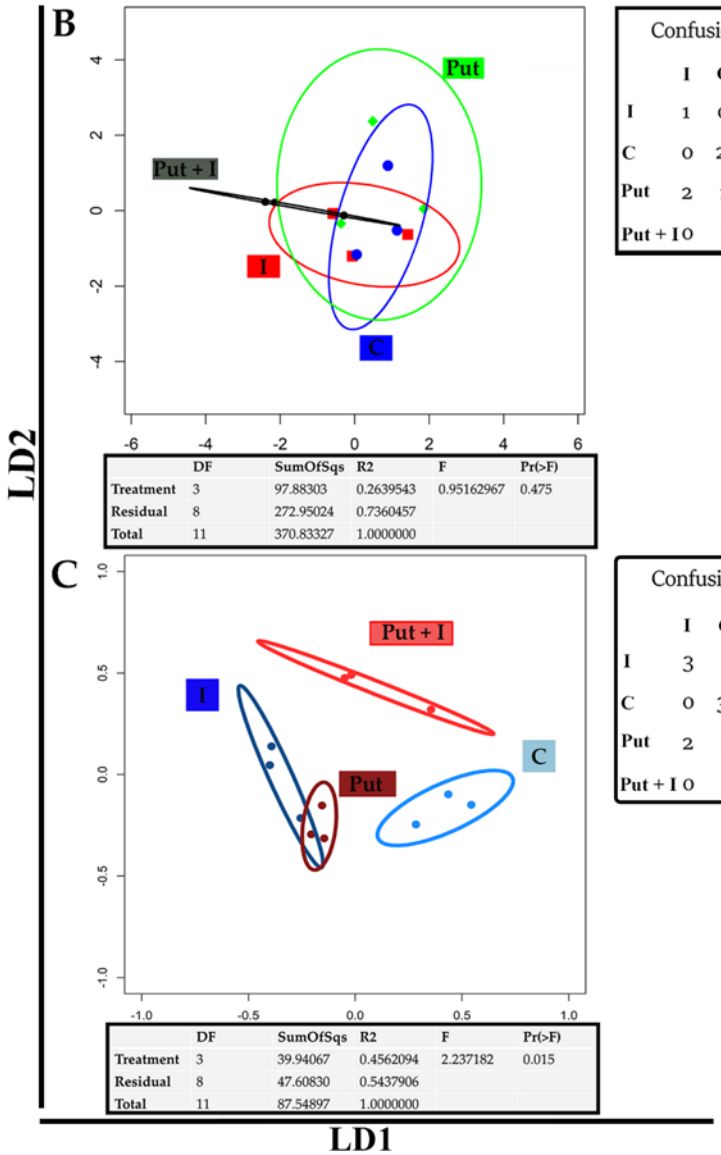


Figure 14. Effect of PA foliar spray (pre-infestation) against Bemisia infestation in cucumber. Impact on Chl and Car content in treated plants. Chl and Car levels, expressed as SPAD and PRI values, respectively, were measured 7 (T7) and 15 (T15) days after PA treatment. Representative images of cucumber plants treated with 5 mM of each PA are shown (**Figure 14A**). Representative images of plants under the different most effective treatments are shown in **Figure 14B**. Data are presented as mean ± SD of five independent replicates (n = 3). Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Differences between treated and C and S plants are highlighted in green or blue, respectively.





	DF	SumOfSqs	R2	F	Pr(>F)
Treatment	3	97.88303	0.2639543	0.95162967	0.475
Residual	8	272.95024	0.7360457		
Total	11	370.83327	1.0000000		

Confusion matrix:

	I	C	Put	Put +I	class. error
I	1	0	2	0	0.6666667
C	0	2	1	0	0.3333333
Put	2	1	0	0	1.0000000
Put + I	0	1	0	2	0.3333333

	DF	SumOfSqs	R2	F	Pr(>F)
Treatment	3	39.94067	0.4562094	2.237182	0.015
Residual	8	47.60830	0.5437906		
Total	11	87.54897	1.0000000		

Confusion matrix:

	I	C	Put	Put + I	class. error
I	3	0	0	0	0.0000000
C	0	3	0	0	0.0000000
Put	2	0	1	0	0.6666667
Put + I	0	0	0	3	0.0000000

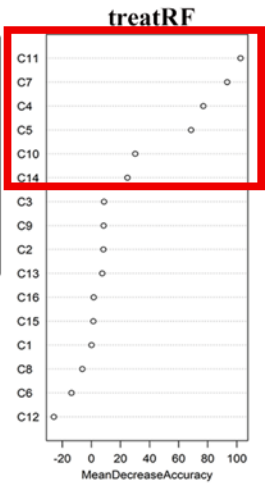


Figure 15. Effect of 5 mM Put foliar spray (pre-infestation) against Bemisia infestation in cucumber. Impact on variation of VOC profiles in treated plants using TD–GC×GC–TOF–MS. (**Figure 15A**) Heatmap of scaled VOC abundances across treatments. The heatmap displays the normalised abundance (area) of VOCs identified in cucumber samples, comparing Put-treated and control plants, with and without infestation. Red indicates higher-than-average abundance, blue indicates lower-than-average abundance, and white represents the mean. Compounds are grouped by chemical family. Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$ (**Figure 15A**). CAP analysis of all detected compounds, with corresponding confusion matrix (**Figure 15B**). CAP analysis based on the reduced compound set selected through random forest, with the corresponding confusion matrix. PERMANOVA analysis of all detected compounds, with corresponding confusion matrix, used to evaluate the overall shift in VOC profiles. CAP plots were generated using two linear discriminants (LD), and all ordination plots (CAP & random forest) include a 95 % confidence interval (**Figure 15C**).

4.11 Put post-infestation treatment mitigates Bemisia damage in cucumber

To evaluate the protective effect of Put applied after Bemisia infestation, cucumber plants at the BBCH 1.5 stage were treated with Put at three concentrations: 5 mM, 1 mM, and 0.5 mM. Pest population dynamics, developmental disruption, and treatment efficiency were monitored over 10 days (**Figure 16**). Only Put was tested in this post-infestation phase.

Bemisia populations were recorded at T0, T5, and T10. At T0, the infected control (C) showed an average of 31.3 individuals per leaf disk, like the other treatment groups. Put 5 mM resulted in a higher reduction, with populations decreasing to 10.7 individuals at T5 and 5.2 at T10, compared to 42.8 and 34 in C, respectively. The 1 mM treatment also showed significant reductions, with the population decreasing to 16.9 individuals at T5 and 9.13 individuals at T10, while the 0.5 mM treatment was less effective, reducing the population to 25.7 individuals at T5 and 16.1 individuals at T10 (**Figure 16A**). Analysis of developmental stages indicated that Put 5 mM significantly slowed down Bemisia development at both T5 and T10, reducing the number of eggs, small nymphs, and large nymphs compared to C (**Figure 16B**).

Treatment efficiency, calculated as % mortality using Abbott's formula at T10, confirmed a dose-dependent effect. In this context, Put 5 mM achieved the highest mortality rate, exceeding 80% (**Figure 16C** and **Figure 16D,** respectively).

Comparison with the preventive pre-infestation treatment demonstrated that the post-infestation foliar application of Put was equally effective, showing no statistically significant difference in efficiency between the two approaches (**Figure 13**).

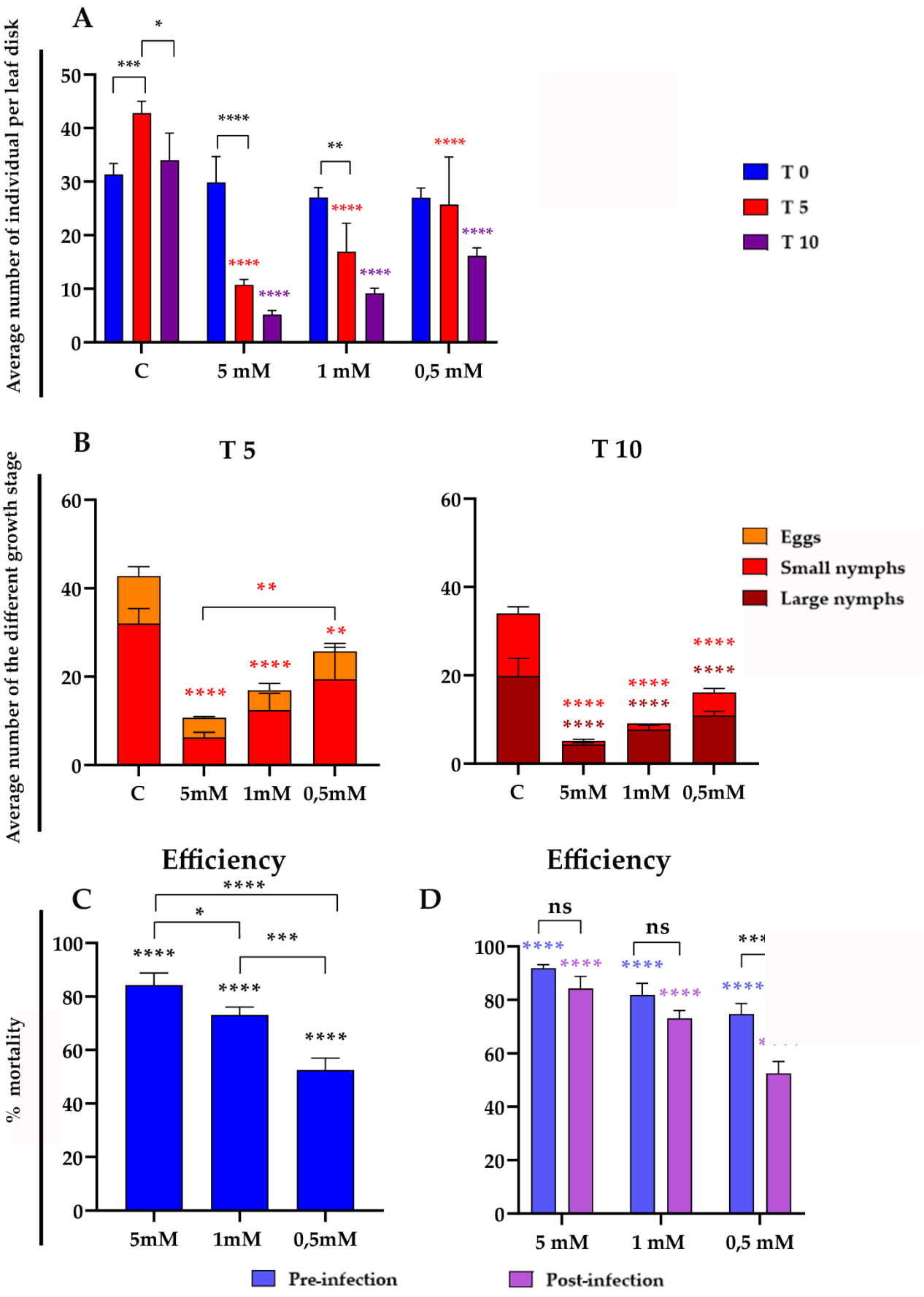

A
Average number of individual per leaf disk
50
40
30
20
10
0
C
5 mM
1 mM
0,5 mM
T 0
T 5
T 10
B
T 5
T 10
Average number of the different growth stage
60
40
20
0
C
5mM
1mM
0,5mM
Eggs
Small nymphs
Large nymphs
Efficiency
Efficiency
C
D
% mortality
100
80
60
40
20
0
5mM
1mM
0,5mM
ns
5 mM
1 mM
0,5 mM
Pre-infection
Post-infection

Figure 16. Effect of Put priming (post-infestation treatment) against Bemisia infestation in cucumber: impact on total pest population and developmental stages at 5 and 10 days after infestation. Average number of Bemisia individuals per leaf disk at T5 and T10, comparing three concentrations (5 mM, 1 mM, 0.5 mM) of Put compared to non-treated control (C). Average number of developmental stages (eggs, small nymphs, and large nymphs) at T5 and T10 (**Figure 16B**). Treatment efficiency (% mortality) calculated using Abbott's formula for the treatments shown in **Figure 16A** at T10 (**Figure16C**). Data are presented as mean ± SD of three independent replicates per concentration/PA (n = 3). Comparison between treatment efficiency at T10 for post-infestation Put treatments (**Figure 16A**) and the pre-infestation PA treatments reported in **Figure 13** (**Figure 16D**). Data are presented as mean ± SD of three independent replicates per concentration/PA (n = 3). For each replicate, data were derived from 5 sampled leaf disks per plant. Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and *** indicates $p \leq 0.001$. Statistically significant differences between each PA treatment and the corresponding time-point control group (C) are shown in red (T5) or purple (T10) in (A). In (B), significant differences for each developmental stage relative to C are shown in orange (eggs), red (small nymphs), and dark red (large nymphs). Significant differences in efficiency between Put treatments and the C are indicated in blue (pre-infestation) and purple (post-infestation; **Figure 16D**). Other significant differences are highlighted in black.

4.12 Put-induced VOCs confer temporary resistance to Bemisia in cucumber plants

In the pre-infestation experiment, control plants neighbouring Put-treated plants exhibited a reduced Bemisia population at T7 (approximately 31 individuals) compared with those adjacent to Spd- or Spm-treated plants (approximately 48 individuals). This difference suggests an indirect protective effect, potentially mediated by VOCs emitted by Put-treated plants, which may influence whitefly host selection or population establishment. The average number of Bemisia per leaf disk and physiological parameters were assessed in: C, isolated control plants, not exposed to external agents; 5 mM Put, plants treated with 5 mM Put but not infested; Bemisia + 5 mM Put, plants directly infested and treated with 5 mM Put; Bemisia near 5 mM Put, infested plants neighbouring those treated with 5 mM Put; Bemisia near Bemisia + 5 mM Put, infested plants neighbouring those treated with 5 mM Put and infested. Pest population dynamics and physiological stress indicators were monitored over 15 days (**Figure 17**). Regarding Bemisia populations, direct treatment of the VOC-donor plants (Bemisia + 5 mM Put) provided the strongest protection, significantly reducing the pest population compared to the infested control (C). At T7, the population on treated plants was 22.6 individuals per leaf disk compared to 49.8 in C, and at T15 it decreased to 11.3 individuals compared to 36 in C (**Figure 17A**).

Plants infested with Bemisia near 5 mM Put directly treated plants exhibited a reduced pest population of 25.6 individuals at T7, which was statistically lower than C with 49.8 individuals. The protective effect was transient, as at T15 neither neighbouring group maintained a pest population significantly lower than C, which reached 36 individuals. Physiological parameters were evaluated by assessing Chl content (SPAD values) and Car content (PRI). VOC exposure maintained SPAD

values at levels statistically comparable to the non-infested control at T7; however, this effect was not sustained at T15. Infestation induced a significant decline in PRI at T15 compared with T7, and VOC-exposed neighbouring plants did not exhibit PRI values higher than their respective controls at the same time point (**Figure 17B** and **17C,** respectively).

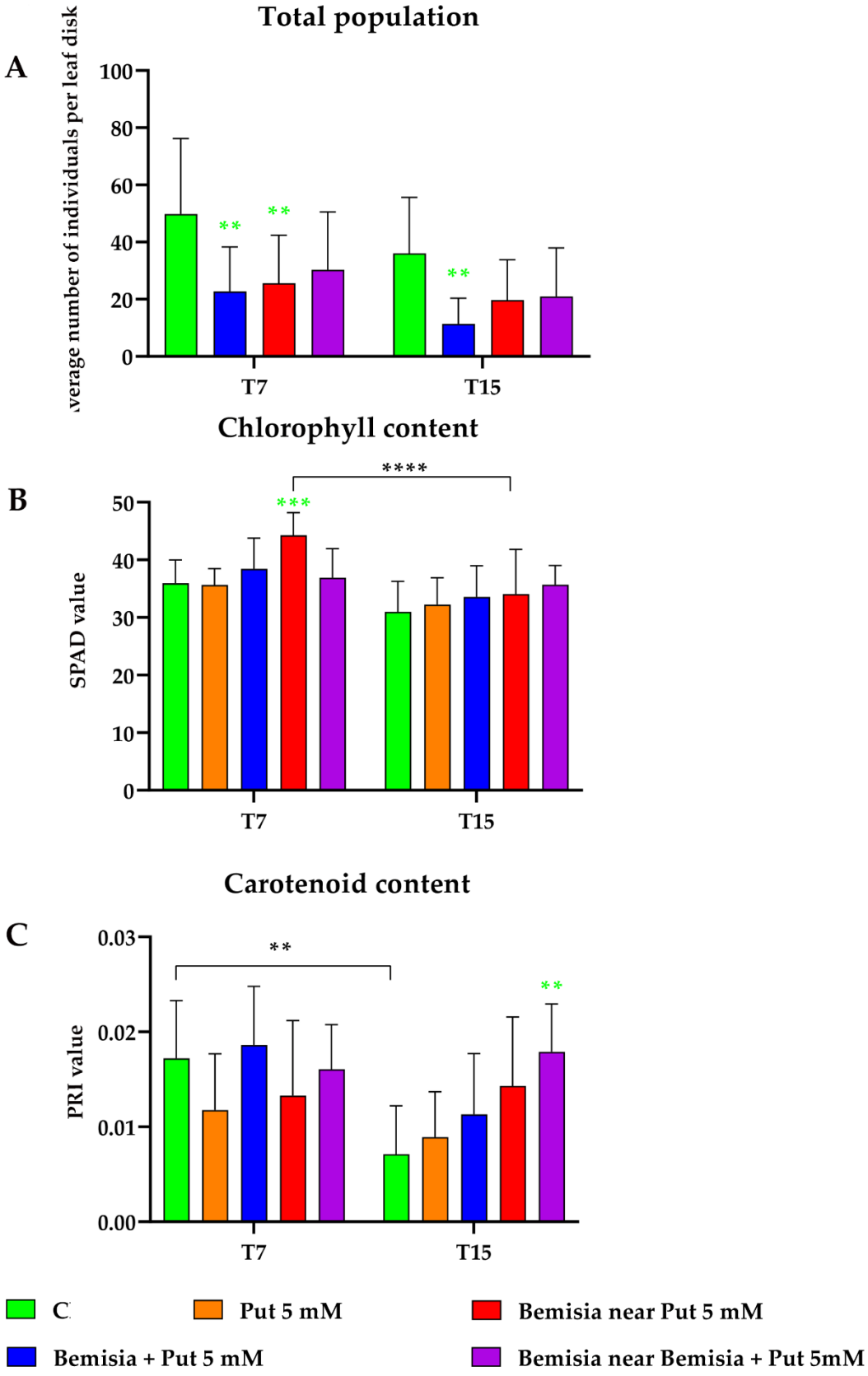

A
Total population
Average number of individuals per leaf disk
100
80
60
40
20
0
**
**
**
T7
T15
B
Chlorophyll content
SPAD value
50
40
30
20
10
0
****
***
T7
T15
C
Carotenoid content
PRI value
0.03
0.02
0.01
0.00
**
**
T7
T15
C
Put 5 mM
Bemisia near Put 5 mM
Bemisia + Put 5 mM
Bemisia near Bemisia + Put 5mM

Figure 17. Effect of VOC priming against Bemisia infestation in cucumber: impact on total pest population and on Chl and Car content. Average number of Bemisia individuals per cucumber leaf disk recorded at 7 and 15 days after treatment (T7, T15; **Figure 17A**). Chl and Car levels of VOC-primed cucumber plants, expressed as SPAD and PRI values, respectively, at T7 and T15. Mean values ± SD of 3 independent replicates were obtained for each VOC-priming treatment (n = 3; **Figure 17B,** and **17C**). Statistical differences were evaluated using one-way ANOVA; p levels > 0.05; *** $p \leq 0.001$. Non-significant differences ($p > 0.05$) are not shown. Statistically significant differences ($p \leq 0.05$) between each treatment and the control group are indicated in green. Other significant differences are highlighted in black.

4.13 Spd foliar spray priming protects cucumber adult plants from *G. cichoracearum* infection

To investigate the protective effects of PA priming against the fungal pathogen *G. cichoracearum*, responsible for powdery mildew (PM), cucumber plants at the BBCH 1.5 stage were treated before infection with 1 mM Put, Spd, and Spm. Azoxystrobin (1.2 mM; Azox.) was used as a positive chemical control. A representative image of infected leaves under the different treatments is shown in **Figure 18**.

Infection level was quantified over 21 days by measuring the percentage of leaf area damaged and the average fungal colony size (**Figure 19**). Regarding leaf damage, Spd priming conferred significant protection that was sustained throughout the observation period at T7, T15, and T21 compared to the infected unprimed control (I). Specifically, the infected control exhibited 47.1 % damaged leaf area at T21, whereas Spd treatment reduced damage to 21.4 %. Spd priming showed a protective effect statistically comparable to the chemical control, Azox. during the early phases at T7 and T15. In contrast, Spm did not provide a statistically significant protective effect, despite a numerical reduction to 33.3 % damaged leaf area at T21. Put treatment did not confer protection and, in fact, increased infection level, reaching 63.5 % damaged leaf area at T21.

Regarding average fungal colony size (**Figure 19B** and **19C**), the infected control exhibited colonies of 0.72 cm^2. Both Spd treatment, with an average colony size of 0.18 cm^2, and Azox., with 0.32 cm^2, significantly reduced colony size compared to the infected control. The effect of Spd was statistically comparable to that of Azox. The treatment, with an average colony size of 0.59 cm^2, and Spm, with 0.68 cm^2, maintained colony sizes statistically like those of the infected control.

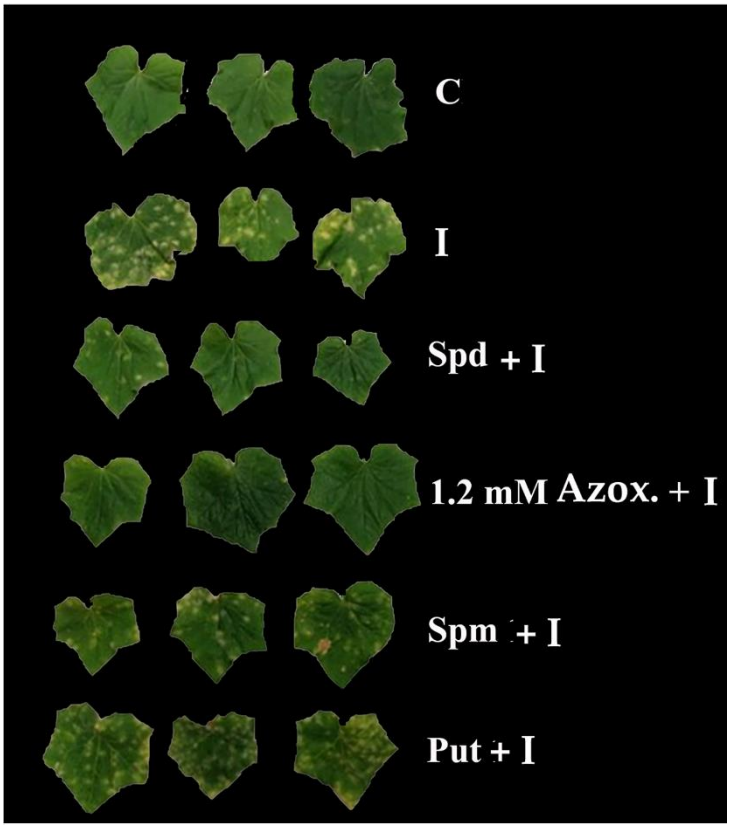


Figure 18. Representative image of percentage of damaged leaf area: effect of *G. chicoracearum* infection on cucumber leaves. C: uninfected control plants, I: plants infected with *G. chicoracearum*, Put + I: Put primed and infected, Spd + I: Spd-primed and infected, Spm + I: Spm-primed and infected, Azox.: azoxystrobin, chemical control.

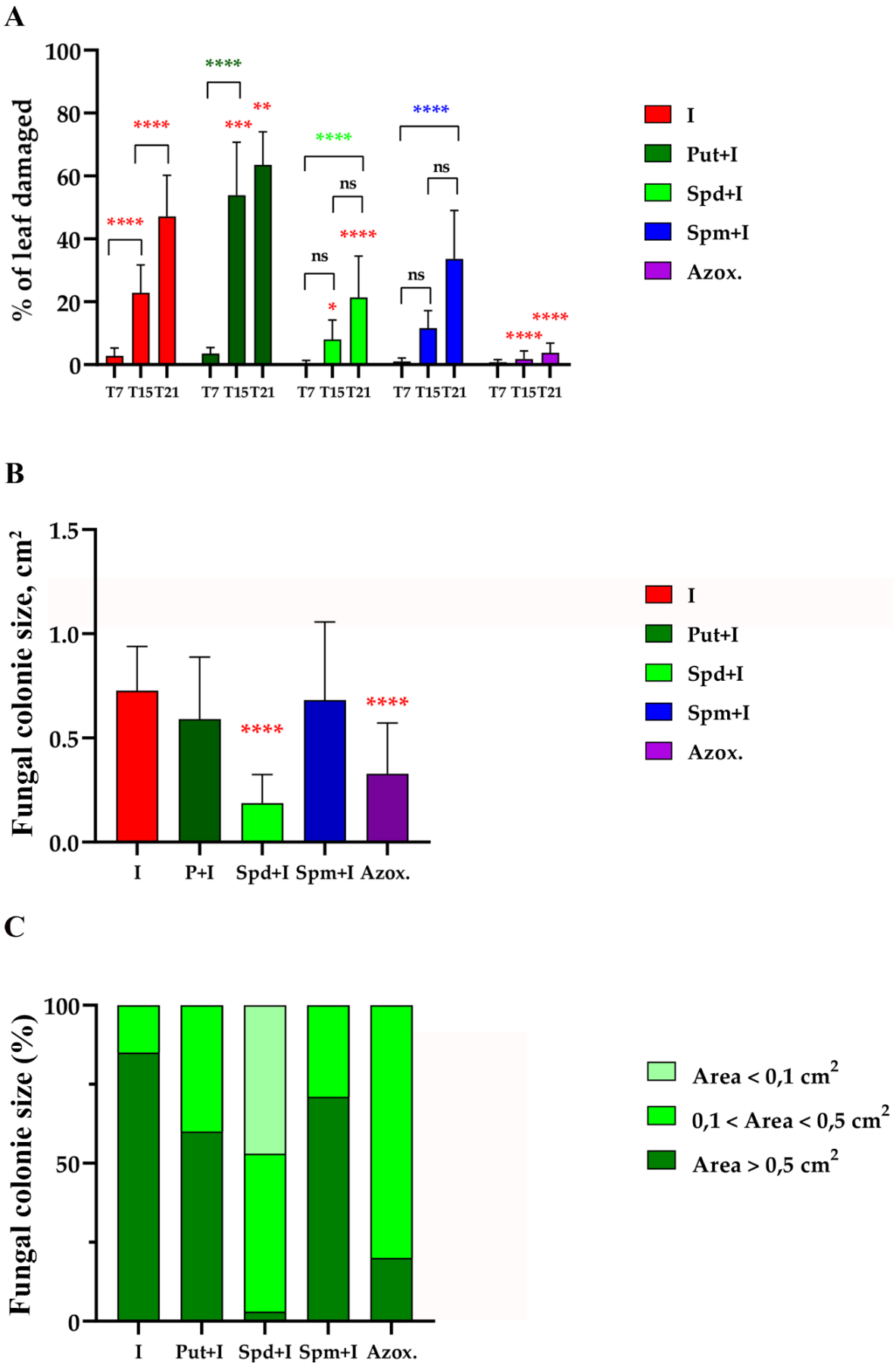

A
% of leaf damaged
100
80
60
40
20
0
T7 T15T21
T7 T15T21
T7 T15T21
T7 T15T21
T7 T15T21
****
****
****
***
**
****
ns
****
ns
*
ns
****
****
****
I
Put+I
Spd+I
Spm+I
Azox.
B
Fungal colonie size, cm²
1.5
1.0
0.5
0.0
I
P+I
Spd+I
Spm+I
Azox.
****
****
I
Put+I
Spd+I
Spm+I
Azox.
C
Fungal colonie size (%)
100
50
0
I
Put+I
Spd+I
Spm+I
Azox.
Area < 0,1 cm²
0,1 < Area < 0,5 cm²
Area > 0,5 cm²

Figure 19. Effect of 1 mM Put, Spd and Spm priming against *G. chicoracearum* infection in cucumber: impact on total fungal presence at 7 (T 7), 15 (T 15), and 21 (T 21) days after treatment. The percentage of fungal damage on leaves **(Figure 19A)** and average fungal colony size (cm^2) were evaluated in I plants and PA-primed infected plants. 1.2 mM azoxystrobin (Azox.) was used as a chemical control (**Figure 19B** and **Figure 19C,** respectively). I: *G. chicoracearum* infection, Put + I: Put primed, infected plants, Spd + I: Spd primed, infected plants, Spm + I: Spm primed, infected plants and P+S: Put-primed under salt stress plants, Azox.: azoxystrobin, chemical control. Mean values ± SD of 3 independent replicates per PA priming treatment (n = 3) are reported. Statistical significance was assessed using one-way ANOVA; $p > 0.05$ is considered not significant (not shown), and **** indicates $p \leq 0.0001$. Differences between treated plants and I plants are highlighted in red.

5. DISCUSSION

The increasing global demand for food poses a significant threat to worldwide food security, a challenge that is further intensified by climate change. Climate-driven alterations are expected to intensify both abiotic stresses, such as soil salinity, and biotic stresses, including pests and pathogens, ultimately leading to significant reductions in crop productivity. In this context, modern agriculture requires innovative and sustainable strategies, including the use of protective compounds to enhance plant resilience. Among these, BSs have gained increasing attention for their ability to mitigate the negative impacts of both abiotic and biotic stresses. Among BSs, PAs have emerged as promising tools to counteract climate-induced yield losses and improve crop performance under adverse conditions (Bulgari *et al*., 2019; Ondrasek *et al*., 2022).

5.1 PA priming enhances germination parameters and seedling development, both in Arabidopsis and cucumber

Seed priming is a well-established pre-sowing strategy that improves germination performance and seedling vigor, particularly under adverse environmental conditions (Farooq *et al*., 2008; Chen & Arora, 2013). By triggering early metabolic activation before radicle protrusion, priming accelerates germination, enhances its uniformity, and increases seedling tolerance to abiotic stresses, among which salinity (Wojtyla *et al*., 2016; Chen *et al*., 2019). In this context, priming with PAs has emerged as an effective priming agent due to their multifunctional roles in osmotic regulation, redox homeostasis, and stress signalling during early developmental stages (Farooq *et al*., 2008; Hongna *et al*., 2021). In this study, the effects of Put, Spd, and Spm priming were evaluated in both Arabidopsis and cucumber plants under optimal and salt-stress conditions.

In Arabidopsis WT seeds, priming with Put significantly accelerated GR and increased the final GI under both control and saline conditions (**Figure 3** and **Figure 4**). The effectiveness of Put priming to enhance germination under salt stress suggests a specific role in Arabidopsis development,

likely through the modulation of osmotic balance and redox signalling during the transition from quiescence to active growth (Chen & Arora, 2013). Among PAs, Put is the most rapidly metabolized and strongly mediates stress-related redox pathways, allowing the fine regulation of ROS dynamics during germination (Tavladoraki *et al*., 2016; Wojtyla *et al*., 2024). In this context, finely regulated ROS accumulation, particularly H_2O_2, has been recognized as a positive signalling component required for dormancy release and successful germination under stress (El-Maarouf-Bouteau & Bailly, 2008; Wojtyla *et al*., 2016). Coherently, Put catabolism represents a source of localized H_2O_2, contributing to the establishment of an optimal redox environment that promotes early metabolic reactivation and stress tolerance (Cona *et al*., 2006; Tavladoraki *et al*., 2016).

In WT Arabidopsis seedlings, Spm priming significantly enhanced post-germinative root elongation, despite having no measurable effect on seed germination (**Figure 7**). This suggests that Spm predominantly modulates associated with cell elongation and early seedling growth. Such functional differences among PAs are likely attributable to a combination of structural and metabolic factors. Higher-order Pas, such as Spm, display greater structural complexity and slower turnover rates, properties that may favor sustained growth-related signalling rather than the rapid responses required for germination (Angelini *et al*., 2010; Wang *et al*., 2019). Moreover, the developmental stage-specific activation of PA-oxidating enzymes, such as CuAOs and PAOs, regulates localized H_2O_2 production, a key signalling molecule involved in redox homeostasis and early metabolic activation. Consequently, the timing and spatial distribution of H_2O_2 generation may be critical in determining whether a given PA preferentially promotes rapid germination or supports post-germinative growth processes (Cona *et al*., 2006; Tavladoraki *et al*., 2016; Wojtyla *et al*., 2016).

Accordingly, with data observed for Arabidopsis seed priming, in cucumber seeds Put priming effectively mitigated the reduction of germination under salinity, whereas 1 mM Spm negatively affected germination parameters (**Figure 5**). The complexity of these PA-mediated responses further highlights the species-specific nature of each PA-mediated priming effects, as previously reported in different crop species, in which specific PA-uptake mechanisms and metabolic regulation pathways have been described (Khan *et al*., 2012; Danalo *et al*., 2018). The improvement of root elongation induced by PA priming was observed only under optimal conditions in cucumber, with the strongest effects induced by Spd (1 mM and 100 µM; **Figure 7**). Under salt stress, however, none of the PA priming have any effect on root elongation in cucumber, suggesting that severe salinity may override PA-mediated growth promotion by imposing dominant osmotic and ionic constraints (Munns & Tester, 2008; Negrão *et al*., 2017).

Analysis of *Atcuao* mutant lines supports a central role for CuAOs in mediating Put-induced priming effects. Both *Atcuaoγ1.1* and *Atcuaoγ2.1* mutants displayed reduced germination parameters

under both Put priming or salt stress compared with WT seeds (**Figure 6**), indicating that CuAO-dependent Put catabolism is required for priming efficacy. CuAO-mediated oxidation of PAs generates localized H_2O_2, which acts as a signalling molecule regulating dormancy release and early seedling metabolism (Cona *et al*., 2006; Wojtyla *et al*., 2016).

Given their expression in root tissues (Fraudentali *et al*., 2021), *AtCuAOγ1 and AtCuAOγ2* likely generate spatially distinct H_2O_2 signals that differentially regulate cell elongation and redox-controlled growth processes. Such localized ROS production can influence the fine-tuning of root growth under both optimal and stress conditions (Angelini *et al*., 2010; Lechowska *et al*., 2021).

Taken together, these findings suggest that Put priming enhances germination parameters and early development of seedling under salt stress through a mechanism that integrates PA molecular structure, rapid metabolic turnover, and CuAO-dependent redox signalling. In contrast, Spd and Spm predominantly influence post-germinative growth processes in a species- and condition-dependent manner. This highlights the necessity of tailoring PA priming strategies to the physiological and metabolic characteristics of the target species to maximize stress resilience during early plant establishment (Lechowska *et al*., 2021).

5.2 Put foliar spray treatment restores growth, photosynthetic performance, antioxidant balance, and modulates gene expression in salt-stressed cucumber plants

Salt stress severely limits plant growth and productivity by inducing osmotic constraints, ionic imbalance and secondary oxidative damage, ultimately resulting in reduced biomass accumulation, impaired photosynthetic performance and yield penalties (Munns & Tester, 2008; Zhu, 2016; García-García *et al*., 2020). In cucumber, exogenous Put has been shown to mitigate salinity-induced damage by supporting growth, preserving photosynthetic function and modulating antioxidant responses (Shu *et al*., 2012; Yuan *et al*., 2014; Yuan *et al*., 2016, Yuan *et al*., 2019). However, most previous studies focused on early developmental stages or on selected physiological traits and were conducted using relatively high Put concentrations and moderate salinity levels. Only a limited number of studies have investigated the effects of low Put concentrations (Yuan *et al*., 2014), and none have examined the integrated physiological responses of adult plants under long-term stress conditions.

From an applied perspective, the use of progressively lower concentrations of BS compounds represents a key objective in sustainable crop management, as it aims to minimize inputs while maintaining efficacy. Nevertheless, the effectiveness of low Put doses under severe salinity conditions remains poorly explored. In the present study, the foliar application of Put, substantially reduced concentration was evaluated under stringent salinity regimes, ranging from 100 mM NaCl

for physiological, transcriptional and yield-related analysis, to 200 mM NaCl for morphological and biochemical traits. This integrated approach enabled the assessment of Put-mediated stress mitigation across multiple organizational levels and throughout the plant development, linking early Put-priming effects to adult plant performance and productivity in cucumber.

Morphological analysis showed that salt stress markedly reduced root length, plant height and biomass accumulation, while increasing leaf damage (**Figure 8**). These effects are consistent with the well-documented inhibitory impact of salinity on cell expansion and growth in glycophytic species, resulting from osmotic constraints and ionic toxicity (Munns & Tester, 2008; Negrão *et al*., 2017). Foliar application of Put partially counteracted these alterations under saline conditions, leading to improved plant fitness and reduced tissue damage. Such growth recovery is consistent with previous studies showing that PAs mitigate salinity-induced cellular damage by preserving membrane integrity and overall cellular structure. Although higher PAs are more effective membrane stabilizers, Put may contribute indirectly to these protective effects, supporting growth recovery under salt stress (Gill & Tuteja, 2010; Shu *et al*., 2012).

Physiological parameters indicated that salt stress significantly impaired RWC, photosynthetic performance, total Chl levels, and stomatal conductance (**Figure 9**). It is well known that salinity negatively affects plant photosynthesis by inducing stomatal closure, resulting in reduced CO_2 availability, as well as chlorophyll (Chl) degradation (Chaves *et al*., 2009). The protective role of Put on physiological parameters is in agreement with previous studies showing that PAs stabilize cellular membranes, protect photosynthetic pigments, maintain stomatal regulation and modulate redox homeostasis under stress conditions (Alcázar *et al*., 2010; Gill & Tuteja, 2010; Shu *et al*., 2012). These protective effects are mediated by electrostatic interactions between PAs and negatively charged membrane phospholipids, which help preserve membrane integrity, together with modulation of redox homeostasis through the regulation of antioxidant enzymes (Alcázar *et al*., 2010; Gill & Tuteja, 2010; Shu *et al*., 2012; Seifi & Shelp, 2019).

Biochemical analysis demonstrated that salt stress induced oxidative damage, as evidenced by increased lipid peroxidation (MDA) and reduced total antioxidant capacity, accompanied by declines in chlorophyll and carotenoid contents (**Figure 10**). These effects are consistent with the well-established generation of ROS under salinity, leading to membrane damage and pigment degradation (Alcázar *et al*., 2010; Gill & Tuteja, 2010; Shu *et al*., 2012). The protective effect of Put is consistent with previous findings in cucumber showing that exogenous Put application reduces lipid peroxidation and preserves photosynthetic pigment integrity under salt stress, thereby contributing to improved antioxidant balance and cellular stability (Yuan *et al*., 2014). Our data demonstrates that the protective effect of Put remains effective also using very low concentrations.

At the molecular level, foliar application of Put appears to prime cucumber plants for improved stress tolerance by selectively modulating stress-responsive gene expression. Put without stress enhanced the expression of osmo-protectors *P5CS* and *ZDH-10*, suggesting that it prepares the plant to better cope with osmotic challenges, while slightly downregulating oxidative stress scavengers *SOS-3* and *SOD-2* to avoid unnecessary ROS signalling in the absence of salt stress (**Figure 11**). These transcriptional adjustments are in line with previous studies demonstrating that exogenous application of PAs can pre-activate specific tolerance pathways and optimize the balance between ROS signalling and osmotic adjustment (Wojtyla *et al., 2016;* Yuan *et al*., 2016; Seifi *et al*., 2019). PAs have been reported to induce *P5CS* expression and proline accumulation under salinity, which contributes to osmotic balance and cellular protection *(Chen et al*., 2019). Moreover, PA-mediated modulation of transcription factors such as NAC proteins is consistent with their function in regulating downstream stress-responsive networks, linking gene expression changes to physiological and biochemical resilience (Nuruzzaman *et al*., 2013; Seifi & Shelp, 2019; Pál *et al*., 2021).

The positive effect of Put priming on fruit development under salinity reflects the cumulative effect of improved plant resilience (**Figure 12**). By enhancing photosynthetic efficiency and maintaining water balance, Put-treated plants are better able to allocate assimilates to reproductive organs, supporting fruit growth even under stress (Munns & Tester, 2008; Zhu , 2016). In addition, Put reduces oxidative damage and preserves Chl and Car content (Yuan *et al*., 2014), which likely protects developing tissues and contributes to improved reproduction. The modulation of stress-responsive gene expression, including osmoprotective pathways and transcription factors, further enhances the plant ability to adjust its metabolic, physiologic, and biochemical plasticity to salinity, creating a molecular environment that favors fruit development (Alcázar *et al*., 2010).

These findings demonstrate that Put not only alleviates vegetative stress responses but also translates into tangible agronomic benefits, enhancing fruit growth and yield potential under saline conditions. Overall, this highlights the role of PA foliar priming as an integrative strategy that links molecular and physiological stress mitigation to improved fruit development and quality in cucumber.

5.3 Put foliar spray treatment enhance cucumber plants' resistance to Bemisia infestation

Biotic stresses, including pest infestations, strongly limit cucumber productivity. Specifically, it has been reported that Bemisia feeding strongly affected photosynthates availability, altering Chl and Car content, impairing photosynthetic efficiency, and increasing ROS production (Oliveira *et al*., 2001; He *et al*., 2022; Morin *et al*., 2024). In this work, among the tested PAs, foliar application of Put resulted the most effective. Indeed, pre-infestation treatment with 5 mM Put

significantly reduced Bemisia populations, delayed insect development, and extended the duration of early instars (**Figure 13**). These findings are consistent with previous reports showing that exogenous PA application modulates redox status and antioxidant activity during pest attack. Specifically, it has been demonstrated that treatment with 2 mM Spm counteracts *T. urticae* attack in cucumber, ultimately reducing pest populations on treated plants (Shahtousi & Talaee, 2023). By limiting oxidative damage and stabilizing cellular metabolism, PAs may indirectly reduce host suitability for herbivores, thereby slowing insect development and limiting population growth, as previously reported for redox-mediated plant–insect interactions (Maffei *et al*., 2007; Kerchev *et al*., 2012).

Physiological analysis further demonstrated that Put preserves photosynthetic functionality under infestation. Although SPAD values were not markedly affected, infestation caused a transient reduction in PRI, an indicator of photosystem activity and light-use efficiency, which was fully restored by Put treatment (**Figure 14**). This response is consistent with a PA-mediated stabilization of the photosynthetic apparatus and mitigation of oxidative stress. PAs are known to protect thylakoid membrane integrity, modulate energy dissipation processes, and regulate ROS signalling, thereby sustaining electron transport efficiency under stress conditions (Navakoudis & Kotzabasis, 2022).

Analysis of VOC profiling showed that Put priming altered volatile emission patterns, resulting in blends comparable to those emitted by infested, untreated plants (**Figure 15**). This indicates that Put priming may activate defence-related pathways typically associated with biotic stress responses. Coherently, PAs are known to interact with JA-dependent signalling and ROS-related pathways, both central to the induction of herbivore-induced plant volatiles (HIPVs; Ozawa *et al*., 2009; Seifi *et al*., 2019). Consequently, Put priming may induce early VOC biosynthesis, resulting in emission profiles typically associated with defence signalling. Such VOCs can act as repellents or deterrents to herbivores, as well as attractants for natural enemies, thereby contributing to indirect plant defence. Moreover, data herein reported show that untreated plants exposed to VOCs emitted by Put-treated donors also exhibited a significant reduction in Bemisia population (**Figure 17**). This indicates that VOCs themselves can function as priming signals, activating defence responses in neighbouring, untreated plants. Similar VOC-mediated defence priming has been observed in crops such as maize and lima bean, where exposure to herbivore-induced volatiles enhanced JA-dependent gene expression, volatile emission, and both direct and indirect resistance (Yi *et al*., 2024). VOC perception likely activates JA and ROS signalling cascades, establishing a primed state that enables faster and stronger defence responses upon subsequent herbivore attack while avoiding the metabolic costs typically associated with constitutive activation of defence responses.

Finally, post-infestation application of Put also effectively reduced Bemisia populations and disrupted insect development (**Figure 16**). This experiment aimed to assess the field-relevant potential of Put, as foliar treatments are commonly applied after pest establishment. The observed efficacy indicates that Put can act not only as a priming agent but also as a post-infestation protective treatment, likely by reinforcing host physiological status, stabilizing photosynthetic parameters, and modulating ROS signalling and antioxidant defences even after herbivore attack. Overall, foliar application of Put emerged as the most effective PA treatment against Bemisia, combining direct suppression of pest fitness, with the preservation of plant physiological function, and the activation of VOC-mediated indirect defence mechanisms.

5.4 Spd foliar spray treatment modulates cucumber plants resistance to *G. cichoracearum*

The effects of foliar application of PAs on cucumber defence responses against the powdery mildew pathogen *G. cichoracearum* were investigated to extend the investigation of PA-mediated effects under different biotic stress, considering their well-known involvement in plant defence mechanisms. (Alcázar *et al*., 2010; Jiménez Bremont *et al*., 2014).

Among the tested PAs, Spd exerted the strongest influence on powdery mildew development. Foliar Spd application resulted in a sustained reduction of powdery mildew-damaged leaf area (**Figure 19A**) and significantly reduced fungal colony expansion (**Figure 19B** and **Figure 19C**). These results indicate that Spd priming enhances host responses that limit both pathogen establishment and subsequent fungal growth. Spd plays a central role in PA homeostasis and has frequently been associated with enhanced resistance to biotic stress, likely acting through similar mechanisms as those described for Put, among which is the fine-regulation of ROS dynamics and the activation of defence-related signalling pathways (Tavladoraki *et al.,* 2016; Seifi *et al*., 2019). Such mechanisms are particularly relevant in interactions with obligate biotrophic fungi, where rapid and localized host responses are critical for restricting fungal development. The relevance of PA metabolism in plant-biotrophic fungal interaction is further supported by previous studies showing that chemical inhibition of fungal PA biosynthesis strongly impairs disease development in wheat (Weinstein *et al*., 1987). Inhibition of ODC, the enzyme that catalyzes the first and unique step in Put biosynthesis in fungi, markedly reduced powdery mildew and rust infections in wheat (Weinstein *et al*., 1987). These findings highlight the strong dependence of obligate biotrophic fungi on an adequate supply of Put for growth and colonization.

Although pathogen PA levels were not directly measured in the present study, data clearly suggest that foliar application of Spd may contribute to limiting plant Put availability or to altering PA homeostasis at the host–pathogen interface, thereby limiting fungal development. In contrast, data

herein reported show that exogenous Put application enhanced powdery mildew susceptibility. Although Put plays a role in plant stress responses, its accumulation does not always correlate with enhanced resistance and appears to be highly modulated by the surrounding physiological and environmental conditions (Jiménez Bremont *et al*., 2014). In interactions with biotrophic fungi, elevated Put levels may be insufficient to sustain the rapid and spatially localized defence responses required to restrict fungal penetration and colony expansion. It can therefore be hypothesized that, in compatible interactions, Put accumulation may even contribute to pathogen development, as suggested by previous reports indicating that Put levels do not consistently correlate with resistance in plant–pathogen systems (Jiménez Bremont *et al*., 2014).

Overall, the differential effects of PAs highlight the critical role of PA specificity and homeostasis in plant immune responses. Rather than acting as general stress mitigators, distinct PAs selectively modulate host responses depending on the biotic challenge. In the cucumber-powdery mildew interaction, Spd emerges as the PA most closely associated with the restriction of fungal growth and disease progression. Together with the distinct role of Put observed in this study under abiotic stress and insect herbivory, these findings support the strategy of targeted application of specific PAs as a promising and sustainable approach to enhance crop performance under multiple stress conditions.

REFERENCE

Abbott, W. S. (1925). A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology*, *18*(2), 265–267.

Abd Elbar, O., Farag, R., & Shehata, S. **(2019)**. Effect of putrescine application on some growth, biochemical and anatomical characteristics of *Thymus vulgaris* L. under drought stress. *Annals of Agricultural Sciences*, *64*.

Alcázar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P., & Tiburcio, A. **(2010)**. Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. *Planta*, *231*, 1237–1249.

Alexandratos, N., & **Bruinsma, J. (2012)**. *World agriculture towards 2030/2050: The 2012 revision*.

Al-Momany, B., & Abu-Romman, S. (2023). Cucumber and salinity. *Australian Journal of Crop Science*, *17*(7), 581–590.

Angelini, R., Cona, A., Federico, R., Fincato, P., Tavladoraki, P., & Tisi, A. **(2010)**. Plant amine oxidases «on the move»: An update. *Plant Physiology and Biochemistry: PPB*, *48*(7), 560–564.

Arimura, G., Kost, C., & Boland, W. **(2005)**. Herbivore-induced, indirect plant defences. *Biochimica Et Biophysica Acta*, *1734*(2), 91–111.

Arnao, M. B., & Hernández-Ruiz, J. **(2019)**. Melatonin: A New Plant Hormone and/or a Plant Master Regulator? *Trends in Plant Science*, *24*(1), 38–48.

Arslan, E., Agar, G., & Aydın, M. **(2019)**. Putrescine as a protective molecule on DNA damage and DNA methylation changes in wheat under drought. *Communications Faculty of Sciences University of Ankara Series C Biology*, *28*(2), 170–187.

Bagni, N., & **Tassoni, A. (2001)**. Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants. *Amino Acids*, *20*(3), 301–317.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. **(2002)**. Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), 1–16.

Basu, R., Maitra, N., & Ghosh, B. **(1988)**. Salinity Results in Polyamine Accumulation in Early Rice (*Oryza sativa* L.). Seedlings. *Australian Journal of Plant Physiology*, *15*(6), 777–786.

Bhat, R. A., Miklis, M., Schmelzer, E., Schulze-Lefert, P., & Panstruga, R. **(2005)**. Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane microdomain. *Proceedings of the National Academy of Sciences*, *102*(8), 3135–3140.

Blázquez, M. A. (2024). Polyamines: Their Role in Plant Development and Stress. *Annual Review of Plant Biology*, *75*(1), 95–117.

Block, C. C., & **Reitsma, K. R. (2005)**. Powdery mildew resistance in the US national plant germplasm system cucumber collection. *HortScience*, *40*(2), 416–420.

Borrell, A., Culianez-Macia, F. A., Altabella, T., Besford, R. T., Flores, D., & Tiburcio, A. F. **(1995)**. Arginine Decarboxylase Is Localized in Chloroplasts. *Plant Physiology*, *109*(3), 771–776.

Bortolotti, C., Cordeiro, A., Alcázar, R., Borrell, A., Culiañez-Macià, F. A., Tiburcio, A. F., & Altabella, T. **(2004)**. Localization of arginine decarboxylase in tobacco plants. *Physiologia Plantarum*, *120*(1), 84–92.

Bouchereau, A., Aziz, A., Larher, F., & Martin-Tanguy, J. **(1999)**. Polyamines and environmental challenges: Recent development. *Plant Science*, *140*(2), 103–125.

Bradshaw, M. J., Boufford, D., Braun, U., Moparthi, S., Jellings, K., Maust, A., Pandey, B., Slack, S., & Pfister, D. H. **(2024)**. An In-Depth Evaluation of Powdery Mildew Hosts Reveals One of the World's Most Common and Widespread Groups of Fungal Plant Pathogens. *Plant Disease*, *108*(3), 576–581.

Breiman, L. (2001). Random Forests. *Machine Learning*, *45*(1), 5–32.

Bulgari, R., Franzoni, G., & Ferrante, A. **(2019)**. Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions. *Agronomy*, *9*(6), 306.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. **(2009)**. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*(4), 611–622.

Chaves, M. M., Flexas, J., & Pinheiro, C. **(2009)**. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany*, *103*(4), 551–560.

Chele, K. H., Tinte, M. M., Piater, L. A., Dubery, I. A., & Tugizimana, F. **(2021)**. Soil Salinity, a Serious Environmental Issue and Plant Responses: A Metabolomics Perspective. *Metabolites*, *11*(11), 724.

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. **(2019)**. Polyamine Function in Plants: Metabolism, Regulation on Development, and Roles in Abiotic Stress Responses. *Frontiers in Plant Science*, *9*.

Chen, K., & Arora, R. (2013). Priming memory invokes seed stress-tolerance. *Environmental and Experimental Botany*, *94*, 33–45.

Choudhary, V., Kumar, K. K., Sahu, B., Muthappa, S.-K., & Das, A. D. A. **(2021)**. Agronomic innovations in biotic stress management and its combined effect with abiotic stresses in crop production. *Indian J Agron*, *66*(5), 237–257.

Cona, A., Rea, G., Angelini, R., Federico, R., & Tavladoraki, P. **(2006)**. Functions of amine oxidases in plant development and defence. *Trends in Plant Science*, *11*(2), 80–88.

Corneo, P. E., Jermini, M., Nadalini, S., Giovannini, O., Nesler, A., Perazzolli, M., & Pertot, I. **(2021)**. Foliar and root applications of the rare sugar tagatose control powdery mildew in soilless grown cucumbers. *Crop Protection*, *149*, 105753.

Cramer, G. R., Urano, K., Delrot, S., Pezzotti, M., & Shinozaki, K. **(2011)**. Effects of abiotic stress on plants: A systems biology perspective. *BMC Plant Biology*, *11*(1), 163.

Danalo, A., Shekari, F., Sabaghnia, N., & Nasiri, Y. **(2018)**. EFFECT OF POLYAMINES APPLICATION ON GERMINATION AND PHYSIOLOGICAL CHARACTERISTICS OF BORAGE (*Borago officinalis* L.). *The Journal Agriculture and Forestry*, *64*(3), 127-140

Dong, S., Qiao, K., Zhu, Y., Wang, H., Xia, X., & Wang, K. **(2014)**. Managing *Meloidogyne incognita* and *Bemisia tabaci* with thiacloprid in cucumber crops in China. *Crop Protection*, *58*, 1–5.

du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3–14.

Dukes, J. S., Pontius, J., Orwig, D., Garnas, J. R., Rodgers, V. L., Brazee, N., Cooke, B., Theoharides, K. A., Stange, E. E., Harrington, R., Ehrenfeld, J., Gurevitch, J., Lerdau, M., Stinson, K., Wick, R., & Ayres, M. **(2009)**. Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America: What can we predict? This article is one of a selection of papers from NE Forests 2100: A Synthesis of Climate Change Impacts on Forests of the Northeastern US and Eastern Canada. *Canadian Journal of Forest Research*, *39*(2), 231–248.

El-Maarouf-Bouteau, H., & Bailly, C. (2008). Oxidative signaling in seed germination and dormancy. *Plant Signaling & Behavior*, *3*(3), 175–182.

Erdogan, C., Toprak, U., & Gurkan, M. O. **(2024)**. Biochemical and molecular analysis of insecticide resistance in greenhouse populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Türkiye. *Phytoparasitica*, *52*(2), 41.

Esfandiari Ghalati, R., Shamili, M., & Homaei, A. **(2020)**. Effect of putrescine on biochemical and physiological characteristics of guava (*Psidium guajava* L.) seedlings under salt stress. *Scientia Horticulturae*, *261*, 108961.

Farooq, M., Basra, S., Rehman, H., & Hussain, M. **(2008)**. Seed Priming with Polyamines Improves the Germination and Early Seedling Growth in Fine Rice. *Journal of New Seeds*, *9*, 145–155.

Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., & Ball, L. A. **(2005)**. *Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*. Academic Press.

Fernández, E., Grávalos, C., Haro, P. J., Cifuentes, D., & Bielza, P. **(2009)**. Insecticide resistance status of *Bemisia tabaci* Q-biotype in south-eastern Spain. *Pest Management Science*, *65*(8), 885–891.

Fraudentali, I., Rodrigues-Pousada, R. A., Tavladoraki, P., Angelini, R., & Cona, A. **(2020)**. Leaf-Wounding Long-Distance Signaling Targets AtCuAOβ Leading to Root Phenotypic Plasticity. *Plants*, *9*(2), 249.

Fraudentali, I., Rodrigues-Pousada, R. A., Angelini, R., Ghuge, S. A., & Cona, A. **(2021)**. Plant Copper Amine Oxidases: Key Players in Hormone Signaling Leading to Stress-Induced Phenotypic Plasticity. *International Journal of Molecular Sciences*, *22*(10), 5136.

Gan, C. M., Tang, T., Zhang, Z. Y., Li, M., Zhao, X. Q., Li, S. Y., Yan, Y. W., Chen, M. X., & Zhou, X. **(2025)**. Unraveling the Intricacies of Powdery Mildew: Insights into Colonization, Plant Defense Mechanisms, and Future Strategies. *International Journal of Molecular Sciences*, *26*(8), 3513.

García-García, A. L., García-Machado, F. J., Borges, A. A., Morales-Sierra, S., Boto, A., & Jiménez-Arias, D. **(2020)**. Pure Organic Active Compounds Against Abiotic Stress: A Biostimulant Overview. *Frontiers in Plant Science*, *11*.

Gill, S. S., & **Tuteja, N. (2010)**. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909–930.

Gilroy, S., Suzuki, N., Miller, G., Choi, W.-G., Toyota, M., Devireddy, A. R., & Mittler, R. **(2014)**. A tidal wave of signals: Calcium and ROS at the forefront of rapid systemic signaling. *Trends in Plant Science*, *19*(10), 623–630.

Gilroy, S., Białasek, M., Suzuki, N., Górecka, M., Devireddy, A. R., Karpiński, S., & Mittler, R. **(2016)**. ROS, Calcium, and Electric Signals: Key Mediators of Rapid Systemic Signaling in Plants. *Plant Physiology*, *171*(3), 1606–1615.

Glawe, D. A. (2008). The Powdery Mildews: A Review of the World's Most Familiar (Yet Poorly Known) Plant Pathogens. *Annual Review of Phytopathology*, *46*, 27–51.

Godoy, F., Olivos-Hernández, K., Stange, C., Handford, M., Godoy, F., Olivos-Hernández, K., Stange, C., & Handford, M. **(2021)**. Abiotic Stress in Crop Species: Improving Tolerance by Applying Plant Metabolites. *Plants*, *10*(2).

Gordon, T. R., & Duniway, J. M. (1982). Effects of Powdery Mildew Infection on the Efficiency of CO2 Fixation and Light Utilization by Sugar Beet Leaves. *Plant Physiology*, *69*(1), 139–142.

Groß, F., Rudolf, E.-E., Thiele, B., Durner, J., & Astier, J. **(2017)**. Copper amine oxidase 8 regulates arginine-dependent nitric oxide production in *Arabidopsis thaliana*. *Journal of Experimental Botany*, *68*(9), 2149–2162.

Hasegawa, P. M., Bressan, R. A., Zhu, J.-K., & Bohnert, H. J. **(2000)**. PLANT CELLULAR AND MOLECULAR RESPONSES TO HIGH SALINITY. *Annual Review of Plant Biology*, *51*(Volume 51, 2000), 463–499.

Hassani, A., Azapagic, A., & Shokri, N. **(2021)**. Global predictions of primary soil salinization under changing climate in the 21st century. *Nature Communications*, *12*(1), 6663.

He, Y., Wei, M., Yan, Y., Yu, C., Cheng, S., Sun, Y., Zhu, X., Wei, L., Wang, H., & Miao, L. **(2022)**. Research Advances in Genetic Mechanisms of Major Cucumber Diseases Resistance. *Frontiers in Plant Science*, *13*.

Hongna, C., Junmei, S., Leyuan, T., Xiaori, H., Guolin, L., & Xianguo, C. **(2021)**. Exogenous Spermidine Priming Mitigates the Osmotic Damage in Germinating Seeds of *Leymus chinensis* Under Salt-Alkali Stress. *Frontiers in Plant Science*, *12*.

Hoegh-Guldberg, O., Jacob, D., Bindi, M., Brown, S., Camilloni, I., Diedhiou, A., Djalante, R., Ebi, K., Engelbrecht, F., Guiot, J., Hijioka, Y., Mehrotra, S., Payne, A., Seneviratne, S. I., Thomas, A., Warren, R., Zhou, G., Halim, S. A., Achlatis, M., Zougmoré, R. B. **(2018)**. Impacts of 1.5°C Global Warming on Natural and Human Systems. In V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P. R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, & T. Waterfield (A c. Di), *Global warming of 1.5°C*, 175–311. IPCC Secretariat.

Hückelhoven, R., & Panstruga, R. **(2011)**. Cell biology of the plant–powdery mildew interaction. *Current Opinion in Plant Biology*, *14*(6), 738–746.

Ivushkin, K., Bartholomeus, H., Bregt, A. K., Pulatov, A., Kempen, B., & De Sousa, L. **(2019)**. Global mapping of soil salinity change. *Remote Sensing of Environment*, *231*, 111260.

Janse van Rensburg, H. C., Limami, A. M., & Van den Ende, W. **(2021)**. Spermine and Spermidine Priming against *Botrytis cinerea* Modulates ROS Dynamics and Metabolism in Arabidopsis. *Biomolecules*, *11*(2), 223.

Jiménez Bremont, J. F., Marina, M., Guerrero-González, M. de la L., Rossi, F. R., Sánchez-Rangel, D., Rodríguez-Kessler, M., Ruiz, O. A., & Gárriz, A. **(2014)**. Physiological and molecular implications of plant polyamine metabolism during biotic interactions. *Frontiers in Plant Science*, *5*.

Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and Proceedings of the Royal Society of New South Wales*, *138*(3–4), 65–75.

Kerchev, P. I., Fenton, B., Foyer, C. H., & Hancock, R. D. **(2012)**. Plant responses to insect herbivory: Interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. *Plant, Cell & Environment*, *35*(2), 441–453.

Khan, H., Ziaf, K., Amjad, M., & Iqbal, Q. **(2012)**. Exogenous Application of Polyamines Improves Germination and Early Seedling Growth of Hot Pepper. *Chilean journal of agricultural research*, *72*, 429–433.

Killiny, N., & Nehela, Y. (2020). Citrus Polyamines: Structure, Biosynthesis, and Physiological Functions. *Plants*, *9*(4), 426.

Kumar, A., Taylor, M., Altabella, T., & Tiburcio, A. F. **(1997)**. Recent advances in polyamine research. *Trends in Plant Science*, *2*(4), 124–130.

Kuznetsov, V. V., Radyukina, N. L., & Shevyakova, N. I. **(2006)**. Polyamines and stress: Biological role, metabolism, and regulation. *Russian Journal of Plant Physiology*, *53*(5), 583–604.

Lamaoui, M., Jemo, M., Datla, R., & Bekkaoui, F. **(2018)**. Heat and Drought Stresses in Crops and Approaches for Their Mitigation. *Frontiers in Chemistry*, *6*.

Lebeda, A., MgGrath, M., & Sedláková, B. (2010). *Fungicide Resistance in Cucurbit Powdery Mildew Fungi*.

Lechowska, K., Wojtyla, Ł., Quinet, M., Kubala, S., Lutts, S., Garnczarska, M., Lechowska, K., Wojtyla, Ł., Quinet, M., Kubala, S., Lutts, S., & Garnczarska, M. **(2021)**. Endogenous Polyamines and Ethylene Biosynthesis in Relation to Germination of Osmoprimed *Brassica napus* Seeds under Salt Stress. *International Journal of Molecular Sciences*, *23*(1).

Lenis, Y. Y., Elmetwally, M. A., Maldonado-Estrada, J. G., & Bazer, F. W. **(2017)**. Physiological importance of polyamines. *Zygote*, *25*(3), 244–255.

Li, S. J., Xue, X., Ahmed, M. Z., Ren, S. X., Du, Y. Z., Wu, J. H., Cuthbertson, A. G. S., & Qiu, B. L. **(2011)**. Host plants and natural enemies of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China. *Insect Science*, *18*(1), 101–120.

Li, Y., Ma, Y., Zhang, T., Bi, Y., Wang, Y., & Prusky, D. **(2019)**. Exogenous polyamines enhance resistance to *Alternaria alternata* by modulating redox homeostasis in apricot fruit. *Food Chemistry*, *301*, 125303.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in enzymology 148*, 350–382.

Lin, D., Xu, Y., Wu, H., Liu, X., Zhang, L., Wang, J., & Rao, Q. **(2019)**. Plant Defense Responses Induced by Two Herbivores and Consequences for Whitefly *Bemisia tabaci*. *Frontiers in Physiology*, *10*.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, *25*(4), 402–408.

Luan, J. B., Wang, X.W., Colvin, J., & Liu, S. S. **(2014)**. Plant-mediated whitefly–begomovirus interactions: Research progress and future prospects. *Bulletin of Entomological Research*, *104*(3), 267–276.

Ma, Q., Bao, A.-K., Chai, W.-W., Wang, W.-Y., Zhang, J.-L., Li, Y.-X., & Wang, S.-M. **(2016)**. Transcriptomic analysis of the succulent xerophyte *Zygophyllum xanthoxylum* in response to salt treatment and osmotic stress. *Plant and Soil*, *402*(1), 343–361.

Maffei, M. E., Mithöfer, A., & Boland, W. **(2007)**. Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry*, *68*(22), 2946–2959.

Mba, C., Guimaraes, E. P., & Ghosh, K. **(2012)**. Re-orienting crop improvement for the changing climatic conditions of the 21st century. *Agriculture & Food Security*, *1*(1), 7.

McGrath, M. T. (2001). Fungicide Resistance in Cucurbit Powdery Mildew: Experiences and Challenges. *Plant Disease*, *85*(3), 236–245.

Meier, U. (2018). Growth stages of mono- and dicotyledonous plants: BBCH Monograph

Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M. A., Shulaev, V., Dangl, J. L., & Mittler, R. **(2009)**. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling*, *2*(84), 45.

Minocha, R., Majumdar, R., & Minocha, S. C. **(2014)**. Polyamines and abiotic stress in plants: A complex relationship1. *Frontiers in Plant Science*, *5*.

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G. A. D., Tognetti, V. B., Vandepoele, K., Gollery, M., Shulaev, V., & Van Breusegem, F. **(2011)**. ROS signaling: The new wave? *Trends in plant science*, *16*(6), 300–309.

Morin, S., Atkinson, P. W., & Walling, L. L. **(2024)**. Whitefly-Plant Interactions: An Integrated Molecular Perspective. *Annual Review of Entomology*, *69*, 503–525.

Mound, L. A., Mound, L. A., Halsey, S. H., & B. M. (Natural History). **(1978)**. *Whitefly of the world: A systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data*. Wiley.

Munns, R., & T**ester, M. (2008)**. Mechanisms of Salinity Tolerance. *Annual Review of Plant Biology*, *59*(1), 651–681.

Mylett, C. (2025). Physiological, Volatilome and Molecular Responses of *Diplotaxis tenuifolia* to Waterlogging Stress and Subsequent Postharvest Storage. *Master's degree thesis*.

Nachtergaele, F., Dudal, R., & Fresco, L. **(2006)**. *The Food and Agriculture Organization of the United Nations (FAO) and Soil Issues*.

Naliwajski, M., Skłodowska, M., Naliwajski, M., & Skłodowska, M. **(2021)**. The Relationship between the Antioxidant System and Proline Metabolism in the Leaves of Cucumber Plants Acclimated to Salt Stress. *Cells*, *10*(3).

Navakoudis, E., & **Kotzabasis, K. (2022)**. Polyamines: A bioenergetic smart switch for plant protection and development. *Journal of Plant Physiology*, *270*, 153618.

Negacz, K., Malek, Ž., De Vos, A., & Vellinga, P. **(2022)**. Saline soils worldwide: Identifying the most promising areas for saline agriculture. *Journal of Arid Environments*, *203*, 104775.

Negrão, S., Schmöckel, S. M., & Tester, M. **(2017)**. Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, *119*(1), 1–11.

Noir, S., Colby, T., Harzen, A., Schmidt, J., & Panstruga, R. **(2009)**. A proteomic analysis of powdery mildew (*Blumeria graminis* f.sp. *Hordei*) conidiospores. *Molecular Plant Pathology*, *10*(2), 223–236.

Novaes, N. S., Lourenção, A. L., Bentivenha, J. P. F., Baldin, E. L. L., & Melo, A. M. T. **(2020)**. Characterization and potential mechanisms of resistance of cucumber genotypes to *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Phytoparasitica*, *48*(4), 643–657.

Nuruzzaman, M., Sharoni, A. M., & Kikuchi, S. **(2013)**. Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Frontiers in Microbiology*, *4*.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevenes, M., & Wagner, H. **(2012)**. *Vegan: Community Ecology Package. R package version 2.0-2*.

Oliveira, M. R. V., Henneberry, T. J., & Anderson, P. **(2001)**. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection*, *20*(9), 709–723.

Ondrasek, G., Rathod, S., Manohara, K. K., Gireesh, C., Anantha, M. S., Sakhare, A. S., Parmar, B., Yadav, B. K., Bandumula, N., Raihan, F., Zielińska-Chmielewska, A., Meriño-Gergichevich, C., Reyes-Díaz, M., Khan, A., Panfilova, O., Seguel Fuentealba, A., Romero, S. M., Nabil, B., Wan, C. (Craig), … Horvatinec, J. **(2022)**. Salt Stress in Plants and Mitigation Approaches. *Plants*, *11*(6), 717.

Ozawa, R., Bertea, C. M., Foti, M., Narayana, R., Arimura, G.-I., Muroi, A., Horiuchi, J. I., Nishioka, T., Maffei, M. E., & Takabayashi, J. **(2009)**. Exogenous Polyamines Elicit Herbivore-Induced Volatiles in Lima Bean Leaves: Involvement of Calcium, H2O2 and Jasmonic Acid. *Plant and Cell Physiology*, *50*(12), 2183–2199.

Pál, M., Szalai, G., Gondor, O. K., & Janda, T. **(2021)**. Unfinished story of polyamines: Role of conjugation, transport and light-related regulation in the polyamine metabolism in plants. *Plant Science*, *308*, 110923.

Pardey, P. G., Beddow, J. M., Hurley, T. M., Beatty, T. K. M., & Eidman, V. R. **(2014)**. A Bounds Analysis of World Food Futures: Global Agriculture Through to 2050. *Australian Journal of Agricultural and Resource Economics*, *58*(4), 571–589.

Paschalidis, K. A., & Roubelakis-Angelakis, K. A. (2005). Spatial and temporal distribution of polyamine levels and polyamine anabolism in different organs/tissues of the tobacco plant. Correlations with age, cell division/expansion, and differentiation. *Plant Physiology*, *138*(1), 142–152.

Pérez-Hedo, M., Alonso-Valiente, M., Vacas, S., Gallego, C., Pons, C., Arbona, V., Rambla, J. L., Navarro-Llopis, V., Granell, A., & Urbaneja, A. **(2021)**. Plant exposure to herbivore-induced plant volatiles: A sustainable approach through eliciting plant defenses. *Journal of Pest Science*, *94*(4), 1221–1235.

Polston, J. E., De Barro, P., & Boykin, L. M. **(2014)**. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science*, *70*(10), 1547–1552.

Prakash, A., Rao, J., Mukherjee, A. K., Jeyaveeran, B., Pokhare, S., Adak, T., Munda, S., & R., S. **(2014)**. Climate Change: Impact on Crop Pests.Rejeb, I. B., Pastor, V., Mauch-Mani, B., Rejeb, I. B., Pastor, V., & Mauch-Mani, B. Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms. *Plants*, *3*(4), 458–475.

Rejeb, I. B., Pastor, V., Mauch-Mani, B., Rejeb, I. B., Pastor, V., & Mauch-Mani, B. **(2014)**. Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms. *Plants*, *3*(4), 458–475.

Rockström, J., Williams, J., Daily, G., Noble, A., Matthews, N., Gordon, L., Wetterstrand, H., DeClerck, F., Shah, M., Steduto, P., De Fraiture, C., Hatibu, N., Unver, O., Bird, J., Sibanda, L., & Smith, J. **(2017)**. Sustainable intensification of agriculture for human prosperity and global sustainability. *Ambio*, *46*(1), 4–17.

Rouphael, Y., & Colla, G. (2020). Biostimulants in agriculture. *Frontiers in plant science*. *11*, 40.

Taha, S. R., Seleiman, M. F., Alotaibi, M., Alhammad, B. A., Rady, M. M., & H. A. Mahdi, A. **(2020)**. Exogenous Potassium Treatments Elevate Salt Tolerance and Performances of *Glycine max* L. by Boosting Antioxidant Defense System under Actual Saline Field Conditions. *Agronomy*, *10*(11), 1741.

Secker, A. E., Bedford, I. D., Markham, P. G., & Williams, M. de. **(1998).** *Squash, a reliable field indicator for the presence of the B biotype of tobacco whitefly, Bemisia tabaci.*

Seifi, H. S., & **Shelp, B. J. (2019)**. Spermine Differentially Refines Plant Defense Responses Against Biotic and Abiotic Stresses. *Frontiers in Plant Science*, *10*.

Seifi, H. S., Zarei, A., Hsiang, T., & Shelp, B. J. **(2019)**. Spermine Is a Potent Plant Defense Activator Against Gray Mold Disease on *Solanum lycopersicum*, *Phaseolus vulgaris*, and *Arabidopsis thaliana*. *Phytopathology®*, *109*(8), 1367–1377.

Shahid, S. A., Zaman, M., & Heng, L. **(2018)**. Soil Salinity: Historical Perspectives and a World Overview of the Problem. In M. Zaman, S. A. Shahid, & L. Heng, *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques* 43–53. Springer International Publishing.

Shahtousi, S., & Talaee, L. (2023). The effect of spermine on *Tetranychus urticae-Cucumis sativus* interaction. *BMC Plant Biology*, *23*(1), 575.

Shrestha, S. (2019). Effects of Climate Change in Agricultural Insect Pest. *Acta Scientific Agriculture*, *3*, 74–80.

Shu, S., Guo, S., Sun, J., & Yuan, L. **(2012)**. Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. *Physiologia Plantarum*, *146*(3), 285–296.

Sitterly, W. R. (1972). Breeding for Disease Resistance in Cucurbits. *Annual Review of Phytopathology*, *10*(1), 471–490.

Slocum, R. D., & Flores, H. E. **(2024)**. *Biochemistry and Physiology of Polyamines in Plants*. CRC Press.

Sotiropoulos, A. G., Arango-Isaza, E., Ban, T., Barbieri, C., Bourras, S., Cowger, C., Czembor, P. C., Ben-David, R., Dinoor, A., Ellwood, S. R., Graf, J., Hatta, K., Helguera, M., Sánchez-Martín, J., McDonald, B. A., Morgounov, A. I., Müller, M. C., Shamanin, V., Shimizu, K. K., … Wicker, T. **(2022)**. Global genomic analysis of wheat powdery mildew reveal association of pathogen spread with historical human migration and trade. *Nature Communications*, *13*(1), 4315.

Spadafora, N. D., Cocetta, G., Ferrante, A., Herbert, R. J., Dimitrova, S., Davoli, D., Fernández, M., Patterson, V., Vozel, T., Amarysti, C., Rogers, H. J., & Müller, C. T. **(2019)**. Short-Term Post-Harvest Stress that Affects Profiles of Volatile Organic Compounds and Gene Expression in Rocket Salad during Early Post-Harvest Senescence. *Plants*, *9*(1), 4.

Takahashi, T., & **Kakehi, J. I**. **(2010)**. Polyamines: Ubiquitous polycations with unique roles in growth and stress responses. *Annals of Botany*, *105*(1), 1–6.

Tavladoraki, P., Cona, A., & Angelini, R. **(2016)**. Copper-Containing Amine Oxidases and FAD-Dependent Polyamine Oxidases Are Key Players in Plant Tissue Differentiation and Organ Development. *Frontiers in Plant Science*, *7*.

Tian, M., Yu, R., Yang, W., Guo, S., Liu, S., Du, H., Liang, J., Zhang, X., Tian, M., Yu, R., Yang, W., Guo, S., Liu, S., Du, H., Liang, J., & Zhang, X. **(2024)**. Effect of Powdery Mildew on the Photosynthetic Parameters and Leaf Microstructure of Melon. *Agriculture*, *14*(6).

Tiburcio, A. F., Altabella, T., Borrell, A., & Masgrau, C. **(1997)**. Polyamine metabolism and its regulation. *Physiologia Plantarum*, *100*(3), 664–674.

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. **(2011)**. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, *108*(50), 20260–20264.

Wang, H. (2021). Cucumber Economic Values and Its Cultivation and Breeding. BoD – Books on Demand.

Wang, W., Paschalidis, K., Feng, J. C., Song, J., & Liu, J. H. (2019). Polyamine Catabolism in Plants: A Universal Process With Diverse Functions. *Frontiers in Plant Science*, *10*.

Wehner, T. C., & Robinson, R. W. **(1991)**. *A brief history of the development of cucumber cultivars in the US.*

Weinstein, L. H., Osmeloski, J. F., Wettlaufer, S. H., & Galston, A. W. **(1987)**. Protection of wheat against leaf and stem rust and powdery mildew diseases by inhibition of polyamine metabolism. *Plant Science: An International Journal of Experimental Plant Biology*, *51*, 311–316.

Whitaker, T. W. (1962). *Cucurbits: Botany, cultivation, and utilization, by Thomas W. Whitaker and Glen N. Davis.* L. Hill.

Wojtyla, Ł., Lechowska, K., Kubala, S., & Garnczarska, M. **(2016)**. Different Modes of Hydrogen Peroxide Action During Seed Germination. *Frontiers in Plant Science*, *7*.

Wojtyla, Ł., Wleklik, K., Borek, S., Garnczarska, M., Wojtyla, Ł., Wleklik, K., Borek, S., & Garnczarska, M. **(2024)**. Polyamine Seed Priming: A Way to Enhance Stress Tolerance in Plants. *International Journal of Molecular Sciences*, *25*(23).

Xu, X., Du, Y., Li, S., Tan, M., Sohail, H., Liu, X., Qi, X., Yang, X., & Chen, X. **(2024)**. A genome-wide association study reveals molecular mechanism underlying powdery mildew resistance in cucumber. *Genome Biology*, *25*(1), 252.

Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., & Brown, P. H. **(2017)**. Biostimulants in Plant Science: A Global Perspective. *Frontiers in Plant Science*, *7*.

Yang, Y., & Guo, Y. **(2018)**. Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytologist*, *217*(2), 523–539.

Yi, Q., Park, M. J., Vo, K. T. X., Jeon, J. S., Yi, Q., Park, M. J., Vo, K. T. X., & Jeon, J. S. **(2024)**. Polyamines in Plant–Pathogen Interactions: Roles in Defense Mechanisms and Pathogenicity with Applications in Fungicide Development. *International Journal of Molecular Sciences*, *25*(20).

Yong, B., Xie, H., Li, Z., Li, Y. P., Zhang, Y., Nie, G., Zhang, X. Q., Ma, X., Huang, L. K., Yan, Y. H., & Peng, Y. **(2017)**. Exogenous Application of GABA Improves PEG-Induced Drought Tolerance Positively Associated with GABA-Shunt, Polyamines, and Proline Metabolism in White Clover. *Frontiers in Physiology*, *8*, 1107.

Yuan, Y., Shu, S., Li, S., He, L., Li, H., Du, N., Sun, J., & Guo, S. **(2014)**. Effects of Exogenous Putrescine on Chlorophyll Fluorescence Imaging and Heat Dissipation Capacity in Cucumber (*Cucumis sativus* L.) Under Salt Stress. *Journal of Plant Growth Regulation*, *33*(4), 798–808.

Yuan, Y., Zhong, M., Shu, S., Du, N., Sun, J., & Guo, S. **(2016)**. Proteomic and Physiological Analysis Reveal Putrescine Responses in Roots of Cucumber Stressed by NaCl. *Frontiers in Plant Science*, *7*.

Yuan, Y., Zhong, M., Du, N., Shu, S., Sun, J., & Guo, S. **(2019)**. Putrescine enhances salt tolerance of cucumber seedlings by regulating ion homeostasis. *Environmental and Experimental Botany*, *165*, 70–82.

Zhang, H., Zhu, J., Gong, Z., & Zhu, J. K. **(2022)**. Abiotic stress responses in plants. *Nature Reviews Genetics*, *23*(2), 104–119.

Zhao, S. X., Wang, S. D., Liu, Y. Q., Pan, L. L., Zhao, S. X., Wang, S. D., Liu, Y. Q., & Pan, L. L. **(2025)**. Modulation of Plant Interactions with Whitefly and Whitefly-Borne Viruses by Salicylic Acid Signaling Pathway: A Review. *Viruses*, *17*(6).

Zhu Z. J. (2016). Abiotic stress signaling and responses in plants. *Cell*, *167*(2), 313-324.

Zhu, X., Wang, L., Yang, R., Han, Y., Hao, J., Liu, C., & Fan, S. **(2019)**. Effects of exogenous putrescine on the ultrastructure of and calcium ion flow rate in lettuce leaf epidermal cells under drought stress. *Horticulture, Environment, and Biotechnology*, *60*.

Zhu, Z., Wei, G., Li, J., Qian, Q., & Yu, J. **(2004)**. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science*, *167*(3), 527–533.