Secondary Metabolites as Biomarkers in Breeding for Biotic and Abiotic Stresses in Plants

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Abstract

Understanding the relationships between metabolite profiles and plant responses to various environmental stresses is a major goal in plant breeding. However, linking molecular markers patterns of plants to environmental pressures and trait variation remains difficult due to ecological driven plasticity. Integrating metabolite profiles as biomarkers alongside molecular markers presents an opportunity to address this challenge. Plants mitigate various biotic and abiotic stresses by activating multiple protective mechanisms. The diversity of secondary metabolites, shaped by environmental selection pressures, plays a critical role in these adaptive responses. Despite their significance, current functional classification systems for plant metabolites remain limited. A more comprehensive understanding of secondary metabolites as integrated components of metabolic networks, shaped by dynamic environmental pressures, can provide insights into plant metabolism and plant-environment interactions across multiple trophic levels. This review explores the complex networks of pathways involved in the production of protective secondary metabolites. These networks represent novel opportunities for crop improvement, particularly in breeding for drought tolerance and insect resistance. By examining key signalling networks and their interactions with abiotic and biotic stress pathways, this paper offers a resource for developing more effective strategies. Integrating metabolite profiling in breeding programs can enhance efforts to select and breed varieties that are more resilient to environmental challenges.

Key Words: Environmental stresses, secondary metabolism, Signalling Pathways, Metabolite Profiles, Biomarkers

Introduction

One of the major factors limiting global crop production is environmental stress, which can be categorized into biotic and abiotic stresses (Zandalinas *et al*., 2021). Biotic stress results from the negative effects of biological organisms, such as fungi, bacteria, arthropods, and herbivores, while abiotic stress is caused by non-living factors, including radiation, extreme temperatures, heavy metals, and drought (Pérez-Jiménez *et al*., 2021). Among these, drought is particularly devastating, reducing crop productivity by up to 50 – 70% (Tron *et al*., 2021). By 2030, an estimated 700 million people could be displace due to drought and its associated impacts on food security (UNCCD, 2021). While solutions such as irrigation and breeding for drought tolerant crops exist, irrigation remains costly option, requiring a consistent and reliable water supply, especially during critical growth stages like flowering and pod filling (Liu *et al.*, 2022). Breeding for drought tolerance and other plant stresses continues to be a more sustainable long-term strategy, especially in regions prone to water scarcity (Kholová *et al*., 2021).

Plants, as sessile organisms, have developed intricate mechanisms to cope with environmental stresses. They detect threat signals through specialized sensors and receptors, triggering multiple response pathways, including the accumulation of secondary metabolites that function as defense agents (Zhou *et al.*, 2021). Biotic stress, such as insect pests, contribute to global yield losses ranging from 16% to 18%, with overall pre-harvest pest damage reducing crop yields by about 35% (Savary *et al.*, 2019). Seasonal fluctuations in pest populations are further complicated by the unpredictability of climate change (Crosby *et al*., 2021) To combat both biotic and abiotic stresses, plants have evolved diverse defence mechanisms (Bialic-Murphy *et al*., 2022).

Various pest management strategies include cultural, biological, and chemical controls, as well as the use of resistant crop varieties (Batista *et al*., 2021). For the past fifty years, synthetic insecticides have been the primary means of pest control, but they pose serious risks to human health, particularly for agricultural workers, and disrupt ecosystem by harming biodiversity, pollinators, and natural enemies of pests (Goulson, 2019). In contrast, plant secondary metabolites provide a natural, eco-friendly alternative by inhibiting pest reproduction and physiology. These compounds are species-specific, biodegradable, and less likely to cause resistance in pests (Isman & Grieneisen, 2014).

Plants synthesise secondary metabolites through several metabolic pathways derived from primary metabolism in response to stress. The shikimate pathway, for example, is crucial for the biosynthesis of aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine, which are vital for secondary metabolite production under stress conditions (Tohge *et al*., 2013). The type and location of the stress, for instance, phytoalexins with antimicrobial properties accumulate in leaves to combat pathogens (Yang *et al*., 2021). Additionally, these metabolites contribute to physical barriers that prevent pathogen entry.

Drought stress has been shown to increase the accumulation of secondary metabolites in plants (Mirniyam *et al*., 2022). For example, drought stress significantly raises the levels of phenolic compounds in *Trachyspermum ammi*. This highlights the dual role of secondary metabolites in both defensive and as biomarkers of plant stress, offering potential opportunities for breeding crops with enhanced resilient to environmental challenges.

Response of Secondary Metabolites to Biotic and Abiotic Stress

Secondary metabolites are critical for plant resistance, serving both as defence mechanisms and potential biomarkers for stress resilience. Their altered production under stress conditions, particularly drought, aids in plant survival by contributing to drought tolerance (Sharma *et al*., 2020). Investigating the plant secondary metabolism helps identify key phytochemicals and their roles in stress adaptation (Zandalinas *et al*., 2021).

Abiotic stress alters plant metabolism by inhibiting enzyme, depleting substrate, and creating demands for specific compounds. To survive, plants undergo metabolic reprogramming, producing compatible solutes such as trehalose, mannitol, and proline to maintain cellular functions (Li *et al*., 2021). However, prolonged drought leads to cytoplasmic ion toxicity and protein denaturation (Hasanuzzaman *et al*., 2019), T requiring crops to crops to balance escape, avoidance, and tolerance while maintaining productivity. While these mechanisms have been described in various crops, their molecular pathways remain under investigation (Sharma *et al*., 2020). Metabolomics, which links metabolite changes to phenotypic shifts, offers insights into stress responses. Studies in model legumes like Medicago and Lotus have demonstrated metabolic reprogramming under drought, revealing complex networks of stress response mechanisms (Li *et al*., 2020; Pereira *et al*., 2022).

Elicitation is a strategy to enhance secondary metabolite production by exposing plants to biotic or abiotic stimuli, mimicking stress responses (Radman *et al*., 2003). Biotic elicitors, such as fungi or bacteria, and abiotic elicitors, like heavy metals or UV radiation, trigger the accumulation of defense-related metabolites, including sesquiterpene lactones, flavonoids, and anthocyanins (Wang & Wu, 2013). Key signalling molecules like methyl jasmonate (MeJA) and salicylic acid (SA) activate secondary metabolite production in response to stress (Wasternack & Hause, 2013). For instance, yeast extract enhances ethylene production in tomato and bacterial resistance in *Phaseolus vulgaris* (Meena *et al*., 2022). Salicylic acid induces systemic acquired resistance (SAR) and promotes the production of compounds such as vinblastine in *Catharanthus roseus* and ginsenosides in ginseng (Hao *et al.*, 2015). Similarly, MeJA has been shown to increase stilbene production in *Vitis vinifera* (Belhadj *et al.,* 2006).

These findings highlight elicitation's potential to sustainably boost secondary metabolite production, enhancing plant resilience to both biotic and abiotic stress. Further research into metabolic pathways could lead to new crop management strategies for environmental adaptability.

Detection of Insect-herbivory damage

While mechanical insect damage is not classified as a true plant disease, plants have evolved sophisticated systems to detect herbivory through herbivore-associated molecular patterns (HAMPs) in insect saliva (Mithöfer *et al*., 2005). These elicitors trigger signalling pathways leading to the production of defensive compounds, including volatile organic compounds (VOCs) like monoterpenoids and sesquiterpenoids. Volatile organic compounds serve multiple ecological functions, repelling herbivores or attracting natural enemies such as parasitoids and predators, which help control pest populations (Heil, 2014). For example, wheat (*Triticum aestivum*) infested by aphids emits VOCs that repel further infestation, while lima beans (*Phaseolus lunatus*) release volatiles that attract predatory mites (Bruce *et al*., 2010; Dicke & Baldwin, 2010).

Volatile organic compounds emissions also "prime" undamaged plant tissues for future attacks and facilitate plant-plant communication, where neighbouring plants activate their defence in response to airborne VOC signals (Karban *et al*., 2014). Though producing VOCs incurs metabolic costs, these defenses contribute to survival and reproduction by mitigating herbivore pressure (Turlings & Erb, 2018). Additionally, plants employ physical defenses such as cell wall reinforcement and production of protease inhibitors and toxic secondary metabolites like alkaloids (Howe & Jander, 2008).

Defense responses are regulated by phytohormonal signalling, particularly involving jasmonic acid (JA) for chewing insects and salicylic acid (SA) for piercing-sucking insects (Koo & Howe, 2009). Recent study also suggests plants detect herbivory through mechanical signals, like vibrations caused by insect movement, broadening the understanding of plant-insect interactions (Appel & Cocroft, 2014).

Secondary Metabolites as Biomarkers in Insect-Pest and Drought Resistance

Secondary metabolites are crucial for plant defense against both biotic stressors, such as insect pests and pathogens, and abiotic stressors, including drought. These compounds, encompassing monoterpenoids, sesquiterpenoids, diterpenoids, phytoalexins, alkaloids, and flavonoids, act as key biomarkers, offering insights into plant resilience under stress.

Monoterpenoids and sesquiterpenoids, the main constituents of essential oils, are highly volatile and serve as insect toxins or repellents while also possessing antifungal or antibacterial properties. For example, mint plants (*Mentha* spp.) produce large amounts of the monoterpenoids menthol and menthone, synthesized and stored in glandular trichomes on the epidermis. These compounds deter herbivores and prevent fungal infections. Pyrethrins, produced by chrysanthemum plants (*Chrysanthemum cinerariifolium*), are potent monoterpenoid esters functioning as neurotoxins in insects, disrupting their nervous systems (Casida & Durkin, 2013). Similarly, gossypol, a diterpenoid produced by cotton (*Gossypium hirsutum*), exhibits strong antifungal properties, vital for cotton's defense against pathogens (Stipanovic *et al*., 2005).

Another class of Secondary metabolites, phytoecdysteroids, mimics insect moulting hormones. Spinach (*Spinacia oleracea*), for instance, produces phytoecdysteroids, which disrupt larval development and increase insect mortality (Schmelz *et al*., 2000). Saponins, glycosylated triterpenoids, are found in cell membranes of many plant species and have detergent-like properties that disrupt the membranes of fungal pathogens. Avenacins, a group of saponins in oats (*Avena sativa*), confer resistance to the soil-borne fungal pathogen *Gaeumannomyces graminis*, reinforcing plant immunity (Papadopoulou *et al*., 1999).

In addition to terpenoids, phenolic compounds, such as phytoalexins and is flavonoids, play critical roles in plant defense. These compounds are synthesized through the shikimate and malonic acid pathways and possess both antibiotic and antifungal properties. Medicarpin, a phytoalexin produced by alfalfa (*Medicago sativa*), and rishitin, found in tomatoes (*Solanum lycopersicum*) and potatoes (*Solanum tuberosum*), are key responses to pathogen attacks (Bennett & Wallsgrove, 1994). Camalexin, a well-studied phytoalexin in *Arabidopsis thaliana*, provides protection from microbial infections (Glazebrook, 2005). Alkaloids like caffeine, found in coffee (*Coffea* spp.), tea (*Camellia sinensis*), and cocoa (*Theobroma cacao*), exhibit broad-spectrum toxicity to insects and fungi (Ashihara & Crozier, 2001), underscoring the diverse roles of SMs in plant defense.

Drought Resistance and Secondary Metabolites

In response to drought stress, plants typically increase the synthesis of secondary metabolites, which serve as osmoprotectants, antioxidants, and signalling molecules. Drought stress triggers the accumulation of flavonoids, terpenoids, and phenolic compounds, which help plants cope with oxidative damage and maintain cellular homeostasis. For example, quercetin, botulinic acid, and artemisinin levels were elevated in plants such as *Hypericum brasiliense* and *Artemisia annua* under drought stress (Verma and Shukla, 2015). Quercetin, in particular, is a flavonoid known for its antioxidant properties and its role in enhancing drought tolerance (Agati *et al*., 2012).

In *Glechoma longituba*, flavonoids were found to accumulate at higher levels under drought conditions, while drought stress in *Ocimum americanum* and *Ocimum basilicum* significantly altered concentrations of macronutrients, essential oils, and proline, indicating a complex response to water deficiency (Ashraf *et al*., 2019). Studies in *Salvia* spp. also revealed that drought stress increases flavonoid and phenolic concentrations, as seen in *Salvia sinaloensis*, where flavonoid levels increased by 101% and total phenol content by 139% under reduced container capacity. Similarly, drought stress in *Salvia dolomitica* and *Salvia officinalis* increased the levels of polyphenols and other secondary metabolites associated with antioxidant activity (Caser *et al*., 2019).

In rice (*Oryza sativa*), a study by Quan *et al*. (2016) found that drought-tolerant cultivars like Q8 exhibited higher levels of secondary metabolites, such as gallic acid and rutin, which contributed to the plant's superior drought resistance. Additionally, artificially induced drought stress using mannitol in *Thymus vulgaris* increased the percentages of thymol and γ-terpinene, two monoterpenoids with antioxidant and antimicrobial properties (Razavizadeh and Komatsu, 2018).

Drought-induced increases in secondary metabolites are not limited to terrestrial plants. In ferns like *Matteuccia struthiopteris* and *Athyrium multidentatum*, drought stress led to enhanced synthesis of flavonoids, total phenols, and proanthocyanidins, along with increased activity of antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase (Wang *et a*l., 2019). These compounds play a key role in mitigating oxidative stress caused by drought. Conversely, *Sesamum indicum* (sesame) exhibited a decrease in sesamin, oil, and quercetin content under drought conditions, although total flavonoids, phenolics, and radical scavenging activity increased, underscoring the diverse responses of secondary metabolites to drought stress (Kermani *et al*., 2019).

Transcription Factors in Regulating Genes Associated with Insect Pest and Drought Resistance

The inducible synthesis of secondary metabolites (SMs) and the transcription of associated biosynthetic genes are significantly influenced at various levels by transcriptional regulation via transcription factors (TFs). These TFs are DNA-binding proteins that attach to the promoter regions of target genes, altering the rate of transcriptional initiation via RNA polymerases. By integrating internal and external signals, TFs can effectively monitor the accumulation of SMs and regulate the expression of enzyme genes. The regulation of genes associated with the SM biosynthesis pathway is influenced by a diverse array of TFs acting at multiple levels (Yang *et al*., 2012). Some identified TFs involved in the regulatory mechanisms of SMs biosynthesis pathways include WRKY, MYB, bHLH, bZIP, and AP2/ERF families.

WRKY Transcription Factors

The inducible expression patterns of WRKY genes support their role in modulating defense-related SM biosynthesis. Members of the WRKY family possess a 60-amino-acid conserved domain responsible for gene regulation through interaction with W-boxes in target promoters. Plant WRKY TFs are primarily involved in stress responses and can be regulated by wound signalling or jasmonic acid. They alter the expression of genes related to the biosynthesis of various SMs, including alkaloids and terpenoids (Phukan *et al*., 2016). For instance, in tobacco, WRKY3 and WRKY6 are linked to volatile terpene biosynthesis (Skibbe *et al*., 2008).

In cotton, WRKY1 regulates gossypol biosynthesis by binding to the promoter region of the gene involved in cadinene synthesis. Furthermore, WRKY1 has been studied in potato plants infected with late blight, where it binds to the promoter of the gene involved in hydroxycinnamic acid amide (HCAA) biosynthesis, modulating the phenylpropanoid pathway (Yogendra *et al*., 2015). In wheat, the TF TaWRKY70 activates genes associated with HCAA when fungal biomass is encountered (Kage *et al*., 2017). *Vitis vinifera*'s VviWRKY24 enhances the expression of the VviSTS29 gene, crucial for resveratrol biosynthesis and antimicrobial resistance (Vannozzi *et al*., 2018). Moreover, SsWRKY18, SsWRKY40, and SsMYC2 regulate abietane-type diterpene accumulation in *Salvia sclarea*, compounds known for their antibacterial and antifungal properties (Singh *et al*., 2019).

In potato, StWRKY8 enhances the expression of TyDC, NCS, and COR2, involved in benzylisoquinoline alkaloid production (Yondra *et al*., 2017). ZmWRKY79 increases phytoalexin accumulation in maize under stress conditions (Fu *et al*., 2020). In *Catharanthus* *roseus*, CrWRKY1, induced by jasmonic acid, plays a significant role in the biosynthesis of terpenoid indole alkaloids and promotes the expression of TDC, a gene in this pathway (Che *et al*., 2013). Overall, a single TF does not solely activate the entire biosynthesis pathway.

MYB Transcription Factors

MYB TFs are involved in SM biosynthesis and various biological processes, including growth, reproduction, and stress responses. Characterized by different DNA-binding domain repetitions, MYB TFs can be classified into four subclasses: R1, R2, R3, and R4. The R2R3 family is significantly associated with regulating various SM pathways across plant species. For instance, AtMYB113, AtMYB114, AtMYB75, and AtMYB90 in *Arabidopsis thaliana* potentially regulate anthocyanin residues via the phenylpropanoid pathway (Gonzalez *et al*., 2013). MYB TFs may also participate in the biosynthesis of glucosinolates, flavonoids, HCAAs, and proanthocyanins. In *Arabidopsis*, MYB29 and AtMYB76 are associated with aliphatic glucosinolate accumulation, while AtMYB34, AtMYB51, and AtMYB122 affect indole glucosinolate accumulation by altering the expression of tryptophan biosynthesis genes (Dubos *et al*., 2010). In citrus plants, CsMYBF1 activates the expression of CHS, a gene involved in flavonoid biosynthesis (Liu *et al*., 2016). Nisha *et al*. (2018) showed that CsMYB2/CsMYB26 from *Camellia sinensis* binds to the promoter regions of CsF30H and CsLAR, enhancing flavonoid accumulation and tolerance against blister blight disease caused by *Exobasidium vexans*. In tobacco, MYB-JS1 regulates the phenylpropanoid pathway, while MYB6 and MYB12 in Asiatic hybrid lily plants are involved in anthocyanin biosynthesis (Yamagishi *et al*., 2011). In poplar trees, PtMYB115 binds to the promoter regions of ANR1 and LAR3, enhancing proanthocyanin accumulation and resistance against *Dothiorella gregaria*, a fungal pathogen (Wang *et al*., 2021).

bHLH Transcription Factors

bHLH TFs are potential regulators of stress response mechanisms, often interacting with MYB proteins to form complexes that enhance the expression of specific genes. These 60-amino-acid proteins contain bipartite conserved domains; the key residue at the N-terminal allows DNA binding, while two alpha helices facilitate interactions with other proteins to form homo/heterodimeric complexes. As important modulators of stress responses, bHLH TFs regulate SM biosynthesis, including anthocyanins, alkaloids, glucosinolates, diterpenoid phytoalexins, and saponins. For example, bHLH04, bHLH05, and bHLH06 interact with MYB51 to regulate GL biosynthesis in *Arabidopsis* (Frerigmann *et al*., 2014). In anthocyanin and flavonoid biosynthesis, bHLHs play significant roles in regulating the phenylpropanoid pathway. The bHLH proteins GL3, eGL3, and TT8 bind with MYB in the presence of TTG1, forming a complex responsible for regulating anthocyanin biosynthesis genes (Dubos *et al*., 2010). MYC2, a key regulator of jasmonic acid signalling, belongs to the bHLH family and is involved in regulating SMs directly or indirectly.

In apple, MdMYC2 induces gene expression following jasmonic acid application, enhancing anthocyanin production in transgenic lines. It also integrates gibberellic acid and jasmonic acid signalling pathways by interacting with DELLA protein, upregulating sesquiterpene genes in *Arabidopsis* flowers. SMs are often regulated by complexes of different TF families, such as the MYB–bHLH–WDR complex, which enhances anthocyanin and proanthocyanin regulation in Arabidopsis (Nemesio *et al*., 2017). In rice, DPF is a bHLH TF that regulates the accumulation of diterpenoid phytoalexin by activating related genes (Yamamura *et al*., 2015). NbbHLH1, NbbHLH2, and NbbHLH3 in tobacco are associated with nicotine biosynthesis; NbbHLH1 and NbbHLH2 are positive regulators, while NbbHLH3 acts as a negative regulator (Toda *et al*., 2011). Additionally, TSAR1 and TSAR2 are bHLH TFs regulating saponin biosynthesis by activating the HMGR gene in *Medicago truncatula* (Mertens *et al*., 2016). Overall, bHLH TFs act independently or interact with other protein families to enhance the biosynthesis of various biotic or abiotic stress-inhibiting SMs in plants.

bZIP Transcription Factors

bZIP TFs are dimeric, transcriptional enhancer proteins with a conserved leucine zipper and a positively charged DNA-binding site. They modulate various plant biological processes. Studies have shown that bZIP and oxidative stress regulate SM biosynthesis in fungi (Hong *et al*., 2014). Specific bZIP proteins, such as SmbZIP20, SmbZIP7, and AabZIP1, regulate pharmaceutically important SMs, including tanshinone and artemisinin in *Salvia miltiorrhiza* and *Artemisia annua*, respectively (Zhang *et al*., 2018). In tomato plants, S1HY5 binds to the G-box and ACGT region in the promoter of CHS and DFR, altering anthocyanin accumulation. It also modulates QH6 expression by binding to its site, enhancing monoterpene biosynthesis (Zhou *et al*., 2015). bZIP proteins are also associated with terpenoid phytoalexins, which provide resistance to blast pathogens in rice. OsTGAP1, a bZIP protein, binds to the promoter regions of OsKSL4 and OsCPS4, enhancing terpenoid phytoalexin biosynthesis and modulating other terpenoid biosynthesis-related genes by influencing the MEP pathway (Yoshida *et al*., 2017).

AP2/ERF Transcription Factors

AP2/ERF transcription factors (TFs) contain a conserved 60-amino-acid AP2 DNA-binding domain first identified in Arabidopsis thaliana's floral homeotic gene APETALA2 (AP2). They are categorized into four subfamilies based on additional conserved domains: AP2 (two AP2 domains), ERF (one AP2 and a B-subfamily), RAV (one AP2 and a B3 domain), and DREB (one AP2 and an A-subfamily) (Zhou and Memelink, 2016). These TFs are crucial in mediating plant stress responses through the regulation of secondary metabolites (SMs) (Wasternack and Song, 2017). For example, ORCA and ORCA2 bind to promoters of genes involved in terpenoid indole alkaloid biosynthesis, while ORCA3 is a jasmonic acid-inducible protein that interacts with the JERE element in the promoters of strictosidine synthase and tryptophan decarboxylase genes. In tobacco, TFs like NtORC1/ERF221 and NtJAP1/ERF10 positively regulate the PMT gene linked to nicotine biosynthesis, with overexpression significantly enhancing nicotine and pyridine alkaloid levels (De *et al*., 2005). The induction of nicotine biosynthesis also involves bHLH proteins, suggesting a combined action of TFs in SM synthesis (Shoji and Hashimoto, 2015).

Additionally, PaNAC03 is a NAC TF that negatively regulates flavonoid pathway genes such as CHS, F30H, and LAR3, enhancing plant tolerance to *Heterobasidion annosum*, a root and butt rot pathogen. ANACO3 similarly downregulates DFR, ANS, and LODX genes, responsible for anthocyanin biosynthesis (Mahmood *et al*., 2016).

To deepen the understanding of how transcription factors (TFs) regulate genes linked to insect pest and drought resistance, it is essential to explore additional TF families and mechanisms, along with emerging insights into combinatorial regulation, cross-talk between signalling pathways, and the role of epigenetic modifications in influencing secondary metabolite (SM) biosynthesis. These includes NAC transcription factors, Ethylene-responsive factors (ERFs), Epigenetic regulation of TFs and cross-talk between pathways.

NAC Transcription Factors

The NAC (NAM, ATAF, and CUC) family of TFs is widely implicated in plant stress tolerance, including responses to both biotic and abiotic challenges. NAC proteins are known to regulate the expression of genes involved in cell wall formation, senescence, and hormone signalling pathways. Studies show that overexpression of TaNAC69 in wheat enhances drought tolerance by upregulating stress-responsive genes such as WHEAT TLP, which modulates SMs involved in osmoprotection and oxidative stress regulation (Xue *et al.*, 2011). Additionally, in rice (*Oryza sativa*), the NAC TF SNAC1 is involved in drought resistance by promoting the expression of stress-responsive genes, leading to improved water-use efficiency and accumulation of osmoprotectants (Hu *et al.*, 2006). NAC TFs can also regulate resistance to insect pests; for instance, GhNAC109 in cotton has been shown to regulate genes associated with SM production that confer resistance to aphids (He *et al.*, 2020).

Ethylene-responsive Factors (ERFs)

Apart from the previously mentioned AP2/ERF TFs, a subclass of ERFs has gained attention for its role in mediating resistance to herbivorous insects and drought stress. For example, SlERF84 from tomato has been implicated in enhancing resistance to the whitefly (*Bemisia tabaci*) by activating SM pathways that produce toxic alkaloids and terpenoids (Zhao *et al.,* 2021). In drought scenarios, ERFs such as OsDREB1A in rice are induced by abscisic acid (ABA) signalling and regulate genes involved in drought adaptation, including those responsible for osmolyte production (Dubouzet *et al*., 2003).

Epigenetic Regulation of TFs

Emerging evidence highlights the role of epigenetic modifications such as DNA methylation, histone acetylation, and chromatin remodelling in regulating TF activity and SM biosynthesis. For example, methylation of the promoter regions of key TFs, such as MYBs and WRKYs, can modulate their expression, which in turn affects downstream genes involved in stress responses. In rice, chromatin remodelling of the OsWRKY45 gene promoter under drought stress enhances its transcriptional activation, leading to the accumulation of phytoalexins (Chujo *et al*., 2013). Similarly, histone acetylation of ZmMYB31 in maize promotes its binding to the promoters of lignin biosynthesis genes, enhancing the plant's resistance to insect pests (Fornalé *et al*., 2014).

Cross-talk Between Pathways

It is also important to note the interactions between different TF pathways that lead to integrated responses to insect pests and drought. Cross-talk between jasmonic acid (JA) and salicylic acid (SA) signalling pathways mediated by WRKY, MYB, and bHLH TFs plays a pivotal role in fine-tuning the plant's response to simultaneous biotic and abiotic stressors. For example, AtWRKY33 in Arabidopsis modulates both JA and SA pathways, enhancing resistance to necrotrophic pathogens and drought stress (Birkenbihl *et al*., 2012). Additionally, MYC2, a key bHLH TF, regulates cross-talk between JA and ABA signaling, balancing SM production and stress tolerance under water deficit and herbivore attack (Kazan and Manners, 2013).

Identification and Quantification of Secondary Metabolites in Insect Pest and Drought Resistance

Metabolomics, defined as the comprehensive profiling of all metabolites within a given sample in a particular physiological state, provide a holistic measure of the global changes occurring post elicitation. Metabolomics has great potential to provide a holistic examination of numerous metabolites' diagnosis and phenotyping of plants (Fernie and Schauer, 2009). Metabolomics has promising prospects to expedite the selection of improved breeding materials and screen elite crop varieties. Metabolic profiling of secondary metabolites provides extensive knowledge of biochemical processes that occur during plant metabolism (Sung *et al*., 2015). The successful detection, identification, assessment, and evaluation of these metabolites is possible through advanced metabolomics tools, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography mass-spectroscopy (LC-MS), and non-destructive nuclear magnetic resonance spectroscopy (NMR) (Che *et al*.,2019).

Recent developments in analytical techniques and bioinformatics tools (Sumner *et al*., 2015) have allowed monitoring metabolites differences among samples in a semi-automated and untargeted manner. Metabolomics makes use mostly of coupled techniques which rely first on chromatographic separation of metabolites followed by mass spectrometry (MS) or nuclear magnetic resonance (NMR) for detection and or structural elucidation of separated peaks. In particular, untargeted ultra-performance liquid chromatography-mass spectrometry (UPLC-MS)-based approach is well suited to reveal the effects of elicitation on samples at metabolite levels (Farag *et al*., 2015).

Metabolomics not only aids in the identification and quantification of secondary metabolites but also plays a critical role in understanding the underlying mechanisms of plant resistance to biotic and abiotic stressors. For instance, secondary metabolites such as flavonoids, terpenoids, and phenolic compounds have been implicated in the plant's defense responses against herbivory and drought stress (Yoshida *et al., 2018*). These compounds can enhance plant resilience by acting as signalling molecules or by directly deterring pests through their toxic or repellent properties (Maffei *et al.,* 2011).

Moreover, the integration of metabolomics with genomics and transcriptomics allows for a comprehensive understanding of the gene-metabolite networks involved in stress responses. This systems biology approach can identify key regulatory genes and pathways, thereby facilitating the development of crop varieties with enhanced resistance traits (Schwacke *et al.*, 2013). For instance, recent studies have shown that metabolites associated with stress responses are often co-regulated with specific gene expression patterns, providing insights into how plants adapt to changing environments (Baxter *et al.,* 2014).

Furthermore, the utilization of machine learning and advanced data analytics in metabolomics can improve the interpretation of complex datasets, allowing for the identification of potential biomarkers linked to drought and pest resistance (Meyer *et al*., 2020). This could significantly accelerate the breeding process by enabling the selection of elite genotypes with desirable traits based on metabolic profiles.

In summary, metabolomics serves as a powerful tool for the identification and quantification of secondary metabolites, offering valuable insights into the biochemical processes underlying plant stress responses. Continued advancements in analytical methodologies and data analysis techniques will enhance the understanding and application of metabolomics in crop improvement strategies.

Conclusion and Future Outlook

In conclusion, the integration of secondary metabolites and transcription factors plays a pivotal role in plants' responses to biotic and abiotic stresses, enhancing their resilience. The potential of metabolomics to bridge the gap between physiological responses and genetic mechanisms is becomes increasingly clear. By elucidating the intricate relationships among metabolites, genes, and environmental factors, metabolomics can significantly contribute to identifying key metabolic markers for stress tolerance and inform precision breeding approaches.

The future of plant metabolomics lies in its ability to create a comprehensive understanding of plant resilience mechanisms through the integration of various 'omics' technologies. This holistic approach can facilitate the identification of multi-trait selection targets, ultimately leading to the development of crops that are not only high-yielding but also resilient to climate change and pest pressures.

As precision agriculture evolves, the application of metabolomics will enhance the capacity to monitor plant health and stress responses in real time, promoting sustainable practices and food security. Additionally, the convergence of metabolomics with synthetic biology and advanced genome editing techniques presents exciting opportunities to manipulate metabolic pathways for improved stress tolerance.

Overall, while the current understanding of metabolomics in plant science is promising, continued research and interdisciplinary collaboration are crucial to unlocking its full potential. Such efforts will significantly advance sustainable crop production and resilience in an increasingly unpredictable environment, positioning the agricultural community to meet future challenges effectively.

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