Genes to Monitor Ecophysiological Parameters for Plants Under Abiotic Stress Conditions

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Abstract

In plants, different mechanisms are activated by gene expression in response to abiotic stresses. The great challenge is to study the physiological patterns and the genes involved in each pathway to evaluate the mechanisms used by plant the overcame de stressfully conditions. To mitigate the negative impact of adverse environmental conditions, the particle films technique has become a potential crop management tool for plants to overcome the adverse environmental conditions. The mean goal of this work is to identify genes expressed and involved in different ecophysiological pathways responding to abiotic stresses. GeneMANIA and Cytoscape software were fundamental in the establishment of gene networks. In addition, a graphical representation encompassed other closely linked genes with similar functions. From the perspective of particle film technology, the genes involved in each ecophysiological parameter may, in the future, elucidate new plant response mechanisms, and therefore, a more adaptive molecular response to adverse environmental conditions.

Keywords: Particle films; gene expression; gene of interest; interaction networks.

1. Introduction

Plants are naturally sessile organisms and, therefore, susceptible to abiotic stress factors such as drought, salinity, high and low temperatures, heavy metals, light and high irradiation. Over the years, several studies have generated substantial knowledge bases and understanding of responses to different environmental stresses, and models to explain mechanisms triggering plant responses [1]. Thus, other physiological, biochemical, and morphoanatomical mechanisms can be activated gene expression in response to abiotic stresses.

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Therefore, understanding the adaptive responses of plants from a physiological perspective in a molecular and genic perspective - in which multiple genes interact in response to the environment - expands the understanding of the adaptation of leaves to abiotic stress. Most adaptive responses, at least in part, occur through the control of gene expression.

Abiotic stresses intensification leads to physiological changes in plants. The metabolism's directional changes and biochemical pathways' reconfiguration result in irreversible damage or physiological adjustment to stressing conditions. Consequently, genes can be differentially expressed in organs, tissues, and different phenological stages. Therefore, plants can trigger several signals through genes expression or repression [2].

One of the main factors is water restriction in plants. This factor triggers alterations in stomatal conductance, photosynthesis, leaf vapor pressure, water use efficiency, and carboxylation, chlorophyll, and fluorescence. Furthermore, in later stages, it reduces vegetative growth [3]. Therefore, understanding the expression of genes involved in control different ecophysiological parameters is essential for plants environmental adaptation.

The technique of real-time PCR, or quantitative PCR (qPCR), has become reliable tool in quantifying the target genes. Transcriptomic improves plant responses to certain stressful conditions and complements the physiological results previously sought in the field [4].

Despite advanced techniques, it is still to access tools to monitor the development of resistant and more productive plants, favoring growth even under stressful conditions [3]. As a short-term strategy, particle films crop management mitigates the negative impact of adverse environmental conditions.

The technique uses inert and specialized minerals nanoparticles that are pulverized to cover leaves' surface. The particle film promotes plant acclimatization and increases the reflection of excess radiation [ultraviolet and infrared (IR)] and reduces photosynthetically active radiation (PAR) [5].

The amount of light reaching the chloroplast reaction centers is reduced, preventing damage to the photosynthetic apparatus of plants. This technology has contributed to maintaining leaf water content, keeping cell division and plant growth active [6]. This review aimed to identify genes involved in different ecophysiological parameters in response to abiotic stresses to create gene networks for studies using particle films in response to advserse environmental conditions.

2. Literature review

2.1 Stomatal conductance (gs)

Understanding the expression of genes related to stomatal conductance in leaves can provide important information about the acclimatization and adaptation of plants to specific environmental changes [7]. The genes differentially expressed on stomatal control affect several physiological mechanisms of autoregulation (Table 1). Furthermore, the combination of these candidate genes allows a gene interaction network with other strongly connected genes with similar functions (Figure 1).

Table 1: Genes involved in stomatal control mechanism

Mechanism	Gene
ABA biosynthesis - regulates stomatal conductance (gs)	CCD1 (NCED1); NCED2
Genes in guard cells regulating ABA-mediated stomatal movement	NF-YB3; SDIR1; CPK9
Transcribe to ABA signaling path	CPK10; SRK2E (OST1); ARK2 (RK2); SLAC1; DREB2A
Promotion of H_2O_2 in response to ABA and regulation of stomatal closure	CDC2.5 (ASR); SRO1
Reduction of (gs) due to lower stomatal density	EPF1; EPF2; EPFL9 (STOMAGEN); CESA3 (ATHB); WOX14
Up-regulation of stomatal opening in response to light	
	APK1b
Regulation of stomatal conductance under drought	LHCB6; SLAC1
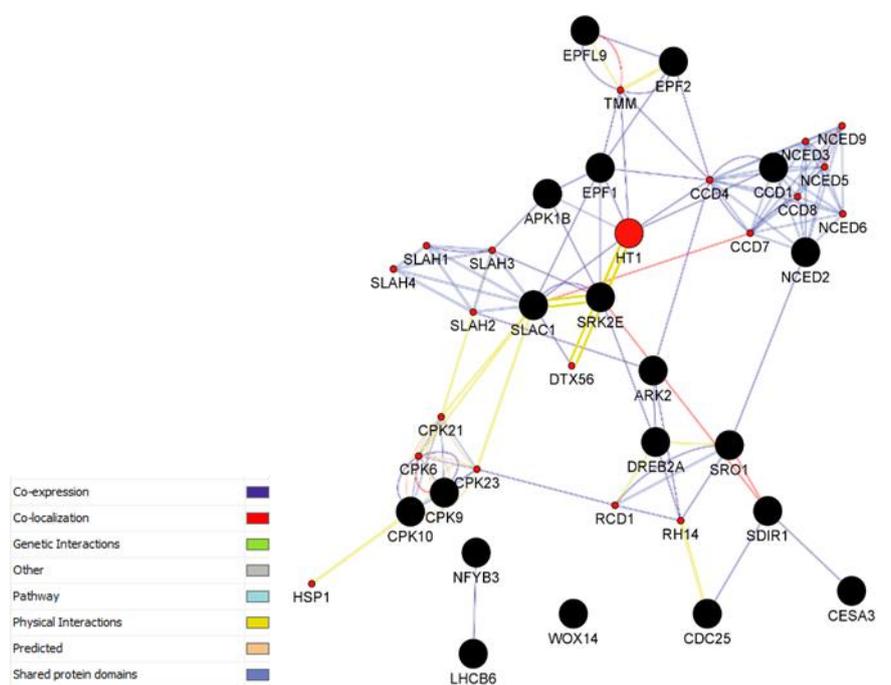
Figure 1: Gene interaction network for the parameter: stomatal conductance (*gs*), with other strongly connected genes with similar functions

Plants in full sun exposed to drought conditions have low stomatal conductance due to ABA biosynthesis. This

hormone plays a role in the stomatal conductance of leaves, optimizing gas exchange in response to soil moisture. Two primary genes associated with ABA, Biosynthesis9-cis-epoxycarotenoid dioxygenase (*NCED1* and *NCED2*), were differentially expressed in the leaf and root of grapevines cultivated under water stress [8].

Reference [9] reported in potato, Nuclear transcription factor Y subunit B-3 (*NF-YB3)*, is overexpression in leaf guard cells. This gene activates several other genes related to the ABA pathway, Calcium-dependent protein kinase (*CPK10*) and Serine (SRK2E - *OST1*), accelerates stomatal closure, Kinesin-like protein (*ARK2 - RK2*), regulates gas exchange and photosynthesis, Slac1 (*SLAC1*) [10]. On the other hand, the Dehydration-responsive element-binding protein 2A (*DREB2A*) has become known to encode a drought-responsive transcription factor and several other components of the ABA signaling pathway in the same crop [11].

Calcium-dependent protein kinase 9 *(CPK9)* present in several vegetative organs of rice under water deficit conditions, mainly in guard cells, indicates a positive role in drought tolerance and improvement in the plant's ability to retain water, basically due to its involvement in osmotic adjustment stomatal limit [12]. Stomatal closure is also the leading cause of reductions in the photosynthetic capacity of plants under water deficit, thus, Sdir1 (*SDIR1)* up-regulated by ABA, expresses vegetative tolerance after drought treatment in *Oryza sativa* [13].

ABA stimulates the generation of H_2O_2 through the enzyme complex NADPH oxidase in guard cells; in this way, the H_2O_2 as essential signaling molecules mediate stomatal closure. Genes involved in this process, such as Dual specificity phosphatase (CDC2.5 – *ASR)* and Probable inactive poly [ADP-ribose] polymerase SRO1 (*SRO1)*, regulate stomatal movement through the H_2O_2 mediated ABA-dependent pathway, decreasing stomatal conductance and contributing to greater drought tolerance [14].

In addition to water deficit, the light intensity directly affects stomatal conductance and opening. The Kinase 1b (*APK1b)* in *Arabidopsis* is available in plant to open its stoma under intense light, promoting improved leaf cooling transpiration. Studies also report that this gene adjusts stomatal closure in response to ABA [15].

The harmful reduction of Chlorophyll *a-b* binding protein, chloroplastic (*LHCB6)* and *SLAC1* may have detrimental effects on stomatal closure during drought, and restoring their functions through overexpression may improve plant tolerance *LHCSB6* encodes an association with photosystem II and, when overexpressed, promotes increased stomatal closure. At the same time, *SLAC*, mainly restricted to guard cells and associated with ABA, increases the sensitivity of plant responses [11].

The stomatal conductance of leaves can be influenced by the size of the guard cell, density, and stomatal opening. It is not clear why plants offer this type of response due to drought. However, several genes involved in these morphological changes are expressed and regulated to modify density and stomatal patterns. Peptide-secreting epidermal pattern factor, EPIDERMAL PATTERNING FACTOR 1 (*EPF1*), is usually expressed in young stomatal guard cells, whereas EPIDERMAL PATTERNING FACTOR 2 (*EPF2)* is expressed in earlier stages. The harmful reduction of *EPF1* results in an increase in stomatal density and grouping in the leaf epidermis [16].

In contrast, the EPIDERMAL PATTERNING FACTOR-like protein 9 (EPFL9 – *STOMAGEN)* down-regulation is described in *Arabidopsis* and reduces stomatal density and opening. Thus, it prevents excessive transpiration, suggesting the cooperation of this gene in controlling stomatal conductance, maintaining the water state of the leaves [17].

Furthermore, the expression of Cellulose synthase A catalytic subunit 3 (*CESA3 – ATHB)* induced by ABA in tomato plants under water deficit reduced stomatal density and stomatal size, consequently reducing transpiration rate and stomatal conductance [18]. The WUSCHEL-related homeobox 14 (*WOX14)* has been reported to regulate stomata density by controlling cell division in leaf tissue in plants under water stress [19].

Even under unfavorable conditions, the particle film on the leaves regulates the high stomatal conductance rates underwater deficit. Probably due to the reductions of ABA inside their stomatal cells and reflection of incident solar radiation also associated with lower leaf internal temperature leading to an improvement in the water potential in the leaves [6].

The responses provided by particle films interfere in the expression of genes that encode and signal ABA in *gs* control, reduce an expression of *APK1b* that is highly expressed in response to high intensity of incident light under the leaves [15]. It is also possible that particle films trigger genes located in guard cells that increase stomatal density and opening, allowing for more efficient gas exchange. In addition, the inhibiting the expression of genes that stimulate ABA-mediated H_2O_2 generation, such as *ASR* and *SRO1* regulate stomatal closure under drought conditions [14].

2.2 Photosynthesis (A)

Photosynthesis is a physiological process that converts light energy into chemical energy used for plant growth and development. Photosynthesis depends on different components of the physiological apparatus, such as PSI and II, and the electron transport system. Moderate heat stress associated with water stress in plants promotes reductions in CO_2 assimilation capacity due to possible damage caused to these photosynthetic components [20].

Therefore, understanding the adaptive responses of plants from a physiological perspective in conjunction with the molecular investigation - in which multiple genes interact with each other in response to the environment - expands the understanding of the adaptation of leaves to abiotic stress. Most adaptive responses, at least in part, occur through the control of gene expression [21].

In this sense, differential gene expression in photosynthesis addressed biochemical changes and transcriptional autoregulation (Table 2). Furthermore, the combination of these candidate genes allows a gene interaction network with other strongly connected genes with similar functions (Figure 2).

Table 2: Genes involved in photosynthesis-related mechanisms

Mechanism	Gene
Regulation of photosynthetic capacity	*NF-YB3*; *HUB1*
Up-regulation of photosynthesis genes in response to light	*NF-YB3*; *NF-YB7*; *APK1B*
ATP synthase subunits	*ATPA*; *ATPD*
Synthesis of chlorophyll a / b and light-collecting complex proteins	*LHCA2*; *LHCA4*; *CHL*; *LHCB5*; *NF-YB3*
Calvin Fructose-1,6-Bisphosphatase Cycle	*CFBP1* (*FBP*); *FBA1*
Plastocyanin	*PETE1*
PSI Subunits	*PSAB*
PSII Subunits	*PSBO2*; *PSBA*; *PSBK*; *PSBI*; *PSBH*
Genes Involved in Electron Transport	*LHCB3*; *LHCB5*; *PETA; PETB*; *ATPA*
HSP-modulated genes involved in photosynthetic acclimation and thermotolerance under drought conditions	*MED37E* (*HSP70-1*); *HSP70-3*; *HSP23.6*

Figure 2: Gene interaction network for the parameter: photosynthetic assimilation (*A*), with other linked genes with similar functions

The nuclear factor NF-Y is composed of three subunits: NF-YA, NF-YB, and NF-YC, each of which is encoded by different families of several specific genes, which go through different evolutionary patterns and result in

similar functions in plants [22].

Among the genes encoding the NF-YB protein in the potato crop database, Nuclear transcription factor Y subunit B-3 (*NF-YB3)* was studied and overexpressed under conditions of abiotic stress, showing a reduction the photosynthetic capacity, changing in few numbers of tubers, and consequently lower productivity [9]. The Nuclear transcription factor Y subunit B-7 (*NF-YB7)* was expressed and light up-regulated, which increased photosynthetic rates and chlorophyll content [23].

The Plastocyanin 1 (*PETE1)* gene also interacts with a calcium-sensing receptor that accelerates stomatal movement and the formation of photosynthetic electron transport, and Cytochrome f (*PETA),* Cytochrome b6 *(PETB),* and ATP synthase subunit alpha, chloroplastic (*ATPA)* that play essential roles in maintaining electron transfer during stress photosynthesis by heat regulating water efficiency and drought tolerance [21].

At the end of the electron transport chain, the ATP synthase protein works as an H+ proton pump, generating an energy gradient coupled to the ATP molecule used in the CBB cycle. The protein is composed of subunits, whose formation results from gene expressions, such as the (*ATPD)* gene, which maintain the fundamental structures for energy generation, electrical potential flow, and acid-base homeostasis in thylakoids and chloroplast stroma [24].

PSI and PSII are two photosystems that catalyze the transport of photosynthetic electrons. Photosystem II reaction center protein K (*PSBK),* Photosystem II protein D1 *(PSBA)*, Photosystem II reaction center protein I *(PSBI),* Photosystem II reaction center protein H *(PSBH)* are expressed in the peripheral core region of the photosystem II reaction center and are involved in the formation and assembly of PSII subunits [24]. Oxygen-evolving enhancer protein 1-2, chloroplastic (*PSBO2)* detects low chlorophyll contents in PSII [25] and Photosystem I P700 chlorophyll *a* apoprotein A2 (*PSAB)* is involved in the maintenance and repair of the same photosystem [24].

Studies with the *Arabidopsis* species showed that when there is greater light intensity in the leaves, the *APK1B* gene acts at the appropriate photosynthetic rate to regulate the tiniest stomatal opening [15]. E3 ubiquitin-protein ligase BRE1-like 1 (*HUB1)* is a gene that regulates photosynthesis due to water stress conditions in Italian ryegrass, making its expression repressed when the availability of water for plants is reestablished [26].

Studies demonstrate that the calcium-sensitive receptor (CAS) is closely related to the formation and improvement of the electron transport system, regulating chloroplast activity and photosynthesis, especially under water stress conditions, modulating an overexpression of Chlorophyll a-b binding protein, chloroplastic (*LHCB3)* and (*LHCB5)*, contributing to drought tolerance and photoprotection, preventing the inhibition of photosynthetic efficiency and degradation of chloroplasts under intense solar radiation [17]. Furthermore, the overexpressed HSP23.6-MITO (*HSP23.6)* protected the NADH: ubiquinone oxidoreductase complex during heat stress in plants. Therefore, an expression of this gene can help protect the mitochondria and reduce, repair, or protect against oxidative damage [27].

Particle films can promote leaf photoprotection, due to the smaller amount of light that reaches the reaction

centers in chloroplasts. In this sense, genes that respond to light and its intensity, in addition to other genes related to protein expression proceeding from the electron transport chain, can be differentially expressed. In this sense, genes that express and encode chlorophyll synthesis Photosystem I chlorophyll *a/b*-binding protein, chloroplastic (*LHCA, LHCA4, LHCB5*) may be induced in plants with particle films, compared to plants grown in full sun or without leaf protection [24].

Films can also protect photosystems II and I under high solar radiation and temperature. Thus, it is likely that genes encoding functional proteins are repressed into PSII and PSI subunits. It is even possible that there is an increase in the expression levels of genes involved in the photosynthetic acclimatization of plants under water stress because the particle film reduces the excessive loss of water over time, contributing to drought tolerance and photochemical efficiency [6].

2.3 Vapor pressure deficit (VPD)

Deficits of water vapor pressure affect species growth as they are directly related to reduced CO_2 assimilation. On the other hand, limiting the transpiration rate of a plant under high VPD translates into better crop yield under conditions of drought. In addition, other molecular mechanisms can also regulate VPD, such as differential gene expression as well as the constant incidence of winds, which promotes a reduction in the % RH of the air and an increase in the deficit of vapor pressure [28].

Responses provided by differential gene expression to VPD involve physiological mechanisms, transcription autoregulation, morpho-anatomical and biochemical changes (Table 3). Furthermore, the combination of these candidate genes allows a gene interaction network with other strongly connected genes with similar functions (Figure 3).

Table 3: Genes involved in vapor pressure deficit (VPD) mechanisms

Mechanism	Gene
Physiological responses under high VPD	*AKRP* (*AKR*); *LEA*; *HSP1*
Genes related to cuticular wax production	*MYB96*
Genes related to high E and VPD values	*PPD*
Involved in the formation of trichomes	*TRY*; *TCL1*; *SHN2*
UR% and VPD responsive ABA tuning	*CCD1* (*NCED1*; *NCED2*; *ETR1*
Number of stomata and stomatal density	*FAMA*; *SPCH*
Transcription factor – related genes of stress responses expressed under different VPD	*AP2*
Reference and Aquaporin-encoding genes involved in the response to elevated VPD	*NAC091* (*TIP*)
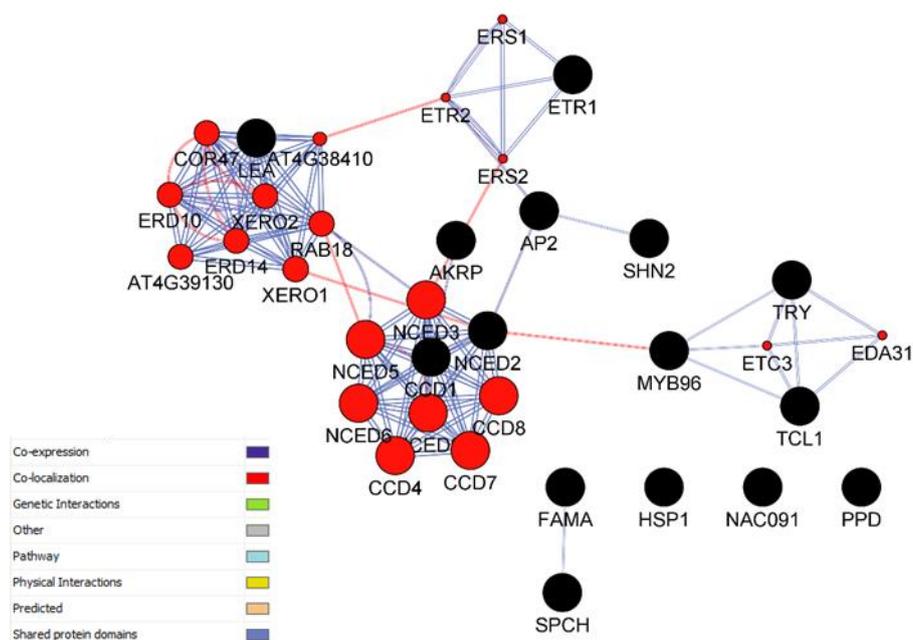
Figure 3: Gene interaction network for the parameter: pressure vapor deficit (VPD), with other genes strongly connected and with similar functions

The levels of *AKRP* (*AKR* transcripts) are light-dependent and are associated with the regulation of chloroplast differentiation [29]. On the other hand, *LEA* is related to the expression of promoter proteins in the stabilization of the membrane structure [27], while the *HSP1* is related to heat shock protein-coding [30]. The gene (*AP2*) that encodes an ethylene binding protein is related to hormonal regulation and non-growth reduction of plants under abiotic stress conditions [27]. Other response mechanisms to elevated VPDs involve ABA signaling via transcription of genes encoding ethylene production pathways. In freshly cut roses, under conditions of high RH% and low VPD, the ethylene response genes Ethylene receptor 1 (*ETR1*) prevent stomatal closure by ABA, leading to water loss under high VPD and low RH%, in the opposite way [31]. The expression of *NCED1* and *NCED2* genes in *Vitis vinifera* L. cv. Cabernet Sauvignon was accentuated and higher in roots than in leaves of plants under water stress and high VPD [8]. On the other hand, plant aquaporin proteins play an essential role in the transport of water across cell membranes, and genes coding for aquaporins such as NAC domain-containing protein 91 (NAC091 - *TIP*) are differentially expressed in higher plants and different tissues subjected to abiotic stress by high VPDs, providing tolerance [27, 28, 32]. The deposition of waxes and cuticles occurs to reflect a greater incidence of sunlight under the leaves, preventing water outflow via the epidermis and consequent dehydration. The increased transcription of the factor MYB96 (*MYB96)* gene contributes to cuticular wax biosynthesis, associated with conditions of water deficit and high VPD. Other morphoanatomical adaptation devices, such as changes in leaf area, were related to the expression of genes *PPD* under high *E* and VPD [28].

Trichomes can also be considered morphoanatomical modifications and can help reduce leaf heat and transpiration rate because they protect the plant against high UV radiation, low temperature, and RH%. Even against the high-added wind, it contributes to excessive heat and transpiration sweating as well as VPD. In *A.*

thaliana, for example, the *NTL8* gene was reported to activate the *TRY* and *TCL1* genes involved in the formation of trichomes, in addition to Ethylene-responsive transcription factor SHINE 2 (*SHN2)* [33, 34]. Other mechanisms involving the expression of *SPCH* and *FAMA* genes to regulate the number of stomata and stomatal density have been reported, preventing transpiration losses in the face of high VPD values [35]. Thus, plants shaded by particle films do not need to develop tolerance anatomical structures, such as greater cuticle thickness or synthesis of waxes that reflect excess sunlight, because the film has a white coloration, which reflects the sun's rays. In addition, particle films have a direct and efficient action to reduce leaf internal temperature, transpiration (*E*), and stomatal conductance (*gs*), favoring the reduction of VPD [6, 36]. In this sense, an expression of genes involved with cuticle production can be reduced, given the protection provided by the films. An expression of genes that code for heat shock proteins can also be reduced in plants with film, given by reducing leaf temperature provided by the films, as observed in mango trees treated with kaolin [36]. The genes involved in the production of trichomes can also be repressed in particle film treatments because there is no need for modifications to maintain the adaptation.

2.4 Water use efficiency (WUE)

The intrinsic water use efficiency can be defined as the proportion between the CO_2 assimilation rate and water lost by the stomata due to stomatal conductance (*A/gs*). In contrast, instantaneous water use efficiency corresponds to the balance between the assimilation rate of CO_2 and water lost by transpiration (*A/E*) [37]. In general, increased water use efficiency (WUE) can improve productivity, biomass generation and reduce water stress in plants grown under drought conditions. Consequently, the reduction of water loss to the atmosphere, later avoiding dehydration, in addition to maintaining leaf water potential Although the regulation of WUE occurs through stomatal pores, molecular mechanisms are still not precise [37]. Understanding the differential expression of genes assisted in the control of WUE contributes even more to better identification of plant tolerance responses. (Table 4). Furthermore, the combination of these candidate genes allows a gene interaction network with other strongly connected genes with similar functions (Figure 4).

Table 4: Genes involved in mechanisms for water use efficiency

Mechanism	Gene
WUE increase by reducing stomatal density	*EPF1*; *EPF2*; *GPA1*; *AT-GTL1* (*GTL1*)
Maintenance of leaf water content even in dry conditions	*ABCG25*; *LEA*; *RAB28*; (*HUB1*)
transmembrane potassium transporter by drought stress	*K + O*
Regulation of WUE in response to drought, VPD and ABA-mediated stomatal movement	*MYB61*; *PSBK*; *PETE1*; *ERECTA*
Synthesis of leaf waxes during dehydration events	*SUD1* (*CER9*); *FATB;* *FATB1*; *LACS1*; *CUT1* (*CER6*); *FAR3* (*CER4*)
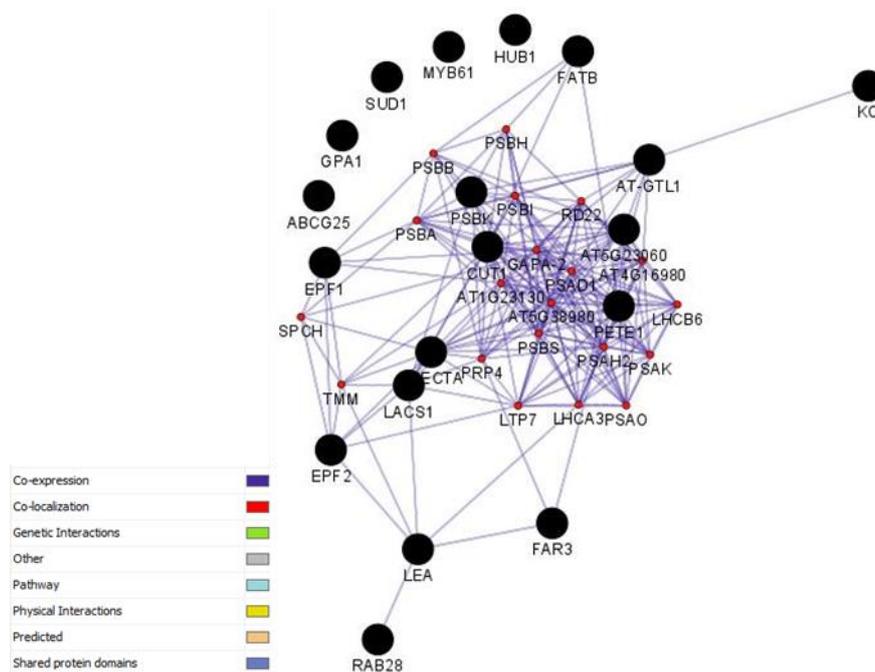
Figure 4: Gene interaction network for the parameter: water use efficiency (WUE), with other linked genes with similar functions

EPF1, EPF2, Guanine nucleotide-binding protein alpha-1 subunit *(GPA1)*, and GT-2-like 1 (*GTL1*) are intimately involved in increasing WUE in plants and related to reductions in stomatal density and *gs* under drought conditions [38]. The reduction of Ent-kaurene oxidase, chloroplastic *(K + O)*, which codes for an osmotic potassium transmembrane transporter, was recorded in different sorghum plants under water deficit at the same time that the vapor pressure deficit (VPD) reached its maximum level, suggesting that this gene promotes tolerance and increased WUE, preventing transpiration and excessive water loss (*E*) through stomatal closure [39].

The role of *MYB61* in improving WUE and drought tolerance was also evaluated in Arabidopsis plants, and coverage in the smaller opening of their stomata in response is to the application of ABA [40].

Thus, since WUE is matched by the ratio between photosynthesis and transpiration rate, candidate genes for WUE are expected to include the one made in photosynthesis. *PSBK* and *PETE1* are up-regulated and involved in the photosynthetic process, with the most significant contribution to WUE. *PETE1* regulates WUE and dry tolerance due to increased interaction with a calcium-sensitive receptor that accelerates stomatal movement and the formation of photosynthetic electron transport [19].

LRR receptor-like serine/threonine-protein kinase *ERECTA (ERECTA)*, on the other hand, is considered a valuable gene used as a proxy for the determination of WUE in Sorghum, and its expression is influenced by the vapor pressure deficit (VPD). Thus, the best time to conduct a destructive sampling for gene expression analysis is when VPD presents the maximum acquired value [39].

Overexpression of ABC transporter G family member 25 (*ABCG25)* also contributes to WUE in Arabidopsis and aids in an ABA-mediated gain in drought tolerance present in guard cells [41]. In maize, the Late embryogenesis abundant protein 31 (*RAB28)* and *LEA* group 5 genes, for example, has a protective function, contributing to the expected growth of plants even in conditions of water deficit. It occurs due to maintaining the water content inside the leaves, which contributes to cell expansion and division [42].

Another WUE candidate gene involved in maintaining leaf water content is which belongs to the *HUB1* subfamily. [26]. Regarding the synthesis of foliar waxes and the formation of the cuticle membrane during dehydration, genes Probable E3 ubiquitin ligase SUD1 (*SUD1 - CER9),* Palmitoyl-acyl carrier protein thioesterase, chloroplastic *(FATB1),* Long chain acyl-CoA synthetase 1 *(LACS1),* 3-ketoacyl-CoA synthase *(CUTI - CER6),* Fatty acyl-CoA reductase 3 *(FAR3 - CER4)* are associated with improving water use efficiency. Plants with Probable E3 ubiquitin ligase SUD1 (*SUD 1 -CER9)* expression, for example, have high cuticle thickness over epidermal cells and cuticular protrusions with increased occlusion of the stomatal pore, delaying the onset of wilting in plants with water deficit, lower transpiration rates [43].

The increase in water use efficiency has been one of the main physiological variables associated with the use of particle film in plants grown under water deficit conditions. Thus, several genes regulating photosynthesis and control water loss can be differentially expressed in plants with particle film treatment in response to drought. Especially, genes involved in the formation and development of stomata (number, cuticle, and density), synthesis of leaf waxes, and control of stomatal movement [6].

2.5 Instant Carboxylation Efficiency (A/Ci)

The carboxylation efficiency is measured by the ratio between the photosynthetic rate (*A*) and the internal carbon concentration (*Ci*) per air mole. This mechanism is involved in the diffusion of CO_2 from the stomatal chamber, towards the chloroplasts via conductance. Different mechanisms facilitate the diffusion of CO_2 to the chloroplasts via mesophiles. However, several barriers can still influence this pathway, including air, cell walls, lipid membranes, cytoplasm, and liquid stroma [44] (Table 5). Furthermore, the combination of these candidate genes allows a gene interaction network with other strongly connected genes with similar functions (Figure 5).

Table 5: Genes involved in instantaneous carboxylation efficiency mechanisms

Mechanism	Gene
Expression of aquaporins	*PIP1*; *PIP2*
Genes involved with stomatal conductance (*gs*)	The same as in table 1.
Genes Involved with Photosynthetic Rate (*A*)	The same as in table 2.
Genes expressed in PEPcase enzyme expression of C4	*PPC3 (PEPC)*
Genes expressed in the expression of RuBisCO units from C3 plants	*RBCL*
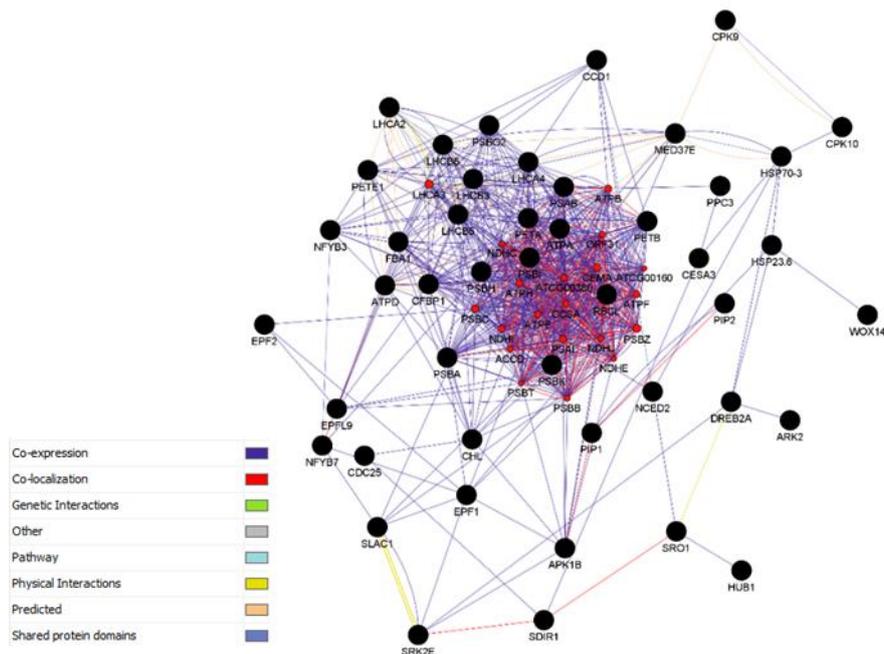
Figure 5: Gene interaction network for the parameter: instantaneous carboxylation efficiency (*A / Ci*), with other strongly connected genes with similar functions

Aquaporins, also marked as Major Intrinsic Proteins (MIPs), are membrane proteins distributed in plant tissues and responsible for water transport and small solutes. In higher plants, such as spermatophytes, aquaporins are divided into five specific groups: PIPs, TIPs, NIPs, SIPs, and XIPs, invitations in the membranes of different organs and plant organelles. Aquaporins have the function of establishing mechanisms for the diffusion of CO_2 from the atmosphere to carboxylation sites in chloroplasts, favoring photosynthetic activity, conductance and permeability in chloroplast membranes [45].

An expression of genes related to the formation of aquaporins has contributed to conductance in the mesophile and permeability in chloroplast membranes. For example, in coffee plants subjected to water restriction, PAMP-induced secreted peptide (*PIP1, PIP2)* was verified. Even so, no relationship was reported with the conductance of CO_2 in the mesophile or membrane of chloroplasts [45].

The phosphoenolpyruvate carboxylase (PEPcase) expressed by the Phosphoenolpyruvate carboxylase 3 (*PPC3 – PEPC)* gene is related to the carboxylation of CBB cycle in C4 plantas and presents higher efficiency for carboxylation [47]. The Ribulose bisphosphate carboxylase large chain (*RBCL)* gene was involved in the expression of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) enzymes in C3 plants, promoting greater use of intercellular carbon (*Ci*), contributing to genetic responses of carboxylation efficiency [17].

Plants shaded by particle films tend to have the greater photosynthetic capacity, verified by *A*'s addition. The film can also control *gs* and *E* for CO_2 diffusion to the intercellular spaces (*Ci*) [5]. Thus, carboxylation efficiency can be favored. The expression of genes coding for RuBisCO can be increased with the increasing of *Ci*.

An expression of aquaporin genes under the effects of particle films related to increases in instant carboxylation efficiency deserves further investigation. There is a double hypothesis that gene expression is reduced considering the films' protection and the water status maintenance. The stomatal opening for CO_2 influx requires the least aquaporins or increased by substrate availability (CO_2).

2.6 Chlorophyll Fluorescence a

The excess light energy for photosynthesis is dissipated in heat conditions through the fluorescence of chlorophyll *a* and represents about 0.5-10% of the energy absorbed by chloroplasts. This dissipation mechanism occurs in photosynthetic tissues and provides photoprotection to leaves when subjected to high sunlight (400-700 nm). Several physiological parameters are involved in the chlorophyll fluorescence signal, making them essential indicators of photosynthetic performance [47].

A combination of these parameters allows identifying possible damage to the photosynthetic apparatus, such as the blocking of the linear flow of electron sheets in the electron transport chain or temporal variations in the photosynthetic efficiency of plants [48].

The ratio between variable fluorescence and maximum fluorescence (Fv / Fm) is one of the main chlorophyll fluorescence parameters used to increase photoinhibition resistance, in addition to acting under the photosynthetic performance index (PIABS), electron transport chain (ETR), and other parameters in the identification of possible blocks and reduction of energy dissipation in photosystems, and NPQ, which dissipates non-photochemical energy in the form of heat, in the xanthophyll cycle [47].

Responses provided by differential gene expression in chlorophyll fluorescence are in Table 6. Furthermore, the combination of these candidate genes allows a gene interaction network with other strongly connected genes with similar functions (Figure 6).

Table 6: Genes involved in chlorophyll fluorescence mechanisms

Mechanism	Gene
Chlorophyll and carotenoid biosynthesis	*CHLD*; *CHLH*; *GUN4*; *CRD1* (*ACSF*); *CHL*; *CHLP*; *CRT2* (*CRTB*); *PDS*; *GPRI1* (*GLK1*); *PORA*
Increased PSII efficiency (Fv/Fm)	*LEA1*; *LEA2*
chlorophyll rates and fluorescence mechanisms	*PPC3* (*PEPC*); *PPDK*
Fluorescence-related photosynthesis genes	*PSBA*; *PSBB*; *PSBC*; *PSBD*
Non-photochemical energy dissipation in the xanthophyll cycle	*CHY1*; *VDE1*; *ZEP* (*ABA1*)
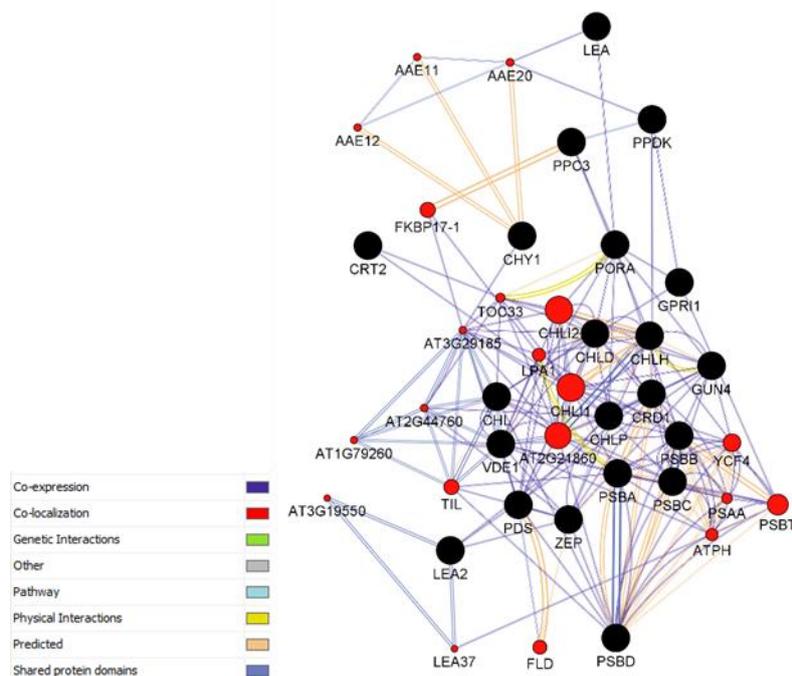
Figure 6: Gene interaction network for the parameter: chlorophyll a fluorescence, with other linked genes with similar functions

In soybeans, Mg-protoporphyrin IX chelatase (*CHLD)*, Tetrapyrrole-binding protein, chloroplastic (*GUN4)*, and Magnesium-chelatase subunit ChlH, chloroplastic (*CHLH)* catalyze the insertion of Mg²⁺ into protoporphyrin IX, which is a rate-limiting step in the rate of chlorophyll biosynthesis [49].

In addition, Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase, chloroplastic (*CRD1 – ACSF)*, *CHL*, Geranylgeranyl diphosphate reductase, chloroplastic (*CHLP)*, Calreticulin-2 (*CRT2 – CRTB)*, 15-cis-phytoene desaturase, chloroplastic/chromoplastic (*PDS)* are also associated with chlorophyll and carotenoid biosynthesis [50]. For example, GBF's pro-rich region-interacting factor 1 (*GPRI1 - GLK1)* plays a role in peanut crops as a transcription factor that positively regulates NADPH-protochlorophyllide oxidoreductase (*PORA)* expression during post-dry recovery, stimulating chlorophyll biosynthesis and photosynthesis [51].

The increase in photochemical efficiency of photosystem II is directly related to the Fv / Fm fluorescence parameter. The *LEA1* and *LEA2* genes that code for LEA proteins dissipated overexpression in *Boea hygrometric* plants and was correlated with increases in Fv/ Fm values [52]. In addition, the *PEPC* and Pyruvate, phosphate dikinase 1, chloroplastic (*PPDK)* genes, were associated with the maintenance of chlorophyll rates and fluorescence mechanisms, possibly due to the efficiency of the PEPcase enzyme in promoting electron flow, preventing thus photooxidation and damage to photosystems [53].

The *PSBA*, Photosystem II CP47 reaction center protein (*PSBB)*, Photosystem II CP43 reaction center protein (*PSBC)*, Photosystem II D2 protein (*PSBD)* genes express proteins that make up an electron transport chain and are directly related to chlorophyll fluorescence and protein D1 in the center of PSII. In this sense, plants subjected to environmental stress start degrading D1 when these genes are down-regulated, later causing

destruction of the PSII reaction center and blocking of electron transfer. Under conditions of plant adaptation, D1 is rapidly synthesized, restoring the values of Fv / Fm, PIABS, and similar to non-photochemical NPQ dissipation [50].

The 3-hydroxyisobutyryl-CoA hydrolase 1 (*CHY1)* gene encodes for an expression of β-carotene hydroxylase that offers essential PSII photoprotection. In addition, its expression can be identified through NPQ readings [54]. The *VDE1* and Zeaxanthin epoxidase, chloroplastic (*ZEP - ABA1)* genes also participate in thermal dissipation, inversely proportional to fluorescence emission [55]. The photoprotection mechanism promoted by the particle film can be proven by the increase in the photochemical efficiency of the photosystem (PSII), assessed by the ratio between variable fluorescence and the maximum fluorescence (Fv / Fm) and by the increase in the electron transport rate (ETR), with reduced NPQ, even under conditions of stress [5, 6].

3. Conclusion

Potential genes have been identified to monitor ecophysiological parameters in plants under abiotic stress. GeneMANIA and Cytoscape software was instrumental in creating gene and graphical representation encompassed other closely linked genes with similar functions. For several studies on transcriptomics, this information becomes a crucial tool in characterizing and identifying genes involved in plant-environment interactions or response pathways under abiotic stress. From the perspective of particle film technology, is possible that under the same environmental conditions, genes express themselves differently in plants with particle films. As it is a recent technology, particle films still require new molecular data, especially in quantifying gene expression in response to environmental conditions.

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