Chapter

Insights into Metabolic Engineering of the Biosynthesis of Glycine Betaine and Melatonin to Improve Plant Abiotic Stress Tolerance

Cisse El Hadji Malick, Miao Ling-Feng, Li Da-Dong and Yang Fan

Abstract

Metabolic engineering in plant can be describe as a tool using molecular biological technologies which promotes enzymatic reactions that can enhance the biosynthesis of existing compounds such as glycine betaine (GB) in plant species that are able to accumulate GB, or produce news compounds like GB in non-accumulators plants. Moreover we can include to these definition, the mediation in the degradation of diverse compounds in plant organism. For decades, one of the most popular ideas in metabolic engineering literature is the idea that the improvement of gly betaine or melatonin accumulation in plant under environmental stress can be the main window to ameliorate stress tolerance in diverse plant species. A challenging problem in this domain is the integration of different molecular technologies like transgenesis, enzyme kinetics, promoter analysis, biochemistry and genetics, protein sorting, cloning or comparative physiology to reach that objective. A large number of approaches have been developed over the last few decades in metabolic engineering to overcome this problem. Therefore, we examine some previous work and propose some understanding about the use of metabolic engineering in plant stress tolerance. Moreover, this chapter will focus on melatonin (Hormone) and gly betaine (Osmolyte) biosynthesis pathways in engineering stress resistance.

Keywords: metabolic engineering, biosynthesis, molecular, abiotic stress, stress tolerance

1. Introduction

The global climate change influence negatively plant growth and development via the increase of the intensity of various abiotic stresses such as drought, chilling, salinity, waterlogging or flooding. Environmental stresses are one of the most threatening factors that can cause massive losses in agricultural crop production, ranging from 50–70% [1]. Plant biotechnology and engineering are promising

platform for exploring the unlimited potential of many various plants species [2]. In recent years, plant metabolism engineering provides successful pathways to increase the production of metabolites that can significantly counterattack the damages caused by diverse abiotic stresses [3]. To improve stress tolerance in plant, various metabolic engineering technologies were used to introduce or increase the synthesis of diverse osmolytes, secondary metabolites or hormones. The adaptation of various plant species to stressful environments can be managed through: (i) the identification of diverse mechanisms developed by plants to counterbalance abiotic stresses (ii) and the improvement of these processes in plants by metabolic engineering [4, 5]. Plant by-products including hormone (melatonin, MT) and osmoprotectant (glycine betaine, GB) that play a prominent roles in plant stress tolerance have been targeting in various plant species to counterattack environmental stresses. The clarification of the biosynthetic pathway of various plant compounds has provided the possibility to metabolically engineer new capabilities in plants as well as successfully engineer whole pathways into microbial systems [6]. Under environmental stresses plant is able to accumulate different molecules such as melatonin or glycine betaine to provide stress tolerance by counteracting with oxidative stress caused by drought, chilling, salinity or heavy metal stresses [7–9]. The protective properties of GB and MT in plant under abiotic stresses had made these substances targets for plant engineering resistance.

The natural biosynthesis of glycine betaine takes place in marine algae and various higher plant species belong to diverse families, counting the Gramineae, Malvaceae, Asteraceae, or Amaranthaceae [10–14]. Glycine betaine accumulation in non-accumulators and accumulators plant species under environmental stresses has long been a target for engineering stress resistance [15, 16]. The biosynthesis of glycine betaine passes by choline → betaine aldehyde → glycine betaine pathways. Most of the enzymes involving in these pathways such as choline monooxygenase (CMO) or betaine aldehyde dehydrogenase (BADH) have been identified, and genes for some of them have been cloned [4, 13].

Indeed, GB as a non-toxic molecule is biosynthesized through two phases of choline oxidation: the first step (Choline → betaine aldehyde) is catalyzed by CMO, and the second step (Betaine aldehyde → glycine betaine) is activated by BADH [13, 17]. The expression of CMO or BADH in tobacco has been done via the cDNA from two natural glycine betaine accumulators; spinach and sugar beet plants. The 35S promoter from plant virus, cauliflower mosaic virus which is a fundamental element of transgenic constructs in the majority of genetically modified plant species was used in transgenic tobacco to control the expression of cDNA for BADH pathway [18]. Also, a crucial tool in metabolism engineering of glycine betaine pathway is the use of a single gene codA from *Arthrobacter globiformis* which is involved in the synthesis of GB [19]. However, GB accumulation in transgenic plants depends on the capacity of endogenous choline uptake, the type of gene that catalyzes the GB biosynthetic pathway, and the localization of the transgene product in a particular cellular compartment [20].

Melatonin a plant hormone identified in a wide variety of animals and plants, has been extensively studied in plants for its properties to counteract with various environmental and biotic stresses [21, 22]. Transcriptome analyses indicated that melatonin primarily affects the pathways of plant hormone signal transduction and biosynthesis of secondary metabolites [23]. In plant the biosynthesis of melatonin is initiated with tryptophan which is converted in serotonin, and between the tryptophan and melatonin, the enzymes hydroxyindole-*O*-methyltransferase and caffeic acid O-methyltransferase (ASMT/COMT) catalyzed a reaction that produce an intermediate molecule named 5-methoxytryptamine [24–26]. The related enzymes involved in melatonin biosynthesis pathway have been targeted

to improve stress tolerance in diverse plant species. The over expression of COMT like gene (TaCOMT) in a transgenic *Arabidopsis* via various metabolic engineering techniques (cloning, transgenesis, genetics or promoter analysis) provided drought tolerance by increasing the concentration of melatonin [27]. Other enzymes such as serotonin N-acetyltransferase (MsSNAT) involve in melatonin biosynthesis have been targeted in rice [28] or *Arabidopsis* [29] to provide stress tolerance, either to clarify the role of melatonin in plant. This chapter will focus on the use of glycine betaine, spermidine and melatonin in plant metabolism engineering, particularly in stress engineering.

2. Glycine betaine and metabolism engineering

Glycinebetaine is a quaternary ammonium compound that appears commonly in a large diversity of plants, animals and microorganisms, the first betaine discovered was trimethylglycine (**Figure 1**) named also N, N,N-trimethylglycine [8, 12]. The glycine betaine as a osmolytes is a crucial non-toxic molecule that is accumulated in various plant species under environmental stresses [15].

2.1 Glycine betaine biosynthesis

GB synthesis begins with an essential molecule named choline, synthesized through three sequential adenosyl-methionine dependent methylations of phospho-ethanolamine catalyzed by the cytosolic enzyme phosphoethanolamine methyltransferase (phosphoethanolamine N-methyltransferase) [30]. In plant, the biosynthesis of GB is two steps of oxidation initiated with choline and then betaine aldehyde (**Figure 2**). In plant such as *Arabidopsis* the biosynthesis of choline can be resume by this following line: L-serine → ethanolamine → *O*-phosphoethanolamine → N-methylethanolamine phosphate → N-dimethylethanolamine phosphate → phosphocholine → choline [31–33]. Pursuing the transformation of N-methylethanolamine phosphate by phosphoethanolamine methyltransferase (PeMt) the byproduct differs according to the plant species, for instance in that stage the spinach produce choline like in *Arabidopsis* choline biosynthesis pathway, meanwhile in tobacco PeMt catalyzed a reaction that synthesize phosphatidylcholine in the first place then metabolized to choline [8, 15]. The first stage of GB biosynthesis is modulated by CMO which is an Fd-dependent monooxygenase with a Rieske-type iron–sulfur (2Fe-2S) cluster-binding motif. The second stage of GB

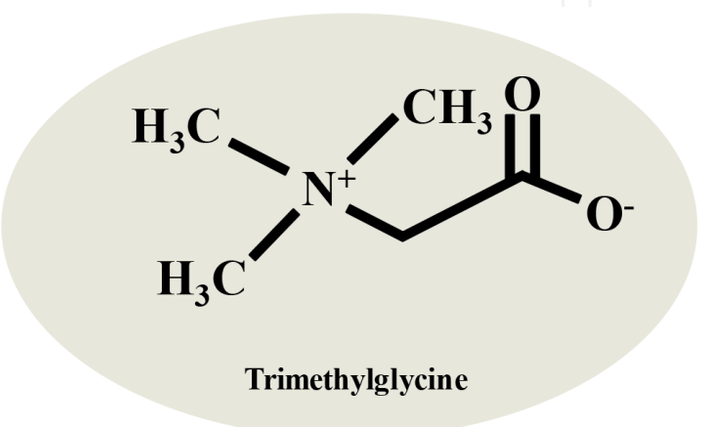

Figure 1.
N,N,N-trimethylglycine.

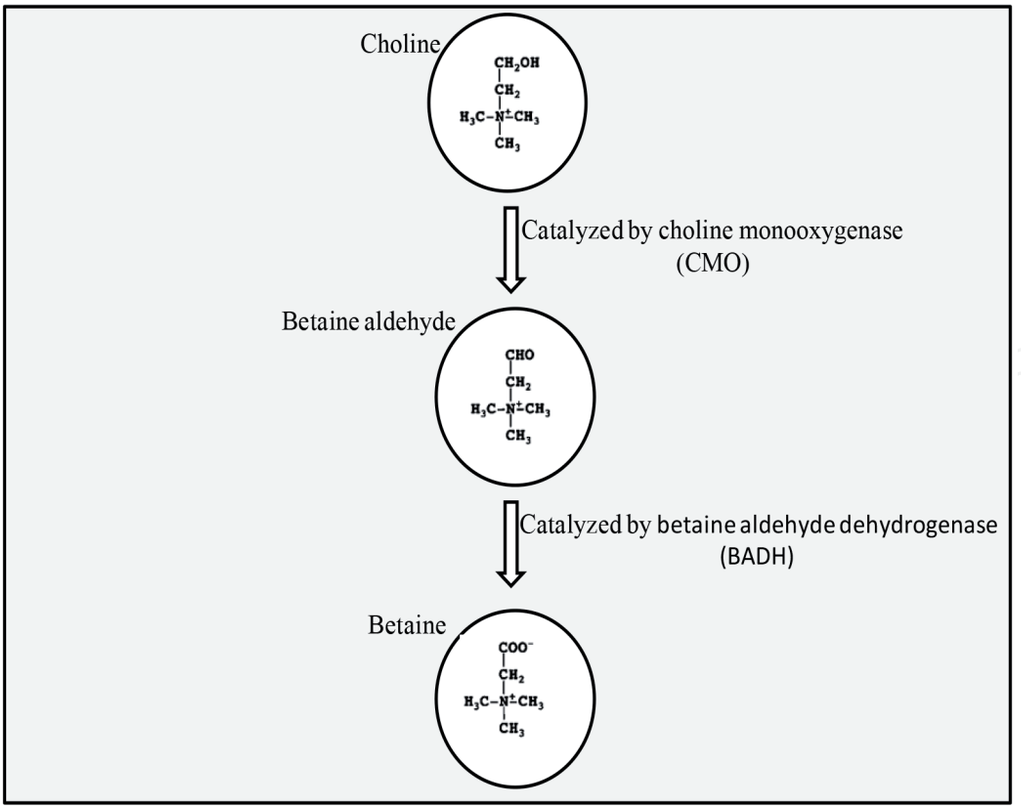

Figure 2.
Diagram of GB biosynthesis in brief.

biosynthesis is catalyzed by BADH, an enzyme belong to the superfamily of aldehyde dehydrogenases which is an NAD^+ or $NADP^+$ dependent [17, 34].

2.2 Glycine betaine and environmental stress

Many plants are able to accumulate naturally GB and diverse osmoprotectants to balance the disruption of plant cell homeostasis caused by environmental stress such as drought, chilling, salinity or high temperature [8, 35, 36]. Many studies have been reported on the positive effect of endogenous GB in plants under abiotic stresses. The role of glycine betaine in osmotic adjustment was related in *Amaranthus tricolor* [37] and *Hordeum maritimum* [38] under salinity. The role of GB against oxidative stress via scavenging the reactive oxygen species and increasing the antioxidant activities was reported in many studies [39–41]. For these reasons, the use of glycine betaine in non accumulator and accumulator plant species become more popular in plant physiology. Indeed, several reports have related the positive effect of GB in transgenic plants (**Table 1**).

2.3 Glycine betaine engineering

The idea of introducing GB pathway and its high accumulation in plant under environmental stresses has long been a target for metabolism engineering stress tolerance. The feasibility of this process was based on comparative physiology and genetic evidence from a maize mutant [15, 54]. Metabolic engineering of the biosynthesis of GB from choline by using various genes such as cod*A* or BADH gene gained more attention to improve stress tolerance in crop and woody plants that are incapable of synthesizing GB under abiotic stresses [8, 18, 55]. Moreover, genetic engineering is also use to increase GB accumulation in various plant species which

Transgenic species	GB Acc./ GB N-Acc.	Type of abiotic stress	Role in stress tolerance	References
Nicotiana tabacum	GB N-Acc.	Salinity	Protection of the photosynthetic apparatus	[42]
Zea mays	GB Acc.	Chilling stress	Protect photosynthesis, Homeostasis	[43]
Synechococcus sp.	GB N-Acc.	Low-Temperature	Enhanced Photosynthesis	[44]
Oryza sativa	GB N-Acc.	Salinity, Chilling stress	Improve photosynthesis and phenotype	[45]
Gossypium hirsutum	GB Acc.	Drought	Osmotic adjustment, enhance yield	[46]
Nicotiana tabacum	GB N-Acc.	Salinity	Phenotypic traits	[47]
Triticum aestivum	GB Acc.	Heat and drought stress	Promoted photosynthesis, antioxidant and water status	[48]
Lycopersicum esculentum	GB N-Acc.	Salinity	Protect photosynthesis and reproductive organs	[49]
Lycopersicum esculentum	GB N-Acc.	High temperature	Enhanced the expression of heat-shock genes	[50]
Oryza sativa	GB N-Acc.	Water stress	Enhance Survival rate and agronomic traits	[51]
Lycopersicum esculentum	GB N-Acc.	Chilling stress	Promoted ROS scavenge	[52]
Brassica chinensis	GB N-Acc.	High salinity and high temperature	Promote photosynthesis	[53]

Table 1.
Reported roles of GB in transgenic plant under abiotic stresses.

produce a low concentration of GB that might not be sufficient for osmoregulation to counteract with abiotic stress [56].

The genes (codA or cDNA BADH) and enzymes involve in GB biosynthesis have been identified and cloned. GB has been successfully synthesized in various targeted organisms and provided stress tolerance via genetic engineering (**Table 2**).

2.3.1 Genetic engineering of GB via codA gene

As shown in **Table 2**, many species that can accumulate or not GB have been targeted via genetic engineering to synthesize or over accumulate GB under both stressed and non-stressed conditions. The choline oxidase (codA) from *A. globiformis* has been widely used in various transgenic plant species to synthesize GB, and *codA* has the ability to convert choline in one reaction [56].

The catalytic activity of choline oxidase (EC: 1.1.3.17) in *A. globiformis* results in this following equation: (Choline + H_2O + 2 O_2 = glycine betaine + H^+ + 2 H_2O_2) [63].

Transgenic species	GB Acc./ GB N-Acc.	Genes targeted	Protein Encoded	Organism sources/ Promoter	Roles in plant	References
Oryza sativa	GB N-Acc.	codA	Choline oxidase	*Arthrobacter globiformis*	Water stress tolerance	[51]
Zea mays/ *Glycine max*	GB Acc.	GB1(novel gene)	GB1 protein	*Zea mays* H-GB genotype / - *Agrobacterium* - Rice actin and - 35S promoter	Enhanced endogenous GB synthesis	[57]
Nicotiana tabacum	GB N-Acc.	cDNA sequence	BADH	*Spinacia oleracea* and *Beta vulgaris*	Betaine aldehyde resistance	[13]
Lycopersicum esculentum	GB N-Acc.	codA	Choline oxidase	*Arthrobacter globiformis*	Modulation of phosphate homeostasis under stress	[58]
Lycopersicum esculentum	GB N-Acc.	codA	Choline oxidase	*Arthrobacter globiformis*	Reproductive organs regulation	[59]
Brassica juncea	GB Acc.	codA	Choline oxidase	*Arthrobacter globiformis*	Photo inhibition tolerance	[11]
Nicotiana tabacum	GB N-Acc.	BADH cDNA	BADH	*Hordeum vulgare*	GB synthesis in non accumulator plant	[60]
Nicotiana tabacum	GB N-Acc.	BADH cDNA	BADH	*Escherichia coli*	Salt tolerance	[47]
Eucalyptus camaldulensis	GB Acc.	codA	Choline oxidase	*Arthrobacter globiformis*/ CaMV 35 promoter	Enhance of GB biosynthesis	[61]
Eucalyptus globulus	GB Acc.	codA	Choline oxidase	*Arthrobacter globiformis*	GB accumulation	[62]
Triticum aestivum	GB Acc.	BADH gene	BADH	*Atriplex hortensis*	Stress tolerance	[48]

Table 2.
Overview of GB genetic engineering in various plant species.

The codA gene is of particular interest with respect to the engineering of desirable productive traits in crop plants and stress tolerance. In transgenic tomato and brown mustard the codA was targeted to the chloroplast and cytosol which allowed GB accumulation for an increase of stress tolerance [19, 59]. Further, transgenic *indica* rice showed a significant increase of water-stress tolerance and transcriptome changes via codA gene expression [51]. One of the advantages of using choline oxidase pathway as a tool for engineering GB synthesis in plant is that the addition of a single gene codA is enough for the conversion of choline to GB [8]. The codA transgenic plant has showed their abilities to counteract with environmental stresses such as salinity, high temperature, high light, cold stress and freezing in different plant growth stages [64].

2.3.2 Genetic engineering of GB via BADH gene

The other pathway that provided successful results in genetic engineering of GB biosynthesis in various transgenic plant species is the BADH pathway (**Table 2**). BADH is one of the most prominent genes involved in the biosynthetic pathway of GB, and its utilization in various plant species has led to an increased tolerance to a variety of environmental stresses [65]. Indeed, the second step of GB biosynthesis is performed by betaine aldehyde dehydrogenase (BADH) that can be encoded by *betB* or *betA* gene from *E. coli*. BADH is an NDA-dependent dehydrogenase that has been characterized and cloned from plants species belong to the Amaranthaceae and Gramineae families [15]. The BADH pathway has been targeted in the chloroplasts in *N. tabacum* [13] and in peroxisomes in Gramineae [60]. Many studies showed positive results in stress tolerance in transgenic plants with genes *betA*, *betB* or both from *Escherichia coli* encoding Oxygen-dependent choline dehydrogenase (CHDH) and BADH [8]. The catalytic activities of CHDH (EC: 1.1.99.1) encode by *betA* from *E. coli* can be resume by this following Eq. (A + choline = AH2 + betaine aldehyde), A (hydrogen acceptor) and AH2 (hydrogen donor) [66]. Meanwhile the catalytic activities of the NAD/NADP-dependent betaine aldehyde dehydrogenase (EC: 1.2.1.8) are done by this equation: (betaine aldehyde + H2O + NAD^+ = glycine betaine +2 H^+ + NADH) [66, 67]. The equation for the catalytic activities is similar for chloroplastic betaine aldehyde dehydrogenase in sugar beet or spinach compared to those of *E. coli*.

3. Metabolism engineering of melatonin

Melatonin (**Figure 3**) as an ancient pleiotropic bio-molecule which can be traced back to the origin of life, is present in both animal and plant organisms [24, 68]. In plant, melatonin has been found in diverse family and at different stage of growth: Asteraceae, papaveracea, apiaceae, linaceae, fabaceae, poaceae, rosaceae, lamiaceae, solanaceae, musaceae or vitacea etc. [69].

Melatonin (N-acetyl-5-methoxytryptamine), a multifunctional plant hormone, was discovered in plants in 1995 [70]. Moreover, the presence of melatonin in plant was confirmed in *Chenopodium rubrum* via chromatography/tandem mass spectrometry and radio-immuno-assays [71]. Melatonin has multi-functional actions

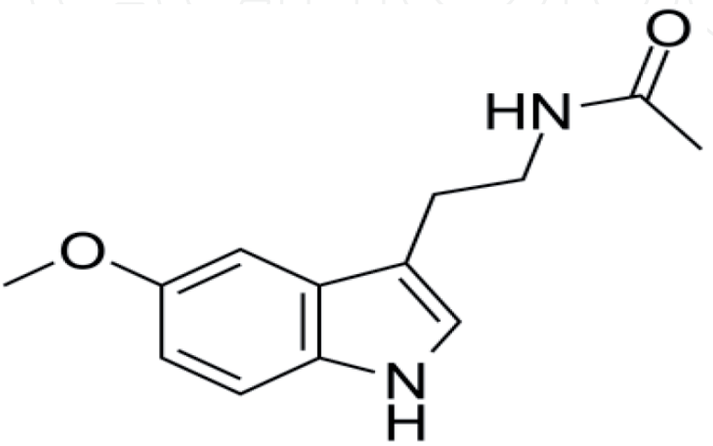

Figure 3.
N-acetyl-5-methoxytryptamine.

that improve cellular and organ health in various plant species and it is a powerful antioxidant in both animals and plants [72].

Melatonin functions as a metabolite with numerous roles in plant, including plant stress responses such as chilling, oxidative stress, drought, salt stress and nutrients deficiency, moreover melatonin can regulates plant growth and development, such as root organogenesis, flowering, and senescence [9, 73, 74]. Plenty of studies have focused on the function and regulation of melatonin in transgenic plants because of its crucial role in plant regulation.

3.1 Melatonin biosynthesis pathways in plant

The **Figure 4** shows a schematic representation of the biosynthesis of MT, in which the tryptophan is synthesized via shikimic acid pathway that is also responsible for the synthesis of vitamins and aromatic amino acids such as phenylalanine and tyrosine. In plants, tryptophan is converted to Tryptamine via a reaction catalyzed by tryptophan decarboxylase (TDC) [75], and the production of serotonin from Tryptamine is activated by tryptamine 5-hydroxylase [76]. The formation of melatonin is preceded by two reactions from serotonin; the first reaction catalyzed by ASMT transform serotonin to 5-methoxytryptamine, and the last step is catalyzed by N-acetyltransferase [77].

As far as we know, there are 6 genes which are involved in plant melatonin biosynthesis: TDC, TPH, T5H, SNAT, ASMT, and COMT [68], and the keys enzymes they encoded are the; L-tryptophan decarboxylase, tryptophan hydroxylase, serotonin-N-acetyltransferase, N-acetylserotonin methyltransferase and hydroxyindole-O-methyltransferase [24].

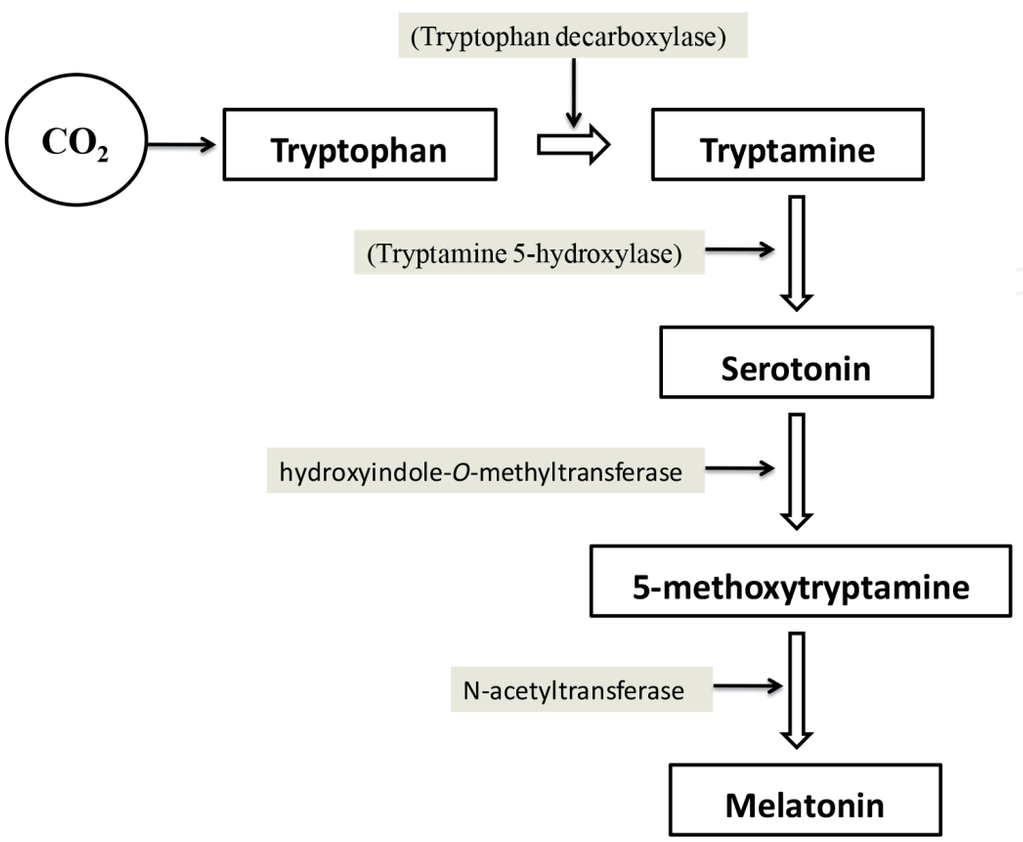

Figure 4.
A schematic representation of melatonin biosynthesis in brief.

3.2 Melatonin involve in abiotic stress tolerance

Melatonin is well know as a hormone which can significantly increase the plant survival rates, photosynthetic efficiency and antioxidant activities in plant under environmental stress [74, 78]. For these reasons, many studies were focused on the effects of exogenous melatonin on various plant species under abiotic stress. Indeed, exogenous melatonin could stimulate the biosynthesis of cold tolerance agents and contribute to increase the plant growth and development under cold stress [79]. As show **Table 3**, the alleviation of environmental stresses by melatonin has been investigated in many plant species: under drought (Zea mays) [89], under heavy metal (Caryaca thayensis) [90], under chilling stress (*Cynodon dactylon*) [91] and under salinity (*Cucumis sativus*) [82]. Compared to glycine betaine genetic engineering in plant under stress, the use of melatonin in transgenic plant to provide stress tolerance is fewer. However, there is several studies that focused on the over expression of melatonin via metabolic engineering (**Table 3**).

In Transgenic Arabidopsis the over expression of N-acetyltransferase gene increased salt tolerance via the increase in autophagy, and the reestablishment of redox and ion homeostasis [29]. Furthermore, increase of over-expressing N-acetyltransferase gene enhances the endogenous content in transgenic rice that provoked pleiotropic phenotypes, including enhanced seedling growth, delayed flowering, and low grain yield [28].

Plant species	Transgenic/exogenous	Stress	Role in stress	References
Oryza sativa	Transgenic	Chilling	Promote photosynthesis	[80]
Malus hupehensis	Exogenous MT	Salt stress	Boost antioxidant system	[81]
Arabidopsis thaliana	Transgenic	Drought	Enhanced melatonin content	[27]
Cucumis sativus	Exogenous MT	Salt stress	Enhanced the rate of germination	[82]
Lycopersicum esculentum	Transgenic	Drought	Enhanced melatonin content	[83]
Oryza sativa	Transgenic	Heavy metal stress (Cadmium)	Enhanced stress tolerance	[84]
Oryza sativa	Transgenic	Herbicide	oxidative stress resistance	[85]
Nicotiana sylvestris	Transgenic	UV-B radiation	Reduced DNA damages	[86]
Phacelia tanacetifolia	Exogenous MT	high temperature and light	Promoted germination	[87]
Lycopersicum esculentum	Transgenic	Salt stress	ROS scavenge	[88]
Arabidopsis thaliana	Transgenic	Salt stress	Increase in autophagy and rebalance homeostasis	[29]

Table 3.
Reported roles of MT exogenously applied and in transgenic plant under abiotic stresses.

Transgenic species	Genes targeted	Protein encoded	Organism source/ transformer/vector	Functions	References
Medicago sativa	*MsASMT*$_1$	N-acetylserotonin methyltransferase	*Alfalfa*/*Agrobacterium* strain EHA105/pZh01-MsASMT1 vector	Ameliorated Plant Growth	[92]
Panicum virgatum	*AANAT and HIOMT*	arylalkylamine N-acetyltransferase / hydroxyindole O-methyltransferase	Ovine/*Agrobacterium*-mediated method / vector Ubi1301	Improved growth and salt tolerance	[93]
Oryza sativa	*ASDAC*	N-acetylserotonin deacetylase	Rice/*Agrobacterium tumefaciens*/ pTCK303:ASDAC RNAi binary and pIPKb002:ASDAC vector	Regulation of melatonin in plant	[94]
Arabidopsis thaliana	*cDNA TaCOMT*	Caffeic acid 3-O-methyltransferase	Wheat/*Agrobacterium tumefaciens* strain GV3101 / pCAMBIA1302-TaCOMT vector	Promoted drought tolerance	[27]
Oryza sativa	*OaSNAT* (*SNAT*)	Serotonin N-acetyltransferase	Sheep/*Agrobacterium*-mediated method	Homeostasic regulation of melatonin	[95]
Arabidopsis thaliana	*MsSNAT*	serotonin N-acetyltransferase	Alfalfa/*Agrobacterium*-mediated method	Salt tolerance	[29]
Arabidopsis thaliana	*MzASMT*$_1$ (*ASTM*)	N-acetylserotonin-O-methyltransferase	Apple/35S promoter	Drought tolerance	[96]
Panicum virgatum	*HIOMT*	hydroxyindole O-methyltransferase	Ovine/*Agrobacterium*-mediated method	biosynthetic and physiological functional networks of melatonin	[97]
Nicotiana sylvestris	*AANAT HIOMT*	arylalkylamine N-acetyltransferase/ hydroxyindole-O-methyltransferase	*Agrobacterium tumefaciens*-mediated transformation	Inhibited UV-B-induced DNA damage	[86]
Lycopersicum esculentum	*SlCOMT1*	caffeic acid O-methyl-transferase	Tomato/*Agrobacterium* LBA4404/pMD18-T cloning, pCXSN-Myc, SlCOMT1-Myc over-expression vectors	Salt tolerance	[88]

Table 4.
Overview of MT metabolic engineering in diverse plants.

3.3 Melatonin in plant metabolism engineering

Previous studies using genetic engineering (transgenic plant) in various plants species with low or high MT accumulation has been achieved to determined the role of MT in plant growth regulation, stress tolerance or MT function in plant (**Table 4**). Indeed it was reported the implication of MT in seed germination, root development, fruit ripening, senescence, yield, circadian rhythm and plant homeostasis [98]. Ectopic over-expression (transgenesis) of human serotonin N-acetyltransferase increased endogenous melatonin that allowed transgenic rice

seedlings to face chilling stress [80]. The increase of endogenous melatonin in various transgenic plant organisms compared to the wild type has been reported in *Arabidopsis thaliana* [29], in *Lycopersicum esculentum* [88] or in *Medicago sativa* [92].

Most of the studies in MT transgenesis are based on the ability of *Agrobacterium* to transfer DNA to plant cells by genetic engineering (**Table 4**). Indeed *Agrobacterium tumefaciens* is a widespread naturally occurring soil bacterium which demonstrated a great ability to introduce new genetic material into diverse plant cell species [99]. The *Agrobacterium*-mediated transformation process can be resumed in this following line: 1- Isolation of the targeted genes → 2- development of a functional transgenic construct → 3- insertion of the transgene → 4- introduction of the T-DNA-containing-plasmid into *Agrobacterium* → 5- mixture of the transformed *Agrobacterium* with plant cells → 6- regeneration of the transformed cells into transgenic plant → 7- testing for trait performance or transgene expression [99–101]. The catalytic activities of different enzymes involved in MT metabolic engineering have been elucidated in various species. The catalytic activity of Acetylserotonin O-methyltransferase (EC: 2.1.1.4) encoded by *ASMT* gene in *Homo sapiens* is done by this following line: (*N*-acetylserotonin + *S*-adenosyl-L-methionine = H^+ + melatonin + *S*-adenosyl-L-homocysteine) [102]. The catalytic activity of Serotonin N-acetyltransferase (EC: 2.3.1.87) from *Ovis aries* (Sheep) encoded by *AANAT* gene is done by this reaction: (2-arylethylamine + acetyl-CoA = CoA + H^+ + *N*-acetyl-2-arylethylamine) [103]. Moreover the catalytic activity of Caffeic acid 3-O-methyltransferase (EC: 2.1.1.68) implicated in many MT genetic engineering manipulations has been decoded in Medicago sativa (Alfalfa): ((*E*)-caffeate + *S*-adenosyl-L-methionine = (*E*)-ferulate + H^+ + *S*-adenosyl-L-homocysteine) [104].

The elucidations of these reactions and techniques provided a huge benefit to increase the use of those compounds in metabolic engineering. There are others areas to explore and clarify to shed light the use of melatonin or glycine betaine metabolic engineering.

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