



Role of GABA in plant growth, development and senescence

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ABSTRACT

The γ -aminobutyric acid (GABA), is a non-proteinogenic, four carbon amino acid having multifaceted roles in both plants and animals. It plays an important role in plant growth and abiotic stress mitigation in plants. It also plays a prominent role in leaf senescence that forms the concluding stage of plant growth and development. Thus, consolidating the advancements in studies pertaining to multifaceted roles of GABA in coordinating plants' growth, development, and senescence to enhance performance in both optimal and stressful conditions is utmost crucial. The present review aims to discuss the significance of GABA molecule in plant growth, development and senescence alongwith the potential role of GABA under abiotic stresses.

1. Introduction

Gamma-aminobutyric acid (GABA) is a non-proteinogenic amino acid that act as a signalling molecule with multifaceted roles in living organisms including both animals and plants (Michaeli and Fromm, 2015; Kamal et al., 2016; Li et al., 2020). GABA mediates various physiological and molecular processes in plants such as regulation of redox status (Jin et al., 2019), regulation of cytosolic pH (Ramesh et al., 2017; Su et al., 2019), osmotic potential (Vijayakumari and Puthur, 2016), modulation of nitrate uptake and its utilization (Zhen et al., 2018), carbon (C) and nitrogen (N) metabolic flux (Fait et al., 2008, 2011; Michaeli and Fromm, 2015; Chen et al., 2020), pollen tube growth during plant reproduction (Priya et al., 2019), cell wall modification (Renault et al., 2011; Renault et al., 2013), adventitious root development (Xie et al., 2020) and plastid associated functions (Xiang et al., 2016; Bashir et al., 2021).

In plants, GABA biosynthesis and metabolism together constitute GABA shunt and mediate plant growth and development (Das et al.,

2016; Ji et al., 2020). GABA shunt consists of cytosolic enzyme glutamate decarboxylase (GAD; EC 4.1.1.15), that catalyses the conversion of glutamate into GABA and two mitochondrial enzymes, GABA-T (EC 2.6.1.19) and succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.16) that participate in GABA metabolism into succinic semialdehyde (SSA) and further into succinate. GABA shunt components have a crucial role in maintaining ion homeostasis (Su et al., 2019), abiotic stress tolerance (Al-Quraan and Al-Share, 2016; Jalil et al., 2017; Sita and Kumar, 2020) and ultimately regulates plant growth, development (Jalil et al., 2017) and plant senescence (Jalil et al., 2019). GABA significantly improves plant morphological and physiological traits during abiotic stress conditions (Li et al., 2016a; Ma et al., 2019) in terms of photosynthetic efficiency, accumulation of osmoprotectants such as proline (Li et al., 2016b; Wang et al., 2017) and polyamine metabolism (Seifikalhor et al., 2020). GABA alleviates abiotic stress induced reactive oxygen species (ROS) accumulation by activating antioxidant defence machinery (Chen et al., 2018; Kumar et al., 2019a). GABA enhance stress tolerance in plants by modulating the expression of

Abbreviations: AKG α , ketoglutarate; ALMT, Aluminium-activated malate transporter; CaM, Calmodulin; CaMBD, Calcium/calmodulin (Ca²⁺/CaM) binding domain; DAO, Diamine oxidase; GABA, Gamma aminobutyric acid; GABA, P GABA permease; GABA-T, Gamma aminobutyric acid Transaminase; GABA-TP, Pyruvate-dependent GABA transaminase; GAD, Glutamate decarboxylase; GDH, Glutamate dehydrogenase; GHB γ , hydroxybutyrate; GHBA γ , hydroxybutyric acid; GHBDH- λ , Hydroxybutyric acid Dehydrogenase; NAD, Nicotinamide adenine dinucleotide; NADH, Nicotinamide adenine dinucleotide dehydrogenase; PAs, Polyamines; PCD, Programmed cell death; RNAi, RNA interference; ROI, Reactive oxygen intermediates; ROS, Reactive oxygen species; SPS, Sucrose-P-synthase; SSA, Succinic semialdehyde; SSADH, Succinic semialdehyde dehydrogenase; TCA, Tricarboxylic acid.

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genes involved in signal transduction, ROS production and related stress responsive processes (Podlešáková et al., 2019). Therefore, GABA-mediated plant responses might assist in formulation of strategies to enhance growth and development in plants optimal and stressful environments and ultimately improving plant productivity.

2. GABA: biosynthesis and signalling in plants

Identification of the biosynthetic pathway of any metabolite and characterising the key regulatory enzymes of that pathway is crucial to determine the metabolite's functional dynamics. GABA biosynthesis and metabolism takes place through a chain of reactions that occur in a sequential manner and are collectively regarded as GABA shunt (Fig. 1; Table 1). GABA is primarily biosynthesised from glutamate in a decarboxylation reaction catalysed by GAD. GAD catalyses the irreversible conversion of glutamate into GABA which involves the addition of a proton and releases CO₂ in the cytosol (Baum et al., 1993; Michaeli and Fromm, 2015). GABA then gets transported to mitochondria where its subsequent catabolism into succinate occurs by two consecutive reactions catalysed by GABA-T and SSADH.

In plants, the GAD gene was isolated from a common petunia along with its Ca²⁺/CaM binding domain (CaMBD) (Baum et al., 1993) later, followed by identification and isolation of several other GAD homologues in different plant species (Ling et al., 1994; Snedden et al., 1995). CaMBD mediated conversion of glutamate into GABA (Baum et al., 1996) indicated the possible role of intracellular Ca²⁺ signalling in GABA biosynthesis. The role of CaM binding domain of GAD in normal GAD activity, glutamate metabolism, GABA biosynthesis, and normal plant growth and development was inferred in vivo analysis of

transgenic tobacco plants having mutant GAD where absence of CaM binding domain led to several growth abnormalities such as short stems due to inhibition of cell elongation in the cortex, abnormal GAD complexes, extreme high levels of GABA and low glutamate levels (Baum et al., 1996). However, first plant GAD isoform i.e., *OsGAD2* that lacks authentic CaM binding ability due to absence of CaMBD at C-terminus was isolated from rice roots where it lacked the ability to bind to bovine CaM in the presence of Ca²⁺ while *OsGAD1* specifically functions in Ca²⁺/CaM dependent manner by binding to the bovine CaM (Akama et al., 2001).

After biosynthesis of GABA in cytosol, it is transported to the mitochondria via GABA carriers and later active GABA metabolism occurs within mitochondria that marks the second step of GABA shunt. The translocation of GABA across the plasma membrane is catalysed by a mitochondrial enzyme i.e. GABA permease (GABA-P) that belongs to APC transporter family (Michaeli et al., 2011). Within the mitochondrial matrix GABA catabolism occurs by GABA-T that catalyses the production of SSA with simultaneous activation of different amino acid acceptors such as pyruvate, α -ketoglutarate (AKG) or glyoxylate (Clark et al., 2009a; Shelp et al., 2012a; Michaeli and Fromm, 2015). Lastly, SSA gets converted into succinate by another mitochondrial enzyme, SSADH. Succinate formed via GABA shunt plays an important role in the TCA cycle and mitochondrial electron transport chain by acting as an electron donor (Barbosa et al., 2010). Biochemical analysis of SSADH, revealed a possible role of CaM mediated calcium signalling in regulation of GABA shunt (Busch and Fromm, 1999). The conversion reaction of SSA to succinate is an oxidation reaction that is NAD-dependent and is catalysed by the help of SSADH enzyme that is active as homo tetramer.

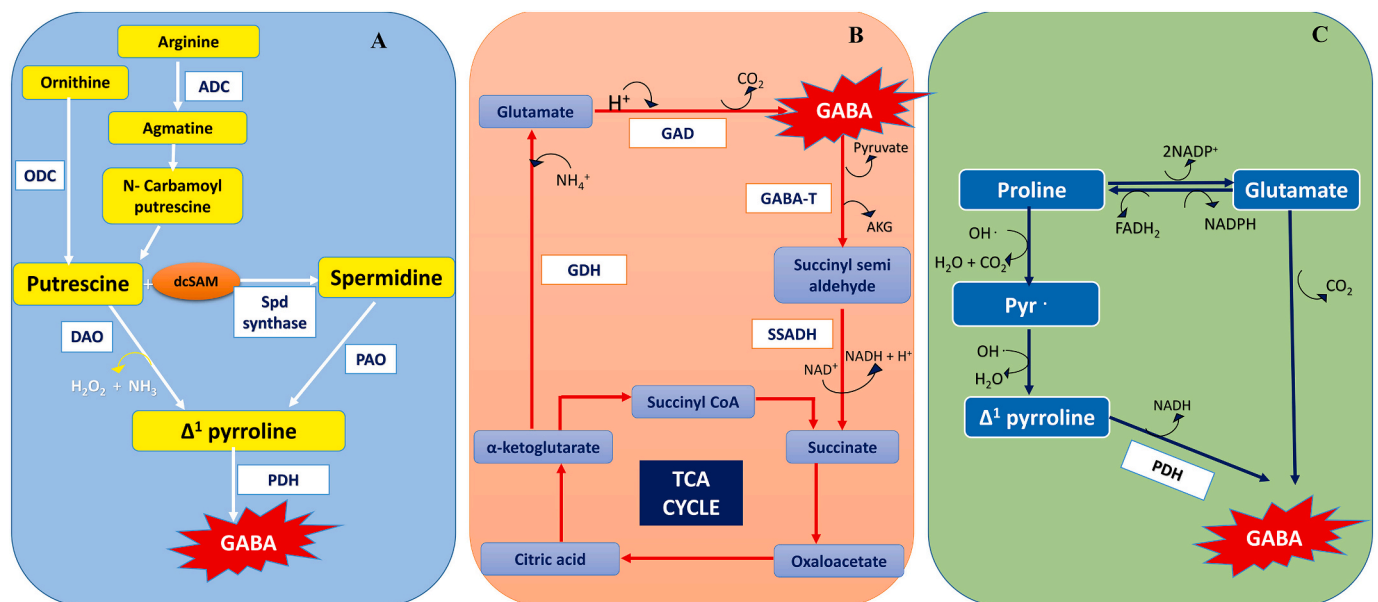


Fig. 1. GABA biosynthesis can possibly occur via three pathways,

A) By polyamine degradation: Polyamines such as put and spd provide an alternate route for GABA biosynthesis. Put is either synthesised directly from ornithine or indirectly from arginine under the enzymatic activity of ODC or ADC respectively. Furthermore, Put undergoes reaction with aminopropyl group derived from dcSAM in the presence of enzyme spd synthase to form another polyamine, spd. Both put and spd are further catabolised to Δ^1 pyrroline via the action of DAO and PAO respectively. PDH, then converts Δ^1 pyrroline into GABA.

B) Via GABA shunt: The biosynthesis of GABA from glutamate via GABA shunt pathway, in an irreversible decarboxylation reaction in cytosol of the plant cell. GABA synthesised from glutamate then gets transferred from the cytosol to mitochondria and gets converted into SSA via GABA-T using either AKG (by GABA-TK) or pyruvate (by GABA-TP) as amino acid acceptors. SSA later gets reduced to succinate via SSADH that acts both as an electron donor to the mitochondrial ETC and as a component of the TCA cycle.

C) By proline: Another alternative route for GABA biosynthesis is via non-enzymatic reactions of proline that is prevalent mainly under oxidative stress conditions due to excessive accumulation of ROS where H abstraction from Pyr leads to the formation of Δ^1 pyrroline which further contributes in GABA biosynthesis.

ADC: Arg decarboxylase, DAO: diamine oxidases, dcSAM: decarboxylated S-adenosyl-Met, ODC: Orn decarboxylase, GAD: Glutamate decarboxylase, GDH: glutamate dehydrogenase, GABA-T: Gamma-aminobutyric acid Transaminase, GABA: gamma-Aminobutyric acid, PAO: polyamine oxidases, PDH: Δ^1 -pyrroline dehydrogenase, Put: Putrescine; Pyr.: pyrroline, Spd: spermidine, Spd synthase: spermidine synthase, SSADH: Succinic semialdehyde dehydrogenase.

Table 1
Involvement of GABA shunt components in scavenging mechanism of ROS for improving tolerance to abiotic stresses

GABA shunt components	Gene name	Plants	Responses	Components determining oxidative stress	References
Glutamate decarboxylase (GAD)	<i>PgGAD</i>	<i>Panax ginseng</i>	Enhanced expression during various abiotic stresses like temperature stress, osmotic stress, anoxia, oxidative stress, and mechanical damage	H ₂ O ₂ activity was regulated by expression levels of GAD gene	Lee et al., 2010
		<i>Triticum aestivum</i>	Protection against salt and osmotic stress	MDA accumulation and seed germination sensitivity	Al-Quraan et al., 2013
GABA transaminase (GABA-T)	<i>γ-vinyl-γ-aminobutyrate (GABA-T inhibitor), ssadh-2</i>	<i>Arabidopsis thaliana</i>	Maintains redox equilibrium in <i>ssadh</i> mutants, inhibits cell death	Altered ROI and GHB levels	Fait et al., 2005
γ-Aminobutyric acid (GABA)		<i>Hordeum vulgare</i>	Alleviates oxidative damage caused by aluminium (Al ³⁺) and proton stresses (H ⁺)	Higher levels of SOD, CAT and POD and reduced levels of f MDA, H ₂ O ₂ and O ₂ ⁻	Song et al., 2010
		<i>Lactuca sativa</i>	Enhances salt tolerance and photosynthetic performance	Reduced proline content and increased CAT, APX and SOD activity	Kalhor et al., 2018
		<i>Trifolium repens</i>	Increased endogenous GABA concentration alleviates the oxidative stress and improves drought tolerance	Pro accumulation and homeostasis	Yong et al., 2017
		<i>Agrostis stolonifera</i>	Exogenous application of GABA (0.5 mM) significantly reduces ROS production and enhances antioxidant enzyme activity that improves heat stress tolerance	O ₂ ⁻ , H ₂ O ₂ , MDA levels reduces with enhanced SOD, POD, APX, Dehydroascorbate reductase (DHAR), ascorbic acid (AsA) and dehydroascorbic acid (DHA)	Li et al., 2016c
		<i>Lycopersicon Esculentum</i>	Decreases chilling stress along with the reduction in H ₂ O ₂ amount.	Reduced MDA and proline levels along with enhanced activity of CAT and SOD	Malekzadeh et al., 2014
		<i>Oryza sativa</i>	Partial protection from heat stress injury	Activities of antioxidant enzymes including CAT, SOD, APX, glutathione reductase (GR) and nonenzymatic antioxidants like ascorbate and glutathione	Nayyar et al., 2014
		<i>Lolium perenne</i>	Mitigates damages caused by drought stress by altering relative water content, turf quality, oxidative stress and lowering wilting.	Enhanced SOD, CAT, APX, and POD (peroxidase) activity	Krishnan et al., 2013
		<i>Triticum aestivum</i>	Alleviate oxidative damage caused by chilling stress in wheat seedlings by activating antioxidant defence responses	Reduced MDA levels and enhanced activities of antioxidant defence enzymes including CAT, APX and SOD.	Malekzadeh et al., 2012
Hydroxybutyrate Dehydrogenase	<i>GHBDH</i>	<i>Caragana intermedia</i>	Inhibits H ₂ O ₂ accumulation brought about by NaCl Stress	expression of genes involved in PA synthesis that acts as radical scavengers	Shi et al., 2010
		<i>Arabidopsis thaliana</i>	Induces oxidative stress tolerance	Redox balance regulated by activity of GHBDH and GHB	Breitkreuz et al., 2003

Table 2
Involvement of GABA Shunt components in plant growth, development and senescence.

GABA Shunt components	Gene	Plants	Responses	References
Glutamate dehydrogenase	<i>GDHI</i>	<i>Arabidopsis thaliana</i>	Increases nitrogen assimilation	Oliveira et al., 1996
	<i>GDH</i>	<i>Nicotiana tabacum</i>	Involved in nitrogen mobilization, are differentially regulated during pathogen attack and by stress hormones (ethylene, jasmonic acid and salicylic acid) and reactive oxygen species	Pageau et al., 2005
Glutamine synthetase	<i>GS1</i>	<i>Nicotiana tabacum</i>	Involved in nitrogen mobilization, are differentially regulated with the supplementation of hormones (ethylene, salicylic acid and jasmonic acid) during pathogen attack	Pageau et al., 2005
	<i>GS-1, GS-2</i>	<i>Lycopersicon esculentum</i>	Remobilization of leaf nitrogen during water stress to developing organs	Bauer et al., 1997
	<i>GS1 and GSr</i>	<i>Triticum aestivum</i>	Roles in assimilating ammonia during the critical phases of remobilization of nitrogen to the grain during grain development and filling and during senescence	Bernard et al., 2008
	<i>GS1</i>	<i>Nicotiana plumbaginifolia</i>	Role in regulating proline production consistent with the function of proline as a nitrogen source and as a key metabolite synthesised in response to water stress.	Brugiere et al., 1999
	<i>GS-1, GS-2</i>	<i>Oryza sativa</i>	Remobilization of Leaf Nitrogen during Natural Senescence	Kamachi et al., 1991

Alternate conversion of SSA into γ-hydroxybutyrate (GHB) catalysed by GHB dehydrogenase (GHBDH) has been reported in animals (Andriamampandry et al., 1998), plants (Breitkreuz et al., 2003) and recently in *E. coli* (Saito et al., 2009). In plant tissues, there are circumstantial reports on alternate catabolism of SSA into GHB in response to unfavourable environment such as oxygen deficient conditions (Allan et al., 2003), high light conditions (Fait et al., 2005) and water as well as heat stress (Allan et al., 2008) have been illustrated. The conversion of SSA into GHB is a reduction process that is prevalent during abiotic

stress conditions indicating the role of GHBDH in abiotic stress tolerance in plants and is putatively regulated by redox status of the cell and NADPH-dependent glyoxylate reductases (Breitkreuz et al., 2003; Allan et al., 2008).

Alternatively, GABA biosynthesis can possibly occur via degradation of polyamines such as putrescine (Put) and spermidine (Spd; Bhatnagar et al., 2002; Shelp et al., 2012b; Fig. 1.A). Put is either produced directly via decarboxylation of non-proteinogenic amino acid, ornithine by the activity of ornithine decarboxylase (EC 4.1.1.17) or indirectly via

essential amino acid, arginine in the presence of arginine decarboxylase (EC 4.1.1.9). However, *spd* is synthesised from reaction of put with decarboxylated S-adenosyl-Met in the presence of enzyme *spd* synthase (EC2.5.1.16). Later, put and *spd* catabolise into Δ^1 -pyrroline via activity of diamine oxidases (E.C.1.4.3.6) and polyamine oxidases (E.C. 1.5.3.3; Mattoo et al., 2010). Δ^1 -pyrroline is then committed to synthesize GABA by the activity of pyrroline dehydrogenase. Alternatively, GABA biosynthesis can probably occur by a non-enzymatic reaction of proline under oxidative stress (Signorelli et al., 2015; Michaeli and Fromm, 2015; Fig. 1). Under stress conditions, proline levels within the cell tremendously increases along with the increased concentration of ROS. OH^\bullet radical abstracts hydrogen from proline which further decarboxylates to form pyrroline. Another hydrogen abstraction from pyrroline leads to the formation Δ^1 -pyrroline that is the substrate for enzyme pyrroline dehydrogenase to form GABA.

Modulation in activity of GABA shunt and its associated components can possibly affect plant resilience during unfavourable conditions and alter plant developmental processes (See Table 2). For instance, in *pop2-1* mutants (GABA-T mutants, the mutation affects the first step of GABA catabolism), GABA accumulation negatively influences plant growth and development by causing cell elongation defects (Renault et al., 2011). The mutation in one of the important enzyme GABA-Transaminase (GABA-T) involved in GABA shunt induces early leaf senescence in Arabidopsis under the influence of various abiotic stresses such as pH, water, light, and cold stress (Jalil et al., 2017). It has been reported that three GABA-T genes isolated from *Solanum lycopersicum* L. (tomato; Akihiro et al., 2008; Clark et al., 2009b; Koike et al., 2013) have distinct subcellular localisation and are characterised as pyruvate-dependent GABA transaminases (GABA-TPs; Clark et al., 2009b). Koike et al. (2013) suggested that the loss-of-function analysis of *SIGABA-T* isoforms using the RNAi lines with suppressed pyruvate and glyoxylate dependent GABA-T gene expression in tomato revealed that GABA-T1 contributes to GABA reduction in the ripening tomato fruits (Koike et al., 2013). GABA content, in *SIGABA-T1*^{RNAi} lines, was higher than in the wild-type. Transgenic plants showed alteration in vegetative growth with severe dwarfism and infertility (Koike et al., 2013). These results demonstrated that impaired functioning of GABA-T1 could lead to a

peculiar GABA accumulation in the cell's cytosol and abnormal plant development suggesting that GABA may be an important player influencing plants developmental processes.

2.1. GABA signalling in plants

Despite the clearest evidences on functions of GABA in regulation of various physiological and metabolic processes in plants, insights to GABA signal transduction have remained vague for a long time and has still not been clearly deciphered. Though, GABA signalling in plants is mediated via aluminium-activated malate transporter (ALMT; Fig. 2), a multigenic anion channel uniquely found in plants (Ramesh et al., 2015). ALMT belongs to a family of anion transporters with several members that show different expressions through various plant tissues and are localised in plasma membrane of the cell (Kollmeier et al., 2001; Pineros and Kochian, 2001). ALMT proteins were genetically identified in wheat (*TaALMT1*; Sasaki et al., 2004) and Arabidopsis (*AtALMT1*) (Hoekenga et al., 2006); and their putative role in regulation of GABA signal transduction was established in wheat roots by Ramesh et al. (2015) that provided the insights to GABA signalling. Here, exposure of wheat roots to pH and Al^{3+} stress revealed that GABA negatively regulates the ALMT activity that further effects plant growth processes including the regulation of pollen tube and root growth. There is an unusual but an interesting interplay between acidosis, Al^{3+} and GABA as acidosis is one among the abiotic stresses that lead to GABA accumulation (Crawford et al., 1994) and Al^{3+} stress co-occurs significantly with acidosis (Sasaki et al., 2004). ALMT channels shows electrogenic activity and have the ability to change electric potential of plasma membrane and tonoplast. GABA negatively regulates anion efflux from ALMT channels for transducing various signals. However, the anion equilibrium potential in plants is highly positive due to which the GABA mediated anion flux results in shift in electric potential of the cell by depolarisation (Ramesh et al., 2015). The proteins that aid in GABA signal transduction in plants are not orthologues to that of animals except a small region of similarity with GABA_A receptor found in mammalian cells (Bergmann et al., 2013). However, no definite receptors involved in GABA signalling within the plants have been

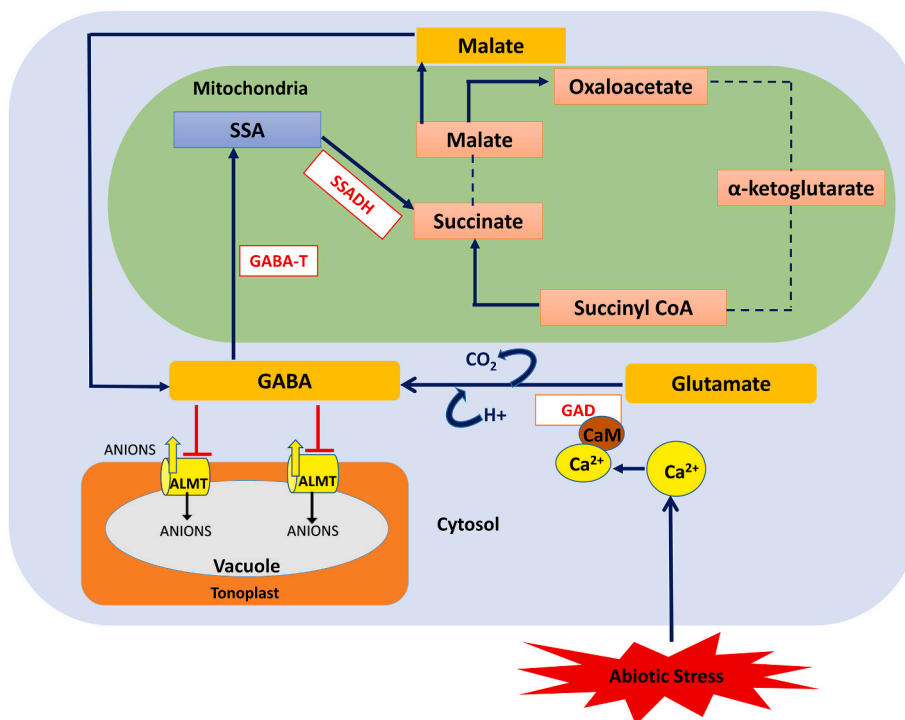


Fig. 2. GABA signalling in plants:

GABA is synthesised via GABA shunt pathway, in turn modulates the activity of ALMT membrane channels. GABA-stimulates anion efflux (yellow arrows) and GABA-inhibits anion influx (red T-bars) in the tonoplast of plant cells. External stimuli such as abiotic stress stimulate Ca^{2+} influx and activate the Ca^{2+} CaM domain of GAD resulting in the increased synthesis of GABA and modulates the activity of ALMT membrane channels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ALMT: Aluminium-activated malate transporter, GABA: γ -aminobutyric acid, GAD; glutamate decarboxylase, GABA-T: γ -aminobutyric acid- transaminase, SSA: succinyl semialdehyde; CaM: calmodulin, TCA cycle: tricarboxylic acid cycle.

characterised yet but it has been found that the activity of ion channels in plants is regulated by plant derived drugs such as muscimol (agonist) and bicuculline (antagonist) that also effects GABA_A receptors in animals (Ramesh et al., 2015). It is still unclear whether these molecules of plant origin show interactions with GABA binding regions of ALMT to mediate GABA signalling in plants. Their interaction with GABA binding sites could further provide a clear insight to various speculations made on GABA receptors. Ca²⁺ plays an important role as ubiquitous secondary messenger in several plant signal transduction processes during various abiotic stresses (Tuteja and Mahajan, 2007). The involvement of Ca²⁺ in GABA signal transduction has also been reported (Ma et al., 2019). During salinity stress (NaCl), GABA significantly resulted in accumulation of phenolics in barley with an indicative involvement of Ca²⁺ in GABA- promoted synthesis of phenolics (Ma et al., 2019). GABA supplementation triggered increase in CaM, enhanced activity of Ca²⁺-ATPase at an early germination stage along with upregulation of calcium binding proteins such as calcium-dependent protein kinase (CDPK), CaM dependent protein kinase (CaMK), and Ca²⁺/CaM dependent protein kinase (CCaMK) in barley. GABA induced Ca²⁺ influx in root tips under salinity, alterations in distribution of Ca²⁺ precipitates and putative role of Ca²⁺ in increasing phenolic accumulation indicates importance of Ca²⁺ in GABA signal transduction against NaCl stress. Identification of suitable GABA receptors, and receptor binding signalling molecules involved in generation of response against several stress conditions can decipher exact mechanism of GABA signalling directly or indirectly involved in stress tolerance. Furthermore, deciphering stress responsive genes induced during GABA signalling, and analysing their expression control network can enhance our understanding about GABA mediated signalling during stress responses.

3. Role of GABA in plant growth and development: an overview

Frequent reiteration of signalling roles of GABA has continuously been linked with plant growth and development. GABA plays a crucial role in mediating plant growth and development (Jalil et al., 2019; Du et al., 2020). Exogenous application of GABA (0, 0.01, 0.1, 1, or 10 mM), overexpression of *GAD2* and exogenous application of GABA-T inhibitor (i.e., vigabatrin; 0, 10, 50, or 100 µM) in *poplar* resulted in endogenous GABA accumulation that negatively regulated adventitious root (AR) growth and inhibited or delayed AR formation (Xie et al., 2020). Elevation in endogenous GABA levels also altered metabolic fluxes such as C: N pools by inhibiting sugar translocation, increasing levels of amino acid modulating hormone homeostasis (increased IAA levels and decreased ethylene levels) and transcriptional modification in hormonal signalling pathways that ultimately affected AR formation in *poplar* (Xie et al., 2020). Hence, it can be interpreted that GABA mediated AR growth inhibition is the result of multidimensional interaction between GABA, metabolic pathways, hormonal homeostasis and signalling responses.

Hyperaccumulation of GABA within the plant cell can misbalance the intracellular amino acid contents resulting in aberrant phenotypes. One such interpretation was obtained from investigations on transgenic lines of rice and tobacco overexpressing rice *GAD2* (Akama and Takaiwa, 2007). It was also shown that the exemplary features of petunia *GAD* are conserved in all *GAD* isoforms (except *GAD2*) of rice. Transgenic lines, *GAD2ΔC* of rice and tobacco overexpressing truncated C-terminal *GAD2* exhibited GABA accumulation due to increased *GAD* activity. In transgenic plants, the levels of other free amino acids simultaneously decreased after the elevation in GABA levels. Furthermore, *GAD2ΔC* lines also exhibited altered phenotypes including small, etiolated and curled leaves, and infertility as in rice and dwarfism with reduced parenchymatous cell size in stem cortex of tobacco (Akama and Takaiwa, 2007). In *Brassica napus* L. (Rapeseed) signals from GABA molecule upregulated nitrate uptake (Beuve et al., 2004). In Arabidopsis, GABA mediates growth of pollen tube and its guidance for targeted entry into the ovary (Palanivelu et al., 2003). However, reduced

expression of *POP2* gene (encoding GABA-T enzyme) decreased GABA-T activity leading to higher GABA accumulation in ovule and aberrant growth patterns in pollen tube (Palanivelu et al., 2003). Hence, it is evident that GABA regulates plant growth related patterns. Studies related to GABA-mediated growth promotion in *Lemna* (duckweed) minor plants revealed a direct correlation between the concentration of GABA in the media and dry weight of duckweed where 10 mM GABA in the media resulted in a 3-fold increase in plant dry weight over control (Kinnersley and Lin, 2000). Statistically significant growth in duckweed was absent during treatment with 0.5 mM or lower levels of GABA and contradictorily, 2-aminobutyric acid and 3-aminobutyric acid despite being isomers of GABA had an inhibitory effect on the growth of plant and plant growth after treatment with 0.1 mM or higher levels (Kinnersley and Lin, 2000). Elevated GABA levels due to exogenous GABA treatments alters dry weight and plant mineral content and thus effects their growth and development. Remarkably, GABA regulated duckweed growth promotion was inhibited by bicuculline (a competitive antagonist) and picrotoxin (non-competitive antagonist) of GABA receptors from mammalian CNS. Exogenous application of GABA (0.5 mM) has shown to alleviate alkaline stress in *Malus hupehensis* (Chinese crab apple) seedlings with significant alterations in growth and developmental processes such as increase in biomass, activation of antioxidant defence system to reduce ROS accumulation and enhancement in root growth (Li et al., 2020). Thus, GABA is a crucial in mediating several plant growth and developmental processes ultimately may affecting plant productivity.

4. Significance of GABA in plants during senescence

Plant senescence is a well-coordinated genetically programmed process in which degradation of functional proteins (Martinez et al., 2008; Buet et al., 2019) and remobilization of nutrients takes place (Himelblau and Amasino, 2001; Maillard et al., 2015) to the young growing parts of the plant (Ansari et al., 2014). Senescence is marked by degradation and redistribution of nutrients, which are synthesised in leaves, from senescing parts to growing parts and reproductive organs resulting in seed and fruit development. In this context, the most important nutrient to be recycled and mobilized from senescing leaves to the young growing parts of the plant during senescence is N (Sakamoto and Takami, 2014; Ansari et al., 2014). Senescence is regulated by various events and also involves signalling molecules such as GABA (Ansari et al., 2005, 2014). It is worth mentioning that, N metabolism involves GABA shunt as its key regulator that mediate its mobilization during leaf senescence which then subsequently enters into TCA cycle (Barbosa et al., 2010; Ansari et al., 2014). During leaf senescence, N present in macromolecules is converted into two amino acids namely glutamine and asparagine and transported through phloem to the growing parts such as seeds and fruits (Feller and Fischer, 1994). These amino acids are also converted into glutamate and α-keto acids with the help of transaminases (Ansari et al., 2014). A part of glutamate formed is also converted into GABA via *GAD* and enters into GABA shunt pathway. Metabolite-recycling routes (*GABA-T* and the TCA cycle), the conversion of glutamate to GABA profoundly affects the C—N balance of the seed (Fait et al., 2011) resulting in altered fatty acid metabolism and storage reserve accumulation. Further, metabolic profiling of glutamate in Arabidopsis established the role of GABA shunt as a nexus between C and N metabolism upon seed imbibition (Fait et al., 2011) before seed germination.

4.1. GABA regulates oxidative stress during stress-induced senescence

Detrimental stress conditions hamper growth, development and productivity of the plant by altering its physiological, biochemical and metabolic processes and by disturbing its osmotic and oxidative homeostasis. Adverse environmental conditions cause disequilibrium in generation and scavenging of ROS simultaneously that leads to oxidative

stress induced damages in plants (Prasad et al., 1994; Choudhary et al., 2020). Chloroplast is the major hotspot for generation of maximal ROS under severe environmental stress conditions (Partelli and Batista, 2001). ROS in plants are also generated in cell wall (Pottosin et al., 2014) mitochondria (Navrot et al., 2007), peroxisomes (Noctor et al., 2002), endoplasmic reticulum (Mittler, 2002) and apoplast (Hu et al., 2006). Enhanced leaf senescence is one of the major plant strategies to escape the drastic effects of oxidative stress caused by excessive ROS accumulation (Kumar et al., 2019b). GABA plays an important role in mediating oxidative stress induced leaf senescence. Deregulated function of GABA shunt component can lead to ROS-induced cell death under heat and light under heat and light stress. Studies carried out on *ssadh* mutants of Arabidopsis showed that the impairment in the last step of GABA Shunt, elevated the levels of reactive oxygen intermediates (ROIs), exhibited phenotypic dwarfness, reduced leaf area, necrotic lesions, lower chlorophyll content, bleached leaves, and fewer flowers in contrast to WT plants and light and heat stress finally led to cell death (Bouche et al., 2003). Based on this study, Fait et al. (2005) observed that treatment of *ssadh* mutants with γ -vinyl- γ -aminobutyrate, a GABA-T inhibitor mitigated ROI and GHB accumulation, inhibited cell death, reduced phenotypic and biochemical defects (such as phenotypic dwarfness, reduced leaf area, necrotic lesions, lower chlorophyll content etc) caused due to deficiency of SSADH and ultimately enhanced plant growth. GABA shunt thus, mitigates oxidative stress by scavenging ROI accumulation. CaM has its role in tolerance against oxidative stress induced by UV treatments (UVA and UVB) and affects seed growth, its germination, ROS accumulation (Al-Quraan, 2015). In Arabidopsis, during seed germination when *cam* mutants with T-DNA insertions of CaM genes were given UVA and UVB treatment inducing oxidative stress, a rapid increase in GABA accumulation along with altered levels of GABA shunt metabolites (i.e. glutamate, alanine) were observed in coordination with the activation of antioxidant defence system to reduce ROS concentration within the *cam* mutants (Al-Quraan, 2015). In *Capsicum annuum* L. (pepper), GABA mitigated oxidative stress damages induced by low light conditions and enhanced stress tolerance via activation of antioxidant defence system and elevation in photochemical efficiency (Li et al., 2017). In this study, exogenous GABA supplementation (20 mM) in pepper reduced oxidative stress via reduction in MDA levels, enhancement in SOD, CAT activity. Exogenous GABA also enhanced endogenous GABA concentration and plant growth related parameters such as chlorophyll content, stomatal conductance, photochemical quenching which increases photosynthesis, gaseous exchange, photochemical activity of PSII respectively (Li et al., 2017). In addition to this, endogenous GABA accumulation suggests the possible metabolism of GABA into succinate which can later enter into TCA cycle and aid in regulating C:N balance in pepper.

It is suggested that exogenous GABA induces tolerance against alkaline stress, regulates antioxidant system, modulates photosynthetic efficiency and elevates activity of TCA cycle enzymes (Li et al., 2020). The altered levels of TCA cycle enzymes on GABA application modulates C: N pool and establish strong links between GABA and plant senescence during abiotic stress conditions. In a recent study, tobacco leaves provided with NaCl treatment (500 mM) reportedly show delayed growth with significant alterations in levels of transamination metabolites such as GABA along with modulations in pathways such as TCA cycle, glycolysis, glutamate-mediated proline biosynthesis (Zhang et al., 2011). The antioxidant defence is an elementary detoxification system of plants to counteract the stress induced oxidative damage thereby delaying plant senescence (Iqbal et al., 2012). Evidence has shown that impairment in the function of GABA shunt relatively leads to elevated levels of ROS under stress conditions (Bouche et al., 2003). Yang et al. (2011) investigated the role of GABA treatment supplemented exogenously (5 mM) on antioxidant enzymes, chilling injury, and energy charge in peach fruit. Further, they concluded that GABA treatment led to inhibition in chilling injury and enhanced activity of antioxidant enzymes and maintained energy level in peach fruits. GABA thus induces

tolerance against abiotic stresses and plays a pivotal role in stress adaptation by delaying plant senescence (Bouche et al., 2003).

4.2. GABA in regulating cytosolic pH during senescence

Plant senescence is characterised as a type of programmed cell death (PCD) which is regulated by many signal molecules. GABA is associated with regulation of plant senescence in many ways, one of which maintaining the cytosolic pH in the cell. Recent works have demonstrated the role of GABA signalling in the plant senescence (Ramesh et al., 2015). In the senescing leaves, the static state of the cells is altered, which adversely affects ion transport in and out of the cell membranes (Del Duca et al., 2014). Cytosolic pH is also altered which hampers normal growth and development of the plant. However, GABA has prominent role in maintaining cytosolic pH homeostasis. It has been shown that reduction in cytosolic pH under low oxygen conditions caused by various abiotic stresses such as flooding (Roberts et al., 1984) in plants accelerates the GABA accumulation (Kinnersley and Turano, 2000). This further caused mechanical damage accompanied by organic acids discharge into the cytosol, due to rupturing of vacuolar membranes resulting in reduced pH levels and increased acidification of cytosol. Conditions like hypoxia are known to reduce cytosolic pH in the plants, leading to low intracellular pH from standard physiological values, stimulates the activity of L-GAD resulting in increased levels of GABA (Crawford et al., 1994). In a study, it was demonstrated that rapid GABA synthesis occurs after addition of a weak acid resulting in a decreased cytosolic pH of 0.60 units having a half-time for the response of approximately 2 s (Crawford et al., 1994). It was also evidently shown that after several minutes of a reduction in pH (0.5 to 0.6 pH units) during hypoxia, GABA synthesis was stimulated (Roberts et al., 1992). Simultaneous elevated levels of cytosolic Ca^{2+} are observed with reduced cytosolic pH values (Gehring et al., 1990). Consequently, elevated levels of Ca^{2+} possibly act as a signal stimulating activity of enzyme L-Glu decarboxylase (Crawford et al., 1994). In *Dacus carota* (carrot) cells, it was demonstrated when the pH decreases, there is an increase in glutamate decarboxylation which is a proton consuming reaction (Carroll et al., 1994). This reaction facilitates GABA accumulation which acts as a sink for the excess of cytosolic protons and maintains pH homeostasis (Carroll et al., 1994).

4.3. GABA involvement in maintaining carbon-nitrogen pool in plants during senescence

The availability of C and N is a crucial factor in sustaining plant growth, development and metabolism (Coruzzi and Zhou, 2001). Adding to this, a significant balance in C and N pools with respect to each other must be highly coordinated (Bao et al., 2015) i.e., balance in C/N ratio which plays a regulatory role in plant growth and development in the standard growth conditions (Martin et al., 2002). The availability C (mostly in the form of sugars) and N in significant amounts maintaining C: N balance is suggested to be an important parameter not only in plant growth and metabolism but also in senescence progression (Aoyama et al., 2014). Deficiency in N leads to increased sugar accumulation leading to elevated C levels in plants (Ono et al., 1996). Explicit partitioning of C and N sources enable plants to sense and adapt to changing C and N availability conditions (Aoyama et al., 2014). Plants also maintain C: N pool balance by significant partitioning of C and N sources and modulations in metabolic activity of C and N within the cell (Sato et al., 2011; Sulpice et al., 2013; Aoyama et al., 2014). The C and N interaction is controlled by an intricate network of signals derived from nitrate, ammonium (Nunes-Nesi et al., 2010), and N-containing metabolites such as glutamate, glutamine, and aspartate (Miller et al., 2008), in other side signals induced from C metabolism via CO_2 (Nunes-Nesi et al., 2010) and C skeletons from TCA cycle which are required for NH_4^+ assimilation (Naliwajski and Skłodowska, 2018). GABA is primarily biosynthesized from glutamate and closely associated with the TCA

cycle in plants (Fig. 3). In *Arabidopsis* seedlings, exogenous application of GABA (50 mM) significantly stimulated root growth (in 1/8 strength Medium); increased nitrate uptake and N accumulation (when NO_3^- levels <40 mM in full strength MS media) along with the enhanced activities of enzymes involved in N metabolism such as nitrate reductase (NR), glutamate synthase (NADH-GOGAT), glutamine synthetase (GS) etc. in the growth medium (Barbosa et al., 2010). This study suggests that based on NO_3^- concentration in the plant, GABA mediates the activity of enzymes involved in N metabolism and speculates a crucial role of GABA in maintaining C:N pools within the plant cells. GABA metabolism is suggested to be the modulator in maintenance of C/N balance in plants (Fait et al., 2008). In a study of postharvest citrus fruit Hirado Buntan Pummelo, GABA shunt catabolises organic acids (Katz et al., 2011) and links organic acid catabolism and amino acid production (Sun et al., 2013). With the construction and screening of transgenic tomato deficient in succinyl-CoA ligase (SCoAL) activity, GABA metabolism was evidently shown to bypass major section of TCA cycle constituting 2-OG to the succinate, in plants (Studart-Guimarães et al., 2007), thereby giving a significant evidence for functional association of GABA metabolism and TCA cycle (Studart-Guimarães et al., 2007). In this study, inconsequential effects of repressed SCoAL activity in tomato such as altered respiration, photosynthesis, and growth rates were observed (Studart-Guimarães et al., 2007). The reduction in SCoAL activity beyond the threshold value resulted in transcriptionally mediated up-regulation of the GABA shunt (Studart-Guimarães et al., 2007). The inhibition of AKG dehydrogenase in *Solanum tuberosum* (potato) slices demonstrated its role in both GABA shunt and TCA cycle (Araújo et al., 2008). Both AKG dehydrogenase and SCoAL enzymes circumvents the GABA shunt and, in both cases, increased activity of GABA shunt compensated for the lost activity of these TCA cycle enzymes (Michaeli and Fromm, 2015). Both at metabolome and transcriptome levels, GABA is associated with primary C metabolism by characterising transcriptional co-response of gene associated with GABA shunt pathways (i.e., GAD2 and SSADH) with that of those associated with primary C metabolism (Fait et al., 2008). GABA-T has been also identified among the SAGs (senescence-associated genes) in leaf senescence (Ansari and Chen, 2009; Ansari et al., 2005; Jalil et al., 2019). Understanding the dynamics of GABA transportation can unravel speculative metabolic and signalling roles associated with GABA in plants. GABA mediated C metabolism

and respiration is suggestively dependent on carrier protein regulated GABA transport from cytosol to mitochondria (Michaeli et al., 2011). AtGABP, a GABA transporter from APC family is suggested to be an important transporter for prominent incorporation of GABA into TCA cycle in *Arabidopsis*. Metabolic profiling of mutant lines of *Arabidopsis* GABA-P *gabp* characterised GABA shunt as a functionally important process in regulation of primary metabolism across mitochondrial compartment, enhancement in activity of the TCA cycle, and in the maintaining C and N pool balance (Michaeli et al., 2011). Abolition of growth defects were also observed after complementing GABP, which is ectopically expressed with the *gabp* mutants under C deficiency supporting growth under limited C availability. Defective growth of *gabp* mutants and efficient growth in WT plants amidst functional AtGABP by sustaining greater C levels were observed respectively. It was also reported that impaired incorporation of GABA into mitochondria can lead to re-allocation of N (Michaeli et al., 2011). The activity of GABA shunt is demonstrated as the connecting bridges between C and N metabolism in leaves of *Xanthium strumarium* (cocklebur) (Tcherkez et al., 2009). 14-3-3 proteins have a crucial regulatory role in the C:N metabolism in plants (Lancien and Roberts, 2006) and these proteins exhibit phosphorylation-dependent interaction with other target proteins to accomplish their function (Lancien and Roberts, 2006). Combined action of GABA with calcium are prominently involved in 14-3-3 genes regulation (Carillo, 2018). It is relevant in C-N balance since 14-3-3 proteins targets several key enzymes of C-N metabolism such as nitrate reductase (Comparot et al., 2003), glutamine synthetase (Moorhead et al., 1999), starch synthase III (Comparot et al., 2003), glyceraldehyde-3-PDH (Lancien and Roberts, 2006).

Role of GABA in regulating cytosolic pH, abiotic stress conditions and primary C and N metabolism which are the major senescence associated processes has been very well exemplified through several studies. This concludes that GABA has a strong connecting link with plant senescence. Further studies on GABA kinetics, its dose responses and GABA-induced changes in senescence associated gene expressions in plants can aid in deciphering hidden links between GABA and senescence. Exploitation of strategies altering GABA regulated dynamics in plant senescence can be very useful in alteration of plant growth and productivity to ensure agronomic sustainability.

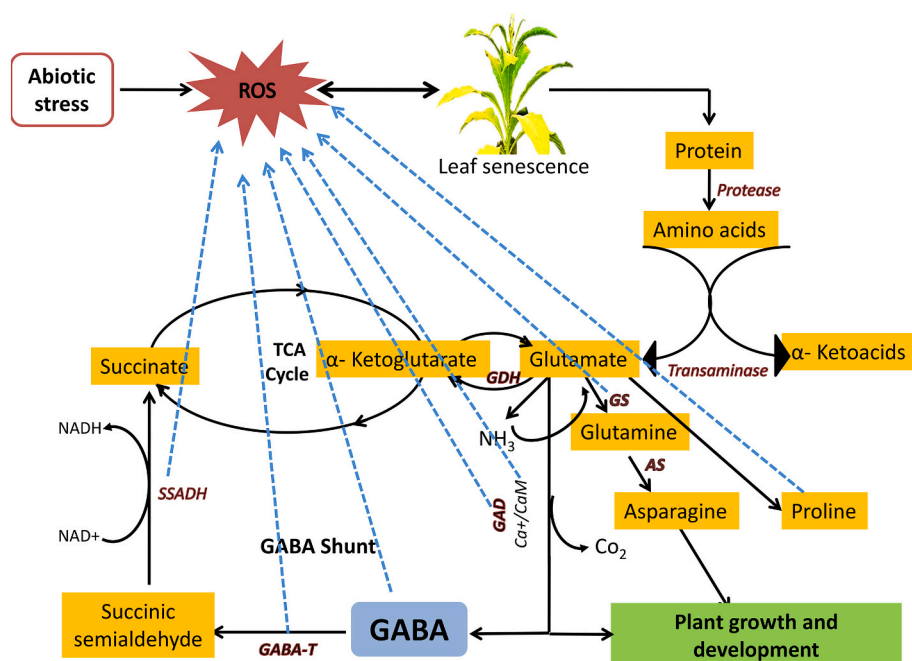


Fig. 3. Involvement of GABA shunt in scavenging mechanism of ROS during leaf senescence process. Leaf senescence can be induced by several factors including stress that synthesize excess amount of ROS due to disruption of cells, during leaf senescence, amino group from most of the amino acids can be transferred to α -ketoglutarate to form glutamate. In GABA shunt, glutamate with the help of GAD is converted into the GABA. GABA-T catalyses the conversion of GABA into succinic semialdehyde, which in turn is converted into succinate by SSADH and goes into TCA cycle. GABA shunt within the mitochondria may provide carbon skeleton to replenish carboxylic acids of TCA cycle. GABA shunt component and GABA metabolites has scavenging ability to reduce the production of free radicals during leaf senescence condition. GDH: Glutamate dehydrogenase, α -KGDH: α -ketoglutarate dehydrogenase, GABA-T: GABA-transaminase, SSADH: Succinic semialdehyde dehydrogenase, SCS: Succinyl CoA synthetase, TCA: Tricarboxylic Acid, ROS: Reactive oxygen species, CaM: Calmodulin (Calcium binding protein). The dark arrow indicates the direction of the reaction during senescence and dotted arrows shows the scavenging activity of GABA shunt components for ROS.

5. GABA in mitigation of abiotic stresses

Plant metabolites accumulate during abiotic stress conditions and among these, GABA kinetics shows rapid increases in endogenous GABA concentration and external supply of GABA under stress conditions such as temperature stress (Priya et al., 2019), salt stress (Xing et al., 2007; Khanna et al., 2021), oxygen stress (Miyashita and Good, 2008), acidosis (Crawford et al., 1994) and mechanical damages (Ramputh and Bown, 1996) and can help in mitigating abiotic stress. Exogenous GABA supplementation significantly reduces ROS accumulation and protects the plant from oxidative damage as observed in *Lycopersicon esculentum* (tomato), *Prunus persica* (peach; Shang et al., 2011), *Triticum aestivum* (wheat; Al-Quraan et al., 2013), *Cucumis melon* (muskmelon; Xiang et al., 2016). Effective role of GABA in mitigating heat stress induced oxidative damages in plants and simultaneous enhancement in vegetative and reproductive growth was observed in *Vigna radiata* L. (mungbean; Priya et al., 2019). Exogenous GABA application substantially reduced MDA levels and H₂O₂ concentration in addition to enhanced antioxidant activities within the leaves and anthers (Priya et al., 2019). In this study, GABA also increased C metabolism and upregulated osmolyte synthesis that improved C fixation and assimilation, and improved leaf water status respectively. Other parameters related to reproductive growth such as pollen germination, stigma receptivity, pollen and ovule viability were also enhanced. An investigation based on GAD2 activity in *Camellia sinensis* (Tea) suggested that unfavourable constraints such as extreme oxygen deficiency and mechanical damage affects expression level of the genes involved in GABA accumulation as hypothesised by high expression of *CsGAD2* during anoxic conditions (Mei et al., 2016). It has also been reported that GABA accumulation in tea plants subjected mechanical damage and anoxic conditions is mediated by combined effects of *CsGAD2* mRNA expression levels and CaM mediated enzymatic activation of *CsGADs* (Mei et al., 2016). GABA mediated abiotic stress responses in plants show significant correlation with oxidative burst that occur due to ROS accumulation (Al-Quraan et al., 2013; Cheng et al., 2018). GABA is a stress responsive metabolite that combats oxidative damages by reducing ROS accumulation within the plants (Carillo, 2018). Significant accumulation of GABA and other metabolites of GABA shunt such as glutamate and alanine occur in CaM mutant lines with T-DNA insertions in Arabidopsis during growth and seed germination due to induced oxidation stress (via treatment with H₂O₂ and paraquat; Al-Quraan et al., 2011). This speculates the role of GABA, Ca²⁺ signalling and CaM in regulating ROS equilibrium, reduction of oxidative stress induced damages in plant cell and involvement in plant growth related mechanisms and developmental processes.

Molecular and metabolic characterisation of GABA during stress conditions can enhance advancements in strategies to induce stress tolerance in the plants. In vitro analysis revealed that during salinity stress, the activity of GAD and GABA-TP markedly increased in Arabidopsis while the transcriptional analysis revealed that GABA metabolism genes significantly upregulated on NaCl treatment (Renault et al., 2010). During salinity stress, GABA accumulation has been observed in various plant species, including muskmelon (Xiang et al., 2016), *Hordeum vulgare* (barley; Widodo et al., 2009), and soybean (Zhang et al., 2011). Metabolites of GABA shunt may also have a decisive role in osmoregulation, including C and N metabolism and signalling in relation to salt and osmotic stress. In response to the mannitol treatments, similar sensitivity patterns were observed concluding that high accumulation of GABA content act as osmo-protectant during oxidative and osmotic stress conditions (Al-Quraan and Al-Share, 2016; Jalil et al., 2017). GABA priming has been effectively used to enhance abiotic stress tolerance in the plants over past few years. GABA primed *Piper nigrum* (black pepper) exhibited osmotic stress tolerance when imparted with PEG stress by reducing the distribution of stress related metabolites and upregulating defence related metabolic activities (Vijayakumari and Puthur, 2016). Synthesis of sugar moieties also increased in the samples primed with GABA (2 mM) along with the

reduced ROS accumulation, maintained cell turgor, reduced chlorophyll degradation and enhanced carotenoid synthesis. Al-Quraan and Al-Share (2016) studied seed germination rate in the Arabidopsis wild type and mutant lines namely *pop2* (line 1), *pop2-1* (line 2) and *pop2-3* (line 3) lacking corresponding transcripts of the *GABA-TA* gene. In this study, mutants showed successful germination. However, oversensitivity in temperature defects in GABA-T led to impaired GABA accumulation, hindering growth and development of the plant (Al-Quraan and Al-Share, 2016). Locy et al. (2000) demonstrated that GABA accumulation during high temperature stress is mediated by the levels of cytosolic Ca²⁺ ions counteracting the possible involvement of calcium transport inhibitors in regulating heat stress induced GABA accumulation in Arabidopsis seedlings. GAD is the key enzyme for GABA accumulation during various abiotic stresses and its activity is mediated by CaM binding to C-terminal regions of the GAD enzyme (Arazi et al., 1995). GAD activity mediated by elevated Ca²⁺ levels lead to increased GABA accumulation which in turn triggers Ca²⁺ extrusion and establishes calcium homeostasis.

GABA also ameliorate roots ability for active extrusion of Ca²⁺ ions that allow the plants to maintain H₂O₂ signalling during flooding (Shabala et al., 2014). GABA significantly reduces salt damage during plant developmental processes such as seed germination by activating enzymes associated with antioxidant defence mechanism such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), guaiacol peroxidase (GPOX), monodehydroascorbate reductase (MDHR) that significantly reduced the oxidative outburst in white clover (Cheng et al., 2018). GABA also mediates plant growth by generating tolerance against toxicity of heavy metal such as cadmium (Seifikalhor et al., 2020). Exogenous application of GABA improved Cd²⁺ toxicity in maize and salinity stress in wheat by elevating antioxidant defence activities via substantial elevations in activity of SOD, APX and CAT. GABA mediated reduction in oxidative stress was accompanied by amelioration in plant growth, cellular metabolism, photosynthetic efficiency, ion homeostasis, cell membrane integrity (Seifikalhor et al., 2020; Khanna et al., 2021). Deciphering molecular and biochemical sensors that regulate GABA induced oxidative stress tolerance in plants can be utilised in strategies for production of tolerant varieties with utmost efficiency. Studies pertaining to signal transduction mechanisms underlying GABA induced stress tolerance will help to determine protective roles of GABA in detail.

6. Conclusion and future prospects

GABA, being a multifunctional molecule acts as both metabolite and signalling molecule. GABA is synthesised and metabolised via GABA shunt pathway which has an important role in plants life span. GABA plays a pivotal role in maintaining C:N pool within the plant cell and is also involved in the N metabolism that is not only important for leaf senescence, but also for whole plant development. Additionally, GABA plays a crucial role in scavenging of ROS generated rapidly due to disruption in intracellular redox equilibrium and hence significantly mitigates oxidative stress induced damages in plants. Therefore, understanding the free radical scavenging mechanism through GABA during plants growth, development, and senescence is very vital for the plant improvement.

Credit

All authors contributed significantly.

Declaration of Competing Interest

None.

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