## REVIEW

# The versatile GABA in plants

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#### ABSTRACT

Gamma-aminobutyric acid (GABA) is a ubiquitous four-carbon, non-protein amino acid. GABA has been widely studied in animal central nervous systems, where it acts as an inhibitory neurotransmitter. In plants, it is metabolized through the GABA shunt pathway, a bypass of the tricarboxylic acid (TCA) cycle. Additionally, it can be synthesized through the polyamine metabolic pathway. GABA acts as a signal in *Agrobacterium tumefaciens*-mediated plant gene transformation and in plant development, especially in pollen tube elongation (to enter the ovule), root growth, fruit ripening, and seed germination. It is accumulated during plant responses to environmental stresses and pathogen and insect attacks. A high concentration of GABA elevates plant stress tolerance by improving photosynthesis, inhibiting reactive oxygen species (ROS) generation, activating antioxidant enzymes, and regulating stomatal opening in drought stress. The transporters of GABA in plants are reviewed in this work. We summarize the recent research on GABA function and transporters with the goal of providing a review of GABA in plants.

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## 1. Introduction

The potential impact of climate change on plant growth poses a serious threat to crop productivity and food security. Being sessile organisms, plants cannot move as animals can to seek more favorable environmental conditions for growth. They have to provide for themselves in one place as best they can to deal with the specific growth conditions they face and to keep pace with environmental change to ensure survival and growth. Gamma-aminobutyric acid (GABA) is thus a molecule for the plant to deal with various growth environments.

GABA is a ubiquitous four-carbon non-proteinogenic amino acid found in both eukaryotes and prokaryotes. In plants, GABA was first found in potato (*Solanum tuberosum*) tubers more than 70 years ago.1 Henceforth, its physiological role has been widely studied<sup>2-12</sup> and to date, it has been confirmed not only as a metabolite, but also as a signal molecule in plants.<sup>13-16</sup> Its functional versatility includes responding to abiotic and biotic stress factors, maintaining carbon/nitrogen (C/N) balance, and regulating plant development. In this review, we discuss GABA metabolism, function, and transporters in plants.

# 2. The metabolic pathway and detection of GABA in plants

In plants, GABA is mainly involved in growth and development through the GABA shunt, a bypass of the TCA cycle. GABA is synthesized from glutamate by irreversible decarboxylation catalyzed by glutamate decarboxylase (GAD) in the cytosol. Subsequently, GABA is transferred into the mitochondria and subjected to transamination to succinic semialdehyde (SSA) by GABA transaminase (GABA-T/POP2). The SSA is then oxidized to succinate by the NAD<sup>+</sup>-dependent succinate semialdehyde dehydrogenase (SSADH), and subsequently succinate feeds into the TCA cycle. Thus, the carbon skeleton of glutamate ultimately enters the TCA cycle by this GABA shunt and recycles. In turn, glutamate can be synthesized from  $\alpha$ -ketoglutarate by glutamate dehydrogenase (GDH). Figure 1 is a simple illustration of GABA metabolism and functions (see review by Bouche & Fromm<sup>14</sup>).

An alternative GABA-synthesis pathway is the polyamine metabolic pathway, where arginine is converted to putrescine through multi-step routes. Putrescine is then converted to 4-aminobutyraldehyde by  $O_2$ -dependent polyamine oxidase or it is converted to spermidine, which degrades to 4-aminobutyraldehyde and in turn is oxidized to GABA by NAD<sup>+</sup>- dependent 4-aminobutyraldehyde dehydrogenase. GABA enters the TCA cycle to be degraded by GABA-T and SSADH (see review by Shelp et al).<sup>17</sup>

Tools to directly detect GABA in vivo have been developed and continue to be improved upon. Some GABA sensors that have been developed are based on Agrobacterium tumefaciens proteins Atu2422 and Atu4243. The Atu2422 protein has low affinity for GABA, and the Atu4243 protein fused with either cpGFP (circularly permuted green fluorescent protein) or cpSFGFP (cp-superfolder GFP) reportedly does not translocate to the membrane surface in HEK293 cells.<sup>18</sup> A more recently developed fluorescent sensor, iGABASnFR, which uses Pf622, a homologue of Atu4243 found in Pseudomonas fluorescens and fused with cpGFP or cpSFGFP, was developed and could be used to detect GABA release in mice and zebrafish in vivo.<sup>18</sup> It is difficult to find in the scientific literature reports of GABA sensors used in plants. Nevertheless, one group, Hijaz and Killiny (2020), reportedly used D<sub>6</sub>-GABA and gas

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**Figure 1.** A simplified diagram of GABA metabolism and its roles in plants. GABA is synthesized from glutamate (Glu) or arginine (Arg) and transferred by GABApermease (GABP) to mitochondria, where GABA is catabolized by GABA transaminase (GABA-T) and succinate semialdehyde dehydrogenase (SSADH) to succinate (Suc). The succinate enters the tricarboxylic acid (TCA) cycle to maintain the C/N balance in cells. Glu can be synthesized by  $\alpha$ -ketoglutarate ( $\alpha$ -KG) via glutamate dehydrogenase (GDH). SSA can also be converted to  $\gamma$ -hydroxybutyrate (GHB) through SSA reductase (SSR) and GHB can be changed to SSA via the GHB dehydrogenase (GHBDH). Diverse biotic and abiotic stress stimuli elicit an increase in cytosolic Ca2<sup>+</sup> levels. A Ca2<sup>+</sup>/calmodulin complex activates GAD in the cytosol and GABA level increases. Transporters control the influx (GAT1, ALMTs, AAP3 or ProT2) and efflux (ALMTs) of GABA. Through various pathways, GABA regulates pollen tube and adventitious root (AR) growth and development and enhances plant stress tolerance.

chromatography-mass spectrometry to investigate the uptake, translocation, and metabolism of exogenous GABA in Mexican lime (*Citrus aurantifolia*) seedlings.<sup>19</sup>

## 3. GABA acts as a signal molecule in plants

## 3.1 Agrobacterium tumefaciens-plant interaction

Agrobacterium tumefaciens is a powerful tool for plant genetic engineering where researchers have taken advantage of its natural ability to transfer DNA within its Ti plasmid to transform plant cells it invades.<sup>20,21</sup> After A. tumefaciens naturally invades a host plant, its T-DNA integrates into the plant's genome and the transformed plant cells produce opines which induce crown gall disease. Opines upregulate the quorum-sensing (QS) system during tumor colonization. N-(3-oxooctanoyl) homoserine lactone (OC8-HSL) is degraded by Agrobacterium lactonase AttM and is the QS signal molecule<sup>22</sup> that enhances conjugation of the Ti plasmid,<sup>23,24</sup> the amplification of the Ti plasmid,<sup>25,26</sup> and the severity of tumor symptoms.<sup>26</sup> GABA rapidly accumulates in wounded plant tissues in response to biotic and abiotic stress conditions.<sup>14</sup> In the Agrobacterium-plant interaction, GABA stimulates the degradation of the OC8-HSL by lactonase AttM<sup>27</sup> and high concentrations of GABA in the tumor suppresses Ti plasmid conjugation.<sup>28</sup> For example, a GABA-rich tobacco line was not as strongly affected by crown gall disease

as the wildtype was affected.<sup>27</sup> Furthermore, the crown gall symptoms were more severe in plants inoculated by an atu2422-mutated A. tumefaciens strain because they were unable to uptake GABA.<sup>29</sup> In tomato, a low-GABA line exhibited greater T-DNA transfer frequency than its control.<sup>30</sup> Moreover, inoculation by an A. tumefaciens strain with GABA-T activity to degrade GABA did not affect ploidy or copy number in two tomato cultivars and Erianthus arundinaceus.<sup>30</sup> These results indicate that GABA inhibits T-DNA transfer and that GABA degradation during cocultivation is an effective method for increasing T-DNA transfer.<sup>30</sup> However, Lang et al. (2016) showed that higher accumulation of GABA in her1 (an Arabidopsis thaliana GABA-T/POP2 mutant line) suppressed vir gene expression, which is essential for T-DNA transfer.<sup>31</sup> Therefore, some high levels of GABA accumulation may inhibit T-DNA transfer via vir gene suppression.

## 3.2 Plant development

In flowering plants, sexual reproduction is important for the completion of the plant life cycle. When a pollen grain lands on the surface of the stigma, polar growth of the pollen tube begins and allows the delivery of sperm cells into the ovule through the pollen tube. Proper guidance of pollen tube elongation is vital for plant mating. The concentration of GABA present in the flower can be a signal for pollen tube growth and

orientation. GABA forms a gradient along the path of the pollen tube through the pistil and reaches the highest concentration near the micropyle, through which the pollen tube penetrates the ovule.13,32,33 POP2 encodes a GABA-T. In the flowers of a pop2 Arabidopsis mutant, growth of many pollen tubes is inhibited concomitantly with the presence of excessive GABA concentration and lack of a GABA gradient.<sup>32,34</sup> Some pop2 tubes can grow toward the ovule, but they are misguided and cannot enter the ovule.<sup>13,32,33</sup> Exogenous GABA can also affect pollen tube elongation in a dose-dependent manner as reported in Picea wilsonii.35 Pollen tube growth in P. wilsonii was observed under low and high concentrations of GABA, where low concentrations prompted pollen tube growth and an overdose of GABA inhibited tube growth.<sup>35</sup> High GABA concentrations can also modulate pollen tube growth by impairing Ca<sup>2+</sup> influx through Ca<sup>2</sup> <sup>+</sup>-permeable channels and GAD functions downstream of the  $Ca^{2+}/CaM$  feedback control on  $Ca^{2+}$ -permeable channels.<sup>36</sup>

GABA is also involved in fruit ripening. Under normal growing conditions, GABA highly accumulates at the mature green stage of fruits with immature seeds and then rapidly declines during ripening, when seeds in tomato fruits become mature.37 Down-regulation of the *SlGAD* genes in tomato reduces GABA levels and has little effect on normal plant growth and development.38 However, up-regulation of tomato *SlGAD2* causes GABA levels to rise and stunts plant growth, delays flowering, and reduces flowering and fruit yield.<sup>39</sup> In contrast, in over-expressing *SlGAD3* tomato lines using the fruit-specific E8 promoter, high GABA levels were observed but no morphological abnormalities were recorded.<sup>40</sup> Further, in C-terminal-truncated *SlGAD3* OX lines, red-ripe fruits fail to develop due to a delay in ethylene production and a reduction of ethylene sensitivity.<sup>40</sup>

GABA is involved in seed gemination and primary and adventitious root growth. GABA levels increase in germination of soybean,<sup>41</sup> oats,<sup>42</sup> barnyard millet,<sup>43</sup> adlay,<sup>44</sup> rice,<sup>45</sup> wheat,<sup>46,47</sup> barley,<sup>48</sup> and Chinese wild rice<sup>49</sup> seeds. GABA activates a-amylase gene expression and promotes seed starch degradation in a dose-dependent pattern in seed germination.<sup>48</sup> Excess GABA inhibits the elongation of primary roots and dark-grown hypocotyls.<sup>34</sup> Exogenous GABA prevents seed germination and primary root growth in the recalcitrant seeds of Chinese chestnut by altering the balance of carbon and nitrogen metabolism to maintain the dormancy and storage of these seeds.<sup>50</sup> Similarly, a high GABA content also inhibits adventitious root growth. For example, adventitious root growth of poplar is inhibited if the GABA level is increased in poplar lines by inhibition of a-ketoglutarate dehydrogenase activity.<sup>51</sup> overexpression of *PagGAD2* <sup>52</sup> or exogen-ous GABA application.<sup>51,52</sup> The changes in GABA shunt activity affect cell-wall carbon metabolism-related genes and phytohormone (IAA, ABA, and ethylene) signaling.<sup>51,52</sup>

## 4. Abiotic and biotic stress

Unlike animals, plants are sessile organisms so they must obtain or produce their own resources to meet abiotic and biotic challenges through immobile means. Plants recruit many materials within themselves to respond to adverse conditions. GABA is one such material that accumulates rapidly in response to abiotic stress factors, such as low or high temperatures, drought, waterlogging, salt, hypoxia, excessive reactive oxygen species (ROS) content, and toxic heavy metals among others.<sup>4,14,53</sup>

#### 4.1 Low temperature

Low temperature is one of the most significant limiting-factors of plant productivity. High concentrations of GABA in plants are often reported in response to cold stress. For example, GABA accumulates to a high extent and the expression of GABA shunt-related genes is induced during exposure of barley or wheat seedlings to cold or freezing temperatures.<sup>54</sup> When hypoxia-treated sprouts were frozen at  $-18^{\circ}$ C for 12 h and thawed at 25°C for 6 h, GABA content increased markedly to 7.21-fold higher levels than that in the unfrozen sprouts.<sup>55</sup> Similarly, GABA increases to high levels in response to freezing stress in the perennial grass *Brachypodium sylvaticum*.<sup>56</sup> Though many studies report high levels of GABA in response to low temperature exposure, GABA content in tea roots, on the other hand, decreases under cold treatment.<sup>57</sup>

High content of GABA is associated with plant tolerance to low temperatures. Exogenous GABA application induces an increase of endogenous GABA and improves cold tolerance in tomato seedlings,<sup>58</sup> banana,<sup>59</sup> anthurium cut-flowers,<sup>60</sup> and tea plants.61 Potential mechanisms by which high levels of GABA alleviate low temperature injury may be due to enhancement of plant antioxidant systems,<sup>58,59</sup> which reduces malondialdehyde (MDA) and ROS contents,58 and proline accumulationmediated osmoregulation.<sup>59</sup> Using iTRAQ-based proteomic analysis, researchers determined exogenous GABA-induced interactions among the biological processes of photosynthesis, amino acid biosynthesis, and C/N metabolism in tea plants.<sup>61</sup> An increased level of GABA-shunt activity allowed GABA to participate in putrescine-induced acclimation to cold storage of zucchini fruit<sup>62</sup> and in salicylic acid-mediated amelioration of postharvest chilling injury in anthurium cut-flowers.<sup>63</sup> In nitric oxide (NO)-induced chilling tolerance, NO treatment increased the activities of diamine oxidase, polyamine oxidase and glutamate decarboxylase while reducing GABA-T activity to lower levels, which altogether resulted in GABA accumulation.64,65

## 4.2 High temperature

High temperature is an important factor that can limit plant growth and development and there are investigations of many different plant species' relationships of heat stress and GABA. For example, in immature seeds of soybean (*Glycine max* L. Merrill) that had been heat-dried at a maximum temperature of 40°C, *GAD* was expressed at high levels and GABA-T and SSADH decreased rapidly during the heat-drying treatment. Consequently, GABA content in the treated seeds increased to more than five-fold (447.5 mg/100 g DW) the content in untreated seeds (79.6 mg/100 g DW).<sup>66</sup> Similarly, GABA increases in ripening grapes<sup>67</sup> and cell suspension cultures originated from pea-size 'Gamy Red' grape berries in response to elevated temperature<sup>68</sup>. Furthermore, heat stress generated an increase of glutamic acid in the cytoplasm<sup>69</sup> that combined with calmodulin to activate GAD, thus producing high temperature-induced accumulation of GABA in *Arabidopsis* roots but not shoots.<sup>70</sup>

Studies indicate that GABA may serve a protective role in plants exposed to heat stress. The exogenous application of GABA to heat-stressed four-day-old rice (Oryza sativa) seedlings significantly increased growth and survival rates by improving leaf turgor and up-regulating osmoprotectants and antioxidants.71 Heat tolerance in creeping bentgrass effectively improved due to exogenous application of GABA, which is involved in regulating photosynthesis, osmotic potential, tricarboxylic acid cycle, metabolic homeostasis,<sup>69</sup> enhancement of antioxidant defense systems,<sup>72</sup> heat shock factors and heat shock proteins.<sup>73,74</sup> Exogenous supplementation with GABA protects the reproductive functions (pollen germination, pollen viability, stigma receptivity, and ovule viability) of heat-stressed mungbean plants, and these plants produce greater weights of pods and seeds in comparison to those of the controls.<sup>75</sup> Moreover, GABA application to heat-stressed plants also improves carbon fixation and assimilation and leaf water status by up-regulating the synthesis of osmolytes and thus reduces the oxidative damage.<sup>75</sup>

## 4.3 Drought

Drought is another highly restrictive factor for crop development and production and, similar to the stresses described above, promotes GABA accumulation. The excised leaves of turnip,<sup>76</sup> bean,<sup>77</sup> soybean<sup>78</sup> and sesame<sup>79</sup> plants subjected to drought stress raised their GABA levels. Drought has also been shown to induce high levels of GABA in tomato,<sup>80</sup> Phyllanthus species,<sup>81</sup> and creeping bentgrass.<sup>82</sup> The Arabidopsis gad1/2 mutant has shown remarkably reduced GABA content, large stomata aperture, and defective stomata closure.<sup>83</sup> Consequently, this mutant wilt earlier than the wildtype during a prolonged drought stress, whereas the functionally complemented gad1/2  $\times$  *pop2* triple mutant exhibits the opposite in phenotype and also produces a higher GABA content.<sup>83</sup> These results indicate that GABA accumulation during drought is a stress-specific response and helps regulate stomatal opening to prevent water loss.83 Increased endogenous GABA content by exogenous application improves white clover drought-tolerance via up-regulation of the GABA shunt, polyamines and proline metabolism.<sup>84</sup> A high level of GABA also increases chlorophyll content, osmoregulation (i.e. soluble sugars, proline), and antioxidant enzyme activity in black cumin subjected to a water deficit.<sup>85</sup> GABA enhancement of drought tolerance is associated with the improvement of nitrogen recycling, the protection of photosystem II, the mitigation of drought-depressed cell elongation, wax biosynthesis, fatty acid desaturase, and the delay of leaf senescence in creeping bentgrass.82 Drought and heat often occur simultaneously; in such case, as well as under drought alone, SSADH was identified as a metabolic quantitative trait loci (mQTL) in the barley flag-leaf.86

## 4.4 Flooding

Flooding severely affects crop yield.<sup>87</sup> Globally, it is estimated that 12% of cultivated land is affected by waterlogging, resulting in a 20% decrease in crop production.<sup>88,89</sup> Waterlogging reduces photosynthetic rate and antioxidant enzyme activity by causing damage to the protective enzymes<sup>90,91</sup> and ultimately limits plant growth.<sup>92,93</sup> In water-logged individuals of soybean<sup>94</sup> and grape plants,<sup>95</sup> GABA markedly accumulates in the nodules of the root systems. Further, GABA promotes the growth of maize seedlings in waterlogged conditions by downregulating reactive oxygen intermediates-producing enzymes, activating antioxidant enzymes, and improving chloroplast ultrastructure and photosynthetic traits.<sup>96</sup>

Hypoxic conditions resulting from soil waterlogging exacerbate the negative effects of the latter on plant growth and crop production. Hypoxia has been shown to induce GABA accumulation in plants.<sup>97–100</sup> When applesand germinating fava bean<sup>101</sup>, Finally, the GABA shunt is considered partly responsible for alanine accumulation under hypoxia.<sup>102</sup>, Finally, the GABA shunt is considered partly responsible for alanine accumulation under hypoxia.<sup>102–106</sup> soybean,, seeds experience hypoxia, GABA content increases greatly and decreases after termination of the stress. Under hypoxic conditions, glutamate decarboxylase and diamine oxidase activities increase, which in turn elevates plant GABA content.

## 4.5 Salt

Soil salinity is a major environmental stress that affects crop yield around the world.<sup>107</sup>Three cellular responses of salt tolerance have been proposed in plants: (i) osmotic stress tolerance, (ii) Na+ exclusion capacity and (iii) tissue tolerance to Na + accumulation. Multiple studies have reported a variety of protective molecules that accumulate in plants and the mechanisms underlying plant response to salinity.<sup>107-109</sup> The GABA-T-deficient pop2-1 mutant is sensitive to salt but not to osmotic stress,<sup>110</sup> and the genes involved in cell-wall and carbon metabolism, particularly sucrose and starch catabolism, increase under salt stress.<sup>111</sup> In contrast to the results observed for pop2-1, the pop2-5 mutant over-accumulated GABA in roots and exhibited salt tolerance rather than salt sensitivity.112 The different results of the different pop2 mutants may be due to their respective GABA levels, where one may have accumulated an excessive amount of GABA beyond a particular threshold that is harmful to plants.<sup>112</sup> Using the mutants pop2-5 and gad1,2 (with reduced ability of GABA production), Su et al. (2019) showed that GABA induces H<sup>+</sup>-ATPase activation and reduces Na<sup>+</sup> uptake, H<sub>2</sub>O<sub>2</sub>-induced K<sup>+</sup> efflux and ROS concentration.112 Consistent with those results, GABA accumulation was also induced in Nicotiana sylvestris and cytoplasmic male sterile (CMS) II plants treated with short- and long-term salt stress, but GAD activity did not correlate with GABA content.<sup>113</sup> Contrary to this study, GABA content in Nicotiana tabacum plants treated with 500 mM NaCl decreased on the first and third day and increased on the seventh day of salt treatment.<sup>114</sup> This may be due to the high level of salinity and a difference in plant development stage.<sup>113</sup> GABA and GAD mRNA levels increase markedly in

five wheat cultivars and poplar under saline conditions.<sup>115,116</sup> In salt-treated wheat leaves, key metabolic enzymes required for the cyclic operation of the TCA cycle reportedly were physiochemically inhibited by salt, but the increase in GABA shunt activity provided an alternative carbon source for the TCA cycle to function in mitochondria and bypassed salt-sensitive enzymes to facilitate the increase in leaf respiration in wheat plants.<sup>117</sup> Further, the application of exogenous GABA to maize,<sup>118</sup> white clover,<sup>119</sup> muskmelon,<sup>120</sup> germinated hull-less barley,<sup>121</sup> and tomato<sup>122</sup> increases endogenous GABA content, activates enzymatic antioxidant activity, alleviates salt damage to plants, and enhances plant salt-tolerance.

GABA accumulation in plants in response to salinity is also associated with other stresses and hormones. GABA accumulated in durum wheat under salinity treatment combined with high nitrogen or high light treatment and GABA could also serve as a temporary place of nitrogen storage.<sup>123</sup> Application of GABA to plants exposed to NaCl affects the production of H<sub>2</sub>O<sub>2</sub>, ABA, and ethylene.<sup>124,125</sup> Moreover, 22 ABA- and 50 ethylene-related genes have been shown to be regulated by exogenous GABA.<sup>125</sup>

## 4.6 Heavy metal

Heavy metals are major pollutants in soils and can contaminate food due to their accumulation in the edible parts of crop plants. Besides ion toxicity, ROS accumulation is a common phenomenon accompanied by heavy metal stress.<sup>126</sup> A metabolome analysis showed that GABA content increases during chromium (Cr) stress in rice roots.<sup>127</sup> Similarly, when soybean is grown under zinc (Zn) and copper (Cu) stress, high levels of GABA have been observed.<sup>128</sup> Nicotiana tabacum plants treated with intermediate (10 µM) Zn concentrations showed highly induced levels of GABA but low levels when treated with high (100 µM) Zn concentrations.<sup>129</sup> When rice seedlings grew under arsenic (As (III)) stress, GABA application induced GABA-shunt-related gene expression, activated the antioxidant enzyme system, and strongly inhibited As accumulation, thus conferring a tolerance to As (III) in the seedlings.<sup>130</sup> Interestingly, long-term accumulation of GABA is more highly efficient in inducing As (III) tolerance than higher GABA levels in the short term, which actually causes toxicity.<sup>130</sup> On the other hand, Cd stress seemingly reduces endogenous GABA content in duckweed, and under Cd stress, exogenous GABA enhanced rhizoid abscission, whereas Glu addition promotes rhizoid abscission.131 GAD genes are uniformly up-regulated in maize and rice roots by Cd stress, and the overexpression of ZmGAD1 and ZmGAD2 in Cd-sensitive yeast and tobacco leaves enhances Cd tolerance in the host cells.132 All of the aforementioned studies indicate that GABA content does not always increase in response to different metal stresses and may be more related to metal concentrations. Furthermore, high GABA content does not always enhance plant tolerance to metal stress.

## 4.8 ROS

A range of abiotic stress conditions can enhance the accumulation of ROS in plants. When the GABA shunt functions in response to abiotic stress, it effectively restricts ROS generation in plant tissues. Succinic-semialdehyde dehydrogenase is the enzyme that catalyzes the last step in the GABA shunt. Four T-DNA insertion mutants of SSADH (ssadh mutants) have been shown to be phenotypically dwarfed with necrotic lesions, bleached leaves, reduced leaf area, lower chlorophyll content, shorter hypocotyls, and fewer flowers.<sup>133</sup> These ssadh mutants are sensitive to heat and UV stress and accumulate high levels of ROS, causing cell death in tissues exposed to the stress.<sup>133</sup> A five-fold greater amount of GHB (y-hydroxybutyrate, a byproduct of SSA) was reported in ssadh mutants than in wildtype Arabidopsis.<sup>134</sup> Treatment with γ-vinyl-γ-aminobutyrate, a specific inhibitor of GABA-T/POP2, or a mutation of the POP2 gene prevents the accumulation of ROS in ssadh mutants, inhibits cell death, and improves growth.<sup>134,135</sup> The phenotype of *ssadh* tomato mutants (*SlSSADH*-silenced plants by the VIGS system) shows stunted growth, curled leaves, and hyper-accumulation of ROS, thus resembling Arabidopsis ssadh mutants.<sup>136</sup> Succinic semialdehyde can be converted into GHB by SSA reductase, and GHB can be converted into SSA by GHB dehydrogenase in animals and plants (Figure 1). Because SSA cannot be converted to succinate by SSADH in ssadh mutants, SSA and/or GHB accumulates.<sup>134,135</sup> In pop2 and pop2ssadh mutants, GABA levels are four and five times higher than that in wild type plants.<sup>135</sup> However, the phenotypes of double pop2ssadh mutants revert to that of the wild type, indicating that the high GABA content is not the cause of the phenotype of ssadh mutants.<sup>135</sup> The ssadh mutants are more sensitive to SSA, and pop2ssadh mutants are more sensitive to SSA or GHB than are wild type or pop2 mutants, indicating that high levels of SSA and/or GHB and not GABA levels cause the observed phenotype of ssadh mutants.135

## 4.9 Biotic stress

During plant growth and development, plants face a diversity of pathogens and pests. Plant GABA levels have also been shown to increase in response to biotic stress.<sup>10,137</sup> For example, in cultured rice cells treated with a cell-wall elicitor of rice blast fungus (Magnaporthe grisea), the level of GABA increased 12.5-fold at 8 hours after treatment.<sup>138</sup> Similarly, GABA production is highly induced in stems of Jatropha curcas L. (Euphorbiaceae) infected with the Jatropha mosaic virus (JMV),<sup>139</sup> in tomato leaves infected by *Botrytis cinereal*,<sup>140</sup> in tobacco leaves infiltrated with a hairpin elicitor,<sup>141</sup> in leaves of potato plants after inoculation with *potato virus* Y,<sup>142</sup> in leaf apoplast of Phaseolus vulgaris inoculated with P. syringae pv. phaseolicola (Pph) 1302Å,<sup>143</sup> in Arabidopsis infected with Fusarium graminearum,<sup>144</sup> in diseased Vitis vinifera,<sup>145</sup> and in lettuce inoculated with gray mold.<sup>146</sup> 'Candidatus Liberibacter asiaticus' and its vector, Diaphorina citri can accelerate cytosolic accumulation of GABA in citrus.<sup>147</sup> Conversely,

a comparative proteomic analysis showed a significant downregulation of GABA biosynthesis in tomato stems inoculated with highly and mildly aggressive Ralstonia solanacearum isolates. 148,149 This result is consistent with a transcriptome profiling showing GABA shunt-related genes GAD2 and SSADH1 knocked-down by VIGS.148 In addition, GABA accumulation decreases the biomass and toxicity of Lasiodiplodia *theobromae* and the metabolites produced by *L. theobromae*.<sup>150</sup> In plants, GABA plays an important role in central C/N metabolism by connecting amino acid metabolism to the tricarboxylic acid (TCA) cycle (see the detailed text in section 5 "C: N balance" below). In plant-microbe interactions, GABA contents increase due to the elevated GAD enzyme activity.-<sup>141,149</sup> Then GABA enters into the TCA cycle to maintain cell viability via alteration in C: N metabolism. 139,140 Consequently, GABA activates antioxidant enzymes (peroxidase, superoxide dismutase, and catalase) and limits the cell death that can be caused by excessive ROS.151 Thus, high GABA levels indicate plant resistance to pathogens.

Reportedly, GABA is also involved in plant defense against herbivorous insects.<sup>152-154</sup> For example, in GABA-reduced (*gad1/2* double mutant) and GABA-enriched (*gad1/2* x *pop2-*5) *A. thaliana* mutants, wounding of plant tissues and cell disruption caused by insect herbivory is sufficient to induce rapid, systemic, jasmonate (JA)-independent GABA synthesis and accumulation.<sup>155</sup> However, in the responses of *Clematis terniflora* D. C. to UVB radiation and darkness, overaccumulation of JA leads to a remarkable increase in GABA content.<sup>156</sup> In addition, high contents of GABA may be a plant defense against insects as GABA is an inhibitory neurotransmitter in invertebrate nervous systems.<sup>137</sup>

In summary, a common response to stress in plants is the immediate elicited increase of  $Ca^{2+}$  concentration. The  $Ca^{2+}$ /calmodulin complex is perceived by GAD in the cytosol and GABA accumulates. Then, GABA is degraded and enters the TCA cycle to maintain C/N balance as a metabolite or GABA inhibits ROS accumulation by activating antioxidant enzymes in plants. GABA may also be a signal to activate other molecules in plant response to stresses. In brief, stress induces high levels of GABA accumulation in plants and high GABA concentrations improve plant resistance to stress.

## 5. C: N balance

Carbon and nitrogen are the major essential elements for plants. Efficient assimilation of C and N is essential for optimal plant growth, productivity, and yield.<sup>157</sup> Carbon skeletons enter the TCA cycle through the GABA shunt, whereby GABA can function as a nitrogen storage metabolite in plants. For example, *A. thaliana* can grow well on a culture medium containing GABA as the sole nitrogen source.<sup>158</sup> Nitrogendeficiency by excision of 50% of the nodules from *Medicago truncatula* causes the concentration of GABA in phloem exudates to almost triple.<sup>159</sup> After artificial petiole-feeding with GABA, the GABA concentration in nodules increases significantly, the concentration of glutamate declines in phloem exudates and N<sub>2</sub> fixation recovers 4–5 days after excision.<sup>159</sup> In the process of seed "maturation-drying" in *Arabidopsis*,

GABA initially accumulates to a high level and then decreases upon germination.<sup>160-162</sup> In truncated-GAD transgenic Arabidopsis, GABA accumulates in dry seeds, while the concentration of a number of sugars and organic acids decrease, and numerous amino acids and total protein significantly accumulate. <sup>162</sup> These results show that deregulated GAD alters the N to C ratio in Arabidopsis seeds.<sup>162</sup> Additionally, the obstruction of the GABA shunt leads to significant changes in sucrose and starch contents and affects carbon metabolism in the cell wall.<sup>111</sup> Therefore, GABA is rightfully considered to represent the central position in the interface between plant carbon and nitrogen metabolisms.<sup>163</sup> Under low-nitrogen conditions, exogenous GABA application increases the nonstructural carbon hydrates and TCA intermediates in the stems of poplar seedlings.<sup>164</sup> Moreover, GABA significantly attenuates the low nitrogen-induced increase of leaf antioxidant enzymes, which suggests that GABA affects the C:N ratio for poplar growth by reducing energy costs under N-deficient conditions.<sup>164</sup>

#### 6. GABA transporters in plants

GABA can be transported across the plasma membrane and organelle membranes. In these processes, both intra- and intercellular transport of GABA is likely required. GABA transporters were first identified in animals<sup>165</sup> and then identified in plants in 1999.<sup>158</sup> Arabidopsis thaliana grows efficiently with GABA as its sole nitrogen source, thereby providing evidence for the existence of GABA transporters in plants.<sup>158</sup> Two low-affinity GABA transporters (amino acid permease 3, AAP3, and proline transporters 2, ProT2) from A. thaliana were identified by heterologous complementation in yeast, and these two GABA transporters can transport proline as well.<sup>158</sup> A high-affinity GABA influx transporter in A. thaliana, AtGAT1, has been characterized heterologous expression systems, through i.e., Saccharomyces cerevisiae and Xenopus laevis oocytes.<sup>166</sup> AtGAT1, localized at the plasma membrane, shares no sequence similarity with any of the non-plant GABA transporters described to date, and it expresses to the highest recorded levels in flowers and upon wounding or during senescence.166 GABA accumulates in the cytosol in response to various stress conditions and is transported into the mitochondria, where it is catabolized. A mitochondrial GABA-transporter (AtGABP, GABApermease) that mediates transport of GABA from the cytosol into the mitochondrion has been functionally characterized in Arabidopsis by complementation in yeast and Arabidopsis gabp mutants.<sup>167</sup> The gabp mutants grow abnormalities under limited-carbon availability on artificial media and in soil under low light intensity.<sup>167</sup>

Although influx transporters of GABA in plants have been characterized as aforementioned, a GABA-efflux transporter that transports GABA from the cytosol to the apoplast was identified recently in wheat.53'168–170 Plant ALMTs (alumi-num-activated malate transporters), classified as anion channels and regulated by diverse signals, are activated by anions and negatively regulated by GABA.168,171 GABA-mediated TaALMT1 activity results in altered root growth and altered

root tolerance to alkaline pH, acid pH, and aluminum ions.<sup>168</sup> Plant ALMTs from wheat, barley, rice, and Arabidopsis can transport GABA into cells.<sup>170</sup> TaALMT1 facilitates GABA efflux and influx at very high rates.<sup>170</sup> Ion of Al<sup>3+</sup> activates malate and GABA efflux at low pH but blocks TaALMT1mediated GABA influx. However, the reduction in GABA content in response to Al<sup>3+</sup> at low pH and to anions at high pH is due to the large GABA efflux caused by activated TaALMT1, bearing no relation to malate efflux.<sup>170</sup> GABA seemingly exerts its multiple physiological effects in plants via ALMT, including the regulation of pollen tube and root growth. The uptake of GABA by AtGAT1 is not reduced in response to Al<sup>3+,170</sup> However, GABA can inhibit anion transport by TaALMT1 from the inside and outside of a cell.<sup>172,173</sup> The molecular mechanism may resemble the conformational transition of GabR when binding to the pyridoxal 5'-phosphate (PLP)-dependent aspartate aminotransferase (AAT) and GABA.<sup>174</sup> It is possible that GABA causes the TaALMT1 active structure to make a conformational transition and render TaALMT1 unable to transport anions.<sup>173</sup>

## 7. Conclusions and future perspectives

Plant GABA was first reported in 1949 from potato tubers.1 As described above, GABA accumulates in response to different kinds of biotic and abiotic stress, and it regulates plant growth and development. Stress factors also rapidly elicit a transient increase of cytosolic Ca<sup>2+</sup> levels. Ca<sup>2+</sup> is the universal second messenger in stress signaling.<sup>109</sup> The Ca<sup>2+</sup>/calmodulin system activates GAD in the cytosol and concomitantly, GABA levels increase.6 Alternatively, GABA is synthesized from arginine through multiple steps. Then, GABA is transported into mitochondria by GABP and enters the TCA cycle to maintain C/N balance in cells. GABA influx is controlled by transporters GAT1, ALMTs, AAP3, or ProT2, and GABA efflux occurs by ALMTs. In cells, GABA facilitates photosynthesis, inhibits ROS generation, and activates antioxidant enzymes. GABA also regulates stomatal opening in drought stress and acts as a signal molecule in plants in order to regulate plant growth and development and elevate stress tolerance.

Recently, considerable progress has been made regarding GABA transporters; GABA regulation of adventitious root growth, primary root growth, and seed germination; and GABA responses to stress. However, many questions remain unclear or controversial. For instance, when plants experience a stress, how do they maintain balance between high GABA levels and plant growth? How do plant hormones interact with GABA? Clearly, GABA plays many roles in plants, but how does the signal pathway(s) of GABA operate(s) in plants? What is the component that senses GABA levels? What is the relationship between GABA and ROS? We hope ongoing and future research will provide the answers to these questions in the near future.

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The authors declare that they have no competing interests.

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## Authors' contribution statement

Chunxia Wu, Li Li and Na Dou wrote this manuscript; Chunxia Wu and Hui Zhang were involved in preparing figures and revising this manuscript. All authors read and approved the final manuscript.

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