4-Aminobutyrate (GABA): a metabolite and signal with practical significance

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3   **4-Aminobutyrate (GABA): a metabolite and signal with practical significance**

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24   **Abstract:** We discuss the origin of 4-aminobutyrate (GABA) from glutamate and

25   polyamines, and its subsequent catabolism to succinic semialdehyde and either succinate or 4-

26   hydroxybutyrate. Promiscuous activities of GABA transaminase, glyoxylate/succinic

27   semialdehyde reductases, and aldehyde dehydrogenase 10As appear to be important

28   determinants of cross-talk among metabolic pathways during stress. Imposition of abiotic stress,

29   as well as genetic or chemical disruption of glutamate decarboxylase, GABA transaminase and

30   tricarboxylic acid cycle reactions, results in non-cyclic carbon flux in the tricarboxylic acid

31   cycle, demonstrating that stress-induced GABA metabolism is strongly linked with respiration.

32   Metabolic generation of 4-hydroxybutyrate is probably linked to the stimulation of succinic

33   semialdehyde reductase activity by an increasing NADPH/NADP$^+$ ratio. We discuss the potential

34   signaling role of GABA in various processes, including pollen tube guidance, interaction with

35   fungal, bacterial and invertebrate pests, and stomatal functioning, and argue that further research

36   on short-term responses to stress is required to determine whether or not GABA functions by

37   binding to or regulating activity of GABA receptor molecules. Finally, we describe how

38   emerging information about the metabolic and signaling roles of GABA is being used to improve

39   plant defense against biotic and abiotic stresses, and benefit human health.

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42   *Key words:* abiotic and biotic stresses, 4-aminobutyrate, metabolism, plant defense, signaling

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46   **Introduction**

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47   4-Aminobutyrate (GABA) is found in virtually all prokaryotic and eukaryotic organisms. In

48   animals, this non-proteinogenic amino acid functions as an inhibitory neurotransmitter through

49   interactions with specialized receptors and transporters. In plants, the role of GABA is less clear.

50   Early research regarded GABA as a temporary N storage compound, which accumulates in

51   response to cold shock, O_2 deficiency and mechanical stimulation, and suggested that insects

52   feeding on leaves could induce the accumulation of GABA, which would in turn influence their

53   feeding habits (Wallace et al. 1984). If so, knowledge about GABA could have important

54   implications for crop resistance and tolerance to stress.

55   Over the past three decades or so, several reviews have summarized advances in our

56   knowledge of GABA in plants, including pathways, biochemical regulation, compartmentation,

57   and role(s) (Bown and Shelp 1989, 1997, 2016; Satyanarayan and Nair 1990; Shelp et al. 1999,

58   2006, 2012*a-d*, 2017; Kinnersley and Turano 2000; Bouché et al. 2003, 2004; Bown et al. 2006;

59   Fait et al. 2008; Allan et al. 2009; Gilliham and Tyerman 2016). In this paper, we discuss: (i) the

60   accumulation of GABA in response to abiotic and biotic stresses; (ii) the properties of the

61   various genes/proteins responsible for GABA metabolism; (iii) the potential crosstalk between

62   GABA metabolism and other metabolic pathways, with an emphasis on their regulation by

63   altered substrate availability and redox and energy balance during stress; (iv) the potential

64   signaling roles of GABA in various physiological, transcriptional and molecular responses; and

65   (v) how emerging information about GABA is being used to improve plant defense against biotic

66   and abiotic stresses, and benefit human health.

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68   **Context: GABA accumulates in response to both abiotic and biotic stresses**

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69   Extensive research has demonstrated that abiotic stresses such as cold, heat, salinity, drought,

70   waterlogging, O_2 deficit, CO_2 enrichment and UV radiation, applied singly or in combination,

71   can lead to GABA accumulation in various plant species, organs/ tissues, cell types and

72   translocation fluids (Table 1). Cytosolic acidification, which reportedly accompanies some

73   abiotic stresses, also increases the GABA level. Furthermore, there have been several reports of

74   GABA accumulation in response to biotic stresses such as fungal and bacterial infections and

75   elicitors, as well as the mechanical stimulation/damage resulting from crawling and feeding

76   insects.

77

78   **GABA metabolism and interactions with other pathways**

79   *GABA Shunt enzymes*

80   The primary source of GABA appears to be glutamate, which is generated via glutamate

81   decarboxylase or GAD (Fig. 1). This reaction consumes a proton, and therefore may mitigate

82   reductions in cytosolic pH. Most plant GADs, unlike bacterial or animal GADs, possess a C-

83   terminal 30-50 amino acid residue calmodulin (CaM)-binding domain, and *in vitro* activity of

84   recombinant GAD is activated by Ca^{2+}/CaM at neutral pH. However, it is not activated by

85   Ca^{2+}/CaM at the acidic pH optimum for the enzyme (Shelp et al. 2012*a*; Trobacher et al. 2013*b*).

86   Multiple GADs are present in most plant species. For example, there are five GADs in

87   *Arabidopsis* (Fig. 1), *Oryza sativa* L. (rice), *Solanum lycopersicum* L. (tomato) and *Zea mays* L.

88   (maize), nine GADs in *Glycine max* [L.] Merr. (soybean), six GADs in *Populus trichocarpa* Torr.

89   et Gray (poplar), three GADs in *Malus* x *domestica* Borkh. (apple) and *Camellia sinensis* (L.)

90   Kuntze (tea), and four GADs in various *Prunus* species (Shelp et al. 2012*a*, *c*; Trobacher et al.

91   2013*b*; Mei et al. 2016; Salvatierra et al. 2016). *In silico* and empirical analyses suggest that the

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92   activities of *At*GAD3/5 (Fig. 1), *Md*GAD2, *Os*GAD2, *Prunus persica* GAD3, and *Cs*GAD2/3 are

93   CaM independent, although *Os*GAD2 and *Cs*GAD2/3 possess a C-terminal autoinhibitory

94   domain. The CaM-binding domain in most GADs therefore provides a causal link between

95   elevated GABA and cytosolic Ca^{2+} levels, both of which occur in response to many stresses (e.g.,

96   Reddy et al. 2011; Shelp et al. 2012*a*). Several recent publications indicate the involvement of

97   stress-induced transcriptional changes in the control of GAD activity. For example, there is

98   evidence for the induced expression of CaM-dependent *AtGAD4* with drought, O_2 deficiency,

99   cold or salinity (Shelp et al. 2012*a*; Zarei et al. 2017*b*), and the simultaneous activation of CaM-

100   dependent *CsGAD1* and induction of CaM-independent *CsGAD2* expression with combined

101   hypoxia and mechanical damage (Mei et al. 2016).

102   Recombinant GABA transaminase (GABA-T) converts GABA to succinic semialdehyde

103   (SSA), with the effective utilization of both pyruvate and glyoxylate, thereby generating alanine

104   and glycine, respectively (Fig. 1) (Shelp et al. 2012*a*; Koike et a*l*. 2013; Shimajiri et al. 2013*b*).

105   This is in contrast to bacteria and animal GABA-Ts which utilize 2-oxoglutarate (and pyruvate to

106   a lesser extent), thereby resulting in the conservation of glutamate during the conversion of

107   glutamate to SSA (Shelp et al. 2012*a*). The activity of plant GABA-T could theoretically be

108   regulated by the availability of pyruvate produced in glycolysis and utilized in alanine

109   production, or the availability of glyoxylate produced in multiple pathways (e.g.,

110   photorespiration and non-photorespiratory serine synthesis, and fatty acid and purine catabolism)

111   and utilized by glyoxylate reductases and hydroxypyruvate reductases (Allan et al., 2009; Shelp

112   et al. 2012*a*; Hoover et al. 2013; Zarei et al. 2017*a*). To date, the potential role of the glyoxylate-

113   dependent reaction has not been closely examined (Renault 2013). Notably, there is only a single

114   GABA-T in Arabidopsis, but many other species have multiple GABA-Ts with similar substrate

115   preference. For example, there are two to four GABA-Ts in tomato, rice and canola (Shelp et al.

116   2012*a*; Shimajiri et al. 2013*b*; Bao et al. 2015; Faës et al. 2015). There is little evidence for

117   induction of GABA-T expression in response to stress (Shelp et al. 2012*c*). Indeed, GABA-T

118   activity is probably limiting during stress, which would contribute to the accumulation of GABA

119   (Simpson et al. 2010).

120   Succinic semialdehyde is oxidized to succinate via a recombinant NAD-dependent SSA

121   dehydrogenase (SSADH), which is regulated by NADH and adenylates (Shelp et al. 2012*a*) (Fig.

122   1). This is significant because redox balance can be modified by numerous stress conditions.

123   There is only a single SSADH in Arabidopsis and tomato. Together these three reactions, from

124   glutamate to GABA, SSA and succinate, are known as the GABA shunt because they bypass two

125   reactions of the tricarboxylic acid cycle (TCAC), 2-oxoglutarate dehydrogenase and succinyl-Co

126   ligase (see Respiratory Processes).

127

128   *Respiratory processes*

129   Dark respiration in aerobic organisms involves the glycolytic production of pyruvate, which

130   is converted to acetyl-CoA via pyruvate dehydrogenase for citrate formation and input into the

131   TCAC. The TCAC includes succinyl-CoA ligase, which generates ATP directly, as well as four

132   oxidation reactions, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase (OGDH), succinate

133   dehydrogenase (SDH) and malate dehydrogenase, which together generate NADH and $FADH_2$.

134   These reduced co-enzymes fuel ATP synthesis by oxidative phosphorylation. The carbon flux

135   under normoxia in the dark is generally considered to be cyclic, proceeding from citrate to

136   oxaloacetate, with the levels of all the C intermediates, including 2-oxoglutarate, succinate and

6

137   malate, constant (Sweetlove et al. 2010). Thus, succinate can be derived from 2-oxoglutarate in

138   the TCAC, as well as the GABA shunt.

139   Several studies have suggested a direct link between the GABA shunt and the TCAC (Shelp

140   et al. 2012*a*). For example, Tuin and Shelp (1994) demonstrated that the metabolism of $[^{14}C]$-

141   glutamate by excised developing soybean cotyledons in the dark results in the rapid production

142   of $[^{14}C]$-labelled GABA, then succinate and other TCAC organic acids. Subsequently, Tcherkez

143   et al. (2009) used $[^{13}C]CO_2$, $[^{13}C]$pyruvate or $[^{13}C]$glucose to demonstrate significant flux

144   through the GABA shunt in illuminated leaves of cocklebur, but little flux between 2-

145   oxoglutarate (or succinate) and fumarate. The cyclic nature of the TCAC was restored during the

146   night. Furthermore, Michaeli et al. (2011) showed that mutants of the mitochondrial GABA

147   permease reduce GABA uptake by mitochondria and increase TCAC activity.

148   Other studies have investigated the impact of metabolic dysfunction on levels of select

149   glycolytic (pyruvate), TCAC (citrate, isocitrate, 2-oxoglutarate, succinate, fumarate, malate) and

150   amino acid (glutamine, glutamate, GABA) metabolites, as well as some associated with

151   fermentation (lactate) and SSA reduction (4-hydroxybutyrate; see GHB Metabolism) (Fig. 2).

152   For example, disruption of NAD-dependent isocitrate dehydrogenase (*nad-idh*) and OGDH

153   activities (i.e., chemical inhibition of OGDH and anti-sense OGDH), respectively, typically

154   results in the accumulation of isocitrate and 2-oxoglutarate (Lemaitre et al. 2007; Araújo et al.

155   2008, 2012). Significantly, the levels of succinate and GABA and occasionally alanine, also

156   increase. Disruption of succinyl-CoA ligase activity decreases the level of succinate, as well as

157   most other TCAC organic acids, and results in the accumulation of GABA (Studart-Guimarães et

158   al. 2007). Anti-sense inhibition of SDH activity causes the accumulation of succinate without

159   increasing the levels of other TCAC organic acids (Araújo et al. 2011). Disruption of glutamate

160 dehydrogenase (i.e., *gdh1/2/3*) decreases the accumulation of 2-oxoglutarate, as well as malate,

161 but does not affect succinate even though GABA accumulates (Fontaine et al. 2012). Inhibition

162 of mitochondrial ATP synthase by RNAi or oligomycin treatment also results in GABA

163 accumulation, as well the accumulation of alanine, lactate and GHB and pyruvate, but not

164 succinate (Geisler et al. 2012). Interestingly, GABA accumulation is associated with both

165 glutamate accumulation and loss in these studies. Anti-sense OGDH for example, displays a

166 reduction in the formation of this 2-oxoglutarate-derived amino acid, as well as alanine, which is

167 derived from pyruvate (Araújo et al. 2012). Overall, the interference of TCAC enzymes,

168 anaplerotic reactions for the TCAC, and ATP synthesis under various conditions is generally

169 associated with non-cyclic carbon flux in the TCAC, and disruption of TCAC reactions upstream

170 of SDH typically stimulates the activity of the GABA shunt.

171 Disruption of the major GAD isoforms in Arabidopsis (i.e., *gad1/2*) decreases the level of

172 GABA, as well as citrate, fumarate and malate; however, surprisingly succinate increases

173 (Mekonnen et al. 2016) (Fig. 2). Disruption of GABA-T function (i.e., *gaba-t*), in combination

174 with salinity, increases GABA and decreases succinate, as would be expected if the shunt

175 produces succinate (Renault et al. 2010, 2013). After 1 d of salinity, malate but not citrate

176 increases, whereas after 3 d of salinity, both 2-oxoglutarate and succinate accumulate, even

177 though GABA presumably does not contribute directly to the succinate pool in this mutant

178 (Renault et al. 2010). Cold stress, as well as waterlogging, flooding and hypoxia, which simulate

179 an O_2 deficit, cause the accumulation of both GABA and succinate, and occasionally citrate,

180 fumarate and malate (Kaplan et al. 2007; Rocha et al. 2010; Komatsu et al. 2011; Antonio et al.

181 2016) (Fig. 2). Notably, pyruvate as well as lactate and alanine may accumulate, as would be

182 expected if glycolysis and related fermentation reactions are stimulated. Overall, these findings

183   indicate that stress-induced GABA metabolism in plants may be associated with various changes

184   in cellular redox and energy balance, which could modify the regulation of the TCAC (e.g.,

185   Sweetlove et al. 2010).

186   Antonio et al. (2016) have used [^{13}C]pyruvate to follow carbon flux through the TCAC,

187   fermentation, alanine metabolism and the GABA shunt in soybean roots under hypoxic

188   conditions, and [^{13}C]glutamate and [^{15}N]ammonium to monitor the metabolism of glutamate to

189   succinate. After 6 h respiratory O_2 consumption is reduced by 40%, and glycolysis stimulated,

190   thereby enhancing the production of ATP and pyruvate. Cytosolic NAD^+ is regenerated from

191   NADH by fermentation reactions such as lactate dehydrogenase-catalysed pyruvate reduction.

192   The activities of pyruvate dehydrogenase and SDH are restricted, so that the direct flux of

193   pyruvate into the TCAC is low and the conversion of succinate to fumarate is markedly

194   decreased. Pyruvate accumulation is reduced via the stimulated formation of alanine via alanine

195   transaminase and GABA-T. The alanine transaminase reaction produces 2-oxoglutarate, which

196   can be used by OGDH and succinyl-CoA ligase to produce another ATP. The mitochondrial

197   NAD^+ that is required to oxidize 2-oxoglutarate is apparently produced by the reversal of the

198   malate dehydrogenase reaction, utilizing oxaloacetate generated via phosphoenolpyruvate

199   carboxylase or aspartate transaminase activity. These data indicate that hypoxia does not

200   completely prevent the C flux from SSA to succinate, even though the redox balance is

201   presumably altered to some degree in response to hypoxia (see Fig. 2). In addition, GABA

202   probably accumulates, at least in part, in response to the stimulation of GAD activity by bound

203   Ca^{2+}/CaM or lower cytosolic pH (see GABA Shunt Enzymes). Thus, with hypoxia both GABA

204   and succinate appear to be temporary storage metabolites, which can readily supply the TCAC

205   when the stress is mitigated. Malate production is also stimulated, and while malate could

206 theoretically be recycled to pyruvate via the malic enzyme, it seems less likely given an

207 unfavourable redox balance.

208

209 *GHB metabolism*

210   Based on earlier studies of bacteria and animals (Valentin 1995; Buckel 2001; Mamelak

211 2012; Salminen et al. 2015: see references therein), it seems likely that stress-induced GABA

212 accumulation and an elevated NADH/NAD ratio would be accompanied by an elevated

213 NADPH/NADP ratio, which could facilitate the operation of an alternative path for SSA

214 metabolism to 4-hydroxybutyrate (GHB) (Fig. 1). Indeed, evidence has shown that GHB

215 accumulates with oligomycin inhibition of mitochondrial ATP synthase (Geisler et al. 2012), as

216 well as cold (Kaplan et al. 2007) (Fig. 2) and various other abiotic stresses, including O_2 deficit,

217 waterlogging, heat, drought and UV (Allan et al. 2003, 2008, 2012; Breitkreuz et al. 2003; Fait et

218 al. 2005). To date, two *GLYR/SSAR* (g*lyoxylate/succinic semialdehyde reductase*) genes have

219 been empirically identified for the irreversible NADPH-dependent reduction of SSA to GHB in

220 Arabidopsis, apple and rice (Brikis et al. 2017; Zarei et al. 2017*a*) (Fig. 1). The encoded enzymes

221 have been designated as GLYRs since the recombinant proteins utilize glyoxylate more

222 efficiently than SSA. However, single *glyr* and *glyr2* knockout mutants of Arabidopsis

223 accumulate less GHB with submergence than the wild type, providing convincing evidence for a

224 role of the GLYRs in SSA reduction *in vivo* (Allan et al. 2012). Recently, Mekonnen and Ludwig

225 (2016) used a *gaba-t x ssadh* double mutant to demonstrate that exogenous GHB can be back-

226 converted to both GABA and succinate, and provided evidence, using a gel-based assay, for the

227 GHB-dependent conversion of NAD^+ to NADH (Fig. 1). Unfortunately, we have not been able to

228 measure NAD^+-dependent GHB dehydrogenase activity in cell-free extracts despite repeated

229   attempts over the last decade. Based on bacterial and human studies, GHB could be back-

230   converted to SSA, as well converted to acetyl-CoA or 2,4-dihydroxbutyrate in the forward

231   direction (Valentin 1995; Buckel 2001; Mamelak 2012; Salminen et al. 2016; see references

232   therein). For the time being, the importance of these reactions in plants exposed to stress is

233   uncertain. It is clear, however, that GLYR/SSAR activity may divert some flux from succinate to

234   GHB during stress, and that this would be accompanied by NADPH oxidation. Indeed, the early

235   growth of a *glyr1/2* knockout or knockdown mutant is more susceptible to SSA toxicity in the

236   cold than WT and *GLYR1* overexpression lines (Zarei et al. 2017*a*). These findings are consistent

237   with an elevated rate of SSA conversion to GHB with cold, and suggest that GLYR1/2 are part of

238   an adaptive response to stress.

239

240   *Polyamine catabolism*

241   GABA can also be derived from polyamines (Shelp et al. 2012*b*; Tiburcio et al. 2014).

242   Indeed, the salinity- and anoxia-induced accumulation of GABA is reduced by 25% to 39% by

243   aminoguanidine, a diamine oxidase inhibitor (Xing et al. 2007; Liao et al. 2017). Recent research

244   has demonstrated that one to two recombinant Cu-amine oxidases and two recombinant

245   ALDH10As, respectively, can convert putrescine to 4-aminobutanal and 4-aminobutanal to

246   GABA in Arabidopsis (Fig. 1) and apple fruit (Planas-Portell et al. 2013; Zarei et al. 2015*a*,

247   2015*b*, 2016). Interestingly, both ALDH10A8/9 prefer 3-aminopropanal as a substrate over 4-

248   aminobutanal, but the root growth of single *ataldh10A8* and *ataldh10A9* knockout mutants is

249   oversensitive to salinity and GABA accumulation in shoots is reduced (Zarei et al. 2015*b*, 2016).

250   Together, these findings indicate that the pathway from putrescine to GABA plays a role in the

251   stress response in dicotyledonous plants and suggest that the carbon flux through this pathway

252   could be regulated by a combination of O_2 availability and redox balance, particularly in bulky

253   fruit (Shelp et al. 2012*b*; Zarei et al. 2015*b*; Lum et al. 2016*b*).

254

255   **Signaling**

256   Various physiological, transcriptional and molecular responses are elicited by changes in

257   plant or tissue GABA status, which can be induced by fungal/bacterial infections, nutrient

258   limitation, exogenous GABA, plant development, and the use of transgenic/gene knockout

259   strategies (see details and citations in Table 2). While these findings might be useful, caution

260   must be exercised in interpreting studies that use very high concentrations of exogenous GABA

261   (e.g., Kathiresan et al. 1997; Barbosa et al. 2011) and/or damage tissue during treatment

262   application (e.g., Sulieman and Schulze 2010), unless appropriate controls are conducted to

263   account for potential osmotic and wounding (see Shelp 2012) effects, respectively.

264   Perhaps the best support for GABA signaling in plants is: (i) the requirement for a GABA

265   gradient to guide the pollen tube through the apoplastic spaces within the pistil to the female

266   gametophyte (Palanivelu et al. 2003); (ii) the upregulation of the KLM operon in *Agrobacterium*

267   by GABA or wounded stems of GAD overexpression (Ox) lines of tobacco, which reduces the

268   quorum-sensing signal and virulence (Chevrot et al. 2006); (iii) the existence of a GABA-

269   binding domain on the plasma-membrane, aluminum-activated malate transporter (ALMT)

270   (Ramesh et al. 2015); (iv) the repression of the type III secretion system in *Pseudomonas* by

271   GABA or by tobacco GAD Ox plants, resulting in a reduction of the plant`s hypersensitive

272   response (McGraw et al. 2016); and (v) the wounding-induced accumulation of GABA in

273   systemic untreated leaves, which is independent of systemic cytosolic Ca^{2+} accumulation and

274   GABA translocation from wounded to systemic leaves (Scholz et al. 2017) (Table 2). Other

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275   research has shown that GABA binds the plasma membrane and changes the levels of cytosolic

276   Ca^{2+} (Yu et al. 2006, 2014), findings which could be explained by the effects of GABA on

277   ALMTs. For example, when ALMTs are closed the plasma membrane hyperpolarizes, thereby

278   increasing the inward-directed gradient for Ca^{2+} influx through either hyperpolarization-activated

279   Ca^{2+} channels or via non-selective cation channels (Ramesh et al. 2015; Gilliham and Tyerman

280   2016).

281   Stress-induced plant GABA can probably bind ALMT, thereby negatively regulating malate

282   efflux and conserving malate within the cell (Fig. 3), as suggested previously (Gilliham and

283   Tyerman 2016). A *gad1/2* double mutant has reduced GABA levels and tolerance to prolonged

284   drought, symptoms that are reversed by crossing this mutant with a *gaba-t* mutant in order to

285   elevate the GABA level (Mekonnen et al. 2016). Since there are multiple *ALMT*s in plants, and

286   they encode anion channels (Ramesh et al. 2015), it has been suggested that GABA accumulation

287   and ALMT are involved in the regulation of stomatal closure by stimulating solute loss and

288   consequent loss of turgor (Mekonnen et al. 2016; also see Bown and Shelp 2016). Notably, the

289   high expression of *TaALMT1* in wheat correlates with elevated GABA accumulation, which is

290   reduced by treatment with aluminum (Ramesh et al. 2016). This has not been explained to date

291   and may be relevant in terms of factors that alter endogenous GABA. Recent research has

292   indicated that up-regulation of *AtGAD4* expression in Arabidopsis plantlets subjected to short-

293   term salinity stress is associated with inducible co-expression of *ALMT2* and *calmodulin-like 37*,

294   as well as the transcription factors *WRKY28, WRKY30, WRKY40, MYB2, MYB15* and *MYB108*,

295   suggesting the involvement of anion transport, protein activation and gene regulation in GABA

296   accumulation (Zarei et al. 2017*b*). Further research should focus on short-term responses to

13

297   stress in order to determine whether or not GABA functions as a signal molecule by binding to

298   or regulating the activity of GABA receptor molecules.

299

300   **The emerging functions of GABA in metabolism and signaling have practical significance**

301   *Defense against bacteria and invertebrate pests*

302   Several studies have suggested that GABA mediates interactions between plants and other

303   organisms such as fungi, bacteria and invertebrate pests (see Bown et al. 2006 and Shelp et al.

304   2006), and consequently defense against such species could be improved by genetic engineering

305   elevated levels of GABA in plants. For example, constitutive GAD Ox lines of tobacco are more

306   resistant to *Agrobacterium* and *Pseudomonas* infection (Chevrot et al. 2006; McGraw et al.

307   2016) (Table 2). Furthermore, constitutive GAD Ox or *gaba-t* lines are more resistant to larvae

308   of the oblique-banded leafroller (Ramputh and Bown 1996; Scholz et al. 2015), the northern

309   root-knot nematode (McLean et al. 2003), the tobacco budworm (MacGregor et al. 2003) and

310   *Spodoptera* (Scholz et al. 2015, 2017) (Tables 2 and 3). These latter findings suggest that

311   wounding due to infestation or herbivory by invertebrate pests disrupts cell structure and

312   stimulates GAD activity and GABA accumulation with the release of hydrogen ions from the

313   vacuole to the cytosol (Bown et al. 2006). They do not establish whether GABA functions by

314   regulating GABA-sensitive neuromuscular junctions or by some other mechanism (Bown and

315   Shelp 2016).

316

317   *Exogenous GABA alleviates stress-induced losses in quality*

318   While it is clear that GAD Ox can enhance the endogenous level of GABA, no one has yet

319   studied the response of such plants to abiotic stress. However, there are several reports on the

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320   impact of exogenous GABA on the plant response to abiotic stress. These have focused on peach

321   fruit, banana peel, tomato seedlings and cut flowers exposed to chilling stress (Shang et al. 2011;

322   Yang et al. 2011; Malekzadeh et al. 2014; Wang et al. 2014*b*; Aghdam et al. 2015, Aghdam et al.

323   2016*a-c*), as well as melon seedlings subjected to hypoxic or saline conditions (Wang 2014*b*; Hu

324   et al. 2015), rice seedlings grown at elevated temperatures (Nayyar et al. 2014), and barley

325   seedlings treated with aluminum (Song et al. 2010). Overall, the application of exogenous GABA

326   appears to alleviate the stress-induced losses in quality such as the incidence of chilling injury

327   and various characteristics associated with membrane deterioration (Fig. 4). This is accompanied

328   by changes in the status of key metabolites such as GABA, proline, and some polyamines, as

329   well as improvements in energy and anti-oxidant systems. Further research is required to

330   establish whether the positive impact of exogenous GABA on the response to abiotic stress is

331   due to its role as a metabolite or signal.

332

333   *Enhancing endogenous GABA and health benefits*

334   GABA is known for its various medicinal properties, such as a reduction in anxiety, depression

335   and insomnia, and for its anti-cancer and antihypertensive activities (Okada 2000;Adham et al.

336   2006). For the last two decades, there has been considerable interest, particularly in Korea, China

337   and Japan, in producing foods with enriched GABA levels (Diana et al. 2014; Cho & Lim 2016).

338   The first main strategy for attaining this outcome in plants is the use of various cultural or abiotic

339   (e.g., hypoxia, heat) stress conditions to enhance GAD activity during the germination of

340   rice/wheat/soybean/fava bean seeds (Matsuyma et al. 2009; Youn et al. 2011; Morrison et al.

341   2013; Yang et al. 2013; Zhang et al. 2014; also see Cho and Lim 2016), or the preparation of

342   dried immature soybean fruits (Takahashi et al. 2013) and fermented green tea (Tsushida et al.

343   1987; Abe et al. 1995; Sawai et al. 2001; Allan et al 2003). GABA levels that are two to 50 times

344   control levels have been reported (Table 4). Some of these treatments can modify the nutritional,

345   organoleptic and functional properties of plants, so there is interest in inducing GABA

346   accumulation in plant-derived foodstuffs using non-thermal technologies such as high-pressure

347   processing (for review see Poojary et al. 2017). The second main strategy involves genetic

348   engineering of GAD Ox and/or GABA-T down-regulation, resulting in GABA levels in rice seed

349   and tomato fruit which are five to 349 times the levels in the wild-type (Akama et al. 2009;

350   Shimarjiri et al. 2013*a*; Nonaka et al. 2017; Takayama et al. 2015, 2017) (Table 4). Some of

351   these GABA-enriched plant foodstuffs have been successfully screened for positive outcomes

352   using spontaneously hypertensive or salt-sensitive rats (Abe et al. 1995; Akama at al. 2009;

353   Yoshimura et al. 2011), providing proof-of-concept for the benefits of plant foodstuffs enriched

354   in GABA.

355

356   **Concluding remarks**

357   Much has been learned about the metabolism and functions of GABA over the past three

358   decades. It is now clear that GABA accumulates in plants in response to both abiotic and biotic

359   stresses. With the identification of many genes responsible for GABA metabolism in various

360   species, including Arabidopsis, it has become possible to generate recombinant proteins for

361   biochemical characterization. These studies have shown that the activity of many plant GADs,

362   unlike bacterial and animal GADs, is activated by bound Ca^{2+}/CaM, thereby linking GABA

363   accumulation to Ca influx resulting from external stimuli. They have also shown that plant

364   GABA-Ts utilize pyruvate and glyoxylate as amino acceptors, rather than 2-oxoglutarate as in

365   bacterial and animal GABA-Ts. These data suggest links between plant GABA metabolism and

16

366   glycolysis, alanine metabolism and photorespiration. Metabolomic and labeling studies, often in

367   combination with mutants, have demonstrated that GABA metabolism does interact with several

368   other pathways during exposure to abiotic stress, including glycolysis, the TCAC and

369   mitochondrial electron transport chain, as well as alanine, GHB and polyamine metabolism.

370   Notably, GLYR prefers glyoxylate as a substrate over SSA, and ALDH10A prefers 3-

371   aminopropanal over 4-aminobutanal. Nevertheless, the use of mutants and transgenic plants

372   confirms that these enzyme activities are physiologically relevant in GABA metabolism during

373   stress, likely due to changing substrate levels and/or redox/energy balance. Therefore, the

374   promiscuous activities of GABA-T, GLYR and ALDH10A appear to be important determinants

375   of cross-talk among metabolic pathways during stress. Overall, these findings suggest that

376   GABA metabolism is an adaptive mechanism for maintaining respiration during and/or

377   following stress, and that the actual diversion of carbon flux from succinate to GHB production

378   depends upon the redox/energy balance. The fate of GHB remains uncertain.

379   Changes in plant or tissue GABA status have been associated with physiological,

380   transcriptional and molecular responses within plants, but many studies are complicated by the

381   use of high concentrations of exogenous GABA, tissue wounding during treatment application,

382   and the inability to distinguish between primary and secondary responses. Nevertheless, strong

383   support exists for a signaling role of plant GABA in pollen tube guidance, interaction of plants

384   with fungal, bacterial and invertebrate pests, anion transport and stomatal functioning. Do these

385   result merely from metabolic adjustments as a consequence of GABA accumulation or from

386   GABA binding to a receptor forming a complex that initiates a signalling cascade? If the

387   function of GABA is solely related to its ability to bind to a receptor, it is possible that multiple

388   binding sites are involved.

389   Our knowledge of GABA metabolism and signaling suggests that there are opportunities for

390   genetically engineering stress-resistant/tolerant plants and plants with enhanced health benefits.

391   If the major role of GABA in plants is as a signaling molecule the regulation of GABA levels

392   within the immediate vicinity of binding sites will be required. Thus the regulation of GABA

393   transport (see Shelp and Zarei 2017), as well as metabolism, would be an important component

394   of the signaling process.

395

396   **Conflict of interest**

397   The authors declare that there is no conflict of interest regarding the publication of this

398   paper.

399

400   **Author contributions**

401   This paper is based on the Gold Medal lecture given by B.J.S at the 2016 meeting of the

402   Canadian Society of Plant Biologists in Kingston, Ontario. A.W.B. and A.Z. contributed

403   significantly to the research presented at that conference and in particular to the preparation of

404   the present manuscript. All authors read and approved the final manuscript.

405

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986   **Table 1.** Abiotic and biotic stresses stimulate GABA accumulation in plants.

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Stress	Species/organ/tissue/cell type/translocation fluid	References
Cold	*Glycine max* and *Arabidopsis thaliana* leaves	Wallace et al. 1984; Kaplan et al. 2007; Allan et al. 2008
	Asparagus sprengeri mesophyll cells	Cholewa et al. 1997
	Hordeum vulgare and *Triticum aestivum* seedlings	Mazzucotelli et al. 2006
Heat	*Vigna unguiculata* cell cultures	Mayer et al. 1990
	A. thaliana leaves	Allan et al. 2008
Salinity	*Solanum lycopersicum* roots and leaves	Bolarin et al. 1995
	T. aestivum seedlings	Bartyzel et al. 2003/4; Al-Quraan et al. 2013
	A. thaliana, *Nicotiana sylvestris* and *S. lycopericum* leaves	Allan et al. 2008; Renault et al. 2010, 2013; Akçay et al. 2012; Bao et al. 2015; Zarei et al. 2016; Zarei et al. 2017*b*
Drought	*G. max* nodules and xylem sap	Serraj et al. 1998
	Brassica napus leaves	Faës et al. 2015
Waterlogging	*N. tabaccum* and *A. thaliana* leaves	Allan et al. 2008, 2012
	H. vulgare roots	Zhang et al. 2015
O_2 deficit	*Oryza sativa*, *A. thaliana*, *Cucumis melo* and *Prunus persica* roots	Reggiani et al. 1988; Aurisano et al. 1995; Miyashita and Good 2008; Mustroph et al. 2014; Wang et al. 2014; Salvatierra et al. 2016
	G. max sprouts and *Commelia sinesis*,	Tsushida and Murai 1987; Allan et al. 2003; Breitkreuz

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	Nicotiana tabaccum and *Arabidopsis* leaves	al. 2003; Allan et al. 2008; Mei et al. 2016;;; ; Liao et al. 2017
	Medicago sativa and *O. sativa* seedlings	Ricoult et al. 2005; Narsai et al. 2009
	O. sativa cotyledons	Kato-Noguchi and Ohashi 2006
	Brassica oleracea var. *italica* florets	Hansen et al. 2001
	G. max roots and nodules	Borella et al. 2017
CO$_2$ enrichment (often in combination with cold and O$_2$ deficit)	*Annona cherimola*, *Malus* x *domestica* and S. *lycopersicum* fruits	Merodio et al. 1998; Makino et al. 2008; Deewatthanawong and Watkins 2010; Deewatthanawong et al. 2010*a*, b; Mae et al. 2012; Trobacher et al. 2013*a*; Deyman et al. 2014*a*, *b*, Chiu et al. 2015; Lum et al. 2016*a*
	B. oleracea. var *italica* florets	Hansen et al. 2001
UV	*A. thaliana* plants	Fait et al. 2005
Cytosolic acidification	*A. sprengeri* mesophyll cells and *Daucus carota* cell suspensions	Carroll et al. 1994; Crawford et al.(1994
Mechanical stimulation	*G. max* leaves and hypocotyls	Wallace et al. 1984; Bown and Zhang 2000
Mechanical damage	*G. max*, *N. tabaccum*, *A. thaliana* and *Camellia sinesi* leaves	Ramputh and Bown 1996; Bown et al. 2002; Hall et al. 2004; Scholz et al. 2015; Mei et al. 2016
	M. sativa and *S. lycopersicum* phloem exudates	Girousse et al. 1996; Valle et al. 1998
Cladosporium fulvum	*S. lycopsericum* cell apoplast	Solomon and Oliver 2001, 2002
Agrobacterium infection	*A. thaliana* tumors	Chevrot et al. 2006; Deeken et al. 2006; Lang et al. 2016

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Pseudomonas infection	*A. thaliana* plants	Park et al. 2010
Harpin (bacterial elicitor)	*N. tabaccum* leaves	Dimlioğlu et al. 2015
Blast pathogen hydrolysate from cell walls	*O. sativa* suspension culture	Forlani et al. 2014
E-2-hexanal (wound-induced volatile organic compound)	*A. thaliana* leaves	Mirabella et al. 2008, 2015

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1000 **Table 2.** Plant GABA acts a signal

Plant species	Experimental details	Physiological, transcriptional or molecular response	References
Crustose red algae	GABA is released from grazed algae	Induces GABA neuronal receptors and triggers metamorphosis in red abalone	Morse and Morse 1979, 1984; Trapido-Rosenthal and Morse 1986
Helianthus annuus	Seedlings treated with 100 mM GABA for 12-16 h	Increases ethylene production and expression of *ACC synthase* and *ACC oxidase*	Kathiresan et al. 1997
Arabidopsis thaliana	*gaba-t* mutant has disrupted GABA gradient along pollen tube path	Arrested or misdirected pollen tube growth reduces seed yield	Palanivelu et al. 2003
	Seedling grown with 10 mM GABA for 7 d	Downregulates expression of 14-3-3 gene family members in a Ca^{2+}-, ethylene- and abscisic acid-dependent manner	Lancien et al. 2004
	gaba-t mutant germinated on 10 mM GABA	White, bleached phenotype with a shorter life cycle than when grown without N	Clark et al. 2009
	gaba-t mutant; seedlings treated with 1-10 mM GABA for up to 6 d	Pollen tube elongation is defective and cell wall-related genes are downregulated; leaf chlorosis and cell elongation is inhibited	Renault et al. 2011
	Seedlings treated with up to 200 mM GABA for up to 15 d	Root length and nitrate uptake are stimulated by GABA at low nitrate, and inhibited at high nitrate; increases amount of nitrate reductase protein at low nitrate	Barbosa et al. 2011

	gaba-t mutant; seedling treated without or with 1.5% (w/v) succinate semialdehyde for 1-2 weeks	Abnormalities in polarity of the adaxial-abaxial axis in leaf primordia; recovered with succinic semialdehyde	Toyokura et al. 2011
	gaba-t mutant; mechanical wounding or *Spodoptera littoralis* feeding	Larvae growth is significantly inhibited with feeding on local or systemic untreated leaves; GABA accumulation in systemic leaves does not depend on GABA translocation or an increase in cytosolic Ca^{2+}	Scholz et al. 2017
Nicotiana tabaccum	*Agrobacterium tumefaciens* culture treated with 1 mM GABA or wounded stems of *NtGADΔC* Ox plants	Stimulates expression of *attKLM* operon and decreases level of quorum-sensing signal in agrobacterium, which reduces virulence	Chevrot et al. 2006
	Detection of binding sites on pollen protoplasts	GABA binds to plasma membrane, triggering an increase in cytosolic Ca^{2+}	Yu et al. 2006
	Pseudomonas syringae DC3000 culture treated with GABA or *NtGADΔC* Ox plants	Represses the expression of type III secretion system in bacterium, which reduces hypersensitive response in plant	McGraw et al. 2016
	In vitro germination of pollen grains with up to 10 mM GABA for 6 h	Stimulates pollen tube growth, activates Ca^{2+}-influx, coupled to outward K+ efflux; modulated by GAD	Yu et al. 2014
Solanum	*Cladopsporium fulvum* infection	Induces expression of *GABA-T* and *SSADH* in	Solomon and Oliver

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lycopersicum	increases apoplastic GABA level from 0.8 to 2-3 mM	fungus	2001, 2002
	Botrytis cinerea infection or treatment with 1-10 mM exogenous GABA	Induces expression of *GAD*, *SSADH* and *GABA-T* genes in host, resulting in partial resistance	Seifi et al. 2013
Capsicum annuum	*Xanthomonas campestris* (Xc) infection; transient co-expression of *ADC1* and *Xc effector* (*AvrBsT*); *ADC1*-silenced leaves; exogenous GABA	Modulates hypersensitive cell death and GABA levels in pepper host; exogenous GABA inhibits avirulent *Xc* growth; results suggest involvement of polyamine-derived GABA in resistance pathway	Kim et al. 2013
Brassica napus	Nitrogen deprivation and growth cycle induces changes in phloem GABA; treatment of plants with 0.1 mM GABA	Positive correlation between phloem-GABA and nitrate influx; elevates expression of plasma membrane-located nitrate transporter and stimulates nitrate influx by root system	Beuvé et al. (2004)
Medicago truncatula	Artificial feeding of 15 mM GABA into phloem	Downregulates symbiotic nitrogen fixation	Sulieman and Schulze 2010
Triticum aestivum	ALMT proteins possess a GABA binding domain, and are activated by anions and negatively regulated by GABA	Modulation of ALMT activity alters root growth and root tolerance to alkaline pH, acid pH and aluminium ions; positive correlation between *ALMT1* expression and aluminum-sensitive GABA accumulation	Ramesh et al. 2015
Prunus spp.	Hypoxia elevates GABA levels	May induce expression of *GAD1*, *GAD2* and *GAD4*	Salvatierra et al. 2016

over 8-d period

1001   Abbrev: ALMT, aluminum-activated malate transporter; ADC, arginine decarboxylase; GABA-T, GABA transaminase; GAD,

1002   glutamate decarboxylase; Ox, overexpression

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1017 **Table 3.** GABA is a defence mechanism against invertebrate pests

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Host plant species/diet	Strategy for elevating GABA in host plant/diet	Biotic agent	Impact on biotic agent	References
Synthetic diet	Increase from 1.6 to 2.6 µmol g^{-1} FM	*Choristoneura rosaceana*	Rates of growth, development and survival of larvae are reduced	Ramputh and Bown 1996
Tobacco	Constitutive *NtGAD or NtGADΔC* Ox; 0.22-3.5 µmol g^{-1} FM root (180-2800% WT) and 0.55 µmol g^{-1} FM shoot (250% WT)	*Meloidogyne hapla*	Nematode egg masses are 50-100% fewer 9 weeks after inoculation	McLean et al. 2003
Tobacco	Constitutive *NtGAD or NtGADΔC* Ox	*Heliothis virescens*	Larval feeding is reduced by 80-90% in preference studies	MacGregor et al. 2003
Arabidopsis	*gad1/2 x gaba-t* triple mutant contains 0.6 µmol g^{-1} FM (5-fold WT)	*Spodoptera littoralis* larvae	Larval weight is reduced by 30% after 7 d of feeding	Scholz et al. 2015
Synthetic diet	Increase from 0 to 1 µmol g^{-1} FM	*Spodoptera littoralis*	Larval weight gain is reduced by 22 % after 7 d of feeding	Scholz et al. 2015

1019 Abbrev: FM, fresh mass; GABA-T, GABA transaminase; GAD, glutamate decarboxylase; Ox, overexpression; WT, wild-type

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1020 **Table 4.** Abiotic stress, breeding and genetic engineering strategies enrich GABA levels in plant-derived foodstuffs and provide

1021 hypotensive benefits

Species/ plant part	Strategy	GABA enrichment	Hypotensive effect	References
		-fold		
Rice grain	Soaking, soaking at elevated temperature and slightly acidic pH with glutamate in dark, or N_2 and CO_2 treatments during germination	up to 14		Zhang et al. 2014; Cho and Lim 2016; Ding et al. 2016
	Seed-specific expression of truncated *OsGAD2*	30	Spontaneously hypertensive rats	Akama et al. 2009
	Seed-specific expression of truncated *OsGAD2* and mild knockdown of *OsGABA-T*	74-349		Shimajiri et al. 2013*a*
Wheat grain	Sequential hydration and germination, followed by N_2 treatment and heat drying	40-57		Youn et al. 2011
Tomato fruit	Genetic variation	10	Spontaneously hypertensive rats	Yoshimura et al. 2011
	Constitutive overexpression of full-length *SlGAD3*	up to 5		Takayama et al. 2015
	Fruit-ripening-specific expression of truncated *SlGAD3*	up to 18		Takayama et al. 2017
	Expression of *SlGAD2* or *SlGAD3* with full or partial deletion of autoinhibitory domain	up to 15		Nonaka et al. 2017

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Soybean seed	Germination or soaking during germination, varying temperature and cultivar	1.5-13		Matsuyama et al. 2009; Morrison et al. 2013
	Genetic variation in meal prepared from dry seed	2		Morrison et al. 2012
	Heat drying seeds within immature fruit	5		Takahashi et al. 2013
Fava bean seed	Germination under hypoxia	2-8		Yang et al. 2013
Green tea leaves	Fermentation under N_2		Salt-sensitive rats	Abe et al. 1995
	Fermentation under N_2	Several		Tsushida et al. 1987; Sawai et al. 2001; Allan et al. 2003

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1038   **Figure legends**

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1040   **Figure 1.** GABA metabolism in Arabidopsis.

1041   The well-known GABA shunt is shown in blue balloons, and auxiliary pathways involving 4-

1042   hyroxybutyrate and polyamines are shown in red and green, respectively; all enzymes are shown

1043   in orange. The dashed lines represent reactions or enzymatic paths which have been

1044   characterized in a preliminary fashion only, if at all, in plants. The dotted lines represent

1045   reactions that are only found in animal and bacterial systems. Three of the five glutamate

1046   decarboxylases possess a calmodulin-binding domain. (See text for more explanation.)

1047   Abbreviations: ABAL, 4-aminobutanal; ALDH, aldehyde dehydrogenase; CaM, calmodulin;

1048   CuAO, copper amine oxidase; DHBA, dihydroxybutyrate; GABA, 4-aminobutyrate; GAD,

1049   glutamate decarboxylase; GABA-T, GABA transaminase; GHB, 4-hydroxybutyrate; GHBDH,

1050   4-hydroxybutyrate dehydrogenase; GLYR, glyoxylate/succinic semialdehyde reductase; SSA,

1051   succinic semialdehyde; SSADH, succinic semialdehyde dehydrogenase.

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1053   **Figure 2.** Metabolic dysfunction and **s**tress stimulates the GABA shunt & non-cyclic flux in the

1054   tricarboxylic acid cycle.

1055   The heat map represents the relative levels of marker metabolites in plants subjected to

1056   chemical, knockout and knockdown strategies, and/or abiotic stresses: red, increased; green,

1057   decreased; yellow, no effect; gray, not determined. Abbreviations: Ala, alanine; as, antisense; Cit,

1058   citrate; Fum, fumarate; GAB, GABA; *gaba-t*, GABA transaminase mutant; *gad*, glutamate

1059   decarboxylase mutant; *gdh*, glutamate dehydrogenase mutant; GHB, 4-hydroxybutyrate; Gln,

1060   glutamine; Glu, glutamate; IC, isocitrate; *nad-idh*, nad-dependent isocitrate dehydrogenase

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1061   mutant; Lac; lactate; Mal, malate; mATPS, mitochondrial ATP synthase; OG, 2-oxoglutarate;

1062   OGDH, 2-oxoglutarate dehydrogenase; Pyr, puruvate, SCL, RNAi, RNA interference; succinyl-

1063   CoA ligase; SDH, succinate dehydrogenase; Suc, succinate;

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1065   **Figure 3.** GABA regulates ALMT membrane channels across the plasmalemma.

1066   GABA-stimulated anion efflux is negatively regulated by GABA (dashed arrows with red bar). ?

1067   represents an unknown transport mechanism. Abbreviations: ALMT, aluminum-activated malate

1068   transporter; GABA, 4-aminobutyrate; GAD, glutamate decarboxylase; GAT, GABA transporter;

1069   TCAC, tricarboxylic acid cycle.

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1071   **Figure 4.** Exogenous GABA alleviates stress-induced losses in quality.

1072   The arrows represent GABA-induced increases or decreases in the levels or status of the

1073   preceding metabolites or enzyme activities during the positive response to stress. Abbreviations:

1074   ADC, arginine decarboxylase; AEC. adenylate energy charge; APX, ascorbate peroxidase; CAT,

1075   catalase; DAO, diamine oxidase; DHAR, dehydroascorbate reductase; GABA, 4-aminobutyrate;

1076   GAD, glutamate decarboxylase; GABA-T, GABA transaminase; GP, glutathione peroxidase;

1077   GSH, glutathione; GST, glutathione S-transferase; LOX, lipoxygenase, MDA, malondialdehyde;

1078   MDHAR, monodehydroascorbate reductase; OAT, ornithine δ-aminotransferase; ODC. ornithine

1079   decarboxylase; P5CS, Δ^1-pyrroline-5-proline carboxylate synthetase; PAO, polyamine oxidase;

1080   PDH, proline dehydrogenase; PLPD, phospholipase D; put, putrescine; ROS, reactive oxygen

1081   species; SAMDC, S-adenosylmethionine decarboxylase; SOD, superoxide dismutase; spd,
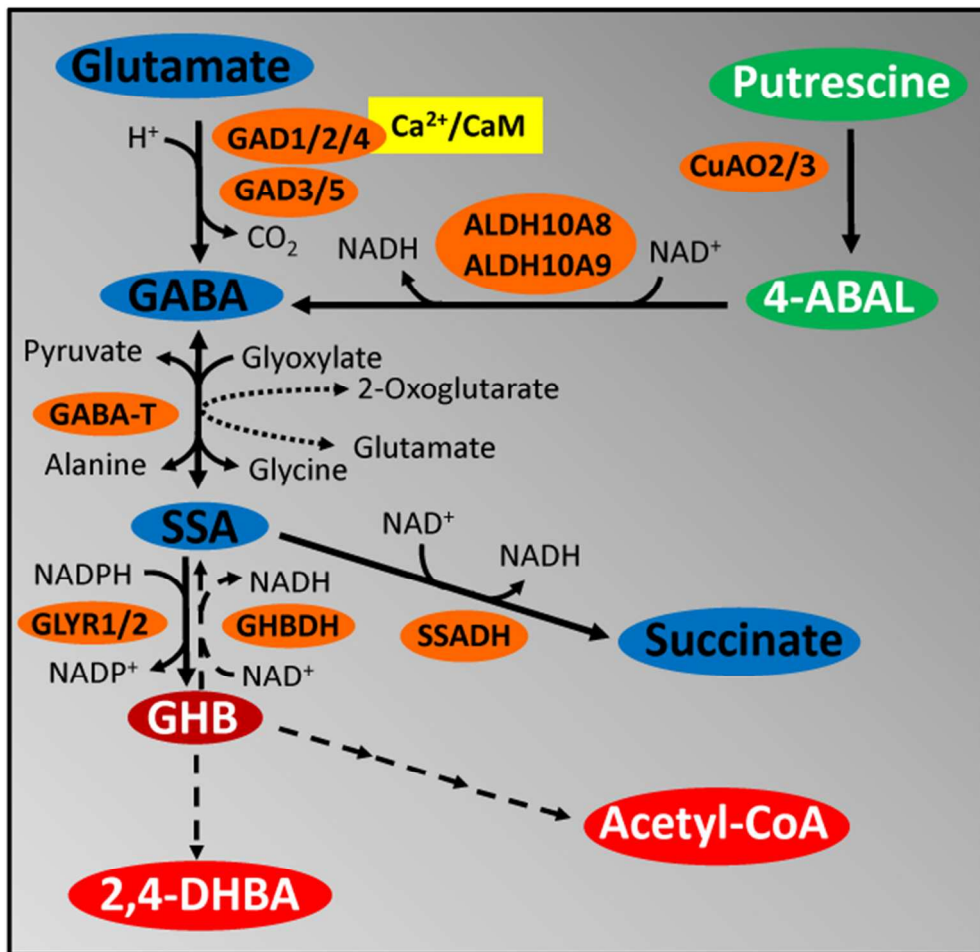
1082   spermidine; spm, spermine;

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Figure 1. GABA metabolism in Arabidopsis

56x54mm (300 x 300 DPI)

Tissue/Conditions	Strategy/abiotic stress	Relative metabolite level
		Lac GHB Pyr Cit IC OG Suc Fum Mal Gln Glu Ala GAB

Illuminated Arabidopsis leaves[1] — *nad-idhii* / *nad-idhiv*
Potato tuber discs[2] — Phosphonate inhibition of OGDH
Illminated tomato leaves[3] — asOGDH14 / asOGDH36 / asOGDH37
Illminated tomato leaves[4] — asSCL
Illuminated tomato leaves[5] — asSDH14 / asSDH43

Arabidopsis roots[6] — *gdh1/2/3*

Arabidopsis plantlets[7] — RNAi mATPS / Oligomycin inhibition of mATPS

Illuminated Arabidopsis shoots[8] — *gad1/2*
Arabidopsis roots[9] — *gaba-t,* 1 d salinity
Arabidopsis roots[10] — *gaba-t ,* 4d salinity

Illuminated Arabidopsis plants[11] — Cold acclimation
Lotus japonica roots[12] — Waterlogging
Soybean roots[13] — Flooding
Soybean root pieces[14] — Hypoxia

Figure 2. Metabolic dysfunction and stress stimulates the GABA shunt & non-cyclic flux in the tricarboxylic acid cycle.
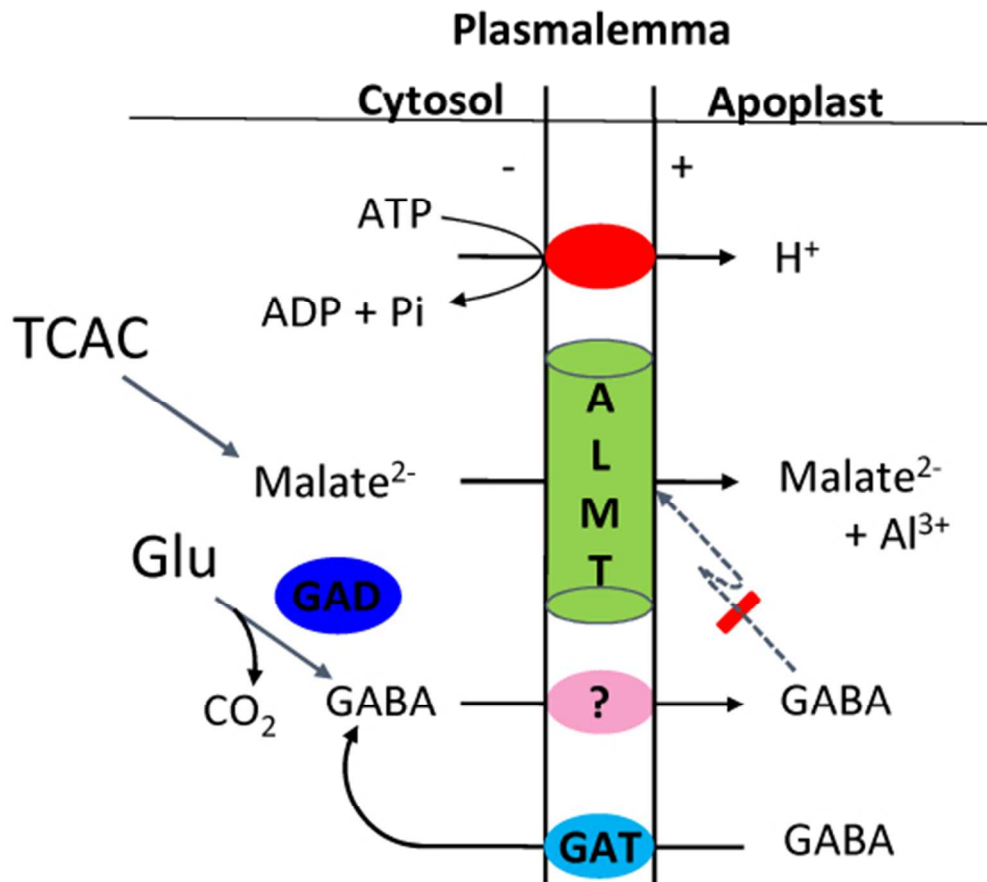
85x51mm (300 x 300 DPI)

Figure 3. GABA regulates ALMT membrane channels across the plasmalemma.
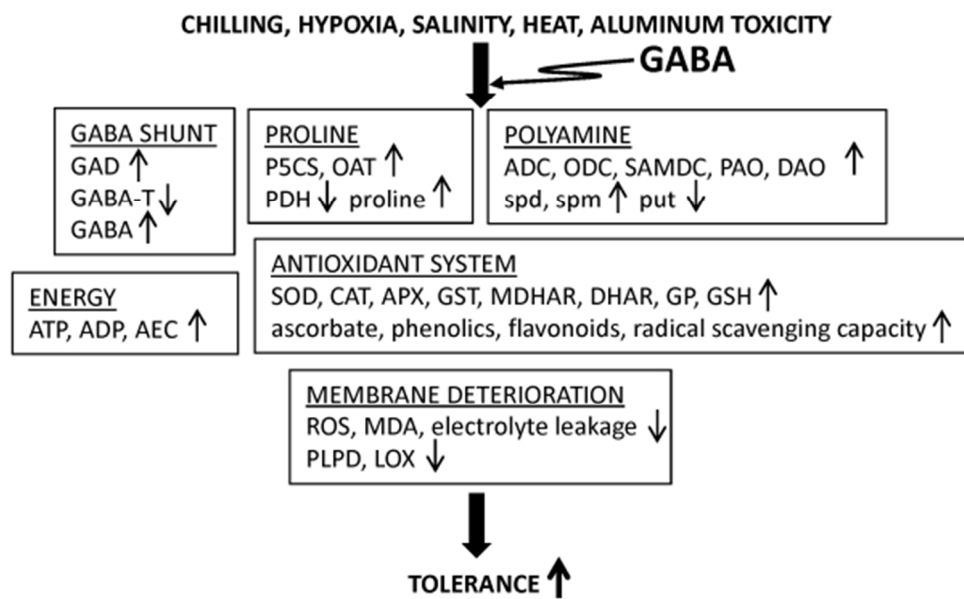
44x40mm (300 x 300 DPI)

Figure 4. Exogenous GABA alleviates stress-induced losses in quality.

52x32mm (300 x 300 DPI)