Critical Reviews in Plant Sciences, 19(6):479–509 (2000)

Gamma Aminobutyric Acid (GABA) and Plant Responses to Stress

Alan M. Kinnersley

Auxein Corporation, 3125 Sovereign Drive, Suite B, Lansing, MI 48911-4240

Frank J. Turano

Department of Biological Sciences, George Washington University, 2030 G Street NW, Washington, D.C. 20052

Referee: Dr. Alan Bown, Brock University, Biology Science Department, 600 Glenridge Ave., St. Catherines, Ontario, L2S-3A1, Canada

* Corresponding author: e-mail: kinnerslya@auxein.com

ABSTRACT: 4-aminobutyrate (GABA) is a non-protein amino acid that is widely distributed throughout the biological world. In animals, GABA functions as the predominant inhibitory neurotransmitter in the central nervous system by acting through the GABA receptors. The neuromuscular system enables animals to escape from environmental stresses. Being nonmotile, plants have evolved chemical responses to mitigate stress. Mechanisms by which GABA may facilitate these responses are discussed in this review. Environmental stresses increase GABA accumulation through two different mechanisms. Stresses causing metabolic and/or mechanical disruptions, resulting in cytosolic acidification, induce an acidic pH-dependent activation of glutamate decarboxylase and GABA synthesis. Extremely marked declines in cytosolic pH occur under oxygen deprivation, which is the primary stress factor in flooded soils, and this stress induces the greatest accumulation of GABA. Other stresses, including cold, heat, salt, and mild or transient environmental factors, such as touch, wind, rain, etc. rapidly increase cellular levels of Ca^{2+}. Increased cytosolic Ca^{2+} stimulates calmodulin-dependent glutamate decarboxylase activity and GABA synthesis. A review of the kinetics of GABA accumulation in plants reveals a stress-specific pattern of accumulation that is consistent with a physiological role for GABA in stress mitigation. Recent physiological and genetic evidence indicates that plants may possess GABA-like receptors that have features in common with the animal receptors. The mechanism of action of animal GABA receptors suggests a model for rapid amplification of ion-mediated signals and GABA accumulation in response to stress. Metabolic pathways that link GABA to stress-related metabolism and plant hormones are identified. The survival value of stress-related metabolism is dependent on metabolic changes occurring before stress causes irreversible damage to plant tissue. Rapid accumulation of GABA in stressed tissue may provide a critical link in the chain of events leading from perception of environmental stresses to timely physiological responses.

KEY WORDS: γ-aminobutyric acid, calcium, GABA receptor, GABA accumulation, stress signals, signal transduction, ethylene.

I. INTRODUCTION

In 1949, a report in *Science* disclosed that GABA (4-aminobutyric acid or γ-aminobutyric acid) had been identified in tuber tissue (Steward et al., 1949). That report is credited with aiding the identification of GABA in brain extracts shortly thereafter (Roberts, 1988). For many years the presence of large amounts of GABA in tissue of the central nervous system (CNS) remained a puzzle. It was unclear whether the unusually high concentrations of GABA in the CNS were connected directly or indirectly with neural transmission (Roberts, 1988). Today, GABA is understood to be the major inhibitory neurotransmitter in the CNS. The present situation regarding the role of GABA in plants is reminiscent of the early confusion concerning the role of GABA in the CNS. Numerous reports over many years have shown that high levels of GABA accumulate rapidly in plant tissues exposed to a variety of different stresses (Table 1). In response to environmental stresses, GABA production often increases so much that cellular levels of this non-protein amino acid exceed that of amino acids involved in protein synthesis. This situation has been reported in

0735-2689/00/$.50

TABLE 1
Stress-Related Kinetics of GABA Accumulation in Plants

Plant	Stress	GABA[a] % of Control	Time	Ref.
Asparagus cells	Acidosis	300	15 s	Crawford et al., 1994
Soybean leaves	Mechanical damage	1800	1 min	Ramputh and Bown, 1996
Soybean leaves	Mechanical damage	2700	5 min	Wallace et al., 1984
Soybean leaves	Cold (6°C)	2000	5 min	Wallace et al., 1984
Asparagus cells	Cold (10°C)	200	15 min	Cholewa et al., 1996
Radish leaves	Anoxia	10,000	4 h	Streeter and Thompson, 1972
Tea leaves	Anoxia	4,000	12 h.	Tsushida and Murai, 1987
Rice root	Anoxia	750	24 h	Aurisano et al., 1995
Rice shoot	Anoxia	1,000	24 h.	Aurisano et al., 1995
Cowpea cells	Heat	1,800	24 h.	Mayer et al., 1990
Bean leaves	Drought	200	3 d	Raggi, 1994
Turnip leaves	Drought	1000	3 d	Thompson et al., 1996
Tomato root	Salt	200	4 d	Bolarin et al., 1995
Tomato leaves	Salt	300	5 d	Bolarin et al., 1995
Tomato leaves	Viral	130	13 d	Cooper and Selman, 1974

[a] For each stress the time to reach the greatest reported GABA accumulation relative to unstressed controls has been shown.

drought-stressed cotton (Hanower and Brzozowska, 1975), heat-stressed cowpea cells (Mayer et al., 1990), and soybeans subjected to cold stress and mechanical damage (Wallace et al., 1984). Despite these reports, a direct role for GABA in mitigating such diverse stresses has not been demonstrated. Earlier reviews on possible functions of GABA have focused on roles in pH regulation, N storage, plant development, plant defense, and a role in carbon metabolism (Bown and Shelp, 1997; Satya Narayan and Nair, 1990). In a more recent review it was suggested that GABA could function as an osmolyte and mitigate water stress (Shelp et al., 1999). A common factor in stresses due to salt, drought, and freezing is cellular dehydration, and as a result the concentration of cellular constituents can increase to levels that cause membrane damage and cell death (Heber et al., 1971). A minimum of 28 to 30% water is required in plant cells for the maintenance and functional integrity of membrane structure (Blum, 1988). Cellular accumulation of GABA could balance the decrease in water potential that occurs during cellular dehydration. In support of such a protective role for GABA, Heber et al. (1971) showed that GABA can protect biological membranes from inactivation during freezing. However, other stresses in Table 1, including mechanical damage, heat, cold, oxygen deficiency, and viral attack, appear unrelated. Is there a mechanism whereby GABA accumulation could mitigate all of the stresses plants encounter in nature? The asking of this question implies a linkage between stress perception, GABA accumulation, and functional physiological responses. In turn, this suggests involvement of a signal transduction pathway and a role for GABA as an intercellular signaling molecule. Labeling studies have demonstrated that newly synthesized GABA is exported from cells (Chung et al., 1992), and GABA efflux in response to anaerobosis has been reported (Crawford et al., 1994). This is consistent with a role for GABA as an intercellular signaling molecule (Crawford et al., 1994; Shelp et al., 1999). Snedden and Fromm (1999) refer to the signaling hypothesis as a "provocative idea", but point out that no direct evidence for plant GABA receptors is available. Signaling molecules have the following characteristics: they (1) alter metabolism and/or growth, (2) alter expression of genes and/or activate enzymes, and (3) have receptor or sensor molecules (Sheen et al., 1999;

Iten et al., 1999; Grill and Himmelbach, 1998; Solano and Ecker, 1998; Walker and Estelle, 1998). GABA has been shown to alter plant growth in duckweed (Kinnersley and Lin, 2000) and tobacco (Baum et al., 1996), stem growth in *Stellaria longipes* (Kathiresan et al., 1998), and root growth in *Arabidopsis* (Locy, 1997). GABA activates arginine decarboxylase in soybean (Turano et al., 1997) and induces gene expression in *S. longipes* (Kathiresan et al., 1998). Until recently, the final criteria for a signaling molecule, the existence of GABA receptors, has remained speculative (Shelp et al., 1999; Snedden and Fromm, 1999). The major purpose of this review is to discuss recent physiological and genetic evidence for the existence of GABA-like receptors in plants, and to consider possible roles for GABA in plant stress responses. This discussion is preceded by a review of the historical record linking GABA accumulation to stress signals and the mechanisms responsible for this association.

II. GABA ACCUMULATION AND STRESS

As discussed by Satya Narayan and Nair (1990), GABA has been found in virtually every plant and plant part that has been examined. This contrasts sharply with the highly restricted distribution of other non-protein amino acids. Plants respond to environmental stresses, and a role for GABA in stress responses would help to explain its ubiquitous distribution. If GABA has a stress-related function, then levels would be expected to be greatest in tissues exposed to stress. The review by Satya Narayan and Nair (1990) reports that Collins and Wilson (1975) showed that the GABA content of aerobically soaked seeds was seven times higher than that of anaerobically soaked seeds. Likewise, the GABA content of aerobically grown tobacco cultures was reportedly higher than that of cells grown anaerobically (Koiwai et al., 1971). We have studied both reports and have not found anaerobic conditions mentioned in either report. Our review of the literature comparing the GABA content of stressed and nonstressed tissues has consistently found higher levels of GABA associated with stressed tissue. In contrast, we find only a few reports that contradict this finding. Shelp et al. (1999) have cited labeling studies indicating GABA does not always accumulate under stress. There are also reports showing increases in GABA accumulation in response to hormone treatments that appear not directly related to environmental stress (Kishinami, 1988; Ford et al., 1996). Pathways by which these reactions could relate to stress responses are discussed in a later section.

A. Kinetics of GABA Accumulation

The first report of a dramatic increase in GABA accumulation in response to stress was made by Naylor and Tolbert (1956). By feeding C^{14} labeled glutamic acid to barley leaves, the effects of oxygen deprivation on glutamate metabolism were determined. Under aerobic conditions 32.2% of the C^{14} was recovered from glutamine and 1.4% from GABA. Under anaerobic conditions, only 0.6% of the C^{14} was found in glutamine and 32.3% was recovered from GABA. Many subsequent studies have confirmed large and rapid accumulations of GABA in response to diverse stresses. Data presented in Table 1 have been selected to illustrate the magnitude of GABA accumulation induced by different stresses over time. The data were obtained from diverse plants and different tissues (seedlings, roots, leaves, and cell cultures). Stresses of different magnitudes were applied for at different times and under different conditions. This may explain some of the discrepancies between results represented in Table 1. To determine whether different stresses induce unique kinetic patterns of GABA accumulation, investigations would need to be performed using the same plant system. In the absence of such definitive studies, Table 1 is presented to show a pattern that is consistent with a physiological role for GABA in stress mitigation.

When the average time of GABA accumulation for each stress is considered, a stress-specific trend is evident (Table 2, Figures 1 and 2). The pattern of GABA accumulation in heat-shocked cowpea cells (Figure 1) and cold-shocked asparagus cells (Figure 2) was different with respect to time. GABA accumulation in cold-shocked cultures occurred in min-

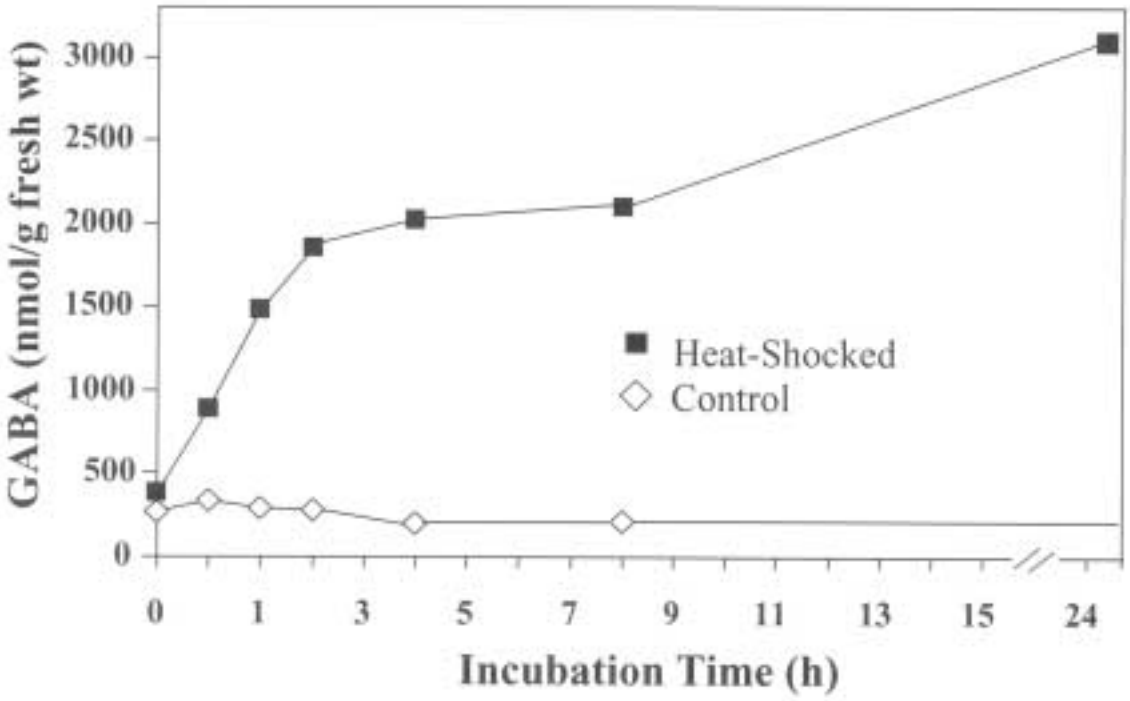

FIGURE 1. The time course of GABA accumulation in heat-shocked (26°C to 42°C) cowpea cell cultures. Heat shock resulted in a fivefold increase in GABA levels in 1 h, and 18-fold increase within 24 h. (Data from Table 2. Mayer, R. R., Cherry, J. L., and Rhodes, D. 1990. *Plant Physiol.* **94**: 796–810.)

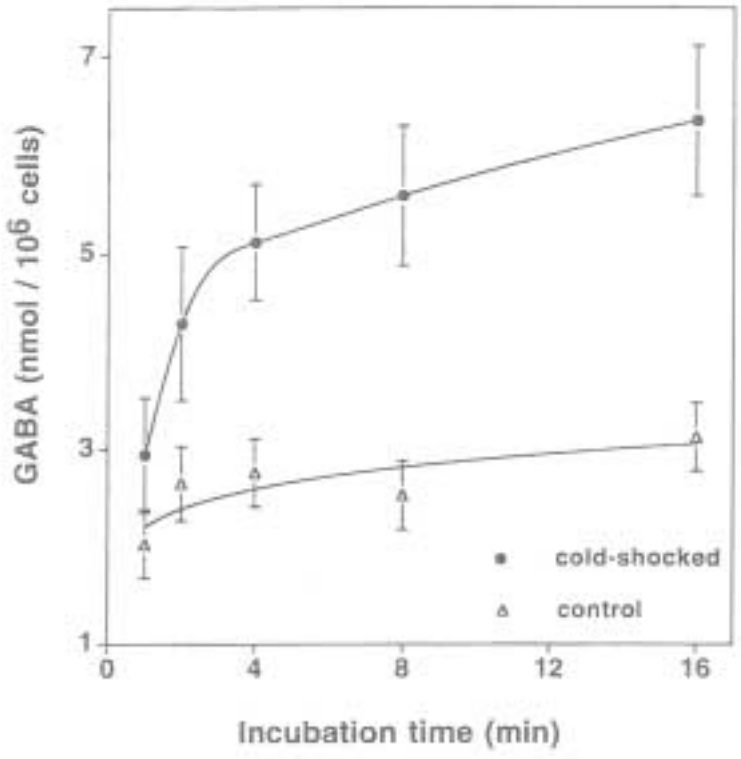

FIGURE 2. The time course of GABA accumulation in cold-shocked (20°C to 1°C) asparagus cell cultures. Cold shock resulted in a twofold increase in GABA levels within 16 min, and after this time the rate of accumulation diminished. (Reprinted with permission of Cholewa, E., Cholewinski, A. J., Shelp, B. J., Snedden, W. A., and Bown, A. W. 1996. *Can. J. Bot.* **75**: 375–382.)

TABLE 2
Stress-Related Trend in Time to Induce Maximum GABA Response

Stress	Time
Mechanical damage	1–5 min
Cold	5–15 min
Anoxia	4–24 h
Heat	1 d
Drought	3 d
Salt	4–5 d
Viral	13 d

utes, whereas in heat-shocked cultures it lasted for hours. If GABA is involved in stress responses, the temporal differences in GABA accumulation may reflect differences in stress-related metabolism. The time period available to elicit effective stress responses against animal feeding, frost or flooding is likely to be shorter than that to protect plants from heat, drought, or salt stress. Differences in patterns of GABA accumulation in Tables 1 and 2, and Figures 1 and 2 appear to reflect this phenomena. Tables 1 and 2 show that the stress that induced the slowest and smallest accumulation of GABA was viral infection. In this study tomato plants infected with TMV virus showed no change in growth rate in the period 7 to 25 days following inoculation (Cooper and Selman, 1974). The ability of many plants to tolerate viral "stress" is evidenced by the belief that all clonally propagated crops today are harboring one or more viral diseases (Hu and Wang, 1983). Although relatively small increases in GABA accumulation were found in tomatoes following TMV infection, GABA was the only amino acid that showed a significant increase 5, 8, and 13 days after inoculation (Cooper and Selman, 1974). Interestingly, drought and salt stress induced patterns of GABA accumulation of similar duration (Tables 1 and 2). Osmotic and salt stress each induce Ca^{2+} signals of similar magnitude and duration (Sanders et al., 1999). Stress-induced changes in Ca^{2+} are related to stress-induced changes in GABA in a manner discussed in the following section.

If GABA is involved in stress responses, the pattern of GABA accumulation in stress-resistant and stress-sensitive cultivars should differ. This question has been investigated in leaves and roots of cultivated tomato (*Lycopersicon esculentum*) and its wild salt-tolerant relative *L. pennellii* (Bolarin et al., 1995). Treatment with 140 m*M* NaCl showed significantly greater GABA accumulation in the salt-sensitive cultivar when compared with the salt-tolerant cultivar (Figure 3). The difference between cultivars can be rationalized on the basis that metabolic differences in the salt-tolerant cultivar made it less sensitive to salt stress. Salinity-reduced plant growth of *L. esculentum* when salt stress was applied for 8 days, while in the salt-resistant relative, *L. pennellii*, growth was increased. Increases in GABA in the cultivated tomato preceded increases in soluble sugars and proline that are known to be associated with salt tolerance (Harborne, 1997). Peak accumulations of soluble sugars and proline in leaves of *L. esculentum* were found 2 days after peak accumulation of GABA that occurred at day 5 (Figure 3). Under salt stress, leaves of *L. esculentum* accumulated three times more soluble sugars then those of the salt-tolerant cultivar by the end of the salinization period. During the same period, roots of *L. esculentum* accumulated over four times more proline than roots of *L. pennellii*.

B. Mechanisms of GABA Accumulation

Glutamate decarboxylase (GAD) catalyzes the formation of GABA from glutamic acid and is activated by increases in the cytosolic concentration of H^+ or Ca^{2+} (Ramputh and Bown, 1996). This dual mechanism of regulation relates cellular accumulations of GABA to the nature and severity of environmental stresses. Cytosolic levels of Ca^{2+} are elevated in response to cold shock, heat shock, salinity, drought, touch, and osmotic stress (Sanders et al., 1999). Increased cytosolic Ca^{2+} forms complexes with calmodulin (CaM) and Ca^{2+}/CaM activates GAD in the physiological pH range. Acidic pH stimulation of GAD

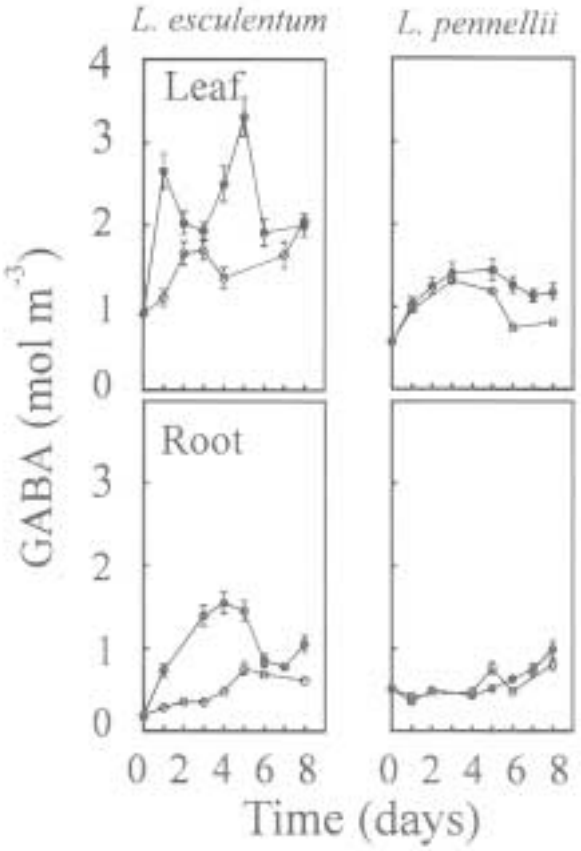

FIGURE 3. The time course of GABA accumulation in leaves and roots of cultivated tomato (*L. esculentum*) and wild salt-tolerant relative (*L. pennellii*). Plants were grown under control medium (open circles) and 140 m*M* NaCl (closed circles). Values are means ± SE of three replicates. (Reprinted with permission of Bolarin, M. C., Santa-Cruz, A., Cayuela, E., and Perez-Alfocea, F. 1995. *J. Plant Physiol.* **147**:463–468.)

occurs in response to stresses that reduce cellular pH. Anoxia occurs under conditions of flooding, and this stress causes cytosolic acidosis (Roberts et al., 1984; Aurisano et al., 1995A), giving the greatest increases in GABA accumulation. The vacuoles in plant cells are reservoirs for organic acids with a pH of 3.5 to 5.5 (Galston et al., 1980). Mechanical damage that ruptures vacuolar membranes will release organic acids into the cytosol, increasing cytosolic acidification and the likelihood of GAD activation.

1. Calcium/Calmodulin

In the last decade several plant GADs have been cloned (Baum et al., 1993; Turano and Fang, 1998; Zik et al., 1998; Yun and Oh, 1998), and recent studies have supported the hypothesis that GAD and GABA may be part of a signal transduction pathway occurring in plants after exposure to stress. The central piece of evidence is the finding that all plant GADs contain calmodulin-binding domains (CaM-BDs). Plant GADs have 22 to 25 additional amino acids at the C-termini when compared with the deduced amino acid sequences of GADs from other kingdoms. These domains have been shown to be sufficient for the binding of CaM in the presence of Ca^{2+} (Arazi et al., 1995; Zik et al., 1998). Several laboratories demonstrated that plant GADs were stimulated by Ca^{2+}/CaM, but not by either Ca^{2+} or CaM alone (Snedden et al., 1995; 1996; Turano and Fang, 1998; Zik et al., 1998; Yun and Oh, 1998).

It is recognized that Ca^{2+} and CaM form complexes, Ca^{2+}/CaM, that can stimulate enzymes to initiate a cascade of biochemical and molecular events in plant cells (for review see Zielinski, 1998; Roberts and Harmon, 1992). Many environmental stresses are known to increase cellular levels of Ca^{2+} (Mahlo et al., 1998; Sanders et al,, 1999). The fact that plant GADs contain CaM-BDs and are enzymatically stimulated by Ca^{2+}/CaM, but not by Ca^{2+} or CaM alone, indicates that GAD isoforms are part of a Ca^{2+}/CaM signal transduction pathway. However, the possibility that GABA may function as a signaling molecule has been neglected until recently. Recent findings by Kinnersley and Lin (2000) and Turano et al. (2000) provide evidence that GABA functions as a signaling molecule in plants.

The hypothesis that GABA functions as a signaling molecule provides a plausible explanation for the existence of a CaM-BD on the enzyme responsible for its biosynthesis. It is likely that the CaM-BD provides a mechanism for the control of GABA biosynthesis at physiological pH. Ca^{2+}/CaM-dependent stimulation provides several levels of control for the activation of GAD activity and thus GABA accumulation. One level of control is stress-induced release of Ca^{2+} into the cytosol that can be from various sources, that is, external or internal (e.g., apoplast, vacuole, and mitochondria). Recent studies have shown that there may be stress-specific routes of Ca^{2+} into the cytosol. For example, anoxia has been shown to release mitochondrial Ca^{2+} into the cytosol (Subbaiah et al., 1998), while wind and cold shock promote Ca^{2+} signaling pathways that are predominantly in the nucleus and cytoplasm, respectively (van der Luit et al., 1999). The other level of control is via the interaction of CaM and the CaM-BD. There appear to be five different GAD genes in *Arabidopsis* (Shelp et al., 1999), each with unique CaM-BDs. The physiological significance for the variability in the CaM region is not known. Because there are at least six CaM and CaM-like genes differentially expressed in *Arabidopsis*; however (Braam and Davis, 1990; Sistrunk et al., 1994; Ito et al., 1995), it is possible that each GAD/CaM-BD may have different affinities for the various CaM and/or CaM-like proteins. This hypothesis is supported by the fact that specific kinases have been shown to be differently activated by the unique affinities of distinct CaMs (Lee et al., 1997; 1999; Liao et al., 1996).

It was reported recently that yeast GAD contains a CaM binding site and that the GABA shunt is required for yeast to resist oxidative stress (Coleman et al., 2000). To our knowledge, this is the first report of a GAD/CaM-BD in eukaryotic organisms other than plants. The fact that this is associated with stress mitigation in yeast lends credence to the thesis that CaM-GAD performs a stress-related function in plants.

2. Acidic pH Stimulation of GAD

In *E. coli* GAD activity is stimulated by low pH with maximal activity at 3.8 (Shukuya and Schwert, 1960). A function of GAD in *E. coli* appears to be the control of cellular pH (Castanie-Cornet et al., 1999). For nearly 20 years, plant GADs were thought to function by reducing cellular acidosis in a manner similar to bacterial GADs (Davies, 1980; Reggiani et al., 1988; Menegus et al., 1989; Satya Narayan and Nair, 1990; Snedden et al., 1992; Crawford et al., 1994). This hypothesis was based on observations that: (1) all plant GADs, tested to date, have maximal activity in the acidic range, at approximately pH 5.8 (for review see Satya Narayan and Nair, 1990; Snedden et al., 1995; 1996; Johnson et al., 1997); (2) elevated GAD activity has been identified in tissues with low cytoplasmic pH (Ramputh and Bown, 1996; Carroll et al., 1994, Crawford et al., 1994); and (3) the synthesis of GABA consumes a proton and raises pH (Davies, 1980). Taken together those data suggest that GABA metabolism regulates cytosolic pH in plant tissues subjected to stress-induced acidosis (Crawford et al., 1994; Carroll et al., 1994; Streeter and Thompson, 1972; Satya Narayan and Nair, 1990).

The isolation of plant GADs and the association of CaM-BDs with plant GADs caused a major paradigm shift. Several models for the activation of GAD activity and GABA accumulation have been proposed (Snedden et al., 1995; Ramputh and Bown, 1996; Bown and Shelp, 1997; Snedden

and Fromm, 1999). Most of these models have focused on different aspects of GAD stimulation mainly via Ca^{2+}/CaM and not via alterations in pH. Despite the fact that it becomes clear that there exists at least two mechanisms, acidic pH and Ca^{2+}/CaM, by which GAD activity can be stimulated *in vitro* and *in vivo*, (Snedden and Fromm, 1999; Shelp et al., 1999), there has been little further discussion concerning the possible physiological role of *in vivo* acid-induced GABA accumulation. In most recent discussions of plant GAD acidic pH stimulation has received little attention, and the evolutionary significance of this mechanism has not been considered.

There are at least two evolutionary perspectives for the presence of pH-mediated GAD stimulation in plants. One is that pH-dependent stimulation of GAD is simply an evolutionary remnant from prokaryotic ancestors. Alternatively, plant GADs have evolved two types of GAD stimulation (biphasic), a Ca^{2+}/CaM- and acidic pH-dependent, and thus acidic pH-dependent stimulation has a physiological role in higher plants. It is apparent that through evolutionary time organisms have lost their acidic pH-stimulated GAD activity. The pH optimum for *E. coli* is approximately 3.8 (Shukuya and Schwert, 1960), in fungi it is 5.0 (Hao and Schmidt, 1991), for plants it is 5.8 (Snedden et al., 1996; Johnson et al., 1997), and mammal GADs have pH optima of 7.0 (Wu et al., 1974). Interestingly, mammalian GADs appear to have lost their acidic pH stimulation capabilities. These data suggest one of the following two hypotheses (1) there was strong selective pressure in mammals against acidic pH stimulated activity, or (2) there was strong selective pressures to maintain the acidic pH stimulation of GAD in plants and other organisms. Although it is difficult to prove either of these hypotheses, from the available data one can draw substantial support for the latter. First, in lower organisms, such as bacteria, GAD does play a role in protection against low pH (Castanie-Cornet et al., 1999), so there is a biological rationale for functional acidic pH-stimulated activity. Second, there are examples of decreases in cytosolic pH due to biotic stresses (Aurisano et al., 1995A, Roberts et al., 1984; Roos et al., 1999) and examples of acidic pH-dependent activation of various enzyme and protein systems in plants (Tognioli and Basso, 1987; Guern et al., 1992; Van der Veen et al., 1992; Blatt and Thiel, 1993; Hedrich and Dietrich, 1996). Third, there are data to support acidic pH stimulation of plant GADs (Ramputh and Bown, 1996; Carroll et al., 1994, Crawford et al., 1994), coupled with the occurrence of maximal GAD activity at pH 5.8, in purified preparations of the enzyme (Snedden et al., 1995; 1996) and in the absence of Ca^{2+}/CaM. In fact, GAD activity at 5.8 is 3 to 9 times higher than activity at 7.3 in the presence of saturating Ca^{2+} and CaM (Snedden et al., 1995, 1996).

3. Bi-Phasic Control of GAD Activity and GABA Accumulation

It is apparent that under some stress conditions, GAD activity has been linked to increases in cytosolic Ca^{2+} concentrations, (Cholewa et al., 1997), whereas in other stress conditions elevated GAD activity has been associated with decreases in pH (Ramputh and Bown, 1996; Carroll et al., 1994; Crawford et al., 1994). It is clear that both types of GAD stimulation have been observed in plants. The question is what are the factors that control the two types of stress-mediated activation of GAD and resulting GABA accumulation? There are several parameters that may control either acidic-pH or Ca^{2+}/CaM GAD stimulation in plants, which can be divided into organism- or environment-dependent factors. In the organism, organ specificity, cell type, developmental stage, and/or metabolic status of the cell that changes cytosolic Ca^{2+} and/or H+ could control GAD stimulation. Environmental factors include type, severity, and/or duration of the stress.

Results from several investigations support a working model for the *in vivo* biphasic stimulation of GAD and control of GABA accumulation in plants exposed to a variety of environmental stresses (Figure 4). For the sake of simplicity, we chose to divide the stimulation of GAD activity into two phases based on Ca^{2+}/CaM-dependent (Phase I) or acidic pH-dependent (Phase II) activity. It is important to emphasize that it is not clear at this time how these phases interrelate, if at all, in the cell (Snedden and Fromm, 1999). Each phase may occur continuously or both phases

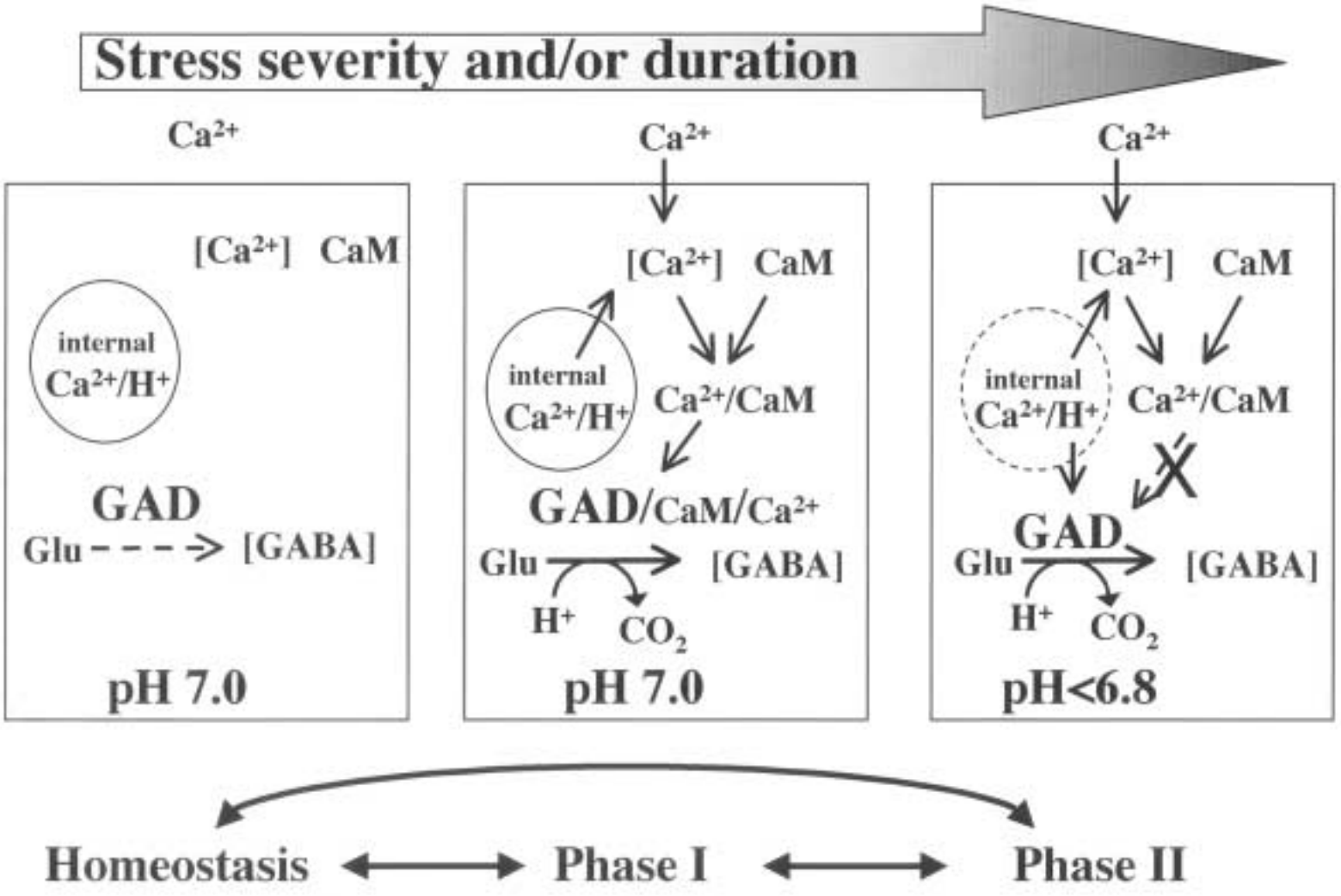

FIGURE 4. Biphasic regulation of GAD activity. At physiological pH, stress-induced increases in cellular Ca^{2+} complex with CaM and GAD is activated by Ca^{2+}/CaM. Intracellular stores of Ca^{2+} release Ca^{2+} into the cytosol further amplifying the stress response. Below pH 6.8, acid pH activates GAD with a pH optima at 5.8, at which GAD activity is 3 to 9 times higher that at 7.3 in the presence of Ca^{2+}/CaM. Stress-induced membrane damage releases H^+ from vacuoles into the cytosol. Several stresses have been shown to markedly reduce cytosolic pH.

may occur as distinct events in no predetermined order, depending on the factors discussed above. The stimulation of GAD by Ca^{2+}/CaM in Phase I may serve as a rapid or initial response to stress and/or a response to a mild or transient stress. As cytosolic pH decreases due to the extended duration and/or severity of the stress, then GAD activity could be stimulated by acidic pH in a Ca^{2+}/CaM-independent manner in Phase II. If the stress were transient and/or mild, however, Phase II may not be reached and the cells would revert to normal metabolism directly from Phase I. Alternatively, under severe conditions Phase II may be the only type of control (pH-dependent) of GAD activity. This is likely under conditions of severe anoxia where pH approaches 6.0 (Roberts et al., 1984), after exposure to a bacterial elicitor that decreases cytosolic pH (Roos et al., 1999), or where cellular damage results in disruption of vacuolar membranes releasing acids into the cytosol.

At homeostasis, cytosolic pH is approximately 6.8 to 7.0 (Felle, 1988; Horn et al., 1992) and cytosolic Ca^{2+} concentrations are low. Estimates of 70 to 250 n*M* and 50 to 150 n*M* Ca^{2+} have been reported in the cytosol of guard cells from *Commelina communis* (McAinsh et al., 1990) and in aleurone protoplasts from barley (Gilroy and Jones, 1992), respectively. Under similar conditions of neutral pH and low Ca^{2+} concentrations, little or no *in vitro* GAD activity has been observed with either pure GAD (Snedden et al., 1995) or pure recombinant GAD (rGADs) isoforms (Snedden et al., 1996; Turano and Fang, 1998; Yun and Oh, 1998; Zik et al., 1998). Thus, under homoeostatic conditions, little or no *in vivo* GAD activity would be expected and GABA biosynthesis would be minimal.

Various external stimuli have been shown to increase cytosolic Ca^{2+}, and the kinetics associated with increased cytosolic Ca^{2+} concentrations are stress-dependent (Knight et al., 1991, 1992, 1993, 1996; Price et al., 1994). Under stress conditions which elevate cytosolic Ca^{2+}, GAD activity would be stimulated and GABA biosynthesis initiated. Ca^{2+}/CaM-dependent activation of GAD has been demonstrated in tissues after exposure to cold (Cholewa et al., 1997), and anoxia (Aurisano et al., 1995). In *Arabidopsis*, experimental results are consistent with the hypothesis that high temperature-induced GABA accumulation is mediated by Ca^{2+}/CaM activation of GAD (Locy et al., 1998, 1999).

If the stress is prolonged and/or severe, the likelihood of metabolic dysfunction, membrane damage and degradation of cellular components increases, and acidification of the cytosol occurs (Ramputh and Bown, 1996). Cytosolic acidification could stimulate GAD activity in a Ca^{2+}/CaM-independent manner. Snedden et al. (1996) demonstrated there was a very clear point, at approximately pH 7.0, where the two types of GAD stimulation, Ca^{2+}/CaM- and acidic pH dependent, abruptly changed. Recently, decreases of 0.3 to 0.7 pH units were observed in plant cells subjected to an elicitor (Roos et al., 1999). Interestingly, the pH optima for other glutamate utilizing enzymes have been shown to be in the neutral range, that is, pH optima for glutamine synthase in the presence of Mg^{2+} is 7.0 to 8.0 (see Stewart et al., 1980 for summary) or alkaline pH 9.0 for cytosolic AAT (see Turano et al., 1990 for recent summary of values). Theoretically, under conditions that acidify the cytosol, most available Glu would be utilized by GAD for GABA synthesis.

The biphasic model of GAD activation accounts for mild and/or transient environmental factors, such as rain, touch, wind, etc., which rapidly increase cytosolic Ca^{2+} levels and may increase GABA accumulation. In addition, Phase I activation could explain the (1) pH-independent accumulation of GABA in maize root tips (Roberts et al., 1992) and root cultures transformed with *Agrobacterium rhizogenes* (Ford et al., 1996); and (2) the Ca^{2+}/CaM-dependent stimulation of GAD in response to cold (Cholewa et al., 1997), heat (Locy et al., 1999), and anoxia (Aurisano et al., 1995).

This model also provides an explanation for acidic pH-dependent (Phase II) activation of GAD that has been reported in several studies (Ramputh and Bown, 1996; Carroll et al., 1994, Crawford et al., 1994). As mentioned by Snedden and Fromm (1999), the results from mesophyll cells may be due to extensive manipulation during the experiments, which results in decreased cytosolic pH. Similarly, naturally occurring damage to cell compartmentalization will result in release of vacuolar H^+ and pH-dependent GAD activation. The idea that extensive manipulation or damage may con-

tribute to acidic-pH GAD stimulation lends credence to the hypothesis that severe conditions may contribute to acidic pH-stimulated (Phase II) GAD activity, as previously discussed. There is a need, however, for continued research to determine the factors that may contribute to acidic pH-stimulated GAD activity and to the factors that interact with Ca^{2+}/CaM-dependent stimulation of GAD activity.

III. EVIDENCE FOR EXISTENCE AND LOCATION OF PLANT GABA-LIKE RECEPTORS

A. Physiological Evidence for GABA-Like Receptors in Plants

Physiological evidence for the presence of GABA receptors in plants was obtained in experiments with duckweed (*Lemna minor* L). *Lemna* provides a simple and rapid whole plant bioassay for investigating GABA biology. *Lemna* cultured in fertilizer solutions with 5 m*M* GABA showed 2- to 3-fold increases in growth over plants grown with fertilizer alone. In contrast, two isomers of GABA were powerful inhibitors of plant growth. Growth promotion by GABA (4-aminobutyric acid) was rapidly terminated by addition of 2-aminobutyric acid to the culture medium. In cultures containing 0.5 m*M* 2-aminobutyric acid, plant growth was inhibited by 97% compared with controls (Kinnersley and Lin, 2000). GABA-mediated growth promotion of *Lemna* contrasts with reports showing that GABA accumulation inhibited cell elongation in tobacco (Baum et al., 1996) and soybean hypocotyl tissue (Bown and Zhang, 2000). In both studies GABA accumulation was 5- to 10-fold higher than that of control plants, and growth inhibition may occur at high levels. Kathiresan et al. (1998) performed a dose response study on the effects of GABA on stem elongation in *Stellaria longipes*. Results showed significant growth promotion when shoot tips were cultured on media with 0.1 to 0.25 m*M* GABA, and significant growth inhibition when media contained more than 1.0 m*M* GABA. Likewise, *Lemna* showed growth promotion and growth inhibition that was dose dependent, but inhibition was not found until the media levels of GABA exceeded 15 m*M* (Kinnersley, unpublished). The dose response growth curves found in *in vitro* studies with *S. longipes* and *L. minor* are consistent with a role for GABA in plant growth. Evidence that this role involves GABA receptors was obtained in experiments with pharmacological agents that have been used to identify GABA receptors in animals. $GABA_A$ receptors in the CNS were first identified by their sensitivity to the convulsant alkaloid bicuculline, a competitive antagonist of GABA at the $GABA_A$ receptor (Krogsaard-Larsen et al., 1997). Baclofen is a specific GABA agonist and the finding that activation of GABA receptors by baclofen was not inhibited by bicuculline led to the identification of the $GABA_B$ receptors (Deisz, 1997). $GABA_C$ receptors are insensitive to both bicuculline and baclofen (Johnston, 1997). $GABA_C$ receptors are sensitive to the antagonist picrotoxin. Picrotoxin is a plant-derived convulsant consisting of equimolar amounts of picrotoxinin and picrotin (Quian and Dawling, 1994).

Results showed that compounds that act as antagonists or agonists of GABA activity in animals acted in a similar manner in *Lemna* (Figure 5). Growth promotion by 5 and 10 m*M* GABA was increased significantly in treatments containing the GABA agonist baclofen. In contrast, the growth promotive effects of 5 m*M* GABA were completely eliminated in treatments containing either of the GABA antagonists 1 m*M* bicuculline or 3 m*M* picrotoxin. The inhibition was not relieved by increasing the GABA concentration to 10 m*M*, suggesting that bicuculline was not acting as a competitive antagonist of GABA activity in plants. In animals, bicuculline competes for GABA at the GABA recognition site on the $GABA_A$ receptor (Young and Penney, 1991).

Baclofen alone promoted *Lemna* growth and showed an optimal growth response at 1 m*M*, a level that was at least 10 times lower than the growth-optimal concentration of GABA. *Lemna* growth was significantly inhibited when plants were cultured in treatments containing bicuculline or picrotoxin (Kinnersley and Lin, 2000). The demonstration that these compounds have physiological activity in treatments without added GABA suggests that they are affecting a growth-related function of endogenous GABA. A role for

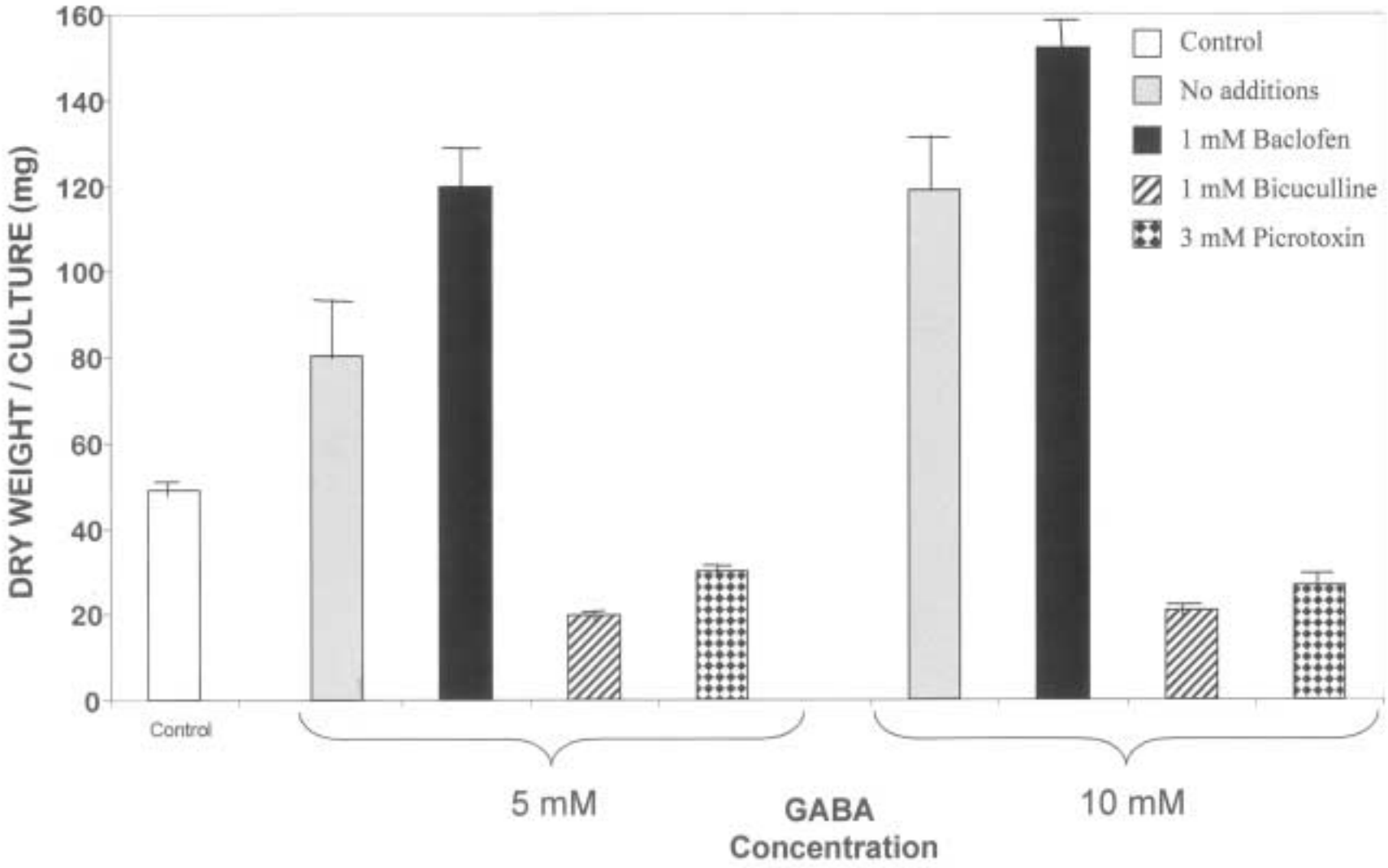

FIGURE 5. Effect of GABA antagonists and a GABA agonist on GABA-mediated growth promotion. Duckweed (*Lemna minor*) was grown in media containing 5 and 10 m*M* GABA with and without the GABA agonist baclofen (1 m*M*) and GABA antagonists bicuculline (1 m*M*) and picrotoxin (3 m*M*). Following culture period, plants were harvested and dry weights determined. Bars show the mean culture dry weight ± SE of six replicate cultures. (Reprinted from Kinnersley, A. M. and Lin, F. 2000. *Plant Growth Regul.* **32**:65–76, Figure 7 with kind permission from Kluwer Academic Publishers.)

GABA in plant development has been suggested by others (Baum et al., 1996).

If the mechanism of action for GABA involves signal transduction and a receptor, it can be predicted that the application of GABA in the field could result in dramatic changes in a variety of plant metabolic processes. This, in fact, has been demonstrated with a novel GABA-containing formulation, AuxiGro™, which has been registered by the United States Environmental Protection Agency (Kinnersley, 1998). AuxiGro contains equal parts by weight of GABA and glutamic acid and was developed after finding that foliar treatments of greenhouse-grown plants with GABA elicited a much smaller growth response than when duckweed and other plants were treated with GABA hydroponically (Kinnersley, unpublished). Glutamic acid, the GABA precursor, was added with GABA to increase endogenous production of GABA in treated foliage. Labeling studies have shown that when glutamic acid was fed to plant leaves, roots, seeds, and tubers, it was rapidly converted to GABA (Sata Narayan and Nair, 1990). AuxiGro has been found to affect the growth, productivity, and quality of a wide variety of crop plants, including root and bulb crops where the site of response is distant from the site of foliar application. On potatoes, for example, application of AuxiGro at the time that the potato tubers are being formed and 21 to 28 days later, results not only in increased yields but in significant increases in tuber quality (i.e., more larger tubers). Application of AuxiGro to snap beans at the time of flowering results in yield increases that can exceed 20% when compared with plants grown under conventional agronomic practices but without the application of AuxiGro. In onions, foliar application of AuxiGro resulted in increased yields of more than 300 bags per acre when compared with yields from areas where the onions had not been treated with AuxiGro. This 30% yield increase ($P \leq 0.01$) is related to increased size and uniformity of the bulbs from plants treated with AuxiGro. In fact, the increase in the largest bulbs (colossals) was increased by five times (Drost, 1999). AuxiGro has also been demonstrated to increase the resistance of plants against various pathogens. In Russet Burbank potatoes, AuxiGro in combination with Dithane®, a standard fungicide treatment, significantly reduced the incidence of both late blight, and early blight resulting in an increase in fresh market value of $506 per acre (Stevenson and James, 1999). AuxiGro has also been used on grape varieties and has been shown to both increase the sugar (Brix) content of fruit and to enhance resistance of the plant against the powdery mildew fungal pathogen (*Erysiphe* spp.). Unpublished studies demonstrate that AuxiGro is neither fungistatic nor fungicidal *in vitro,* and other evidence supports that the product may induce resistance to the fungal pathogen. This latter mechanism strongly supports a GABA receptor and signal transduction to enable the plant to respond with its own resistance mechanisms more rapidly to the attack by the fungal pathogen. GABA-mediated responses, which could increase disease resistance through mineral acquisition and/or biosynthesis of defensive compounds, are discussed in later sections of this review.

B. Molecular Evidence of GABA-Like Receptors

Plant genes encoding for proteins with a high degree of amino acid sequence homology to the superfamily of animal ionotropic glutamate receptors (iGLRs) have been identified (Lam et al., 1998). These genes have been designated the putative plant glutamate receptors (GLRs) based on their phylogenetic and structural similarities to animal iGLRs. The GLRs contain six signature domains characteristic of iGLRs, including three transmembrane, one pore forming and two putative glutamate-binding domains in the C-terminal regions of the proteins. Hydrophobicity analysis and transmembrane prediction models indicate the topologies of the putative *Arabidopsis* GLRs are similar to those of the iGLRs (Lam et al., 1998; Chiu et al., 1999). The topology predictions are supported by phylogenetic analyses of the six signature domains (Chiu et al., 1999), which demonstrates that the plant GLRs are evolutionarily related to iGLRs, and the periplasmic binding proteins of bacteria.

A similar approach was used to examine the evolutionary relationships between eight

Arabidopsis GLR sequences and the iGLRs and family 4 of the G-protein-coupled receptors (GPCRs), specifically the metabotropic glutamate receptors (mGLR), and γ-aminobutyric acidB receptors ($GABA_B$-Rs) (Turano et al., 2000). Based on the results from preliminary BLAST searches and incongruency analysis, they considered the possibility of a recombination event at *GLR* loci during evolutionary history. The peptides in the phylogenetic analyses were separated into two general regions. One contained approximately the first one-third (N-terminal regions) and the last two-thirds (C-terminal regions) of the peptides.

As expected, the C-terminal regions of the plant GLRs were related to members of the iGLRs superfamily and not to members of the GPCRs. Surprisingly, it was found that the N-terminal regions of the plant GLRs (within broken circle, Figure 6) are related to members of GPCRs superfamily, specifically the GABAB-Rs and not to members of the iGLRs. The phylogenetic relationship of the N-terminal domains is illustrated in Figure 6. Results from experiments with iGLR and GABA antagonists indicate that plants contain both iGLR-like (Lam et al., 1998) and GABA-like receptors (Kinnersley and Lin, 2000), respectively. *Arabidopsis* genes homologous to iGLRs have been reported (Lam et al., 1998; Chiu et al., 1999). Genes or peptides with homology to $GABA_B$-Rs have not been reported in plants. We have searched the *Arabidopsis* database for $GABA_A$ or $GABA_C$ -like receptors without success. Similar searches with regions of the $GABA_B$-Rs identified homology with the N-terminal regions of the putative GLRs.

To date, there are no other GABA-R-like sequences in the *Arabidopsis* genome and most of the *Arabidopsis* genome, is sequenced and the sequence of the entire genome should be completed by 2001 (Chaudhury et al., 1999). If other GABA-like receptors are not identified in the genome, we would propose that the GABA antagonists are interacting with the GABA-like domains of the putative GLRs in plants. This hypothesis is supported by results from experiments with antagonists and agonists to animal $GABA_B$-Rs, which demonstrated that the N-terminal domains of animal $GABA_B$-Rs are sufficient to specifically bind to GABA antagonists and agonists (Malitschek et al., 1999). These findings suggest that some of the putative GLRs in plants are fundamentally and perhaps functionally different from the iGLRs in animals. Perhaps some of the putative GLRs may bind GABA and function as GABA-like receptors.

Other preliminary data suggest that the putative GLRs may be functionally different from the iGLRs. In animals the iGLRs are exclusively associated with the plasma membrane. Protein sorting analysis was used to predict the likely location of two putative GLR sequences (2a and 2b) with N-terminal homology to the $GABA_B$-Rs in animals (Turano et al., unpublished results). Results indicated mitochondria as being the most likely location of GLR2a and the plasma membrane of GLR2b (Turano et al., 2000).

V. POSSIBLE ROLES FOR GABA IN PLANT STRESS RESPONSES

Circumstantial evidence gathered over more than 40 years suggests that GABA performs a stress-related function (Naylor and Tolbert, 1956; and Table 1). Physiological and genetic evidence have indicated the existence of GABA-like receptors in plants (Kinnersley and Lin, 2000; Turano et al., 2000). Hypotheses that relate the function of receptors to the role of GABA as a signaling molecule in plant stress responses are discussed below. The interconnection between GABA, environmental signals, and stress responses that may involve GABA receptors are shown in Figure 7. Nonsignaling pathways through which GABA could mitigate plant stress are also indicated in this figure.

A. GABA as an Amplifier of Stress Signals

The amino acid sequences in the N-terminal domains of the putative GLRs in *Arabidopsis* have domains in common with $GABA_B$ receptors in animals. GABA receptors function by modulating ion channels. This suggests a possible role for GABA in amplification of stress perception signals in plants (Kinnersley and Lin, 2000). A mechanism by which GABA may amplify stress-

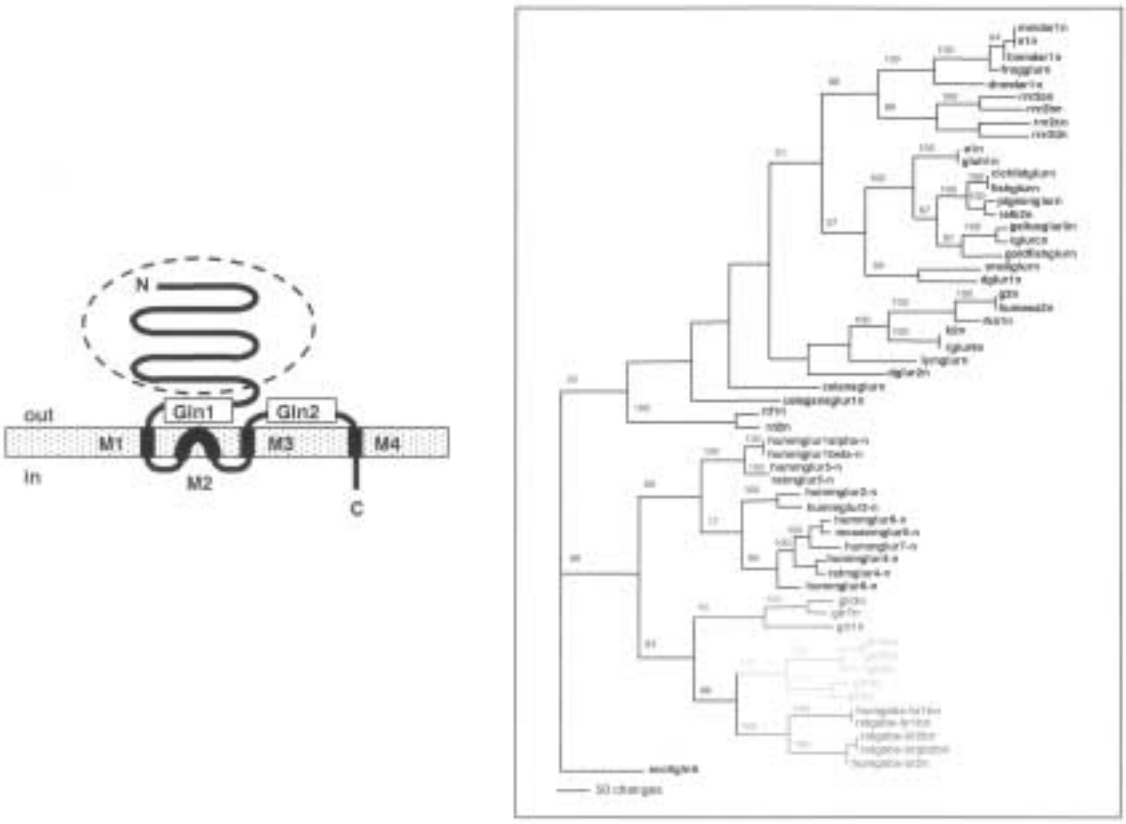

FIGURE 6. Evolutionary relationship of N-terminal domains of putative plant GLR receptors. Phylogeny of bacterial periplasmic binding proteins and eukaryotic receptors based on parsimony analysis of the N-terminal (circled, left) amino acid sequences. Sequences included in the phylogentic reconstruction (right) were a bacterial periplasmic binding protein, the animal ionotropic glutamate (iGLR), metabotropic glutamate (mGLR), and γ-aminobutyric acidB (GABA-BR) receptors, and putative plant glutamate receptors (GLR). The tree is one of four equally parsimonious trees generated from heuristic analysis (length = 6186 steps, consistency index = 0.649, retention index = 0.753, and rescaled consistency index = 0.488). A strict consensus tree generated from the four equally parsimonious trees was identical to the tree shown with the exception that hummglur7n was placed between hummglur6n and ratmglur4n. Support of the more important clades is indicated by bootstrap values using 500 permutations of the aligned data set. Ecoliginh was used as an outgroup. Similar results were obtained with nearest neighbor analyses (not shown). The abbreviations and accession numbers for the bacterial periplasmic binding proteins, animal iGLRs, and plant GLRs (1, 3, and 4) sequences are identical to those used by Chiu et al. (1999). The abbreviations and accession numbers for the mGLRs, $GABA_B$-BRs, and the remaining plant GLRs are as follows: human metabotropic glutamate receptor 1 alpha (hummglur1alpn, ACC# U31215), human metabotropic glutamate receptor 1 beta (hummglur1betn, ACC# U31216), human glutamate receptor, metabotropic 5 (hummglur5n, ACC# NM000842), rat metabotropic glutamate receptor mGluR5 ratmglur5n, ACC# D10891), human glutamate receptor, metabotropic 2 (hummglur2n, ACC# NM000839), human glutamate receptor, metabotropic 3 (hummglur3n, ACC# NM000840), human glutamate receptor, metabotropic 8 (hummglur8n, ACC# U92459), mouse metabotropic glutamate receptor 8 (mousemglur8n, ACC# U17252), human metabotropic glutamate receptor 7 (hummglur7n, ACC# U92458), human metabotropic glutamate receptor 4 (hummglur4n, ACC# U92457), rat metabotropic glutamate receptor 4 (ratmglur4n, ACC# M90518), human glutamate receptor, metabotropic 6 (hummglur6n, ACC# NM_000843), human $GABA_B$-B receptor subunit 1a (hum $GABA_B$-BR1an, ACC# AJ012185), rat $GABA_B$-B receptor subunit 1a (ratGABA-B1an, ACC# Y10369), human $GABA_B$-B receptor subunit 1b humGABA$_B$-BR1bn, ACC# AJ012186), rat GABA-B receptor subunit 1b (rat GABA-B1bn, ACC# Y10370), human $GABA_B$-B receptor subunit 2 (humGABA-BR2n, ACC# AJ012188), rat $GABA_B$-B R2 receptor (rat $GABA_B$-br2n2, ACC# AJ011318), rat $GABA_B$-B R2 receptor (rat GABA-br2n1, ACC# AF07442), *Arabidopsis* putative glutamate receptor 2a (glr2an, ACC# AF079999), *Arabidopsis* putative glutamate receptor 2b (glr2bn, ACC# AF038557), *Arabidopsis* putative glutamate eceptor 5 (glr5n, ACC# AL022604), *Arabidopsis* putative glutamate receptor 6 (glr6n, ACC# AL022604), and *Arabidopsis* putative glutamate receptor 7 (glr7n, ACC# AL031004). Lower case n designates N-terminal sequences from amino acid residues 80 to 320. GLR2a, 2b, 5, 4 and 6 are shown faded to highlight the closest similarity of these receptors to animal $GABA_B$ receptors.

induced Ca^{2+} signals is shown in Figure 7. Immediate increases in cytosolic Ca^{2+} occur in response to stress (Knight et al., 1992; Malho et al., 1998). Stress-induced increases in cytosolic Ca^{2+} stimulate CaM-dependent GAD activity that increases GABA biosynthesis (Aurisano et al., 1995; Cholewa et al., 1996). GABA binds to a receptor that releases Ca^{2+} from an intracellular Ca^{2+} store. The increase in cytosolic Ca^{2+} binds to more CaM increasing GAD activity and amplifying the GABA accumulation/Ca^{2+} release cycle. The major store of intracellular Ca^{2+} in most plants is the vacuole, and this organelle has been identified as a source of increases in cytosolic Ca^{2+}. A review of ion transport across the vacuolar membrane (Barkla and Pantoja, 1996) discusses voltage-dependent Ca^{2+} channels and ligand-gated Ca^{2+} channels that are proposed to function as release mechanisms for Ca^{2+}. The fact that concentrations of Ca^{2+} in the vacuole may exceed 100 m*M* (Trewavas and Malho, 1997) provides a strong argument for an important role of the vacuole in calcium signaling. A recent review discusses mechanisms by which this organelle regulates calcium levels in plant cells (Sze et al., 2000).

Despite the prominence of the vacuole as an intracellular store of Ca^{2+}, Sanders et al. (1999) have counseled that the possible participation of other intracellular compartments in Ca^{2+} signaling should not be ignored. Mitochondria are both a likely store of Ca^{2+} and another possible site of GABA receptors in plants. It is known that animal mitochondria have an important role in cellular Ca^{2+} signaling through their ability to accumulate and release large quantities of Ca^{2+} (Babcock et al., 1997). Calcium release channels have been identified in the inner membrane of animal mitochondria (Bernadi and Petrinelli, 1996). Evidence that plant mitochondria may perform a similar role has been obtained in studies showing stress-induced release of Ca^{2+} from maize mitochondria (Subbaiah et al., 1998). In response to anoxia, Ca^{2+} released from mitochondria made a significant contribution to the stress-related elevation of cytosolic Ca^{2+}. The possibility that oxygen deprivation damage was causing mitochondria to release Ca^{2+} was investigated by measuring changes in membrane potential during anoxia. At least 30 min or more of oxygen deprivation were required before any changes in potential, indicating a loss of membrane integrity, were found. Substantial decreases in mitochondrial Ca^{2+} were found 10 min after anoxia, well before any changes in membrane potential. The proposal that mitochondria are a source of Ca^{2+} for the rapid elevations of cytosolic Ca^{2+} during anoxia has implications for signal transmission. Regenerative Ca^{2} release from organelles that serve as intracellular Ca^{2+} stores has been suggested as a mechanism for transmission of Ca^{2+} signals to different cell regions (Malho, 1999). *In vitro* studies with isolated animal mitochondria have shown that calcium signals can propagate from one mitochondria to another generating traveling waves of Ca^{2+} and depolarization (Ichas et al., 1997). Results of protein sorting analysis (Nakai and Kanehisa, 1992), as described earlier, indicate that some GABA-like receptors in *Arabidopsis* may be located on mitochondrial membranes (Turano et al., unpublished results). In animals, mitochondrial membranes contain benzodiazepine receptors (Parola et al., 1993) and benzodiazepine receptors are associated with GABA-Rs ($GABA_A$ receptors) in the CNS (Young and Penney, 1991). Plant enzymes that catabolize GABA are associated exclusively with mitochondria (Breitkreuz and Shelp, 1995), and evidence for transport of GABA from the cytosol across mitochondrial membranes has been found (Shelp et al., 1999). It is possible that membrane transport of GABA is associated with Ca^{2+} release, and that these events involve the GABA-Rs. Evidence for an association between Ca^{2+} and a GABA-like receptor was reported recently (Kim et al., 2000). The receptor gene from *Arabidopsis* was identified as a glutamate receptor, GLR2, and is the same as the GLR2 loci described by Lam et al. (1998). As described in Section II.B of this review, closer analysis of the N-terminal domain of this sequence has revealed that it has sequence similarity to the animal GABAB receptor (Turano et al., 2000). This GABA-like receptor, has been designated GLR2a due to RNA editing (Turano, unpublished), and is shown in Figure 6. Kim et al. (2000) have now reported that this receptor regulates utilization of Ca^{2+} in *Arabidopsis.*

As indicated in Figure 7, there is evidence that Ca^{2+} plays a direct role in eliciting certain

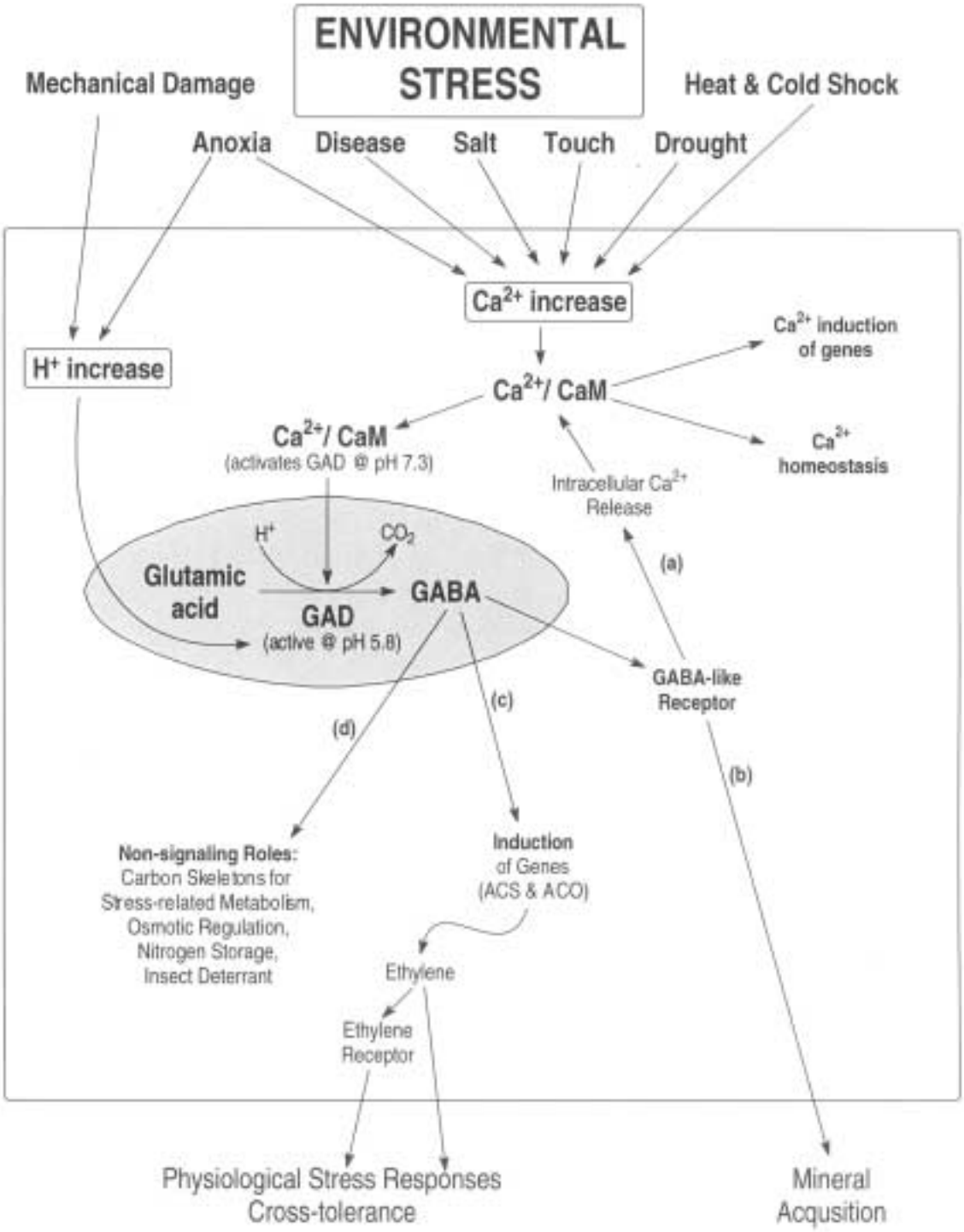

FIGURE 7. Proposed roles of GABA in plant stress responses. Hypothetical pathways by which GABA may function as a cellular barometer and transducer of environmental stress signals. The nature and severity of stress is sensed through cellular changes in Ca^{2+} and/or H^+ that activate GAD (shaded) producing GABA. GABA binds to a GABA-like receptor, which releases Ca^{2+} from an intracellular store (a). An increase in cytosolic Ca^{2+} amplifies the Ca^{2+}/CaM stress response signal and induces stress response genes (cold acclimation/anoxia). GABA-like receptors may also be involved in acquisition of minerals (b) that activate enzymes in stress-related metabolic pathways. GABA-mediated activation of genes for ethylene biosynthesis (c) induces physiological responses associated with "stress" ethylene. Non-signaling stress-related roles for GABA (d) include possible functions as an osmolyte, as an insect deterrent and within mitochondria GABA catabolism may provide carbon skeletons to replenish carboxylic acids depleted as a result of stress-related metabolism.

stress responses. Investigations with alfalfa protoplasts showed that the influx of 45 Ca^{2+} was nearly 15 times greater at 4 than at 25°C. The Ca^{2+} influx was associated with the induction of cold acclimation-specific genes and gene expression and cold acclimation were blocked by calcium channel blockers and Ca^{2+} chelators (Monroy and Dhindsa, 1995). In maize seedlings similar studies have shown that intracellular Ca^{2+} is involved in the expression of anaerobic genes under conditions of oxygen deficiency. In these studies, gene expression was blocked by ruthenium red, an inhibitor of organellar Ca^{2+} fluxes, indicating that release of Ca^{2+} from an intracellular store was required for gene expression (Subbaiah et al., 1994). Increased levels of cytosolic Ca^{2+} have also been implicated in the hypersensitive response (HR) associated with disease resistance to fungal infection (Xu and Heath, 1998).

B. GABA as an Inducer of Stress Ethylene

Stress generally promotes ethylene production in plants (Morgan and Drew, 1997). The response is so evident that the term "stress ethylene" was coined by Abeles (1973) to describe the phenomenon. The association between GABA and ethylene has received little attention despite the fact that nearly all of the signals that induce GABA accumulation (Table 1) also increase production of ethylene; this includes chilling, high temperature, salinity, flooding and anoxia, drought, mechanical damage, and bending (Abeles et al., 1992; Morgan and Drew, 1997). In addition, tobacco mosaic virus infection increased GABA accumulation in tomato leaves (Cooper and Selman, 1974) and ethylene production from tobacco plants (Balazs et al., 1969). Plant hormones that increase GABA accumulation also increase ethylene production. Thus, abscisic acid increased GABA accumulation in wheat roots (Reggiani et al., 1993) and has been shown to promote ethylene production by leaves (Aharoni, 1989), explants (Abeles, 1967), flowers (Koning, 1986), fruit (Plich, 1987), and buds (Goren et al., 1979). Likewise, auxins increased GABA accumulation in rice root tips (Kishinami, 1988) and root cultures of *Datura stramonium* (Ford et al., 1996). Auxin-induced ethylene production has been demonstrated in a wide variety of different plants and tissues (Abeles and Rubenstein, 1964; Abeles et al., 1992).

The only signal in Table 1 that has no effect on ethylene production is acidosis, despite the fact that acid rain (pH 2.8) induced lesions on treated oat leaves (Abeles, 1992). Acidosis can be relieved with simple proton-consuming reactions that occur in ethanol production (Roberts, 1984) or GABA formation, as described previously in this review article and in GABA reviews (Satya Narayan and Nair, 1990; Bown and Shelp, 1997; Snedden and Fromm, 1999).

Establishing a role for GABA in ethylene production would provide a physiological stress-related function for GABA accumulation in plants, as illustrated by the following stresses that all occur with GABA accumulation.

Oxygen deprivation is the primary stress factor in flooded soils. In response to flooding and anoxia, promotion of adventitious rooting by ethylene provides a replacement root system for roots damaged by extreme oxygen deficiency in waterlogged soil (Morgan and Drew, 1997). This increases survival of plants under flooded conditions, and the formation of adventitious roots is correlated with flooding resistance of plant species (Voesenek and Van der Veen, 1994).

Mechanical damage, which induces very rapid accumulation of GABA (Table 1), can result from fungal, insect, and herbivore attack. In conifers, the terpenoid oleoresin is induced by wounding and has antibacterial, antifungal, insect repellant, and insecticidal properties, and it also deters herbivores. Lewinsohn et al. (1999) have reviewed the role of ethylene in this defensive response. Exogenous ethylene had a marked effect on oleoresin biosynthesis in grand fir, and promoted the emission of monoterpenes from cyprus seedlings. Oleoresin consists of approximately 50% by weight of monoterpenes. Intriguingly, the review of the ethylene response in cyprus seedlings suggests a survival-related rationale for ethylene being a gaseous signaling molecule. Defensive responses in plants could be amplified through interplant communication mediated by ethylene.

The term thigmomorphogenesis was introduced by Jaffe (1973) to describe the changes in

plant development induced by the action of mechanical stimuli such as wind or rubbing on plant stems. In response to mechanical stimuli, plants undergo morphological changes, including shortening and thickening that enhance resistance to subsequent mechanical stresses such as windy environments. Thigmomorphogenetically hardened plants can survive and produce viable seed where winds can be in excess of 200 mph (Jaffe and Forbes, 1993). Jaffe and Biro (1979) identified ethylene as the phytohormone-mediating thigmomorphogenesis in kidney bean plants on the basis of three independent lines of evidence. First, when various phytohormones were applied to bean plants, only ethylene was found able to mimic the shortening and swelling of stems that is associated with mechanically stimulated plants. Second, ethylene production in bean plants was increased by mechanical stimulation. Finally, thigmomorphogenesis was partially inhibited when ethylene gas was removed from the tissues of mechanically stimulated plants by placing the plants in a partial vacuum. The involvement of ethylene in the developmental and molecular responses to mechanical stimulation has been questioned on the basis of kinetic data, and the ability of ethylene insensitive mutants of *Arabidopsis* to respond to mechanical stimuli similar to the response of wild-type plants (Johnson et al., 1998). The failure to find an association between ethylene and thigmomorphogenesis in *Arabidopsis* mutants does not disprove the role of ethylene in mediating these responses in other species. A surprising dissociation between ethylene and wound-inducible gene expression has been observed in *Arabidopsis* (Reymond et al., 2000). Abeles et al. (1992) have speculated about compensating mechanisms that could account for the ability of ethylene-insensitive mutants of *Arabidopsis* to complete their life cycle under laboratory conditions. It seems important to distinguish between rapid effects of mechanical stimuli on cell elongation and longer term changes in cell number and structure that constitute the kind of thigmomorphogenesis changes shown in Figure 8. Recently, Bown and Zhang (2000) have shown that rapid growth inhibition in response to mechanical stimulation of soybean hypocotyl tissue is associated with rapid GABA synthesis. By manipulating tissue GABA levels and using modulators of Ca^{2+} homeostasis, they concluded that the elevation in GABA levels alone was not sufficient to cause growth inhibition. Therefore, results of studies by Johnson et al. (1998) and Bown and Zwang (2000) indicate that GABA alone and ethylene do not mediate the rapid changes in cell elongation induced by mechanical stimuli. Thigmomorphogenesis, however, has been observed in 50 species in over 20 families (Abeles et al., 1992). The evidence suggests that hormones play a key role in the sequence of events induced by stress (Itai, 1999). If ethylene does not mediate the thigmomorphogenetic response to mechanical stress shown in Figure 8, which other phytohormone is better qualified to fulfill this role? In their study of thigmomorphogenesis, Jaffe and Biro (1979) measured extensive changes in cell number and structure days after the increase in ethylene production and the mechanical stimulation that induced the response. Such stress-induced morphological changes are generally regulated by phytohormones (Itai, 1999).

A linkage between stress-induced increases in Ca^{2+}, GABA, ethylene, and resulting thigmomorphogenetic responses remains to be established. Nevertheless, the indications are that thigmomorphogenesis could provide an early example of how stress-induced changes in Ca^{2+} are coupled to physiological responses. Less than 1 s after mechanical stimulation was given to bean plants, there was a dramatic drop in electrical resistance of the tissue which was interpreted as an increase in membrane permeability to electrolytes (Jaffe and Biro, 1979). The earliest thigmomorphogenetic events seem to involve membrane changes that allow Ca^{2+} to act as a secondary messenger, probably via CaM. Evidence for the involvement of CaM comes from the demonstration that thigmomorphogenetic responses have been blocked by CaM inhibitors (Jaffe and Forbes, 1993). In this later review of thigmomorphogenesis, Jaffe and Forbes (1993) conclude that the strongest evidence indicates that ethylene mediates thigmomorphogenetic responses. The review ends by anticipating that the future will show how early changes in Ca^{2+} are coupled to ethylene production.

FIGURE 8. Thigmomorphogenesis in kidney beans. Appearance of the first internode of control (left) and rubbed (right) plants. Mechanically stimulated plants were rubbed 10 times once each day for 5 days. (Reprinted with permission of John Wiley and Son, New York. From Jaffe, M. J. and Biro, R. 1979.)

GABA is metabolically positioned to link stress-induced changes in Ca^{2+} to thigmomorphogenetic responses through an effect on ethylene biosynthesis. Direct evidence for an effect of GABA on ethylene production has been obtained *in vitro* using *Stellaria longipes* (Kathiresan et al., 1998). High concentrations of GABA induced ethylene evolution and inhibited stem elongation in shoot tip cultures of *S. longipes*. In parallel to the increase in ethylene production, there was an increase in mRNA for 1-aminocyclopropane, 1-carboxylase (ACC) synthase in GABA-treated plants. ACC synthase plays a major role in regulating ethylene biosynthesis (McKean and Yang, 1988), and in most plant tissues this is considered the rate limiting step in ethylene production (Kende, 1993). Ethylene production was increased significantly when cultures were grown in media containing 750 μ*M* GABA and ethylene was increased threefold in treatments with 10 m*M* GABA. The GABA-mediated inhibition of stem elongation was significantly reduced by aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, and silver thiosulfate (ITS) an inhibitor of ethylene action. In separate studies with sunflower cotyledons exogenous applications of GABA caused up to a 14-fold increase in ethylene production (Kathiresan et al., 1997). The effects of GABA on ethylene production could not be mimicked with equimolar treatments of either L-glutamate, L-alanine, or succinic acid. The findings that GABA induces ethylene biosynthesis may have been facilitated by the long exposure times of plant tissue to GABA. Shoot tip cultures of *S. longipes* were cultured for 21 days (Kathiresan et al., 1998), and sunflower cotyledons were treated for 6 h before any significant increase in ethylene was detected (Kathiresan et al., 1997).

The effects of GABA on ethylene biosynthesis were investigated in detached soybean leaves

by Turano et al. (1997). GABA did not increase ethylene production, but the exposure time to GABA treatments (6 h) would not have been sufficient time to induce a response in sunflower cotyledons (Kathiresan et al., 1997). Interestingly, in soybean leaves 20 m*M* GABA treatments inhibited ethylene biosynthesis more than treatments with AVG and cobalt, an inhibitor of ACC oxidase activity. This result is consistent with an inhibiting effect of high levels of GABA on the activity of enzymes for ethylene biosynthesis.

If GABA-induced ethylene production can be confirmed in other plants, it would connect stress-induced changes in intracellular Ca^{2+} to functional stress-related responses. There is, as yet, no case where the entire series of events can be traced from perception of environmental stress to hormonal and functional physiological responses (Morgan and Drew, 1997; Itai, 1999). If GABA-induced ethylene production is a common element in stress transduction pathways, how do plants differentiate between different stress signals? Sometimes it seems they do not. Mechanical stress (brushing) applied to tomato leaves increased chilling tolerance in tomato plants (Keller and Steffen, 1995). Heat shock induced disease resistance in tomato plants (Stermer and Hammerschmidt, 1987) and increased salt tolerance in cotton plants (Kuznetsov et al., 1993). Mechanical stimulation increased drought tolerance (Figure 9) and freezing tolerance in kidney beans (Jaffe and Biro, 1979). In these examples of cross tolerance to stress, GABA and ethylene are common molecules having the characteristics of signaling molecules discussed in the introduction.

C. GABA-Mediated Mineral Acquistion in Stress-Related Metabolism

An effect of GABA on mineral acquisition in plants was found in experiments with duckweed (*Lemna minor L)*. When plants were cultured in media containing 1 and 10 m*M* GABA, plant growth and plant mineral content were increased in a dose-dependent manner (Kinnersley and Lin, 2000). Plants treated with 10 m*M* GABA had higher levels of macronutrients and higher levels of Mn, Zn, and B than untreated plants, and the increases in tissue Mn content of GABA-treated plants were more than twofold higher than that of other micronutrients.

Observations of improved tolerance to powdery mildew in wheat plants treated with a foliar GABA-containing formulation (AuxiGro™) suggested a mechanism by which GABA-mediated mineral acquisition could promote stress-related metabolism. In a greenhouse experiment, the AuxiGro-treated plants were significantly less infected, yielded 35% more grain, and plant tissue had 32% higher levels of manganese (Mn) than untreated plants (Kinnersley, 1998). Graham and Webb (1991) have discussed the way that manganese availability can increase tolerance to powdery mildew disease in cereals. The importance of this element in disease resistance is indicated by the distribution of powdery mildew problems in wheat, which is closely correlated with the distribution of Mn-deficient soils. Manganese-containing foliar fertilizers have been used to control powdery mildew in wheat. Manganese is an essential element for activating enzymes in the lignin biosynthetic pathway, and an increased capacity to synthesize lignin has been correlated to disease resistance in cereals (Hammerschmidt, 1984). Lignin is a complex polymer and cellular deposition of lignin in cell walls acts as a physical barrier to the penetration of fungal hyphae.

Distinct metabolic pathways are associated with plant response to specific stresses (Harborne, 1997). The extent to which tissue resources of micronutrients are sufficient to support the sudden needs of stress-induced metabolic pathways is unknown. The effectiveness of Mn-containing fertilizers for treating powdery mildew disease shows that there are times when the availability of Mn is limiting the stress response. At such times, GABA may promote stress-related metabolism through mineral acquisition.

The mineral acquisition hypothesis merits consideration on at least three accounts. First, it has been a common observation that tissue samples from GABA/AuxiGro-treated plants have higher amounts of minerals than tissue from untreated plants. As described above, this has been found in duckweed and greenhouse-grown wheat. It has also been observed in field grown crops. In tomatoes the AuxiGro treatment that gave the greatest increase in plant nu-

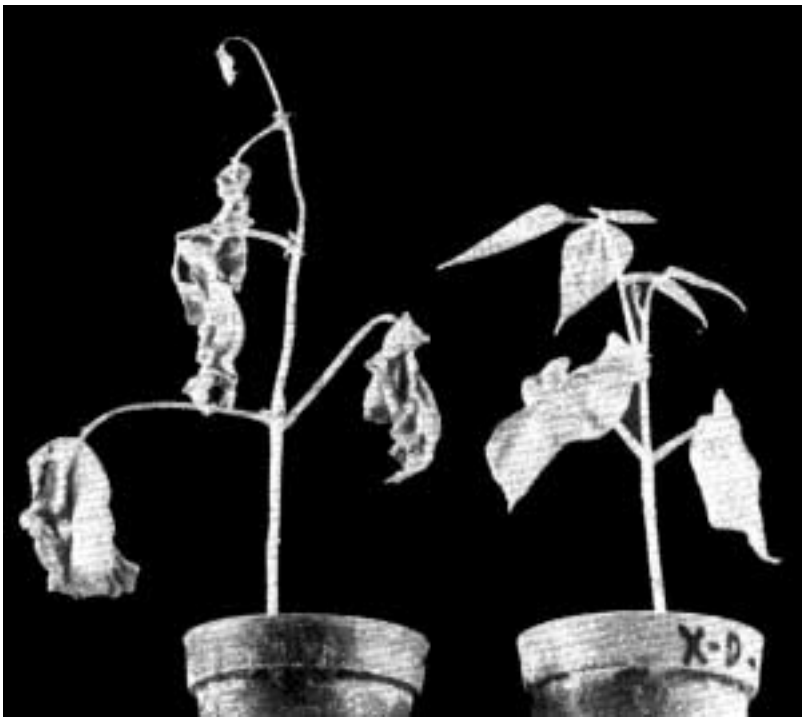

FIGURE 9. Example of cross-tolerance to stress. The effect of drought on control (left) and rubbed (right) kidney bean plants. Plants that had been rubbed or not rubbed for 8 days were placed in the growth chamber without irrigation. The picture was taken 5 days later. (Reprinted with permission of John Wiley and Son, Inc. New York, N.Y. 10158. From Jaffe, M. J. and Biro, R. 1979.)

trients also gave the best increase in tomato yield and solids content (Kinnersley, 1998). Second, this hypothesis provides a mechanism whereby GABA-mediated increases in nutrient availability could promote both stress-related metabolism and the increased crop growth and productivity described in Section III.A. Finally, the mineral acquisition hypothesis relates GABA effects in plants to known mechanisms of action in animal systems. $GABA_A$ and $GABA_C$ are members of the ligand-gated ion-channel superfamily of receptors (Johnston, 1997). Activation of $GABA_B$ receptors involves a different mechanism, mediated through G-proteins that modulate ion channels (Bowery and Brown, 1997). Direct evidence that one of the GABA-like receptors we have identified may regulate ion channels was reported recently. Results from *Arabidopsis* overexpressing a putative glutamate receptor gene suggested that GLR2 may play a regulatory role in ion utilization during normal development (Kim et al., 2000). The GLR2 gene that was overexpressed in transgenic plants corresponds to the GABA-like loci shown in Figure 6, (GLR2a) which has sequence similarity in the N-terminal domain to animal $GABA_B$ receptors.

The observations that samples of tissue from crops given foliar applications of AuxiGro™ had higher levels of minerals than tissue from untreated plants suggests that GABA mediates a root-specific response and raises the question of long distance transport of GABA. Intercellular GABA transport and the possible involvement of stress-induced GABA transporter proteins has been discussed by Shelp et al. (1999).

D. Anaplerotic Role in Stress-Related Metabolism

Anaplerotic reactions ("filling up reactions", Kornberg, 1966) are metabolic processes that

ensure that the intramitochondrial concentrations of the citric acid cycle intermediates remain constant with time (Matthews and Van Holde, 1996). Hill (1997) has discussed the fundamental difference in the role of the TCA cycle in animals and plants and the metabolic consequences of this for plants. The TCA cycle in plants performs an anabolic role, and consequently intermediates are constantly being drained from the cycle for biosynthesis. A primary anabolic role of TCA cycle intermediates is the formation of amino acids. Transamination converts the keto acids, pyruvic, oxaloacetic, and α-ketoglutaric to alanine, aspartic, and glutamic acids, respectively. The crucial role of these anabolic reactions in stress-related metabolism can be appreciated by considering the role of aspartic acid in the biosynthesis of stress metabolites. Aspartic acid and the derivative amino acids lysine, threonine, methionine, and isoleucine are building blocks for stress-specific proteins. Such proteins include heat-shock proteins (for review see Waters et al., 1996), anti-freeze proteins (Antikainen and Griffith, 1997) and low-temperature-induced proteins (Boothe et al., 1997), anaerobic proteins (Sacks et al., 1980), and pathogenesis-related proteins (PR-proteins, Kuc, 1997). In addition, methionine, derived from aspartic acid, is a precursor in the synthesis of polyamines and ethylene, the formation of which is associated with numerous environmental stresses (Bouchereau et al., 1999) and Section IV.B of this review.

The foregoing has been described, with reference to a single amino acid, to indicate the magnitude of the demand for TCA cycle intermediates to support stress-related metabolic pathways. This poses a problem because if intermediates are removed and not replaced, the cycle will ultimately stop. In plants the filling up or anaplerotic role is provided by oxaloacetic acid that is synthesized from phosphoenolpyruvic acid (PEP) by PEP carboxylase (Plaxton, 1996; Hill, 1997). Evidence that anaplerotic carbon fixation occurs in plants comes from the demonstration that plant tissues incorporate label from $^{14}CO_2$ into organic acids in the dark. Initially, the label is confined to oxaloacetate, malate, and aspartate, but after further metabolism labeling of all the intermediates of the TCA cycle and many amino acids is found (Hill, 1997). Interestingly, PEP carboxylase, the enzyme that mediates dark CO_2 fixation, is absent from animal tissues (Conn and Stumpf, 1973). Presumably, this reflects the fact that heterotrophic organisms obtain the carbon skeletons of amino acids from their diet.

The question whether GABA serves an anaplerotic role may be dependent on the extent to which PEP carboxylase is able to supply sufficient amounts of oxaloacetate from PEP to replace carbon lost from the cycle. When the availability of PEP is limited, the capacity of PEP carboxylase to fulfill its anaplerotic role will also be limited. Under a variety of biotic and abiotic stresses, PEP is also required to support stress-induced phenylpropanoid biosynthesis, which at times consumes as much as 20% of total photosynthetic carbon (Dennis and Blakely, 1995). Quantitatively, the most important product of phenylpropanoid metabolism is lignin. It is estimated that 15 to 20% of the carbon fixed in the biosphere each year is eventually incorporated into this cell wall polymer (Ellis, 1997). The defensive function of lignin in disease resistance was discussed in the preceding section of this review. Many other stress metabolites are derived from phenylpropanoid metabolism (Figure 10). In addition to lignin, stress-induced phenylpropanoids include antimicrobial compounds such as phytoalexins and compounds such as coumestrol and coumarin, which are toxic to potential herbivores and induced in response to herbivore feeding. All these defensive compounds are derived from cinnamic acid, which is formed from phenylalanine. Synthesis of phenylalanine requires equimolar amounts of PEP and D-erythrose-4-phosphate from the pentose phosphate pathway. Stress-induced phenylpropanoid metabolism therefore will divert carbon away from the PEP anaplerotic pathway, which is the primary route to replenish TCA cycle intermediates. How much carbon is likely to be diverted away from anaplerotic reactions and into synthesis of antifeeding compounds such as coumestrol and coumarin when an otherwise defenseless plant is being eaten by a herbivore? The answer to that question will determine the extent to which carbon in the TCA cycle must be replenished to maintain a constant concentration of carboxylic

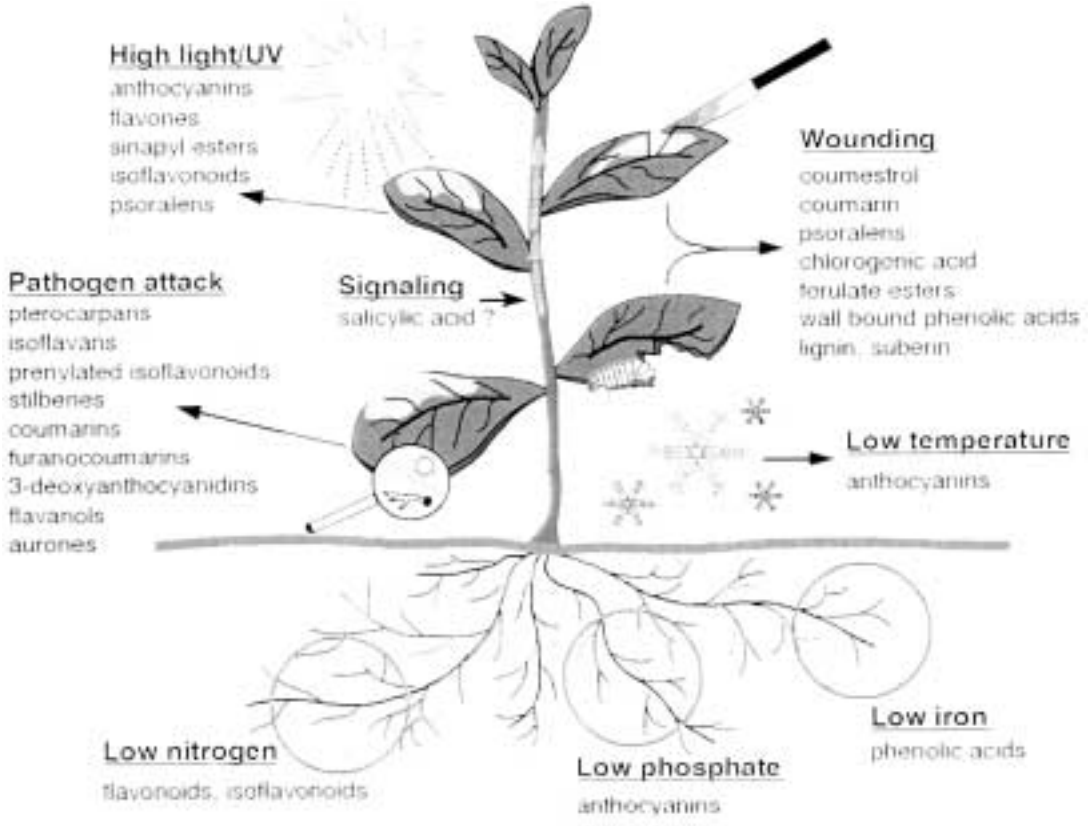

FIGURE 10. Examples of phenylpropanoid compounds induced in plants by various biotic and abiotic stresses. (Reprinted with permission of the American Society of Plant Physiologists, Rockville, MD. From Dixon, A. and Paiva, N. L. 1995. *The Plant Cell,* **7**:1085–1097.)

acid intermediates. In such circumstances the GABA shunt may perform an anaplerotic role by providing carbon to the cycle through GABA catabolism to succinic acid. Shelp et al. (1999) have provided evidence for transport of GABA from the cytosol into the mitochondria, where the enzymes for GABA catabolism are located. Interestingly, the last step of GABA catabolism involves an irreversible oxidation of succinic semialdehyde to succinate, catalyzed by succinic semialdehyde dehydrogenase (Shelp et al., 1999). In his seminal essay on anaplerotic sequences, Kornberg (1966) notes that a distinguishing feature of anaplerotic enzymes is that the reactions they catalyze are irreversible under physiological conditions. The likelihood that GABA is able to provide significant amounts of anaplerotic carbon is strengthened by considering a similar metabolic role for proline. This amino acid accumulates in plants in response to drought and cold stress then disappears rapidly when the stress is removed (Stewart and Voetberg, 1985). Proline is oxidized via a three-step process that produces 2-oxyglutarate that feeds into the TCA cycle. In barley leaves recovering from drought, the oxidation of proline can account for up to 20% of the rate of respiration (Stewart and Voetberg, 1985). As noted in the introduction, the levels of GABA that accumulate under stress frequently exceed that of amino acids involved in protein synthesis. Levels of GABA were more than threefold higher than levels of proline in leaves of cotton (Hanover and Brzozowoska, 1975) and bean (Raggi, 1994) exposed to drought stress. Recent reviews on GABA have suggested that GABA produced during stress can provide a source of carbon skeletons for the TCA cycle *after* stress has been relieved (Snedden and Fromm, 1999; Shelp et al., 1999; Bown and Shelp, 1997). The metabolic considerations discussed above suggest that stress-induced phenylpropanoid metabolism may create an immediate need for an alternative source of

carbon to provide for the uninterrupted functioning of the TCA cycle. GABA is well positioned metabolically to fulfill this need.

V. CONCLUDING REMARKS

The discovery of glutamate receptor-like genes in plants and their physical similarity to animal ionotropic glutamate receptor genes led to the conclusion that both evolved from a primitive signaling mechanism that existed before plants and animals diverged (Chiu et al., 1999). In this review, molecular and physiological studies have been described that show that plants possess domains that are similar to $GABA_B$ receptors and respond to pharmacologically active agents that affect animal GABA receptors. Glutamate and GABA play antithetical roles in the transmission of systemic signals in animals. Establishing the existence of GABA-like receptors in plants would open up the intriguing possibility of finding functional similarities among GABA receptor domains in receptor molecules found in both kingdoms. In plants and animals the GABA signaling system may perform a similar role in survival by facilitating responses to stress. The role of GABA in neural mechanisms that underlie survival-related motor functions in animals is clear. The role of GABA in functionally analogous survival activities in plants is not. "There is as yet no case where the entire series of events can be traced from stress perception to response" (Morgan and Drew, 1997). However, GABA is connected to environmental stress signals through GAD activation, which reflects both intensity and duration of environmental stress. GABA is connected to intracellular signal transduction pathways mediated by Ca^{2+}, and to physiological responses mediated by "stress" ethylene. Elucidating the function of GABA-like receptors in plants may help clarify the precise roles of GABA in plant responses to stress.

ACKNOWLEDGMENTS

We thank Dr. John L. McIntyre, Auxein Corporation, for his critical review of the manuscript, Robert E. Venable, Auxein, for insightful discussions on the mechanism of action of GABA, and Sue Ann Walker, Auxein, for managing all the revisions of this manuscript. AK gratefully acknowledges the philanthropy of Dr. Harry W. Galbraith, Galbraith Laboratories, Knoxville, TN. This support enabled the effects of GABA on plant growth to first be discovered at Galbraith Laboratories in 1993.

REFERENCES

Abeles, F. B. and Rubinstein, B. 1964. Regulation of ethylene evolution and leaf abscission by auxin. *Plant Physiol.* **39**: 963–969.

Abeles, F. B. 1967. Mechanism of action of abscission accelerators. *Physiol. Plant.* **20**: 442–454.

Abeles, F. B. 1973. In: *Ethylene in Plant Biology*. p. 302. Academic Press, New York.

Abeles, F. B., Morgan, P. W., and Saltreit, M. E. 1992. In: *Ethylene in Plant Biology, 2nd ed.* Academic Press, New York.

Aharomi, N. 1989. Interrelationship between ethylene and growth regulators in the senescence of lettuce leaf discs. *J. Plant Growth Regul.* **8**: 309–317.

Antikainen, M. and Griffith, M. 1997. Antifreeze protein accumulation in freezing-tolerant cereals. *Physiol. Plant* **99**: 423–432.

Arazi, T., Baum, G., Snedden, W. A., Shelp, B. J., and Fromm, H. 1995. Molecular and biochemical analysis of calmodulin interactions with the calmodulin-binding domain of plant glutamate decarboxylase. *Plant Physiol.* **108**: 551–561.

Aurisano, N., Bertani, A., and Reggiani, R. 1995. Involvement of calcium and calmodulin in protein and amino acid metabolism in rice roots under anoxia. *Plant Cell Physiol.* **36**: 1525–1529.

Aurisano, N., Bertani, A., and Reggiani, R. 1995A. Anaerobic accumulation of 4-aminobutyrate in rice seedlings; causes and significance. *Phytochemistry* **38**: 1147–1150.

Babcock, D. F., Herrington, J., Goodwin, P.C., Bae Pank, Y., and Hille, B. 1997. Mitochondrial participation in the intracellular Ca^{2+} network. *J. Cell Biol.* **136**: 833–834.

Balazs E., Gaborjanyi, R., Toth, A., and Kiraly, Z. 1969. Ethylene production in Xanthi tobacco after systemic and local virus infections. *Acta Phytopath. Acad. Sci. Hung.* **4**: 355–358.

Barkla, J. B. and Pantoja, O. 1996. Physiology of ion transport across the tonoplast of higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Bio.* **47**:159–184.

Baum, G., Chen, Y., Arazi, T., Takatsuji, H. and Fromm, H. 1993. A plant glutamate decarboxylase containing a calmodulin binding domain, cloning, sequence, and functional analysis. *J. Biol. Chem*. **268**: 19610–19617.

Baum, G., Lev-Yadun, S., Fridmann, Y., Arazi, T., Katsnelson, H., Zik, M., and Fromm, H. 1996. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. *EMBO J.* **15**: 2988–2996.

Bernardi, P. and Petronilli, V. 1996. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. *J. Bioenerg. Biomemb*. **28**: 131–138.

Blatt, M. R. and Thiel G. 1993. Hormonal control of ion channel gating. *Annu. Rev. Plant Physiol.* **44**: 543–567.

Blum, A. 1988. *Plant Breeding for Stress Environments*. CRC Press, Boca Raton, FL.

Bolarin, M. C., Santa-Cruz, A., Cayuela, E., and Perez-Alfocea, F. 1995. Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings under salinity. *J. Plant Physiol.* **147**: 463–468.

Boothe, J. G., Sönnichsen, F. D., de Beus, M. D., and Johnson-Flanagan, A. M. 1997. Purification, characterization, and structural analysis of plant low-temperature-induced protein. *Plant Physiol.* **113**: 367–376.

Bouchereau, A., Aziz, A., Larher, F., and Martin-Tanguy, J. 1999. Polyamines and environmental challenges: recent developments. *Plant Sci.* **140**: 103–125.

Bowery, N. G. and Brown, D. A. 1997. The cloning of $GABA_B$ receptors. *Nature* **386**: 223–224.

Bown, A. W. and Shelp, B. J. 1997. The metabolism and functions of γ-aminobutyric acid. *Plant Physiol.* **115**: 1–5.

Bown, A. W. and Zhang, G. 2000. Mechanical stimulation, 4-aminobutyrate (GABA) synthesis and growth inhibition in soybean hypocotyl tissue. *Can. J. Bot* **78**: 119–123.

Braam, J. and Davis, R. W. 1990. Rain, wind, and touch-induced expression of the calmodulin and calmodulin-related genes in *Arabidopsis. Cell* **60**: 357–364

Breitkreuz, K. E. and Shelp, B. J. 1995. Subcellular compartmentation of the 4-aminobutyrate shunt in protoplasts from developing soybean cotyledons. *Plant Physiol.* **108**: 99–103.

Bush, D. S. 1995. Calcium regulation in plant cells and its role in signaling. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. **46**: 95–122.

Carroll, A.D., Fox, G. G., Laurie, S., Phillips, R., Ratcliffe, R. G., and Stewart, G. R. 1994. Ammonium assimilation and the role of γ-aminobutyric acid in pH homeostasis in carrot cell suspensions. *Plant Physiol.* **106**: 513–520.

Castanie-Cornet, M. P., Penfound, T. A., Smith, D., Elliott, J. F., and Foster, J. W. 1999. Control of acid resistance in *Escherichia coli. J. Bacteriol.* **181**: 3525–3535.

Chaudhury, A., Okada, K., Raikhel, N. V., Shinozaki, K., and Sundaresan, V. 1999. A weed reaches new heights down under. *Plant Cell* **11**: 1817–1826.

Chen, Y., Baum G., and Fromm, H. 1994. The 58-kilodalton calmodulin-binding glutamate decarboxylase is a ubiquitous protein in petunia organs and its expression is developmentally regulated. *Plant Physiol*. **106**: 1381–1387.

Chiu, J., DeSalle, R., Lam, H-M., Meisel, L., and Corunzi, G. 1999. Molecular evolution of glutamate receptors: A primitive signaling mechanism that existed before plants and animals diverged. *Mol. Bio. Evol*. **16**: 826–838.

Cholewa, E., Cholewinski, A. J., Shelp, B. J., Snedden, W. A., and Bown, A. W. 1997. Cold-shock-stimulated γ-aminobutyric acid synthesis is mediated by an increase in cytosolic Ca^{2+}, not by an increase in cytosolic H^+. *Can. J. Bot.* 75: 375–382.

Chung, I., Bown, A. W., and Shelp, B. J. 1992. The production and efflux of 4-aminobutyrate in isolated mesophyll cells. *Plant Physiol.* **99**: 659–664.

Collins, D. M. and Wilson, A. T. 1975. Embryo and endosperm metabolism of barley seeds during early germination. *J. Exp. Bot*. **26**: 737–740.

Coleman, S. T., Fang, T. K., Rovinsky, S. A., Turano, F. J., and Moye-Rowley, W. S., 2000. Expression of a glutamate decarboxylase homologue is required for normal oxidative stress tolerance in *Saccharomyces cerevisiae. J. Biol. Chem.* (in press).

Conn, E. E. and Stumpf, P. K. 1973. *Outlines of Biochemistry*. p. 340, 4th ed. John Wiley & Sons, New York.

Cooper, P. and I. W. Selman. 1974. An analysis of the effects of tobacco mosaic virus on growth and the changes in the free amino compounds in young tomato plants. *Ann Bot.* **38**: 625–638.

Crawford, L. A., Bown, A. W., Breitkreuz, K. E., and Guinel, F. C. 1994. The synthesis of γ-aminobutyric acid in response to treatments reducing cytosolic pH. *Plant Physiol*. **104**: 865–871.

Davies, D. D. 1980. Anaerobic metabolism and the production of organic acids. In*: The Biochemistry of Plants.* Vol. 2. pp. 581–611. Davies, D. D., Ed., Academic Press, New York.

Deisz, R. A. 1997. Electrophysiology of $GABA_B$ receptors. In: *The GABA Receptors.* pp. 157–207, 2nd ed. Enna, S. J. and Bowery, N. G., Eds., Humana Press, Totowa, NJ.

Dennis, D. T. and Blakely, S. D. 1995. The regulation of carbon partitioning in plants. In: *Carbon Partitioning and Source-Sink Interaction in Plants. Current Topics in Plant Physiology*, Vol. 13, p. 260. American Society of Plant Physiologists, Rockville, MD.

Dixon, R. A. and Paiva, N. L. 1995. Stress-induced phenylpropanoid metabolism. *The Plant Cell* **7**: 1085–1097.

Drost, D. 1999. Reports on drip irrigation, the use of AuxiGro and storage challenges. *Onion World.* March 1999. Columbia Publishing, Yakima, WA.

Ellis, B. E. 1997. Metabolism of defense and communication. In: *Plant Metabolism,* 2nd ed. Dennis, D. T.,

Turpin, D. H., Lefebre, D. D., and Layzell, D. B., Eds. Longman Publishing, Harlow, England.

Felle, H. 1988. Short-term pH regulation in plants. *Physiol. Plant*. **176**: 583–591.

Ford, Y. Y., Ratcliffe, R. G., and Robins, R. J. 1996. Phytohormone-induced GABA production in transformed root cultures of *Datura stramonuim*: an *in vitro* ^{15}N NMR study. *J. Exp. Bot*. **299**: 811–818.

Galston, A. W., Davies, P. J., and Satter, R. L. 1980. In: *The Life of the Green Plant,* 3rd Ed., p. 45. Prentice Hall, Inc. Englewood, NJ.

Gilroy, S. and Jones, R. L. 1992. Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barely aleurone protoplasts. *Proc. Natl. Acad. Sci. USA.* **89**: 3591–3595.

Goren, R., Altman, A., and Giladi, I. 1979. Role of ethylene in abscisic acid-induced callus formation in citrus bud cultures. *Plant Physiol*. 63: 280–282.

Graham, R. D. and Webb, M. J. 1991. In: *Micronutrients and Disease Resistance and Tolerance in Plants*, pp. 331–339. Mortvedt, J. J., Cox, F. R., Shuman, L. M., and Welch, R. M., Eds. *Micronutrients in Agriculture*. Soil Society of America, Inc., Madison, WI.

Grill, E. and Himmelbach, A. 1998. ABA signal transduction. *Curr. Opin. Plant Biol*. **1**: 412–418.

Guern, J., Mathieu, Y., Thomine, S., Jouanneau, J. P., and Beloeil, J. C. 1992. Plants counteract cytoplasmic pH changes but likely use these pH changes as secondary messages in signal perception. *Curr. Top. Plant Biochem. Physiol*. **11**: 249–269.

Hammerschmidt, R. 1984. Rapid deposition of lignin in potato tuber tissue as a response to fungi non-pathogenic on potato. *Physiol. Plant Path.* **24**: 33–42.

Hanower, P. and Brzozowska, J. 1975. Influence d'un choc osmotique sur la composition des feuilles de cotonnier en acides amines libres. *Phytochemistry* **14**: 1691–1694.

Hao, R. and Schmit, J. C. 1991. Purification and characterization of glutamate decarboxylase from *Neurospora crassa* conidia. *J. Biol. Chem*. **266**: 5135–5139.

Harborne, J. B. 1997. Biochemical plant ecology. In: *Plant Biochemistry*. pp. 503–516, Dey, D. M., and Harborne, J. B., Eds., Academic Press, London, New York.

Heber, U., Tyankova, L., and Santarius, K. K. 1971. Stabilization and inactivation of biological membranes during freezing in the presence of amino acids. *Biochem. Biophys. Acta.* **241**: 578–592.

Hedrich, R. and Dietrich, P. 1996. Plant K^+ channels: similarity and diversity. *Bot. Act*. **105**: 94–101.

Hill, A. A. 1997. Carbon metabolism in mitochondria. In: *Plant Metabolism*, 2nd ed., pp. 181–199, Dennis, D. T., Turpin, D. H., Lejebone, D. D., and Layzel, D. B., Eds. Addison Wesley Longman, Ltd., Harlow, Essex, England.

Horn, M. A., Meadows, R. P. M., Apostol, I., Jones, C. R., Gorenstein, D. G., Heinstein, P. F., and Low, P. S. 1992. Effect of elicitation and change in extracellular pH on the cytoplasmic and vacuolar pH of suspension-cultured soybean cells. *Plant Physiol*. **98**: 680–686.

Hu, C. Y. and Wang, P. J. 1983. Meristem, shoot tip, and bud cultures. In: *Handbook of Plant Cell Culture*, Vol. 1., p. 191. Evans, D. A., Sharp, W. R., Ammirato, P. V., and Yamada, Y., Eds. Macmillan, New York.

Ichas, F., Jouaville, L. S., and Mazat, J. P. 1997. Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals. *Cell* **89**: 1145–1153.

Itai, C. 1999. Role of phytohormones in plant responses to stresses. In*: Plant Responses to Environmental Stresses*. pp. 287–301, Lennar, H. R., Ed., Marcel Dekker, New York, Basel.

Iten, M., Hoffmann, T., and Grill, E. 1999. Receptors and signaling components of plant hormones. *J. Recept. Signal Transduct. Res.* **19**: 41–58.

Ito, T., Hirano, M., Akama, K.,Shimura. Y., and Okada, K. 1995. Touch-inducible genes for calmodulin and a calmodulin-related protein are located in tandem on a chromosome of *Arabidopsis thaliana*. *Plant Cell Physiol*. **36**: 1369–1373

Jaffe, M. J. 1973. Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation: With special reference to *Bryoria dioria*. *Planta* **114**: 143–157.

Jaffe, M. J. and Biro, R. 1979. Thigmomorphogenesis, the effect of mechanical perturbation on the growth of plants, with special reference to anatomical changes, the role of ethylene and interaction with other environmental stresses. In: *Stress Physiology in Crop Plants.* pp. 25–69, Mussell, H. and Staples, R. C., Eds. John Wiley & Sons, New York.

Jaffe, M. J. and Forbes, S. 1993. Thigmomorphogenesis: The effect of mechanical perturbation on plants. *Plant Growth Reg.* 12:313–329.

Johnson, B. S., Singh, N. K., Cherry, J. H., and Locy, R. D. 1997. Purification and characterization of glutamate decarboxylase from cowpea. *Phytochemistry* **46**: 39–44.

Johnson, K. A., Sistrunk, M. L., Polisensky, D. H., and Braam J. 1998. *Arabidopsis thaliana* responses to mechanical stimulation do not require ETRI on EIN2. *Plant Physiol.* **116**: 643–649.

Johnston, G. A. R. 1997. Molecular biology, pharmacology, and physiology of $GABA_C$ Receptors. In: *The GABA receptors*, 2nd Ed. p. 298, Enna, S. J. and Bowery, N. G., Eds., Humana Press, Totowa, NJ.

Kathiresan, A., Tung, P., Chinnappa, C. C., and Reid, D. M. 1997. γ-aminobutyric acid stimulates ethylene biosynthesis in sunflower. *Plant Physiol.* **115**: 129–135.

Kathiresan, A., Miranda, J., Chinnappa, C. C., and Reid, D. M. 1998. γ-aminobutyric acid promotes stem elongation in *Stellaria longipes*: the role of ethylene. *Plant Growth Regul*. **26**: 131–137.

Keller, E. and Steffen, K. L. 1995. Increased chilling tolerance and altered carbon metabolism in tomato leaves following application of mechanical stress. *Physiol. Plant*. **93**: 519–525.

Kende, H. 1993. Ethylene biosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol.* **44**: 283–307.

Kim, A. S., Wang, M-H., Jae, S-K., Kwak, T. M., Schroeder, J. I., and Nam, H. G. 2000. Over expression of AtGLUR2, an *Arabidopsis* homolog of mammalian ionotropic glutamate receptor renders transgenic plants inefficient in ion utilization. Abstr. #1030. *Plant Biol. 2000*. American Society of Plant Physiologists, Rockville, MD.

Kinnersley, A. M. 1998. Bioactivity of AuxiGro™ Plant Metabolic Primer, a formulation containing GABA and glutamic acid. In: *Proc. 25th Ann. Mtg. Plant Growth Regulation Soc. of America*, pp. 89–94. July 7–10, 1998.

Kinnersley, A. M. and Lin, F. 2000. Receptor modifiers indicate that GABA is a potential modulator of ion transport in plants. *Plant Growth Regul.* **32**: 65–76.

Kishinami, I. 1998. Effect of auxins (2,4-dichlorophenoxyacetic acid, indole-3–acetic acid and naphthaleneacetic acid) on the accumulation of γ-aminobutyric acid in excised rice root tips. *Plant Cell Physiol*. **29**: 581–585.

Knight, H., Trewavas, A. J., and Knight, M. R. 1996. Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signaling after acclimation. *Plant Cell* **8**: 489–503.

Knight, M. R., Campbell, A. K., Smith, S. M., and Trewavas, A. J. 1991. Transgenic plant acquorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature* **352**: 524–526.

Knight, M. R., Read, N. D., Campbell, A. K., and Trewavas, A. J. 1993. Imaging calcium dynamics in living plants using semi-synthetic recombinant aequorins. *J. Cell Biol*. **121**: 83–90.

Knight, M. R., Smith, S. M., and Trewavas, A. J. 1992. Wind-induced plant motion immediately increases cytosolic calcium. *Proc. Natl. Acad. Sci. USA* **89**: 4967–4971.

Koiwai, A., Noguchi, M., and Tamaki, E., 1971. Changes in the amino acid composition of tobacco cells in suspension culture. *Phytochem*istry **10**: 561–566.

Koning, R. E., 1986. The role of ethylene in corolla unfolding in *Ipamvea nil*. *Amr. J. Bot*. **73**: 152–155.

Kornberg, H. L. 1966. Anaplerotic sequences and their role in metabolism. In: *Essays in Biochemistry*, Vol. 2, pp. 1–31, Academic Press, London.

Krogsgaard-Larsen, P., Frolund, B., and Ebert, B. 1997. $GABA_A$ receptor agonists, partial agonists, and antagonists. In: *The GABA Receptors*, 2nd ed., pp. 37–81, Enna, S. J. and Bowery, H. G., Eds., Humana Press, Totowa, NJ.

Kuc, J. 1997. Molecular aspects of plant responses to pathogens. *Acta Physiol Plant* **19**: 551–559.

Kurkdjian, A. and Guern, J. 1989. Intracellular pH: measurement and importance in cell activity. *Annu. Rev. Plant Physiol*. **40**: 271–303.

Kuznetsov, V. V., Rakitin, V. Y., Barisara, N. N., and Rotschupkin, B. V. 1993. Why does heat shock increase salt resistance in cotton plants? *Plant Physiol. Biochem*. **31**: 181–188.

Lam, H. M., Chiu, J., Hsieh, M. H., Meisel, L., Oliveira, I. C., Shin, M., and Corruzzi, G. 1998. Glutamate-receptor genes in plants. *Nature* **396**: 125–126.

Lee, S. H., Seo, H. Y., Kim, J. C., Heo, W. D., Chung, W. S., Lee, K. J., Kim, M. C., Cheong, Y. H., Choi, J. Y., Lim, C. O., and Cho, M. J. 1997. Differential activation of NAD kinase by plant calmodulin isoforms. The critical role of domain I. *J. Biol. Chem*. **272**: 9252–9259.

Lee, S. H., Kim, M. C., Heo, W. D., Kim, J. C., Chung, W. S., Park, C. Y., Park, H. C., Cheong, Y. H., Kim, C. Y., Lee, K. J., Bahk, J. D., Lee, S. Y. and Cho, M. J. 1999. Competitive binding of calmodulin isoforms to calmodulin-binding proteins: implication for the function of calmodulin isoforms in plants. *Biochim. Biophys. Acta* **1433**: 56–67.

Leionsohn, E., Sadanobu, S., and Grotear, R. 1999. Conifer chemical defense against bark beetles: the modulations of monoterpene biosynthesis by wounding, environmental stress and ethylene. In: *Plant Responses to Environmental Stress*, p. 659–681. Lenner, H. R., Ed., Marcel Dekker, New York, Basel.

Liao, B., Gawienowski, M. C., and Zielinski, R. E. 1996. Differential stimulation of NAD kinase and binding of peptide substrates by wild-type and mutant plant calmodulin isoforms. *Arch. Biochem. Biophys*. **327**: 53–60.

Locy, R. D., Cherry, J. H., and Singh, N. K. 1997. The effects of GABA on the growth of *Arabidopsis* seedlings. *Plant Physiol*. 114 (Suppl): Abstr. 501.

Locy, R. D., Wu, S-J., McNabb, D., Cherry, J. H., Singh, N. K., and Knight, M. R. 1998. The heat-induced accumulation of GABA involves the generation of cytoplasmic calcium signal, and enhances the thermotolerance of *Arabidopsis* seedlings. *Plant Physiol*. **116**: Abstr. 99.

Locy, R. D., Wu, S-J., Singh, N. K., and Cherry, J. H. 1999. The heat-induced accumulation of GABA is correlated with heat-induced cytoplasmic calcium level in *Arabidopsis* seedlings. Abstract #154. *Plant Biology 99*. American Society of Plant Physiologists, Rockville, MD.

Lynch, J., Polito, V. W., and Luchli, A. 1989. Salinity stress increases cytoplasmic Ca^{2+} activity in maize root protoplasts. *Plant Physiol*. **90**: 1271.

Malho, R. 1999. Coding information in plant cells: the multiple roles of Ca^{2+} as a second messenger. *Plant Biol*. **1**: 487–494.

Malho, R., Moutinho, A., Van der Luit, A., and Trewavas, A. J. 1998. Spatial characteristics of calcium signaling: The calcium wave as a basic unit in plant cell calcium signaling. *Philos. Trans. R. Soc. Lond. B.* **353**: 1463–1473.

Malitschek, B., Schweizer, C., Keir, M., Heid, J., Froestl, W., Mosbacher, J., Kuhn, R., Henley, J., Joly, C., Pin, J. P., Kaupmann, K., and Bettler, B. 1999. The

N-terminal domain of γ-aminobutyric acid (B) receptors is sufficient to specify agonist and antagonist binding. *Mol. Pharmacol*. **56**: 448–454.

Mathews, C. K. and van Holde, K. E. 1996. Anaplerotic sequences: the need to replace cycle intermediates. In: *Biochemistry*, 2nd ed., pp. 503–506, Benjamin/ Cummings Publishing, USA/UK.

Mayer, R. R., Cherry, J. L., and Rhodes, D. 1990. Effects of heat shock on amino acid metabolism of cowpea cells. *Phytochemistry* **94**: 796–810.

McAinsh, M. R., Brownlee, C., and Hetherington, A. M. 1990. Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomal closure. *Nature* **343**: 186–188.

McKean, T. A. and Yang, S. F. 1988. Biosynthesis and metabolism of ethylene. In: *Plant Hormones and Their Role in Plant Growth and Development*, p. 97. Davies, P. J., Ed. Kluwer Academic Publishers, Dordrecht, Boston, London.

Menegus F., Cattaruzza., L., Chersi, A., and Fronza, G. 1989. Differences in the anaerobic lactate-succinate production and in the changes of cell sap pH for plants with high and low resistance to anoxia. *Plant Physiol*. **90**: 29–32.

Monroy, A. F. and Dhindsa, R. S. 1995. Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25°C. *The Plant Cell*. **7**: 321–331.

Morgan, P. W. and Drew, M. C. 1997. Ethylene and plant responses to stress. *Physiol. Plant*. **100**: 620–630.

Naylor, A. W. and Tolbert N. E. 1956. Glutamic acid metabolism in green and etiolated barley leaves. *Physiol. Plant*. **9**: 220–229.

Nakai, K. and Kanehisa, M. 1992. A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics* **14**: 897–911.

Parola, A. L., Yamamura, H. I., and Laird, H. E., II. 1993. Minireview. Peripheral-type benzodiazepine receptors. *Life Sci*. **52**: 1329–1342.

Plaxton, W. C. 1996. The organization and regulation of plant glycolysis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:185–214.

Plich, H. 1987. Regulating factors of ethylene production in apple fruits. *Fruit Sci. Rpts*. 14: 57–63.

Price A. H., Taylor, A., Ripley, S. J., Griffiths, A., Trewavas, A. J., and Knight, M. R. 1994. Oxidative signals in tobacco increase cytosolic calcium. *Plant Cell* **6**: 1301–1310.

Qian, H. and Dowling, J. E. 1994. Pharmacology of novel GABA receptors found on rod horizontal cells of the white perch retina. *J. Neurosci*. **14**: 4299–4307.

Raggi, V. 1994. Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. *Physiol. Plant*. **91**: 427–434.

Ramputh, A. I. and Bown, A. W. 1996. Rapid γ-aminobutyric acid synthesis and the inhibition of the growth and development of oblique-banded leaf-roller larvae. *Plant Physiol*. **111**: 1349–1352.

Reggiani, R., Aurisano, N., Mattana, M., and Bertani, A. 1993. ABA induces 4-aminobutyrate accumulation in wheat seedlings. *Phytochem*istry **34**: 605–609.

Reggiani, R., Cantu, C. A., Brambilla, I., and Bertain, A. 1988. Accumulation and interconversion of amino acids in rice roots under anoxia. *Plant Cell Physiol*. **29**: 981–987.

Reymond, P., Weber, H., Diamond, M., and Farmer, E. E. 2000. Differential gene expression in response to mechanism wounding and insect feeding in *Arabidopsis*. *The Plant Cell* **12**: 707–719.

Roberts, D. M. and Harmon, A. C. 1992. Calcium-modulated proteins: targets of intracellular calcium signals in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **43**: 375–414.

Roberts, E. 1988. The establishment of GABA as a neurotransmitter. In: *GABA and Benzodiazepine Receptors*, Squires, R. F., Ed., pp. 1–21, CRC Press, Boca Raton, FL.

Roberts, J. K. M., Callis, J., Wemmer, D., Walbot, V., and Jardetzky, O. 1984. Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. *Proc. Natl. Acad. Sci. U.S.A.* **81**: 3379–3383.

Roos, W., Evers, S., Hieke, M., Tschope, M., and Schumann, B. 1999. Shifts of intracellular pH distribution as a part of the signal mechanism leading to the elicitation of benzophenanthridine alkaloids. Phytoalexin biosynthesis in cultured cells of *Eschscholtzia californica*. *Plant Physiol*. **118**: 349–364.

Sacks, M. M., Freeling, M., and Okimoto, R. 1980. The anaerobic proteins of maize. *Cell* **20**: 761–767.

Sanders, D., Brownlee, C., and Harper, J. F. 1999. Communicating with calcium. *The Plant Cell* **11**: 691–706.

Satya Narayan, V. and Nair, P. M. 1985. Purification and characterization of glutamate decarboxylase from *Solanum tuberosum*. *Eur. J. Biochem*. **150**: 53–60.

Satya Narayan, V. and Nair, P. M. 1990. Metabolism, enzymology and possible roles of 4-aminobutyrate in higher plants. *Phytochemistry* **29**: 367–375.

Shelp, B. J., Bown, A. W., and McLean, M. D. 1999. Metabolism and functions of γ-aminobutyric acid. *Trends Plant Sci*. **41**: 446–452.

Sheen, J., Zhou, L., and Jang, J. C. 1999. Sugars as signaling molecules. *Curr. Opin. Plant Biol*. **2**: 410–408.

Shukuya, R. and Schwert, G. W. 1960. Glutamic acid decarboxylase. I. Isolation procedures and properties of the enzyme. *J. Biol. Chem.* **235**: 1649–1652.

Sistrunk, M. L., Antosiewicz, D. M., Purugganan, M. M., and Braam, J. 1994. *Arabidopsis TCH*3 encodes a novel Ca^{2+} binding protein and shows environmentally induced and tissue-specific regulation. *Plant Cell*. **6**: 1553–1565.

Snedden, W. A. and Fromm, H. 1999. Regulation of the γ-aminobutyrate-synthesizing enzyme, glutamate decarboxylase, by calcium-calmodulin: a mechanism for rapid activation in response to stress. In: *Plant Re-*

sponses to Environmental Stresses, pp. 549–576. Lerner, H. R., Eds., Marcel Dekker, New York.

Snedden, W. A., Arazi, T., Fromm, H., and Shelp, B. J. 1995. Calcium/calmodulin activation of soybean glutamate decarboxylase. *Plant Physiol*. **108**: 543–549

Snedden, W. A., Chung, I., Pauls, R. H., and Bown, A. W. 1992. Proton/L-glutamate symport and the regulation of intracellular pH in isolated mesophyll cells. *Plant Physiol*. **108**: 543–549.

Snedden, W. A., Koutsia, N., Baum, G., and Fromm, H. 1996. Activation of a petunia glutamate decarboxylase by calcium/calmodulin or by a monoclonal antibody which recognizes the calmodulin binding domain. *J. Biol. Chem*. **108**: 543–549.

Solano, R. and Ecker, J. R. 1998. Ethylene gas: perception, signaling and response. *Curr. Opin. Plant Biol. 1998*. **1**: 393–398.

Stermer, B. A. and Hammerschmidt, R. 1987. Association of heat shock induced resistance to disease with increased accumulation of insoluble extension and ethylene synthesis. *Physiol. Mol. Plant Path.* **31**: 453–461.

Stevenson, W. R. and James, R. V. 1999. Evaluation of fungicides to control early blight and late blight of potato. In: *Vegetable Disease Control Trials 1999*, pp. 1–14. Department of Plant Pathology, University of Wisconsin–Madison.

Steward, F. C., Thompson, J. F., and Dent, C. E. 1949. γ-aminobutyric acid: a constituent of the potato tuber? *Science* **110**: 439–440.

Stewart, C. R. and Voetberg, G. 1985. Relationship between stress-induced ABA and proline accumulations and ABA-induced proline accumulation in excised barley leaves. *Plant Physiol*. **79**: 24–27.

Stewart, G. R., Mann, A. F., and Fentem, P. A. 1980. Enzymes of glutamate formation: glutamate dehydrogenase, glutamine synthetase, and glutamate synthase. In: *The Biochemistry of Plants, Vol 5*, Miflin, B. J., Ed., pp. 271–327, Academic Press, New York.

Streeter, J. G. and Thompson, J. F. 1972. *In vivo* and *in vitro* studies on γ-aminobutyric acid metabolism with the radish plant (*Raphanus sativus* L.). *Plant Physiol.* **49**: 572–584.

Subbaiah, C. C., Zhang, J., and Sacks, M.M. 1994. Involvement of intracellular calcium in anaerobic gene expression and survival of maize seedlings. *Plant Physiol*. **105**: 369–376.

Subbaiah, C. C., Bush, D. S., and Sacks, M. M. 1998. Mitochondrial contribution to the anoxic Ca^{2+} signal in maize suspension-cultured cells. *Plant Physiol.* **118**: 759–771.

Sze, H., Liang, F., Hwang, I., Cunran, A. C., and Harper, J. F. 2000. Diversity and regulation of plant Ca^{2+} pumps: insights from expression in yeast. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**: 433–462.

Thompson, J. F., Stewart, C. R., and Morris, C. J. 1966. Changes in amino acid content of excised leaves during incubation. I. The effect of water content of leaves and atmospheric oxygen level. *Plant Physiol.* **41**: 1578–1582.

Tognioli, L. and Basso, L. 1987. The fusicoccin-stimulated phosphorylation of a 33–kDa polypeptide in cells of *Acer pseudoplatanus* as influenced by extracellular and intercellular pH. *Plant Cell Environ.* **10**: 233–239.

Trewavas, A. J. and Malho, R. 1997. Signal perception and transduction: the origin of the phenotype. *The Plant Cell* **9**: 1181–1195.

Tsushida, T. and Murai, T. 1987. Conversion of glutamic acid to γ-aminobutyric acid in tea leaves under anaerobic conditions. *Agric. Biol. Chem.* **51**: 2865–2871.

Turano, F. J. and Fang, T. K. 1998. Characterization of two glutamate decarboxylase cDNA clones from *Arabidopsis thaliana*. *Plant Physiol*. **117**: 1411–1421.

Turano, F. J., Kramer, G. F., and Wang, C. Y. 1997. The effect of methionine, ethylene, and polyamine catabolic intermediates on polyamine accumulation in detached soybean leaves. *Physiol. Plant.* **101**: 510–518.

Turano, F. J., Panta, G. R., Allard, M. W., and van Berkum, P. 2000. The plant glutamate receptors are the progenitors to two superfamilies of animal neurotransmitter receptors. *Mol. Bio. Evol.*, in press..

Turano, F. J., Wilson, B. J. and Matthews, B. F. 1990. Purification and characterization of aspartate aminotransferase isoenzymes from carrot suspension cultures. *Plant Physiol.* **92**: 587–594.

Van der Luit, A. H., Olivari, C., Haley, A., Knight, M. R., and Trewavas, A. J. 1999. Distinct calcium signaling pathways regulate calmodulin gene expression in tobacco. *Plant Physiol.* **121**: 705–714.

Van der Veen, R., Heimovaara-Dijkstra, S., and Wang, M. 1992. The cytosolic alkalinization mediated by abscisic acid is necessary but not sufficient for abscisic acid-induced gene expression in barley aleurone protoplasts. *Plant Physiol.* **100**: 699–705.

Voesenek, L. A. C. J. and Van Der Veen, R. 1994. The role of phytohormones in plant stress: too much or too little water. *Acta. Bot. Neerl*. **43**: 91–127.

Walker, L. and Estelle, M. 1998. Molecular mechanisms of auxin action. *Curr. Opin. Plant Biol.* **1(5)**: 434–439.

Wallace, W., Secor, J., and Schrader, L. E. 1984. Rapid accumulation of γ-aminobutyric acid and alanine in soybean leaves in response to an abrupt transfer to lower temperature, darkness, or mechanical manipulation. *Plant Physiol*. **75**: 170–175.

Waters, E. R., Lee, G. J., and Vierling, E. 1996. Evolution, structure and function of the small heat shock proteins in plants. *J. Exp. Bot.* **47**: 325–338.

Wu, J. Y., Matsuda, T., and Roberts, E. 1974. Purification and characterization of glutamate decarboxylase from mouse brain. *J. Biol. Chem*. **245**: 3029–3034.

Xu, H. and Heath, M.C. 1998. Role of calcium in signal transduction during the hypersensitive response caused

by basidiospore-derived infection of the cowped rust fungus. *The Plant Cell* **10**: 585–597.

Young, A. B. and Penney, J. B. 1991. Benzodiazepine, GABA and glutamate receptors in cerebral cortex, hippociampus, basal ganglia, and cerebellum. In: *Receptors in the Human Nervous System*, p. 9. Mendelsohn F. A. O. and Paxinos, G., Eds., Academic Press.

Yun, S. J. and Oh, S-H. 1998. Cloning and characterization of tobacco cDNA encoding calcium/calmodulin-dependent glutamate decarboxylase. *Mol. Cells* **8**: 125–129.

Zielinski, R. E. 1998. Calmodulin and calmodulin-binding proteins in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **49**: 497–725.

Zik, M., Arazi, T., Snedden, W. A., and Fromm, H. 1998. Two isoforms of glutamate decarboxylase in *Arabidopsis* are regulated by calcium/calmodulin and differ in organ distribution. *Plant Mol. Biol.* **37**: 967–975.