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Research Article

EFFECT OF GAMMA-AMINOBUTYRIC ACID (GABA) ON ROOT GROWTH OF *ALLIUM SATIVUM*

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ABSTRACT
GABA (Gamma-Aminobutyric Acid), a non-protein amino acid is primarily known as a neurotransmitter in vertebrates. GABA is also present in bacteria and plants. GABA accumulates in plants under several stress conditions. It also plays several roles in plants including pollen tube growth, root and shoot development etc. This study reports that GABA inhibits the root growth in *Allium sativum*. It was also observed that root inhibition was highest in 1000 µM initially. As the time progressed, 200µM concentration of GABA showed higher inhibition. However, higher concentration of GABA imparted lesser impact on root growth. Different concentrations of GABA imparted different level of root inhibition as the time progressed.

Key Words: *Amino Acid, Neurotransmission, Signal Molecule, Inhibition, Stress*

INTRODUCTION
Gamma-Aminobutyric acid (GABA) is a non-protein amino acid present ubiquitously in bacteria, plants and vertebrates (Bouché and Fromm, 2004). In animals and plants, GABA is mainly metabolized via a short pathway called GABA shunt which is composed of three enzymes: glutamate decarboxylase (GAD), GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) (Bouché and Fromm, 2004). GABA shunt attracted much attention as it has been observed that GABA is rapidly and largely produced in plants in response to different biotic and abiotic stresses (Shelp *et al.*, 1999; Kinnersley and Turano, 2000). However, function of GABA was mainly highlighted in animals as it plays a major role as a signaling molecule in neurotransmission in animal brains (Bouché and Fromm, 2004). It also plays important role in brain network development (Represa and Ben-Ari, 2005). In addition, GABA is known to impact development of other organisms like *Agrobacterium* (Chevrot *et al.*, 2006) and planktonic larvae (Morse *et al.*, 1979). Park *et al.*, (2010) showed that high GABA levels could inhibit bacterial growth. However, the role of GABA in plant development has gained little attention (Renault *et al.*, 2011). It has been suggested that GABA act as a maternal signal in the directional growth of pollen tubes to the ovule (Palanivelu *et al.*, 2003). Walch-Liu *et al.*, (2006) Reported that 0.05-0.5mM concentration of GABA had no effect on the growth of primary roots. On the contrary, Renault *et al.*, (2011) observed growth inhibition of primary root of *Arabidopsis thaliana* upon exposure to exogenous GABA at a concentration of 10mM. However, the same study reported that no growth inhibition occurred when the plants were exposed to 1mM GABA. These results suggest that concentration of GABA is a crucial factor for controlling the root development in plants. Beuve *et al.*, (2004) suggested that GABA might operate as a putative long-distance inter-organ signal molecule in plants in conjunction with negative control exerted by Glutamine.

The above studies showed that GABA has a role in root development in plants. However, more plants should be studied for better understanding the role of GABA in root development. Additionally, the effect of different concentration of GABA should also be studied. Thus, I have selected *Allium sativum* for this purpose. Thus, the objective of the present study is to investigate the effect of different concentration of GABA on the root growth of *Allium sativum*.

MATERIALS AND METHODS

Allium sativum was selected as the plant material because a single clove can produce several roots and the root growth is also very rapid. A single bulb with many cloves was selected to nullify the effect of genetic variation among the plant materials. Healthy cloves that had not started the formation of green leaves and root growth were chosen. Before starting the experiment, the dry scales from the bulb were removed. Cloves were soaked in water for 5 hours. They were allowed to produce roots. Then they were treated with different concentrations of GABA (and a control without GABA). Three replicates were made with the cloves from the same bulb.

Aqueous solutions of 0.1M GABA was prepared as stock the solution and subsequently diluted to different concentrations. Five different concentrations were prepared: 200µM, 400µM, 600µM, 800µM and 1000µM. A control was also made without GABA. The GABA solutions of different concentrations and the control were poured into different collection tubes. Cloves were placed on the collection tubes in such a way that the bottoms of the cloves were immersed in the solution. A scale was also placed alongside the collection tubes. Roots have emerged from the cloves on the second day. Regular pouring of solutions has been done when the solution level decreased due to evaporation and absorption by roots. Every day, the roots have been photographed with a Canon powershot A550 digital camera (Canon India Pvt Ltd, India). Root lengths were measured with ImageJ software (Abramoff *et al.,* 2004) downloaded from http://rsbweb.nih.gov/ij/download.html. Statistical analyses and graphs were prepared using Open Office 4.0 downloaded from http://www.openoffice.org/.

RESULTS AND DISCUSSION

The results show that GABA inhibited root growth in *Allium sativum*. Figure 1 shows the root growth of the cloves under control and different concentration of GABA solution after 5 days.

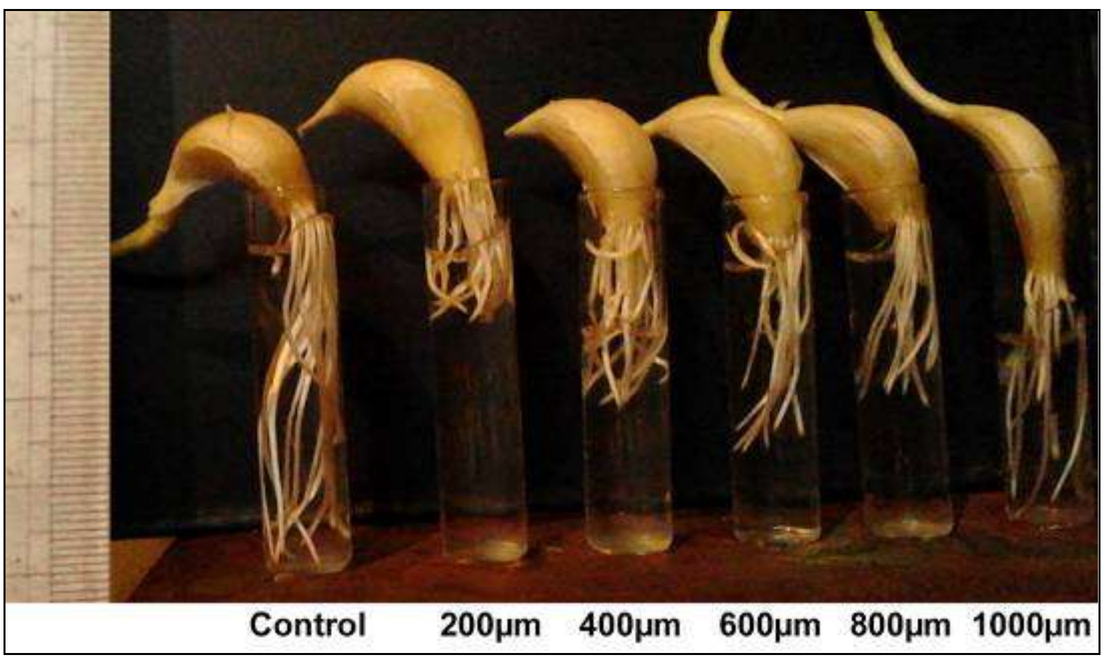

Figure 1: Growth of roots of *Allium sativum* in different concentrations of GABA after five days of treatment

It was observed that initially 1000µM (1mM) GABA showed highest effect on root growth as the mean root length was shortest at day one under this concentration of GABA followed by 200µM solution (table

1, figure 2). However, all the concentrations of GABA had some effect on root growth as the mean root length of all the treatments was shorter than the control (without GABA) (table 1, figure 2). However, on day two, 200 µM of GABA treatment showed maximum inhibition followed by 1000µM (table 1, figure 2). From day 3 to day 5, root growth inhibition was lowest in 1000µM GABA concentration as the mean root length was highest among the cloves under different GABA concentrations.

Table 1: Day wise growth of roots in different concentrations of GABA

Days	Length of root in mm under different concentrations					
	control	200µm	400µm	600µm	800µm	1000µm
Day 1	7.500857	4.775571	6.137857	6.831	5.616	4.111714
Day 2	17.616	9.670286	11.52786	13.34571	13.48843	10.01114
Day 3	19.21743	11.40829	11.86057	13.39771	16.46	16.56771
Day 4	35.11814	14.76243	15.69629	19.50871	17.38014	20.21629
Day 5	41.96529	16.55029	21.77957	24.54186	21.18457	26.63971

Renault *et al*., (2011) reported that in *Arabidopsis*, 1mM GABA showed no difference in root growth after 6 days of treatment. This study, however, showed that in *A. sativum*, 1mM GABA caused root inhibition. It is interesting that percentage root inhibition was time dependent. At day 1, 45.18% root inhibition was reported in 1mM GABA compared to the control (figure 3). However, that decreased in the following days and day 3 showed lowest root growth inhibition (13.78%). It was also important to note that except 400µM and 600µM of GABA treatment, all other treatments showed the lowest root growth inhibition in the third day (figure 3). The highest root inhibition (60.56%) was reported in 200µM GABA concentration after 5 days. At day 4, 57.96% root inhibition was reported under the 200µM GABA concentration (figure 3). Thus, 200µM GABA was found to be most effective in root inhibition as the time progressed. On the other hand, 1000µM GABA showed lowest root inhibition in 4^{th} and 5^{th} day (42.43% and 36.51%, respectively) among all the treatments. It is also notable that in the control solution, the root growth from 2^{nd} to 3^{rd} day was the lowest while after the third day, rapid root growth occurred (figure 4). However, this rapid growth of roots after the 2^{nd} day was not observed in cloves under GABA treatment (figure 4). Although increased root growth was observed after the second day in cloves under treatment of 400µM and 600µM of GABA solutions (figure 4), growth was not very rapid.

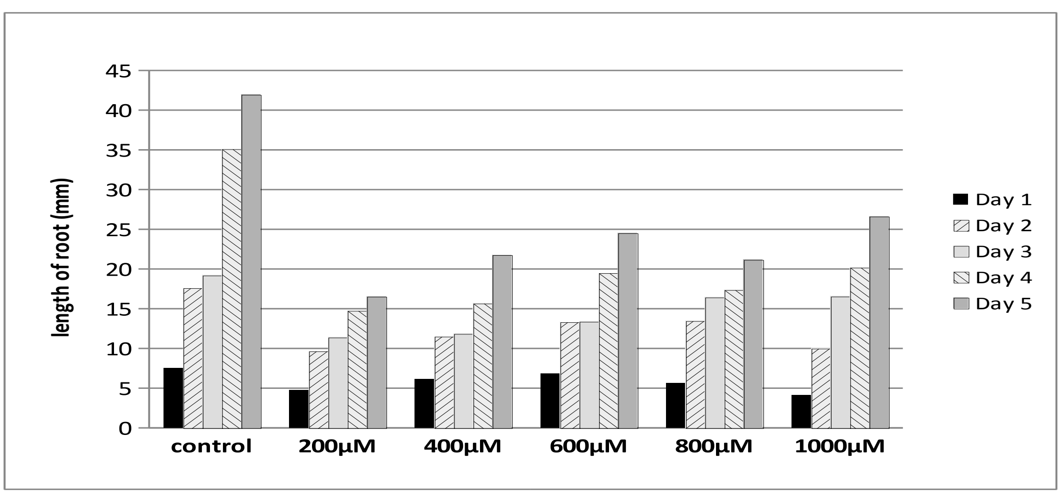

Figure 2: Graphical representations of the root growth under different concentrations of GABA and the control in five days

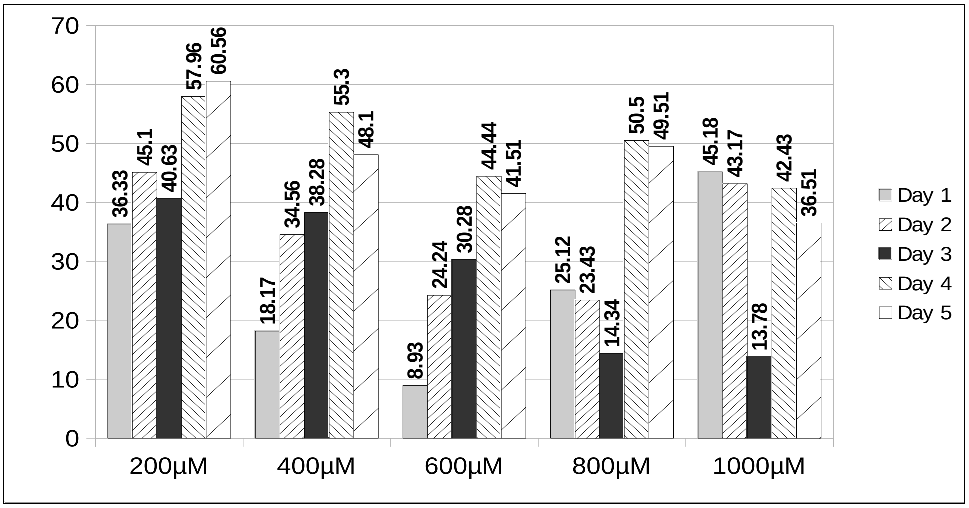

Figure 3: Percentage root growth inhibition of *Allium sativum* roots under different GABA concentrations compared to the control

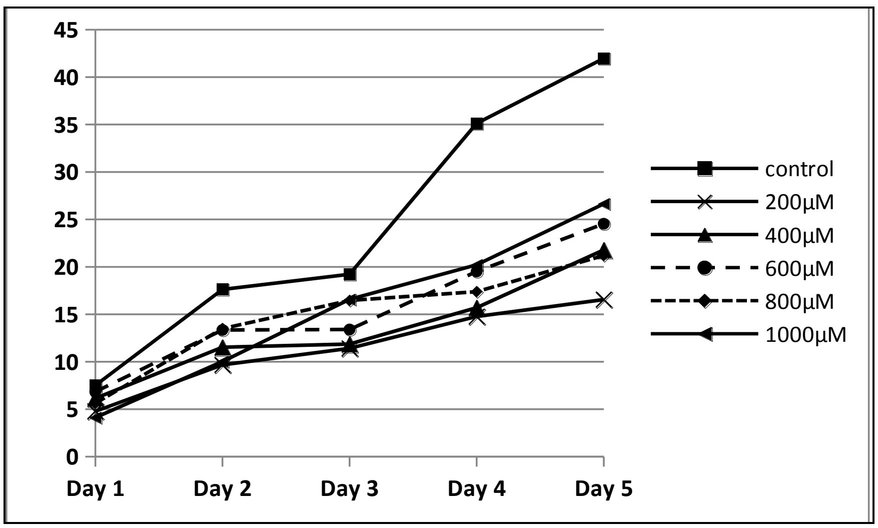

Figure 4: Trend of root growth of different concentrations of GABA and the control in five days

These results showed that root growth of *Allium sativum* is sensitive to exogenous GABA. Kathiresan *et al.*, (1998) found that in case of stem of *Stellaria longipes* (Caryophyllaceae), GABA had biphasic response *i.e.* lower concentrations of GABA (upto 500µM) promoted stem elongation and higher concentrations inhibited stem elongation. They have also found that 250µM of GABA concentration caused maximum stem elongation. Thus, GABA was effective at lower concentration in case of stem growth. Our results showed that at lower concentrations, GABA was most effective in inhibiting the root growth of *A. sativum*. However, the effect was not biphasic as GABA did not show root growth promotion in any of the treatments.

Kathiresan *et al.*, (1997) previously observed that exogenous GABA stimulated ethylene production in Sun-flower. They have also suggested that GABA stimulated ethylene production by acting as a signaling regulatory molecule, not as a precursor of ethylene. Ethylene influences many aspects of plant development including root development (Abeles *et al.*, 1992; Růžička *et al.*, 2007). Ethylene has been shown to strongly inhibit the elongation of root apical meristematic cells (Le *et al.*, 2001). Thus, it is possible that GABA inhibits root growth by stimulating ethylene production. However, it has to be investigated in future.

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In conclusion, this study showed that GABA inhibits root growth of *Allium sativum* and lower concentration of GABA has more effect on root inhibition as the time progresses.

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