PUBLISHED ON 29TH FEB 2012

CHANGES IN PHENOL METABOLISM AND IAA OXIDASE ACTIVITY OF BRINJAL (*SOLANUM MELONGENA* L.) PLANT IN RESPONSE TO FOLIAR APPLICATION OF DIFFERENT B CONCENTRATIONS

URVI GUPTA AND *HITESH SOLANKI

DEPARTMENT OF BOTANY, UNIVERSITY SCHOOL OF SCIENCES, GUJARAT UNIVERSITY, AHMEDABAD

urvi8198@gmail.com

ABSTRACT:

Boron is an essential micro nutrient required for nutrition and growth of all plants. Adequate Boron nutrition is critical for high yield and quality of crop. Experiments were carried out in vivo to find out the effect of foliar application of boron on physical and chemical parameters of brinjal. In chemical parameters changes in phenol content, polyphenol oxidase activity and IAA Oxidase activity were studied after the foliar application of Boron. Four treatments comprising of foliar application of boron at four concentrations viz. 5ppm, 10ppm, 15pm and 20ppm with one control were checked and parameters were studied with the use of standard methods. Higher phenol concentration was recorded in the controlled plants and decreased with the higher B concentration. This indicates phenol accumulation induced by the minimum B treatment. And the exact opposite results were found in polyphenol oxidase and IAA oxidase activities where the least enzyme activities were recorded in controlled plants and it goes on increasing with the higher B concentration, which indicates the oxidation of the phenols and IAA induced by the higher B treatments.

KEY WORDS: *Phenol Metabolism, Iaa, Brinjal, Foliar, B Concentrations.*

INTRODUCTION:

Boron (B) is an essential element for plants and the only non-metal among the eight plant micronutrients. The requirement of boron for plant growth was demonstrated in the early 1920s (Marschner 1995; Warington, 1923). It plays an important role in plant metabolism and there is a long list of roles of boron (Parr and Loughman, 1983). Many experimental reports have revealed its involvement and importance in sugar and starch metabolism, cell division, cell wall synthesis, lignifications, cell wall structure integrity, RNA metabolism, nucleic acid metabolism, protein metabolism, flower formation, seed production, membrane function, IAA and phenol metabolism (Barker and Pilbeam, 2007; Li and Ling, 1997).

Role of boron in indole-3-acetic acid (IAA) metabolism was first proposed by Eaton in 1940. It has been reported that boron has an effect on the transport metabolism and activity of auxin it controls the level of IAA in plants. (Robertson and Loughman, 1974) The role of boron in IAA metabolism is well studied. It has been revealed that sunflowers with boron deficiency contained more IAA than control group. Also it has been proposed that IAA-oxidase was inhibited due to high level of phenolic acid (Cohen and Bandurski, 1978). Bohnsack and Albert (1977) demonstrated a severe inhibition in root growth of squash and increase in IAA oxidation by boron deficiency. Resupply of boron to boron deficient squash plants rapidly stimulated root growth and reduced IAA oxidation. Rajaratnam and Lowry (1974) reported that IAA content in palm trees increased in the presence of boron deficiency.

Few studies also revealed the fact of positive co-relation between phenols and IAA content. Decreased content of phenol and IAA was recorded with the raised level of boron (Cohen and Bandurski, 1978; Shkolnik, 1984; Mazher *et al.,* 2006).

Thus the present study aimed to investigate the changes in the phenol and IAA metabolism and their correlation on *Solanum melongena* plant by application of different boron concentrations. Brinjal, *Solanum melongena* L. is an important and well-accepted vegetable crop of sub-tropics and tropics. The brinjal is of much importance in the warm areas of Asia, parts of Europe and Africa (Karihaloo and Gottileb, 1995), being grown extensively in India, Bangladesh, Pakistan, China and the Philippines. It is also well-liked in Egypt, France, Italy and United States. In India, it is one of the most widespread, popular and primary vegetable crops grown all over the country except higher altitudes.

MATERIALS AND METHODS:

For the experiments seeds of *Solanum melongena* L. cultivar longum were utilized, purchased from an agricultural product supplier in Ahmedabad. These seeds were grown in pots at green house of Botany Department of Gujarat University. Pots were filled with farm yard manure and soil. After the one month of seed sowing small saplings were transplanted in other pots containing 4 plants each pot.

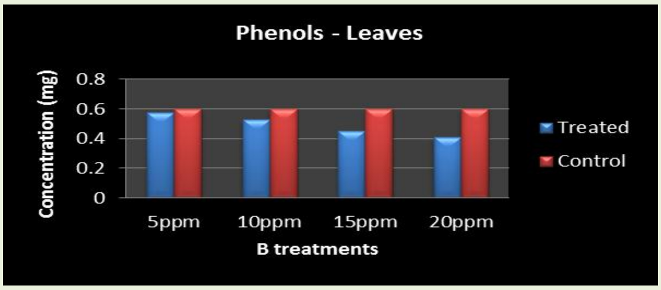
As borax is the most suitable B salt for foliar application, it was selected as the B source for treatments. Four different B concentrations were used for foliar application viz. 5ppm, 10ppm, 15ppm, 20ppm. One set of plants were kept as control i.e. without any B application. Due to the immobility of B, repeated applications are necessary. Because of this, foliar feeding of different B concentrations was given to the plants at the interval of 15 days.

Metabolites and biochemical parameters were studied in different plant parts like leaves, stem, buds, flowers and fruits. These plant parts were collected at the different stages of plant life and taken to laboratory for analysis. Changes in various bio-chemicals and metabolites of brinjal in response to different concentration of B were studied using spectrophotometric quantification. Total phenols were determined by using method of Bray *et al*. (1954). Polyphenol oxidase activity was determined by the method given by Kar *et al*. (1976). IAA Oxidase activity was determined using the methodology given by Mahadevan (1964).

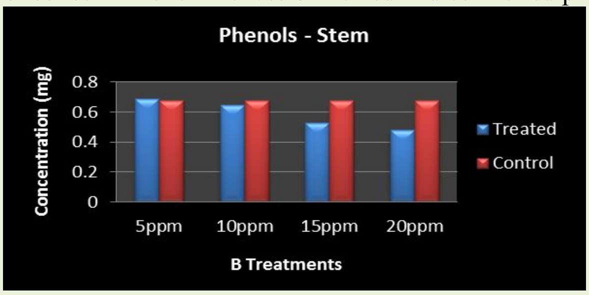
RESULT AND DISCUSSION:

Total Phenols:

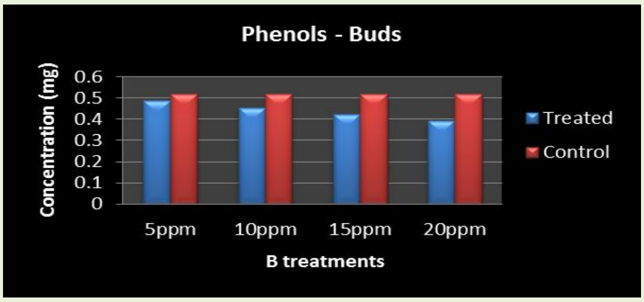
Phenol content was determined in different plant parts i.e. leaves, stem, buds, flowers and fruits and compared with the phenol content of plants kept under controlled condition. Lower phenol content was determined in all the plants treated with boron concentrations while the plants kept under controlled conditions possesses the highest amount of phenol content. It has been observed that phenol content was decreased with the increased B concentrations (Graph 1-5).



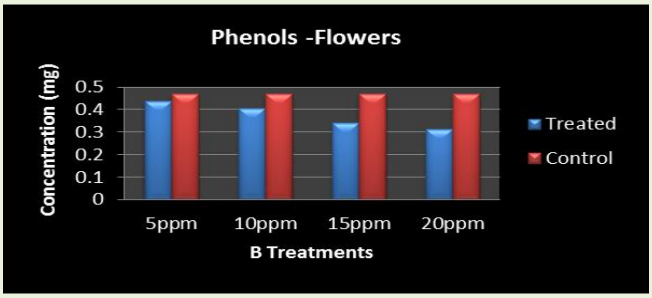
Graph 1 - Comparison between phenol concentrations in leaves of treated and controlled plants for 5-25ppm treatment of Boron



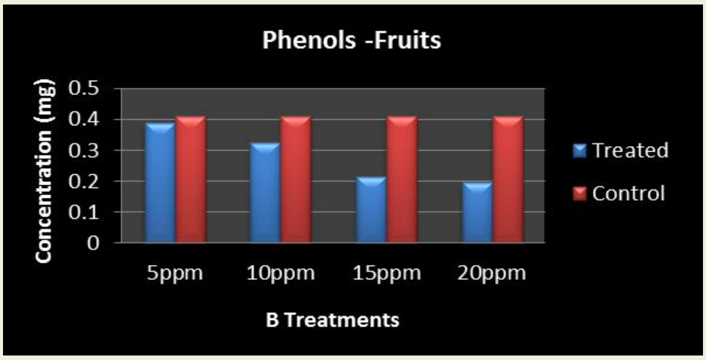
Graph 2 - Comparison between total phenol concentrations in stem of treated and controlled plants for 5-25ppm treatment of Boron



Graph 3 - Comparison between phenol concentrations in buds of treated and controlled plants for 5-25ppm treatment of Boron



Graph 4 - Comparison between phenol concentrations in flowers of treated and controlled plants for 5-25ppm treatment of Boron

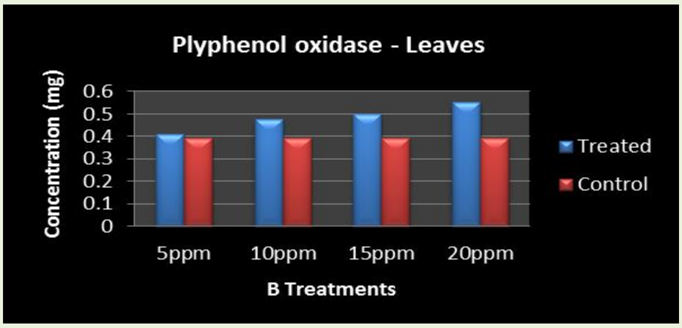


Graph 5 - Comparison between phenol concentrations in fruits of treated and controlled plants for 5-25ppm treatment of Boron

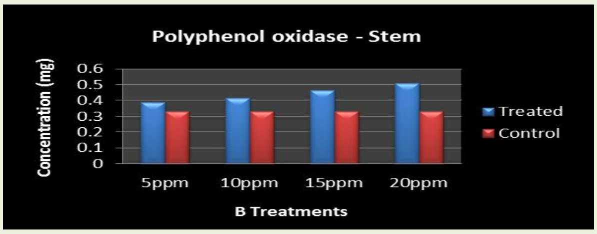
All the B treated plants recorded with lower phenol content in compare to control. Many reports are available which shows that phenol level increases in B deficient plants and this accumulation is form due to the stimulation of the enzyme phenylalanine-ammonium lyase (PAL) (Zehirov and Georgiev, 2000; Cakmak *et al*. 1995; Ruiz *et al*. 1998; Camacho-Cristóbal *et al*. 2002). Results of present study support the above mentioned statement as all the plants which received regular B treatments have lower phenol content. While plants kept under controlled conditions shows the maximum content of phenols. This may be result of borate complex formation which develops to control the rate of free phenols (Lewis, 1980; Pilbeam and Kirkby, 1983; Shkolnik, 1984). In fact, it is well known that B deficiency causes an accumulation of phenolics through the stimulation of the enzyme phenylalanine-ammonium lyase (PAL) (Cakmak *et al*. 1995; Ruiz *et al*. 1998b; Camacho-Cristóbal *et al*. 2002). Zehirov and Georgiev (2000) reported that the long-term absence of B was found to increase total soluble phenol content in root apoplast exudates.

Polyphenol Oxidase activity

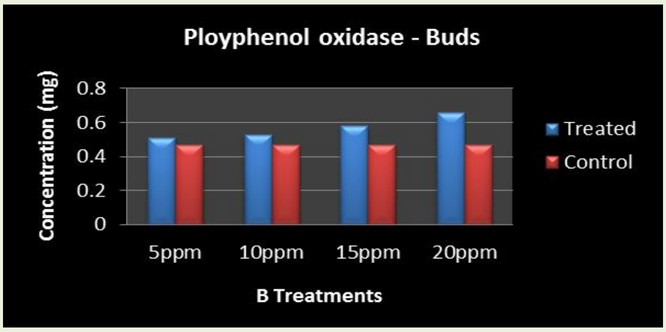
Least polyphenol oxidase activity was recorded in plants kept under controlled conditions in compared to treated plants. While plants treated with 20ppm B concentration i.e. highest B concentration recorded with maximum activity which shows the highest rate of degradation of phenolic compounds. (Graph: 6-10)



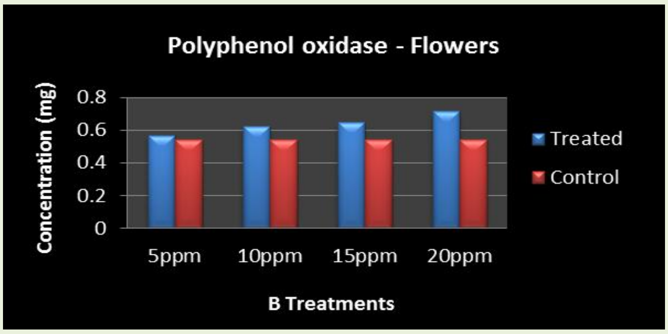
Graph 6 - Comparison between polyphenol oxidase activities in leaves of treated and controlled plants for 5-25ppm treatment of Boron



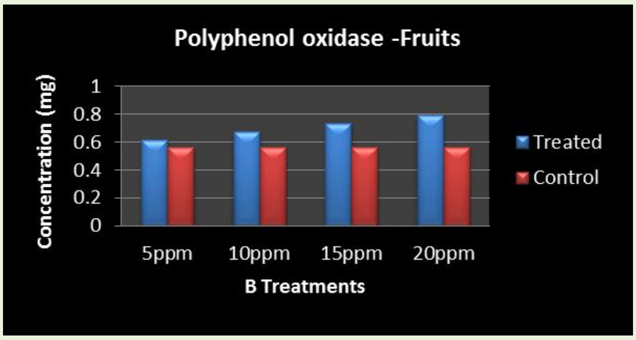
Graph 7 - Comparison between polyphenol oxidase activities in stem of treated and controlled plants for 5-25ppm treatment of Boron



Graph 8 - Comparison between polyphenol oxidase activities in buds of treated and controlled plants for 5-25ppm treatment of Boron



Graph 9 - Comparison between polyphenol oxidase activities in flowers of treated and controlled plants for 5-25ppm treatment of Boron



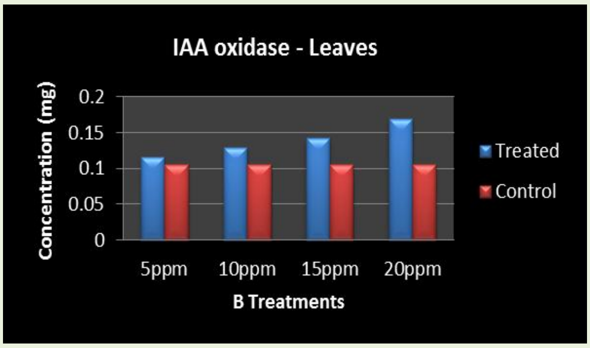
Graph 10- Comparison between polyphenol oxidase activity in fruits of treated and controlled plants for 5-25ppm treatment of Boron

Polyphenol oxidase activity was recorded highest in plants treated with 20ppm B concentration and phenol content was recorded the least phenol content in these plants. This shows the maximum degradation of phenols in the plants treated with 20ppm B concentration. It is known that micronutrient deficiency often leads to increased content of phenols and decreased lignifications. Shkol'nik *et al.* (1981) related an increased content of phenols to boron (B) deficiency. Results of present study supports the above statement as phenol content was recorded highest in controlled plants i.e. without B treatments. And polyphenol oxidase activity is recorded lowest in controlled plants which shows lower degradation rate of phenols. Some studies also reported that B deprivation also increased the activity of polyphenoloxidase activity (PPO) (Pfeffer *et al.,* 1998; Camacho-Cristóbal *et al*., 2002), enzyme that catalyses the oxidation of phenolic compounds into quinones. But the results of present study are not supported by this.

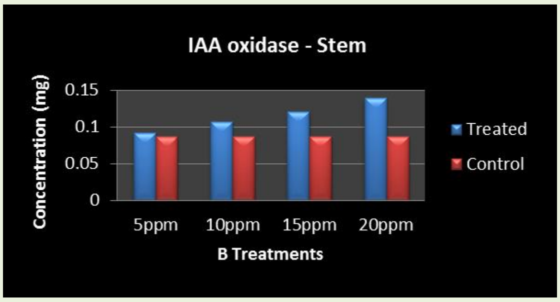
IAA-Oxidase activity

When the result of IAA oxidase activity of controlled plants was compared with the treated plants it have been noticed that IAA Oxidase activity increases with the increasing B concentrations. The controlled plants showed the lowest rate of IAA Oxidase activity while all the plants treated with B showed the higher IAA Oxidase activity in compare to the controlled plants. IAA oxidase activity took the same trend obtained previously on polyphenole oxidase activity. They increased as the B concentration level rising upwards.

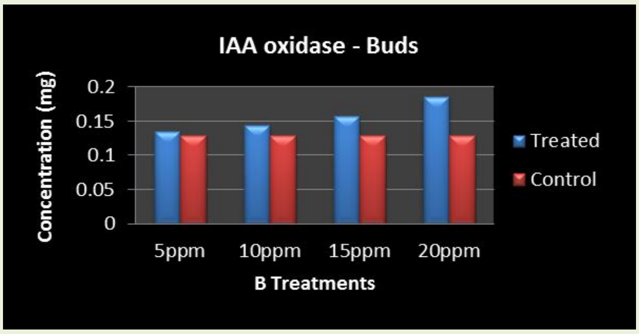
Plants kept under controlled condition showed the least IAA oxidase activity which means reduced oxidation of the growth hormone IAA. While plants treated with 20ppm have the highest IAA oxidase activity i.e. higher rate of IAA oxidation. IAA oxidase activity has been reported to be decreased in plants subjected to several days of boron deficiency as free auxin level decreases and bound auxin level increases (Shkol'nik et.al, 1964; Cohen and Bandurski, 1978). Also it has been suggested that increased levels of IAA associated with boron deficient plants are not the result of a decrease in IAA oxidase activity, but IAA apparently causes the induction of this enzyme (Bohnsack and Albert 1977). In addition, it has been observed that, depending on a reduction of IAA-oxidase activity, an increase is observed in the IAA level of the plant (Paul *et al*., 1992; Cohen and Bandurski, 1978; Dugger, 1983). This shows that controlled plants and plants treated with 5ppm B concentration had the higher IAA level. Graphs 11-15 show the IAA oxidase activity in the different plant parts.



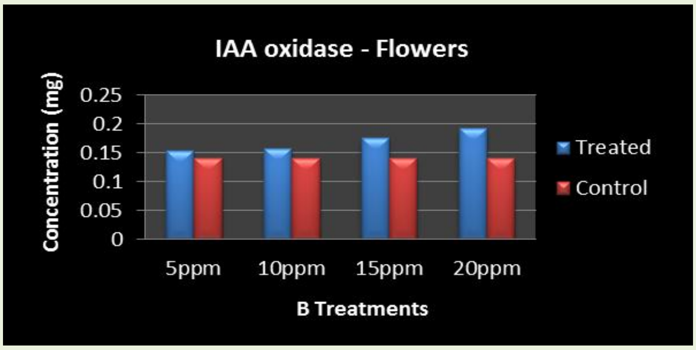
Graph 11 - Comparison between IAA Oxidase activities in leaves of treated and controlled plants for 5-25ppm treatment of Boron



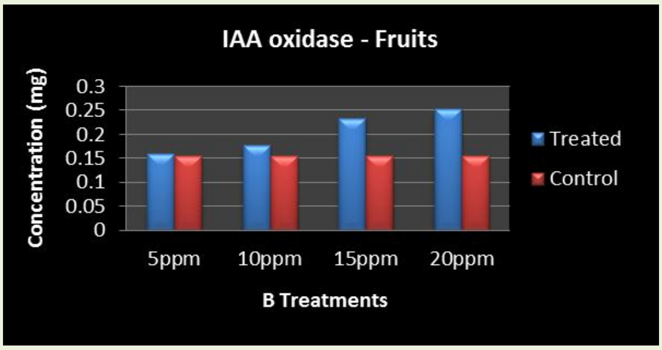
Graph 12 - Comparison between IAA Oxidase activities in stem of treated and controlled plants for 5-25ppm treatment of Boron



Graph 13 - Comparison between IAA Oxidase activities in buds of treated and controlled plants for 5-25ppm treatment of Boron



Graph 14 - Comparison between IAA Oxidase activities in flowers of treated and controlled plants for 5-25ppm treatment of Boron



Graph 15 - Comparison between IAA Oxidase activities in fruits of treated and controlled plants for 5-25ppm treatment of Boron

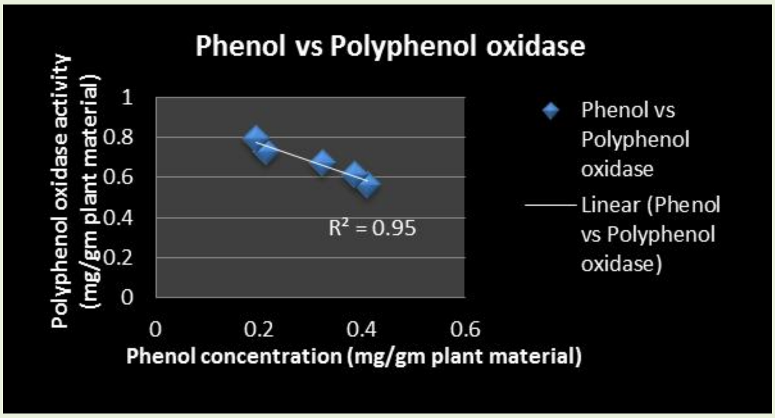
CONCLUSION:

Correlations

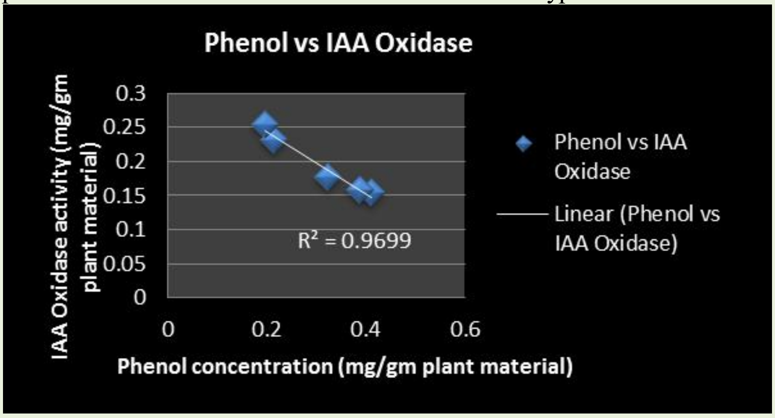
			Phenol	PPO	IAAOxidase
Studied Parameters		Correlation Coefficient	1.000	-1.000*	-1.000*
		Sig. (2-tailed)	.	.	.
		N	5	5	5
		Correlation Coefficient	-1.000**	1.000	1.000*
		Sig. (2-tailed)	.	.	.
		N	5	5	5
	IAA Oxidase	Correlation Coefficient	-1.000**	1.000**	1.000
		Sig. (2-tailed)			.
		N			5

*. Correlation is significant at the 0.05 level (2-tailed).

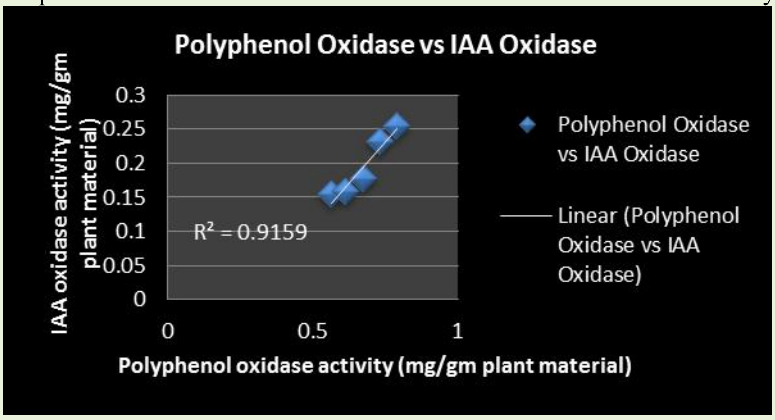
**. Correlation is significant at the 0.01 level (2-tailed).



Graph 16: Correlation between Phenol content and Polyphenol Oxidase activity



Graph 17: Correlation between Phenol content and IAA Oxidase activity



Graph 18: Correlation between Polyphenol Oxidase and IAA Oxidase activity.

From the correlation studies between phenol and IAA metabolism (Graph 16-18), it has been found that phenol and IAA shares positive correlation and content of both were decreased with the increased B level. In 2006 Mazher *et al.* studied the impact of B fertilizer on growth and chemical constituents of *Taxodium distichum* and found the same correlation between IAA and phenols in the response of different B levels. This might be possible that interaction between boron and IAA is secondary event caused by primary effect of boron on phenol metabolism (Lewis 1980; Pilbeam and Kirkby 1983). Also it has been proposed that that IAA-oxidase was inhibited due to high level of phenolic acid (Cohen and Bandurski, 1978). Furthermore, Shkolnik [1974] could explain the physiological role of boron with the fact that auxin and phenolic compounds exhibit accumulation at the absence of boron. For this reason, the results we obtained seem to support the findings of other researchers that phenol and IAA content are decreased with the higher level of B. Thus IAA and phenol metabolism is affected by B.

ACKNOWLEDGEMENT:

We are thankful to Prof. Dr. Y. T. Jasrai, Head of Botany Department, School of Sciences, Gujarat University, Ahmedabad for providing all facilities and encouragement.

REFERENCES:

Barker AV, Pilbeam DJ (2007). *Handbook of Plant Nutrition*. Pp; 241-268. CRC Press. ISBN: 0-8247-5904-4.

Bohnsack CW, Albert LS (1977). Early effects of boron deficiency on indoleacetic acid oxidase levels of squash root tips. *Plant Physiol*.; 59:1047–1050.

Bray HG and Thorpe WVT (1954). Analysis of phenolic compounds of interest in metabolism. *Biochemical Analysis*; 1: 2752.

Cakmak I, Kurz H and Marschner H (1995). Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. *Physiol. Plant*.; 95: 11–18.

Camacho-Cristóbal JJ, Anzelotti D, González-Fontes A (2002). Changes in phenolic metabolism of tobacco plants during short-term boron deficiency. *Plant Physiol. Biochem.*; 40: 997-1002.

Cohen JD and Bandurski RS (1978). The bound auxins: Protection of indole-3 acetic acid from peroxidase-catalyzed oxidation. *Planta*; 139: 203-208.

Dugger WM (1983). *Boron in plant metabolism*. In: Lauchli A, Bieliski RL (eds.) Inorganic Plant Nutrition, Encyclopedia of Plant Physiology, New Series; 15B: 626-650. Berlin: Springer-Verlag.

Eaton F (1940). Interrelations in the effects of boron and indoleacetic acid on plant growth. *Bot. Gaz.*; 101:700-705.

Kar M and Mishra D (1976). Catalase, Peroxidase and polyphenol oxidase activities during leaf senescence. *Plant Physiology;* 57: 315-319.

Karihaloo JL and Gottlieb LD (1995). Allozyme variation in the eggplant, *Solanum melongena* L. (Solanaceae). *Theor Appl Genet*; 90: 578-583.

Lewis OH (1980). Boron, lignification and the origin of vascular plants:a unified hypothesis. *New Phytologist*; 84: 209-229.

Lewis OH (1980). Are there inter- relation between the metabolic role of boron. Synthesis of phenolic phytoalexins and the the germination of pollen! *New Phytol*.; 84: 261-270

Li Y and Ling H (1997). Soil boron content and the effect of boron application on yield of maize, soybean, rice and sugar beet in Heilonjian Province, P.R. China. In: *boron in soil and plants*, RW Beli and B Perksem (Eds), pp: 17-21.

Mahadevan S. (1964). Enzymes involved in the synthesis and breakdown of IAA. Modern Methods of *Plant Physiology* 7:233-259. (eds.) Linskons MF, Sanwar BD and Tracey MV.

Marschner H (1995). Boron. *Mineral Nutrition of Higher Plants*.; 2: 379 - 396. Academic Press San Diego.

Mazher AAM, Zaghloul SM, Yassen AA (2006). Impact of Boron fertilizer on growth and chemical constituents of *Taxodium distichum* grown under water regime. *World journal of agricultural Sciences*; 2(4): 412-420.

Parr AJ and Loughman BC (1983). *Boron and membrane function in plants*. In Metals and Micronutrients. Uptake and Utilization by Plants. Edited by Robb DA and Pierpoint WS: 87 – 107. Academic Press, New York.

Pfeffer H, Dannel F, Römheld V (1998). Are there connections between phenol metabolism, ascorbate metabolism and membrane integrity in leaves of borondeficient sunflower plants? *Physiol. Plant*.; 104: 479-485.

Pilbeam DJ and Kirkby EA (1983). The physiological role of boron in plants. *Journal of Plant Nutrition*; 6: 563-582.

Paull JG, Nable RO and Rathjen AJ (1992). Physiological and genetic control of the tolerance of wheat to high concentrations of boron and implications for plant breeding. *Plant and Soil*; 146: 251–260.

Rajaratnam JA, and Lowry JB (1974). The role of boron in the oil-palm (*Elaeis guinensis*). *Ann Bot*; 38: 193-200.

Robertson GA and Loughman BC (1974). Response to boron deficiency: a comparison with responses by produced by chemical methods of retarding root elongation. *New Phytol*; 73: 821-832.

Ruiz JM, Bretones G, Baghour M, Ragala L, Belakbir A, Romero L (1998) Relationship between boron and phenolic metabolism in tobacco leaves. *Phytochemistry*; 48: 269-272.

Shkolnik MY (1984). *Trace elements in plants*. Amsterdam: Elsevier: pp-463.

Shkol'nik MY, Krupnikova TK and Dmitrieva NN (1964). Influence of boron deficiency on some aspects of auxin metabolism in the sunflower and corn. *Sov Plant Physiol*.; 11: 164-169

Shkol’nik MY, Krupnikova TK, and Smirnov YS (1981). Activity of polyphenol oxidase and sensitivity to boron deficiency in monocots and dicots. *Sov. Plant Physiol*.; 28: 279-283.

Shkolnik MY (1974). General conception of the physiological role of boron in plants. *Fiziolog Rast*; 21: 174-186.

Warington K (1923). The effect of boric acid and borax on the broad bean and certain other plants. *Annals of Botany*; 37: 629-672.

Zehirov G and Georgiev GI (2000). Effects of boron deficiency on the contents of lignin, soluble phenols, sugars and water related to nodulation and N2 fixation of soybean plants, Compt. rend. *Acad. bulg. Sci*.; 53(10): 55–58.