Indian Journal of Agriculture and Allied Sciences
A Refereed Research Journal

ISSN 2395-1109
e-ISSN 2455-9709
Volume: 2, No.: 2, Year: 2016

www.mrfsw.org

Received: 03.06.2016, Accepted: 10.06.2016

IMPACT OF MICRONUTRIENTS BORON AND IRON APPLICATION ON MORPHOLOGICAL, BIOCHEMICAL ATTRIBUTES IN TOMATO (*Lycopersicon esculentum* mill. var. Punjab chhuhara)

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Abstract: *An experiment was conducted to find out the response micronutrients application on morphological, biochemical attributes in variety Punjab chhuhara of Tomato. The treatments consisted of boron, iron, combination and control. All the treatments resulted in improvement of plant growth characteristics viz. plant height, Total leaf area, Total dry matter yield, net assimilation rate chlorophyll content total carotenoids, total protein, sugar content, nitrate reductase activity, and proline content in the variety. Growth, biochemical parameters and yield attributes of tomato was significantly improved by the application of boron and iron either individually or in combination up to different concentrations. The plant height was found to be significantly reduced under depleted iron combined with higher boron (50 mM). NR activity and toatal sugar content increases with increase in boron concentration with different iron levels. The treatment records maximum value for total sugar at pre-flowering stage with B1F2, i.e., 9.66 % increase over control at flowering and post-flowering stages both. The results regarding NAR well explains the role of B for efficient sink-source relationship in case of tomato, which on the one hand improved total leaf area and on the other hand accumulated optimum dry matter in respect to control*

Key words: *Tomato (Lycopersicon esculentum), Boron, Iron.*

Introduction: Tomato (*Lycopersicon esculentum* Mill.) is one of the most commonly grown vegetable crops of the world due to its wide adaptability under various agro climatic conditions. It is an important mineral and vitamin rich vegetable crop playing a vital role in Indian economy by virtue of its various uses as vegetable and processed forms as well as industrial product. The production and productivity of crop is being adversely affected in different areas due to deficiencies of micronutrients [1]. Micronutrients like Zinc, Iron, Manganese, Copper, Boron and Magnesium play an important role in physiology of tomato crop and these are being a part of enzyme system or catalyst in enzymatic reactions. They are required for plant activities such as aspiration, meristematic development, chlorophyll formation, photosynthesis, energy system, protein and oil synthesis, gossypol, tannin and phenolic compounds development [2]. For harnessing the higher yield potential, supplementation of micronutrients is essential.

Boron (B) is an essential micronutrient required for normal plant growth and development. It is a very sensitive element, and plants differ widely in their requirements: the range of deficiency and toxicity level are narrow. Boron management is challenging as the optimum B application range is narrow [3], and optimum B application rates can differ from one soil to another [3,4]. Although the precise function of boron in plant metabolism is unclear, evidence suggests that it plays roles in cell elongation, nucleic acid synthesis, hormone responses, and membrane function [5]. Boron deficient plants may exhibit a wide variety of symptoms, depending on the species and the age of the plant.

Iron has an important role as a component of enzymes involved in the transfer of electrons (redox reactions), such as cytochromes. In this role, it is reversibly oxidized

from Fe^{2+} to Fe^{3+} during electron transfer. As in magnesium deficiency, a characteristic symptom of iron deficiency is interveinal chlorosis. In order to study the effect of different micro nutrients viz., zinc, boron, molybdenum, copper, iron and manganese, application on tomato on growth, seed yield and yield contributing parameters, the present investigation was initiated.

Materials and Methods

Tomato (*Lycopersicon esculentum* mill.) plants genotype cultivar Punjab Chhuhara was selected for study. Observations on different morpho-physiological and biochemical parameters were done and recorded at regular intervals (35, 56 and 84 Days after germination). The treatments were B1F0 [Iron deficient solution] (B 25 mM Fe 0 mM); B1F1 [Balanced nutrient solution] (B 25 mM Fe 20 mM); B1F2 [Iron solution] (B 25 mM Fe 20 mM); B2F0 [Boron combined with deficient iron solution] (B 50 mM Fe 0 mM); B2F1 [Boron oolution] (B 50 mM Fe 20 mM); B2F2 [Boron and Iron solution] (B 50 mM Fe 40 mM).

Plant height (cm) was measured from the base of the plant to the top of the main stem with meter scale and expressed in centimeter. The total leaf area of all the counted leaves was measured with the help of leaf area meter (Systronics 211). The total dry weight (g) of the whole plant is recorded by gently removing the whole plant from soil without damaging the roots.

Net Assimilation Rate (NAR) is the rate of dry weight increase per unit leaf area per unit time (mg/cm^2 /day) and was calculated as suggested by Gregory (1926)[6]. Chlorophyll content in the leaf was determined by the method of Lichtanthaner (1987)[7]. Carotenoids were estimated by method of Venkatarayappa *et al.* 1984. Total soluble protein and total soluble sugar content were estimated by Bradford, MM (1976)[8] and Morris (1948)[9] respectively. Nitrate reductase activity free proline content in the leaves were determined by the method of Srivastava (1975)[10] and Bates et al. (1973)[11] respectively.

Results and Discussion

The morphological parameters of mungbean plants include root length, plant height, leaf number, leaf area, total fresh weight and total dry weight under normal and saline conditions after various plant treatments are presented in different tables and figures.

Fig 1: Tomato plants added with (A)normal boron with normal iron (B1F1) showing healthy plant with normal fruit growth; (B) with normal boron with iron deficiency (B1F0) showing chlorosis and necrosis affecting growth with small sized fruits; (C) with double dose of boron and iron (B2F2) sowing rapid chlorosis and rapid leaf tip burning, which may be considered the toxic effect of higher B and Fe.

A perusal fig 2 visualizes significant differences among the various treatments and when plant height compared with control (F1B1), it was found to be significantly reduced under depleted iron combined with higher boron (F0B2) level at all the three stages, viz., pre-flowering (6.54 %), flowering (1.8%) and post-flowering (2.15%) stages, closely followed by the depleted or no iron combined with normal boron level (F0B1) of nutrition with the reductions being 6.1 %and 1.7 % at pre-flowering and post-flowering stages of growth respectively, and at the level F2B1 with a drop of 3.5 % at the flowering stage. However, plant height increased by 4.5 % at the flowering stage with F0B1 level of treatment. While the treatment showing a significant enhancement in plant height at pre-flowering stage with higher level of iron combined with higher level of boron (F2B2) (8.5 %). Increased plant height with respect to boron prompts to understand role of boron in efficient translocation of photosynthates from source to sink as boron is involved in many processes including sugar transport, cell wall synthesis and maintenance, membrane integrity, and RNA, indole acetic acid (IAA) and phenol metabolism [12].

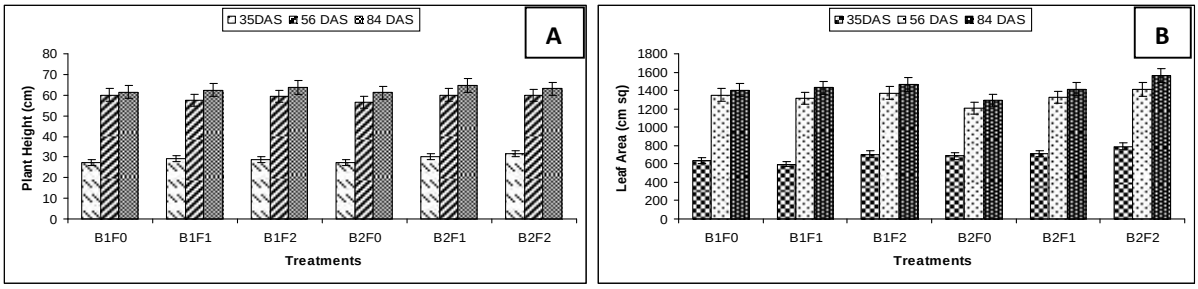

Fig 2: Effect of boron in combination with iron on **(A)** plant height (cm) and **(B)** leaf area (cm sq.) in tomato genotype at different growth stages (Data are expressed as mean ± SEm, n=3).

It was the level F2B2 with 32.2 % increase compared to the control set of plants in leaf area at the pre-flowering level, but it came down to 7.6 % at flowering stage for the same treatment and rose up to a 9.5 % increase at post-flowering stage of growth (Fig 2). Total leaf area, which increased with double doses of boron and iron concentration against normal boron and iron, is supported by Kastori et al. (1995)[13] who observed reduced leaf area in boron deficient plants. Sinha (2000)[14] also reported that leaves were reduced in size, become thick, brittle and unshaped under B- deficient condition.

The treatments F0B1 and F0B2 showed a 17.9 and 11.9 % decline respectively in root dry weight as against the control at pre-flowering stage; while it showed a 5.8 % increase in the same for this growth stage. At flowering stage, the treatment F0B2 recorded a 4 % decline in root dry weight, while it was a 14.14 % rise in the same for F2B2 treatment. The tally for the post-flowering stage of growth was found to be at an 11.61 % decline in root dry weight for F0B2 treatment while it was a 25.16 % high rise at F1B2 level of treatment (Table 1).

Table 1: Effect of boron in combination with iron on shoot dry weight (g) and root dry weight (g) in tomato genotype at different growth stages.

Treatments	Shoot dry weight (g)			Root dry weight (g)		
	35DAS	56 DAS	84 DAS	35DAS	56 DAS	84 DAS
B1F0	0.99	1.66	1.89	0.55	0.96	1.48
B1F1	1.36	1.82	2.67	0.67	0.99	1.55
B1F2	1.39	1.67	2.41	0.63	0.97	1.69
B2F0	1.33	1.70	2.23	0.59	0.95	1.37
B2F1	1.41	1.71	2.37	0.71	1.05	1.94
B2F2	1.43	1.73	2.42	0.62	1.13	1.71
SEm+	0.01	0.01	0.01	0.00	0.02	0.01
CD at P<0.05%	0.02	0.02	0.05	0.01	0.07	0.02

Table 1 reveals that there was a sharp decline of 27.2 % in shoot dry weight for the F0B1 treatment as compared to the control. While treatment with both iron and boron (F2B2) in 'toxic or higher' concentrations was found to cause an elevation of 5.1 % in shoot dry matter at the pre-flowering stage. This is again showing plant's higher capability to utilize these micronutrients at this stage; whereas the control (F1B1) was found to be the one with maximum dry weight accumulation at flowering and post-flowering stages. Boron may contribute to biological cell activities at both the transcription and translation levels that might improve protein synthesis of cell and increase plant dry weight [15].

In biochemical parameters, chlorophyll "a" content was decrease at depleted iron levels both in combination with normal and toxic boron levels. It was found to decrease at successive growth stages at 35, 56 and 84 DAS. There was a significant decrease of chlorophyll a content at pre-flowering and post-flowering stages at toxic or higher boron level in combination with depleted iron (B2F0) as compared to B1F0. There were a 15.48, 57.69 and 33.33 % rise in chlorophyll a content respectively at the three successive growth stages for B2F1 whereas chlorophyll b content indicates significant differences among treatments at various growth stages (Table 2). Treatment B1F0 recorded the minimum level showing a decline of 57.69, 64.00 and 64.29 % over control for the three respective growth stages, which showed an increasing trend for the same over time. Chlorophyll "a" content decreased in B-deficient with iron and excess boron with iron. It is reported to increase chlorophyll content [16]. B deficiency decreases the chlorophyll content of leaf [13]. B toxicity causes chlorosis marginal chlorosis and necrosis of older leaves [14]. Besides this, B toxicity decreased chlorophyll

concentration, followed by reduced growth and decreased photosynthesis [17]. Boron accumulation is known to be greatly influenced by transpiration rates [18] and shading to reduce transpiration rate decreased B absorption by kiwifruit plants. The role of Fe is more important for biosynthesis and integration of the prime pigment chlorophylls.

Table 2: Effect of boron in combination with iron on chlorophyll 'a' content (mg g^{-1} fresh weight) and chlorophyll 'b' content (mg g^{-1} fresh weight) in leaves tomato genotype at different growth stages.

Treatments	Chlorophyll *'a'* (mg g^{-1} fresh weight)			Chlorophyll 'b' (mg g^{-1} fresh weight)		
	35 DAS	56 DAS	84 DAS	35 DAS	56 DAS	84 DAS
B1F0	0.39	0.22	0.21	0.22	0.18	0.15
B1F1	0.84	0.52	0.48	0.52	0.50	0.42
B1F2	0.97	0.82	0.64	0.68	0.58	0.52
B2F0	0.30	0.21	0.16	0.52	0.50	0.28
B2F1	0.42	0.23	0.22	0.60	0.51	0.36
B2F2	0.59	0.46	0.35	0.62	0.57	0.39
SEm+	0.02	0.02	0.01	0.01	0.01	0.01
CD at P<0.05%	0.06	0.05	0.04	0.04	0.04	0.05

Data on carotenoid content of leaves at the control was found to be non-significantly differing with the minimum value at pre-flowering stage with B1F0 treatment (Table 3). The highest values were recorded in B2F2 with 59.62 and 34.15 % rise over control at pre-flowering and flowering stages, respectively. Yamouchi et al. (1986)[19] reported that boron helps maintain membrane stability.

Table 3: Effect of boron in combination with iron on carotenoid (mg g^{-1} fresh weight) nitrate reductase (NR) activity (µ moles NO_2 $hr^{-1}g^{-1}$) in leaves of tomato genotype at different growth.

Treatments	Carotenoid (mg g^{-1} fresh weight)			Nitrate reductase (NR) activity (µ moles NO_2 $hr^{-1}g^{-1}$)		
	35 DAS	56 DAS	84 DAS	35 DAS	56 DAS	84 DAS
B1F0	0.49	0.33	0.12	14.36	39.85	16.56
B1F1	0.52	0.41	0.17	16.25	51.00	38.21
B1F2	0.66	0.43	0.18	17.74	50.54	37.11
B2F0	0.71	0.49	0.14	17.55	47.33	32.30
B2F1	0.77	0.52	0.17	17.76	48.17	37.19
B2F2	0.83	0.55	0.20	18.47	49.49	38.18
SEm+	0.01	0.01	0.01	0.42	0.36	0.26
CD at P<0.05%	0.03	0.04	0.03	1.29	1.12	0.81

The minimum values were invariably recorded at B1F0 for nitrate redcutase activity at all flowering stages (Table 3). The maximum values showed some interesting trends. For the pre-flowering stage, the maximum value was recorded with B2F2, which registered a higher value in the range of 8.00-13.66 %; whilst these were differing non-significantly from one-another. For the flowering stage the highest valued treatment B1F2 was at a non-significantly higher level from the control. Similarly, at post-flowering stage, it was control as the highest valued treatment, closely followed by B2F2 with a value non-significantly less than it. In the present enquiry, maximum NR activity was observed with normal boron with iron applied through balanced nutrient solution to plants. NR activity increases with increase in boron concentration with different iron levels.

Table 4 depicts values for total soluble protein. There was a decline of 25.76 % at the pre-flowering stage for B2F2 over control for the total soluble protein content. The decline was 22.77 and 31.53 % respectively for B2F0 over control at the flowering and post-flowering stages.

Table 4: Effect of boron in combination with iron on total soluble protein (µg g^{-1} fresh wt.) and proline content (µ mole g^{-1} fresh wt.) in tomato genotype at different growth stages.

Treatments	Soluble protein (µg g^{-1} fresh weight)			Proline content (µ mole g^{-1} fresh weight)		
	35 DAS	56 DAS	84 DAS	35 DAS	56 DAS	84 DAS
B1F0	308.50	320.63	690.43	14.21	19.92	21.84
B1F1	328.27	393.33	894.00	12.12	11.94	19.19
B1F2	340.03	390.13	803.07	12.92	19.14	20.74
B2F0	313.70	303.73	612.13	29.40	34.68	33.81
B2F1	297.40	317.03	649.53	29.25	33.18	34.19
B2F2	243.70	380.83	793.30	32.17	37.95	36.40
SEm+	1.74	1.89	2.28	0.23	0.16	0.14
CD at P<0.05%	3.79	4.12	7.04	0.71	0.50	0.44

Contrary to the general trend of successive growth stages showing a rise in values of proline over treatments, the control showed a drop at flowering stage, along with B2F2. The

maximum value was recorded at B2F2 with a substantial rise of 165.43, 217.84 and 89.68 % respectively at successive stages of growth (Table 4). Proline is one of the most common compatible osmolytes in drought stressed plants. The acceleration of the activities of antioxidant enzymes and increased accumulation of proline resulted in an increase in the capacity of tolerance to B-stress [20] as found in the present experiment.

Table 5 shows minimum value for total sugars with B2F2 at all the three stages, with respective fall of 22.16, 19.39 and 9.12 % over control. The treatment records maximum value for total sugar at pre-flowering stage with B1F2, i.e., 9.66 % increase over control at flowering and post-flowering stages both. B2F2 treatment showed a meager but significant decline of 1.13 % over control.

Table 5: Effect of boron in combination with iron on total soluble sugar (µg g^{-1} fresh wt.) in leaves and Net Assimilation Rate (NAR) (mg/cm^2/day) of tomato genotype at different growth stages.

Treatments	Total soluble sugar (µg g^{-1} fw)			Net Assimilation Rate (NAR)(mg/cm^2/day)		
	35 DAS	56 DAS	84 DAS	35 DAS	56 DAS	84 DAS
B1F0	260.27	603.90	1393.13	0.0237	0.0114	0.0237
B1F1	266.67	621.40	1411.93	0.1257	0.0174	0.1257
B1F2	292.43	502.83	1396.00	0.1623	0.0167	0.1623
B2F0	240.80	558.63	1406.93	0.1449	0.0202	0.1449
B2F1	222.40	581.07	1372.20	0.1267	0.0169	0.1267
B2F2	207.57	500.93	1283.17	0.0614	0.0144	0.0614
SEm+	0.83	1.88	2.62	0.0237	0.0114	0.0237
CD at P<0.05%	2.56	5.80	8.07	0.1257	0.0174	0.1257

Net Assimilation Rate (NAR) for the 25 days period from the pre-flowering to the flowering stage, treatment B1F2 recorded a 26.29 % decline over the control (Table 5). The treatment B2F0 showed a 22.74 % rise over control for the pre-flowering to the flowering stage duration. While it was B2F2 with a 51.62 % decline over control for flowering to post-flowering stage period. Besides this, for the longest duration of 60 days from pre-flowering to post-flowering stage, the minimum value was registered by B1F0 with a 34.43 % decline over control, and it was B2F0, like for the earliest duration of pre-flowering to flowering, registering a 17.41 % rise over the control for this duration once again. The results regarding NAR well explains the role of B for efficient sink-source relationship in case of tomato, which on the one hand improves total leaf area and on the other hand accumulates optimum dry matter.

Conclusion: The findings notice significance of the present investigation, which was especially planned to evaluate the potential of boron and iron under deficient and toxic conditions besides effects of individual normal and higher doses of B and Fe and their combinations on the important vegetable crop tomato. These findings up to post-flowering stage are of relevance that develops insight that additional amount of micronutrients boron and iron might be beneficial in increasing vigour of tomato plants for bearing healthy and quality fruits. This experiment could have been extended up to yield studies, which could not become possible due to making critical studies on a number of aforementioned morpho-physiological and biochemical parameters in relation to B and Fe for coming to a few conclusive notes of significance and/or for the suggestion to our farmers who in some way or other way are ignorant of the fact for adding certain micronutrients to the cultivable lands except for addition of N, P and K fertilizers. Therefore, it may be suggested that B and iron in sand culture command a great significance in maintaining the "physiological balance in tomato".

This work, indeed, leaves enough scope to study micronutrient availability and their roles at cellular and molecular levels in diverse plant groups under changing climate, which is the need of time.

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