



Effect of boron on yield and physiological properties in rape (*Brassica campestris*)

MITALI MANDAL¹ and DILIP KUMAR DAS²

Bidhan Chandra Krishi Viswavidyalaya, PO Mohanpur, Nadia 741 252

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ABSTRACT

The results of the present investigation show that the physiological properties as well as yield of rape (*Brassica campestris* L.) have been found to be affected by integrated applications of B and well rotten farmyard manure. The activity of indole acetic acid (IAA) oxidase was recorded a lowest value in the treatment T₄ where recommended N: P₂O₅: K₂O (80:40:40), boron at 0.5 kg/ha as calbor and well rotten farmyard manure at 5 tonnes/ha was applied. The IAA was highest in control treatment suggesting crops may suffer due to boron deficiency with an increase in IAA oxidase activity. The highest value of polyphenol oxidase (PPO) with no boron application exhibited boron deficiency while that of the same recorded a lowest value with the application of boron at 0.5 kg/ha along with 5 tonnes/ha well rotten farmyard manure did not show any boron deficiency. The highest and lowest polyphenol activity were recorded in the treatment T₁ and T₄ where no boron and boron at 0.5 kg/ha along with 5 tonnes/ha well rotten farmyard manure was applied respectively.

Key words: Boron deficiency, IAA oxidase, PP Oxidase, Rape, Yield

Micronutrients especially the application of boron (B) have received a great deal of importance in high responsive oil seed crops during the last few years, because of widespread occurrence of deficiency from different parts of India as well as in West Bengal. Significant response of oil seed crops to the application of boron containing fertilizers have also been reported (Chakraborty and Das 2000, Mandal *et al.* 2009). Boron is one of the essential micronutrients required for normal growth of plants and it has a marked effect on plants from the stand point of both nutrition as well as toxicity because of its narrow range between deficiency and toxicity levels in soils (Warington 1923). Boron deficiency is associated with a range of morphological modifications and changes in differentiation of tissues similar to those induced by either suboptimal or supraoptimal levels of IAA. It is evident that auxins accumulated in boron deficient tissues. Several physiological impairments are known to be caused by boron deficiency such as inhibitions of mitosis and cell elongation, along with of cell differentiation and development, suppression of respiration, photosynthesis, an increase in auxin content (Cohen and Bandurski 1978). It is reported that the activity of IAA oxidase, polyphenol oxidase, ribonucleose are known to influence due to B application. It has been also reported

that an increase in IAA oxidase activity to be an index of B deficiency in plants (Das 2007). However, very little or no information is still available on these aspects and hence it is undertaken in the present study.

MATERIALS AND METHODS

Field experiments were conducted during the winter seasons of 2006-2007 and 2007-2008 with rape (*Brassica campestris* L.) (cv B 9) in an Aeric Endoaquept (pH-6.8; organic carbon-0.52 %; CEC-12.50 c mol (p+)/kg and hot calcium chloride extractable boron-0.23 mg/kg) at the Instructional Farm, Jaguli, Bidhan Chandra Krishi Viswavidyalaya (22.93°N latitude and 88.53°E longitude, average altitude of 9.75 m above mean sea level), West Bengal, India. The treatments were Control, only recommended dose of fertilizer (80:40:40) (T₁); N, P₂O₅ and K₂O as recommended + Well rotten farmyard manure (FYM) @ 5 tonnes/ha (T₂); N, P₂O₅ and K₂O as recommended + B as calbor (1 kg B/ha) (T₃); N, P₂O₅ and K₂O as recommended + B as calbor (0.5 kg B/ha) + Well rotten farmyard manure (FYM) @5 tonnes/ha (T₄); N, P₂O₅ and K₂O as recommended + B as borax (1 kg B/ha) (T₅); N, P₂O₅ and K₂O as recommended + B as borax (0.5 kg B/ha) + Well rotten farmyard manure (FYM) @5 tonnes/ha (T₆). Experiments were laid out in a randomised block design (RBD), and the respective treatments were applied to each plot. Each treatment was replicated thrice. Calbor (4.5 % B, 1.7 % K as K₂O, 11% Ca as CaO, 12 % S, 1%

¹e mail: mitali_mandal@rediffmail.com; ²e mail: dkdas1231@rediffmail.com, Department of Agricultural Chemistry and Soil Science

Mg) and borax (10.5 % B) were used as a source of B. Well rotten farmyard manure (FYM) (0.28 mg/kg B) was incorporated to the soil at the time of final land preparation. The size of each plot was 5 m × 2 m.

Soil samples were collected periodically at an interval of 20 days from five to six randomly selected locations in each plot up to 90 days of sowing. Boron was determined spectrophotometrically using modified Azomethine – H method after extracting the soil with hot CaCl₂ solution (Parker and Gardner 1981). The plant samples were collected periodically at regular intervals and analyzed for total B content by the Azomethine –H method (Wolf 1971) after digesting the samples with tri acid mixture (HNO₃:HClO₄:H₂SO₄ in -10:4:1); (Jackson 1973). Indole acetic acid oxidase was determined by measuring residual IAA following dark incubation with shaking at 30° C. The activity of IAA oxidase was determined by Salkowski reaction (Byrant and Lane 1979).

With regards to the determination of polyphenol oxidase activity the intensely yellow 2-nitro-5-thiobenzoic acid (TNB) with an absorption maximum at 412 nm was made to react with the quinones generated through enzymatic oxidation of 4-methylcatechol (catechol oxidase) and 1, 4 dihydroxybenzene (laccase) to yield colourless adducts. The decrease in the absorbance of yellow-colour due to enzyme activity is measured (Esterbaner *et al.* 1977). Yields were recorded after harvest of the crop. The percentage of oil content of rapeseed was determined by adopting Soxhlet's Ether Extraction method (AOAC 1984). The crude protein of rape seed determined by microkjeldahl digestion method (Jackson 1973). The amount of protein content was obtained by multiplying the nitrogen content with a constant factor of 6.25.

All replicated data of 2 years were pooled statistically. Univariate ANOVA technique for RCBD followed by Duncan's test at 5% level of significance was used for comparing day-wise mean boron concentration. General Linear Model technique repeated over 4 DAS was used to compare the main effects further. All main effects means were subjected to Post –hoc tests like Duncan's test and day means by LSD (Least Significant Difference) to identify the homogeneous means at 5% level of significance

RESULTS AND DISCUSSION

Hot CaCl₂ extractable boron (HCC)

The results (Table 1) showed that the amount of HCC extractable B (non-specifically adsorbed B, NSA-B) was higher in all the treatments compared to control. The mean percent increase of HCC extractable B (SPA-B) was recorded as 19.31 in the treatment when the recommended NPK and well rotten farmyard manure at 5 tonnes/ha was applied while such increase was further in the treatment T₄ (118.69) where recommended NPK (80:40:40), B at 0.5 kg/ha as calbor sources and farmyard manures were applied together. This might be explained partly by the use of organic manure and also partly by the calcium content in the calbor exhibiting

Table 1 Effect of organic manure and boron application on the changes in hot CaCl₂ (HCC) extractable boron (mg/kg) in soil during the year 2006-2007 and 2007-2008 (pooled)

Treatment	HCC (mg/kg)	Total B in plant (mg/kg)	(IAA) oxidase (mg/g fwt./h)	PPO (μmol caffeic acid oxidized/mg protein/min)
T ₁ : control (only NPK recommended)	0.321 ^E	7.12 ^F	430.25 ^A	5.36 ^A
T ₂ : NPK as recommended + organic manure @5 tonnes/ha	0.383 ^D	8.08 ^E	370.25 ^B	5.14 ^B
T ₃ : NPK as recommended + B as calbor (1 kg B/ha)	0.586 ^B	12.61 ^B	335.17 ^C	3.50 ^D
T ₄ : NPK as recommended + B as calbor (0.5 kg B ha ⁻¹)+ organic manure @5 tonnes/ha	0.702 ^A	14.30 ^A	307.33 ^D	3.22 ^E
T ₅ : NPK as recommended + B as borax (1 kg B/ha)	0.592 ^B	10.06 ^D	315.34 ^D	3.75 ^C
T ₆ : NPK as recommended + B as borax (0.5 kg B ha ⁻¹)+ organic manure @5 tonnes/ha	0.511 ^C	10.91 ^C	311.50 ^D	3.61 ^{CD}

Within a column :means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's multiple range test (DMRT)

a positive role in releasing B in the soil solution (Bose *et al.* 2002). With regards to sources of B, it was found that the amount of HCC extractable B did not vary significantly between calbor and borax applied as their equivalent levels of B along with NPK fertilizers, but differed significantly between these two sources of B when applied with well rotten farmyard manure at 5 tonnes/ha in addition to recommended NPK and B with two sources at 0.5 kg/ha.

Total boron content in plant

B content in plant (Table 1) has recorded a significant increase with the application of B as its different sources as well as organic manure along with recommended doses of NPK fertilizers, being a greater magnitude with B application at 1 kg/ha as calbor compared to equivalent level of B as borax. Significantly higher B contents was recorded in plants receiving treatment T₄ where 0.5 kg B/ha was applied along with recommended dose of fertiliser. This increased B content might be due to the presence of Ca in calbor since B is reported to be positively related to the presence of Ca (Bose *et al.* 2002).

Table 2 Effect of boron and organic matter application on the changes in oil and protein content (%) and yield (tonnes/ha) in rape (pooled of two years)

Treatment	Yield	Oil content	Protein content
T ₁ : Control (only NPK recommended)	5.59 ^E	36.58 ^C	20.29 ^D
T ₂ : NPK as recommended + organic manure @5 tonnes/ha	6.84 ^D	38.36 ^B	21.01 ^D
T ₃ : NPK as recommended + B as calbor (1 kg B/ha)	8.12 ^B	41.16 ^A	25.05 ^{AB}
T ₄ : NPK as recommended + B as calbor (0.5 kg B/ha) + organic manure @5 tonnes/ha	8.95 ^A	41.65 ^A	25.52 ^A
T ₅ : NPK as recommended + B as borax (1 kg B/ha)	7.26 ^C	40.11 ^A	23.71 ^C
T ₆ : NPK as recommended + B as borax (0.5 kg B ha ⁻¹) + organic manure @5 tonnes/ha	7.82 ^B	40.89 ^A	24.23 ^{BC}

Within a column : means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's multiple range test (DMRT)

The results also indicated that such mean increased in B content has been recorded a further enhanced (14.30 mg/kg) with the application of well rotten farmyard manure at 5 tonnes/ha along with recommended NPK (80:40:40) and B as calbor at 0.5 kg/ha compared to equivalent level of B as borax source. Sharma *et al.* (1999) also reported similarly who showed that the combined application of boron and

Table 3 Component matrices with all variable loadings for first two components and corresponding eigen values with variance extracted by each component

Variable	Component	
	1	2
SOIL_B	0.98	0.09
TOT_B_30	0.96	0.27
TOT_B_50	0.92	0.36
TOT_B_70	0.96	0.26
TOTB_90	0.96	0.28
IAA_30	-0.94	0.30
IAA_50	-0.92	0.34
IAA_70	-0.93	0.36
IAA_90	-0.94	0.33
PPO_30	-0.98	0.03
PPO_50	-0.98	0.00
PPO_70	-0.99	0.05
PPO_90	-0.99	0.01
seed yield	0.97	0.11
oil content	0.99	-0.05
protein content	0.99	0.08
Eigen value	14.85	0.82
% of Variance	92.82	5.09
Cumulative %	92.82	97.91

FYM significantly affect the B concentration in stalk as well as total B accumulation in sunflower. Pakrashi and Haldar (1992) and Sakal (2001) also reported that the application of organic matter to B deficient soil increased the utilisation and recovery of applied B by maintaining relatively higher proportion of B in soils and extent of increase was more with increasing levels of organic matter.

Indole acetic acid oxidase (IAA)

The magnitude of IAA oxidase activity varied with treatments, with maximum increase in T₁ where only recommended NPK fertilizer was applied (Table 1).

Lowest value of IAA oxidase was recorded in the treatment T₄ where recommended NPK, boron at 0.5 kg/ha as calbor and farmyard manure at 5 tonnes/ha was applied. The results suggest that boron deficiency might not be exhibited on the plant when the activity of IAA recorded a

Table 4 Chi-square tests with successive roots removed

Root removed	Canonical R	Canonical R-sqr.	Chi-sqr.	Df	P	Lambda Prime
0	1.000000	1.000000	308.13	39	0.00	0.00
1	0.999997	0.999994	173.28	24	0.00	0.00
2	0.999886	0.999772	71.29	11	0.00	0.00

Table 5 Factor structure, left set

Variable	Root 1	Root 2	Root 3
SOIL_B	-0.942	-0.257	-0.003
TOT_B_30	-0.959	-0.256	-0.066
TOT_B_50	-0.903	-0.343	-0.166
TOT_B_70	-0.921	-0.360	-0.103
TOTB_90	-0.933	-0.330	-0.090
IAA_30	0.783	0.266	0.133
IAA_50	0.747	0.386	0.036
IAA_70	0.768	0.345	0.015
IAA_90	0.792	0.330	0.007
PPO_30	0.959	0.116	0.025
PPO_50	0.967	0.106	0.039
PPO_70	0.954	0.127	0.025
PPO_90	0.960	0.135	0.048
<i>Variance Extracted (Proportions), left set</i>			
Variance extractd	0.801	0.076	0.006
Reddncy.	0.801	0.076	0.006

Table 6 Factor structure, right set

Variable	Root 1	Root 2	Root 3
SEED_YIE	-0.854	-0.519	-0.038
OIL_CONT	-0.830	-0.451	0.328
PROTEIN	-0.948	-0.263	0.176
<i>Variance extracted (Proportions), left set</i>			
Variance extractd	0.773	0.181	0.047
Reddncy.	0.773	0.181	0.047

lowest value. Hassanein *et al.* (2001) reported that the foliar application of boron up to 250 mg/litre increased the yield attributes with resultant increase in the activities of IAA oxidase, catalase and peroxidase in *Vigna sinensis*. In the control treatment, the value of IAA was recorded highest suggesting crops may suffer due to boron deficiency. The present study also confirms the result of Kavitha *et al.* (2000) who reported that the combined foliar application of boron (0.1%) and zinc (0.5%) led to lowest IAA oxidase activity while the IAA activity was higher in control treatment receiving neither B nor Zn. Cohen and Banburski (1978) also reported in boron deficient plants, the activity of IAA oxidase increased.

Polyphenol oxidase activity (PPO)

Polyphenol oxidase (PPO) followed a similar trend of changes to that of IAA oxidase (Table 1). The highest and lowest polyphenol oxidase activity were recorded in the treatments T₁ and T₄ where no boron and boron at 0.5 kg/ha along with 5 tonnes/ha well rotten farmyard manure was applied respectively. The results suggest that the highest value of PPO was corresponded to lower value of B. The results also suggested that the greater absorption of boron within the plant resulting from its higher rate of application might have interference with the metabolic processes of the PPO activity causing lowest value of PPO in boron treated soils (Olcer and Kocacalskan 2007).

Yield of rape

The yield of rape seed increased with treatments and was highest (8.95 q/ha) in the treatment T₄ where NPK (80:40:40), B at 0.5 kg/ha as calbor and well rotten farmyard manure at 5 tonnes/ha was applied (Table 2). Lu *et al.* (2000) and Salroo *et al.* (2002) also reported that integrated boron management practices increased the yield of brown sarson. The percent increase of rape seed yield in the treatment T₄ over control was (60.10) followed by the

treatment T₃ (43%). The yield of rape seed followed order - T₄ > T₃ > T₆ > T₅ > T₂ > T₁. Zou *et al.* (2008) also reported similarly who showed that the application of boron increased the yield and yield components of rape.

Oil content and protein content

The result showed that the oil and protein content increased significantly with the application of different treatments compared to control (Table 2). The highest mean oil (41.65 %) and protein content (25.52 %) was recorded in the treatment T₄. The oil and protein contents of rape and soybean have been found to be increased with different levels of boron fertilizers (Deosarkar *et al.* 2002 and Hu *et al.* 1994). However, the oil and protein content in rape followed the order - T₄ > T₃ > T₆ > T₅ > T₂ > T₁.

The highest per cent increase in oil (13.86) and protein content (25.77) over control was recorded in the treatment T₄. Li *et al.* (2005) reported that the oil and protein content in rape seed was in the order of N > B > P₂O₅ > K₂O suggesting a greater role played by boron compared to phosphate and potash.

The Principal component analysis (PCA) showed that first two components could extract 97.91% of total variance (Table 3 and Fig 1). First component explained 92.82% of total variance where all enzyme activities at all days of observations were negatively loaded in contrast to the positively loaded variables like soil boron, plant uptake of boron at all days and all yield attributing characters. For this component it is clear that treatment 4 followed by 3, 6 and 5 are desirable treatments in comparison to treatment 1 and 2 respectively. Second component explained only 5.09% of total variance further, where except oil content all other positively loaded. Considering both components it can be concluded that treatment 4 followed by 3 are optimum treatments.

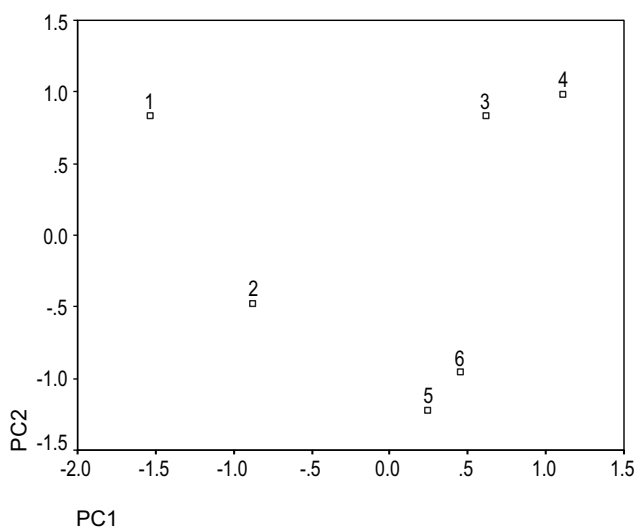


Fig 1 Scatter diagram using regression factor scores resulted by PCA for first two components

Table 7 Canonical weights, left set

Variable	Root 1	Root 2	Root 3
SOIL_B	-0.187	-0.235	3.979
TOT_B_30	-1.054	2.396	-10.152
TOT_B_50	0.262	2.558	-15.333
TOT_B_70	-0.487	-3.092	7.741
TOTB_90	1.004	-3.431	15.057
IAA_30	-0.014	-0.244	0.531
IAA_50	-0.330	1.131	0.359
IAA_70	-0.429	0.850	2.445
IAA_90	0.325	-0.584	0.067
PPO_30	-0.100	-1.857	9.055
PPO_50	0.695	-0.848	-3.704
PPO_70	0.336	-0.521	-3.055
PPO_90	-0.031	0.617	-3.610

Canonical weights, right set

SEED_YIE	0.079	-1.882	-2.387
OIL_CONT	1.162	-2.137	3.056
PROTEIN	-2.143	3.566	-0.527

From the present investigation, it may be concluded that with an increased activity of both IAA and PP oxidases, boron deficiency likely to be increased and widespread. Based on the principal component analysis and canonical correlation studies, the application of recommended doses of N, P₂O₅ and K₂O along with 0.5 kg B as Calbor and 5 tonnes well rotten FYM/ha followed by the recommended doses of N, P₂O₅ and K₂O and 1 kg B as calbor were found to be optimum treatments for increasing yield and quality of rape.

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