Response mechanism of *Oryza sativa* to Boron and NaCl stresses

Dominic V J and T Jithin

Department of Botany, Sacred Heart College, Thevara, Cochin-682 011, Kerala
domvempala@gmail.com

ABSTRACT

With the rapid growth in population consuming rice as staple food and deteriorating soil and irrigating water quality around the globe, it is necessary to understand the response of this crop towards these environmental abuses. The present study was carried out on unique type of rice variety (Pokkali VTL-4) cultivated in acid saline soils of Kerala. *Oryza sativa* (Pokkali VTL-4) plants were cultivated under different boron concentrations. Percentage of germination and symptoms on the plant generated under high and low boron concentrations were monitored. A double stress condition of boron toxicity and salinity were introduced to the plants to monitor their growth and development. It was observed that membrane permeability of the plants was increased in the presence of salinity by raising levels of boron. Also, salinity intensified boron toxicity effects by increasing soluble boron concentration in inter and intra cellular compartments. Total chlorophyll was also extracted from leaves of *O. sativa* (Pokkali VTL-4) plants cultivated under different boron concentration. The result clearly indicated that, boron toxicity and deficiency significantly affected photosynthesis.

Key words: *Oryza sativa*, Pokkali, NaCl stress, Boron toxicity.

INTRODUCTION

The Pokkali system of rice (*O. sativa*) cultivation is a unique method of rice production in acid saline soils of Kerala. Most rice crops growing under field conditions are often exposed to various abiotic stresses such as high or low temperature, drought and salinity, which influence plant metabolism directly or indirectly, thereby affecting plant growth, development, and finally productivity (Shylaraj *et al*, 2005). Soil problems resulting from either excess or shortage of certain elements as a consequence of specific soil properties are widespread globally and significantly constrain agriculture production. Boron is an essential micronutrient for plant growth, but soils derived from marine or volcanic sediments can have high concentrations of Boron, and on these soils Boron can accumulate in plants to levels that are phytotoxic. (Chesworth, 1991).

Boron deficiency physiologically affects *O. sativa (*Pokkali VTL-4) plants at vegetative stage. Boron deficiency inhibits root elongation (Marschner, 1995) and that root growth is more sensitive than shoot growth. This primary effect of boron seems to be a direct consequence of losing the cell wall plasticity, whereas the inhibition of cell division of the root meristematic region is a secondary effect of boron deficiency (Hu and Brown, 1997). Kastori *et al*, (1995) stated that Boron deficiency affects photosynthesis reducing the photosynthetic oxygen evolution rate and efficiency of photosystem -2. However, Goldbach and Wimmer (2007) revealed that the mechanism of primary role of boron in photosynthesis is unknown and therefore effects of boron deficiency on photosynthesis are secondary in nature.

For crop production, boron toxicity is more difficult to manage than its deficiency, latter can be prevented by fertilization. However, fertilization with boron to avoid deficiency can result in toxicity, since the concentration range between boron deficiency and toxicity is narrower than for any other plant essential nutrient (Goldberg, 1997). In soils where B toxicity occurs, B concentrations are highest in the subsoil (Yau and Ryan, 2008) which may limit root growth deep in the soil profile (Holloway and Alston, 1992). By restricting the effective rooting depth of crops, high soil Boron may reduce grain yield by limiting water use and water use efficiency (Holloway and Alston 1992).

The present work is an attempt to investigate the effect of Boron and sodium chloride stress on the response mechanism of *O. sativa* (Pokkali VTL-4).

MATERIALS AND METHODS

The *Oryza Sativa* (Pokkali VTL-4) grains were provided by Vytila Rice Research Station (VRRS, Cochin, Kerala: India. Soils samples were collected from different regions in Ernakulum and Alappuzha districts of Kerala (Thevara, Kumbalangi, Chellanam, Cheranellur, Vayalar and Nedumudi). The grains were sown in different plastic pots consisting of different concentration of boron, ranging from 2.2 mg/kg to 22.0mg/kg.

Plastic pots were filled with 6 inches of potting soil. The containers were filled with water, up to 2 inches above the top of the soil. Forty VTL-4 rice seeds were sawed in each container, and seeds were scattered evenly. Labeled containers were kept in the laboratory, providing necessary lighting and temperature.

Water level was maintained in the containers at 2 inches above the soil level until the sprouts reach 5 to 6 inches in height. Water level was increased in all the containers to 4 inches deep to ensures the sprouts have enough water to complete their growth process. Water level was allowed in the container to dissipate gradually. Little or no standing water was left in the container when the plants reached their harvest stage, typically in four months. Rice stalks were harvested when they changed from green to gold.

Soil Boron Estimation

In order to estimate the range of optimum boron concentration, which enhances the growth of *O. sativa* (VTL-4 Pokkali) variety, it was estimated from the soils used for cultivation. Soil boron concentrations of various other pokkali fields were also estimated. Soil boron concentration was estimated using Hot Water extraction procedure.

Plant Boron Estimation and application of NaCl stress

Boron in plant samples was measured by dry ashing and subsequent measurement of Boron was done by Atomic Spectroscopy. *O. sativa* (VTL-4 Pokkali) varieties were treated for 24 and 48 hrs with 150 mM NaCl, in order to create a double stress condition. Hence, the plant boron was estimated before the application of NaCl and after the treatment of 150 mM NaCl. The amount of sodium accumulated in the VTL-4 variety was also estimated, both after 24 and 48 hrs NaCl treatment.

Sample preparation for Boron estimation by Atomic spectroscopy

Dry Ashing: 1 g dry, ground plant material was weighed in porcelain crucible and ignited in a muffle furnace by slowly raising the temperature to 550°C. Ashing was continued for 6 hours after attaining 550°C. Wet the ash with five drops DI water, and then 10 mL 0.36 N sulfuric acid solution was added into the porcelain crucibles. It was allowed to stand at room temperature for 1 hr and stirred occasionally with a plastic rod to break up ash. Later, the solution was filtered through Whatman No.1 filter paper into a 50-mL polypropylene volumetric flask and brought to volume. Filtrate was used for Boron determination.

Sample preparation for Sodium (Na) estimation by Atomic Spectroscopy

The procedure adopted was that of Chapman and Pratt (1986) with slight modifications, *i.e*. the cooled ash was dissolved in 5-ml portions 2 N hydrochloric acid (HCl) and mixed with a plastic rod; after 15 - 20 minutes, volume was made up to 50 mL using DI water. After mixing well, it was allowed to stand for about 30 minutes, and was filtered through Whatman No. 42 filter paper, discarding the first portions of the filtrate.

Chlorophyll Extraction by Acetone method

About 1g of finely cut sample of leaf was taken into a clean mortar. The Tissue was ground to a fine pulp using 20 ml 80% acetone. After centrifugation (5000 rpm for 5 minutes), the supernatant was transferred to a 100ml volumetric flask. Later, residue was ground with 20 ml of 80% acetone, centrifuged and supernatant transferred to the same volumetric flask. Procedure was repeated until the residue became colorless. Mortar and pestle were washed thoroughly with 80% acetone and clear washings were collected in volumetric flask. Volume was made up to 100 ml with 80% acetone. Absorbance of solution was then noted at 645, 663 nm against the solvent (80% acetone) blank. Chl-a, Chl-b and total Chlorophyll were estimated in the months of August, September and October.

RESULTS AND DISCUSSION

The sown grains were germinated after 3-4 days. The percentage of germination was very low in pots having high concentration of Boron. The germination was late in pots having high Boron concentration. It was observed that in pots having high Boron concentration, i.e. above 11 mg/kg, the percentage of germination was comparatively less than those with low boron concentration. Germination was delayed for about 3 to 5 days in pots having high Boron concentration i.e. above 15.4 mg/kg. The maximum percentage of germination was noted for samples treated with a medium boron concentration (8.8 to 13.2 mg/kg). The percentage of germination was very low in plants with very high boron concentration and with very low boron concentration.

Fig. 1: *O.sativa* (Pokkali VTL-4) leaf tip showing symptoms of B toxicity, Fig-2 : Elliptical dark brown blotches appear in the discolored area, Fig-3: Progression of Boron Toxicity symptoms 7-8 weeks old *O. sativa* (Pokkali VTL-4), Fig-4: Leaf tips and margins turn yellow spindle like appearance at the tips.

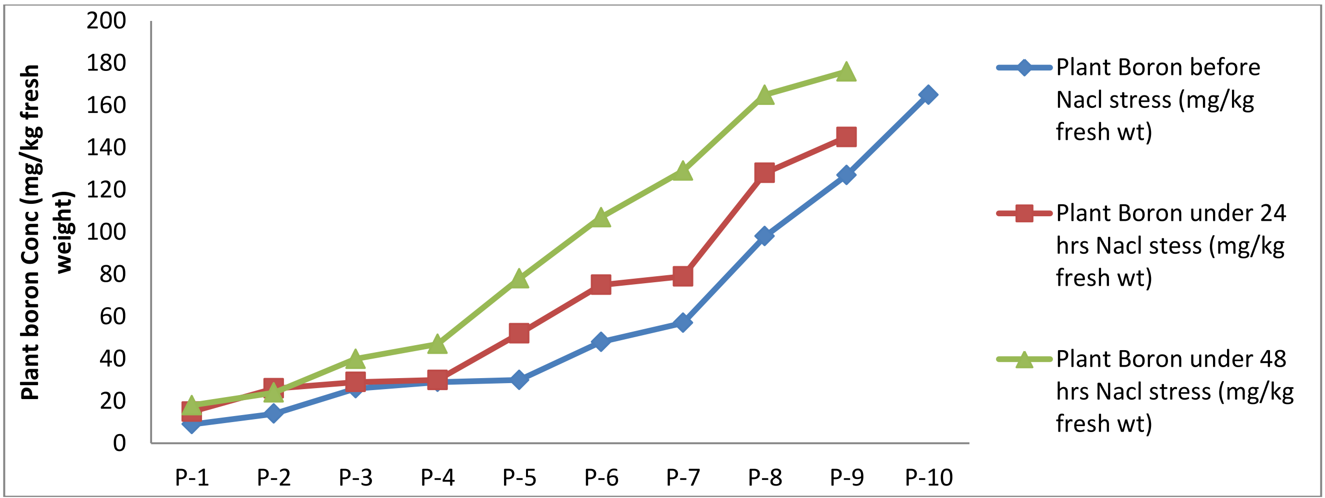

Fig-5: Plant Boron concentration of 8 weeks old *O. sativa* (Pokkali VTL-4) plants subjected to 150 mM NaCl stress.

This indicates that Boron concentration varying from 8.8 to 13.2 mg/kg soil is found to be the optimum range for proper germination of *Oryza sativa* (Pokkali VTL-4).

Soil Boron Estimation (Hot water extraction method)

The level of boron was around 0.223, 0.275 and 0.262ppm in Thevara, Kumbalangi and Chellanam respectively. But the boron concentrations of Alapuzha district were comparatively less. It was estimated to be around 0.192 and 0.170ppm at Vayalar and Nedumudi respectively.

In areas with high rainfall, there occurs a gradual decrease in the subsoil Boron concentration due to leaching (Roessner *et al*, 2006). In Alapuzha district this may be the reason why, soil boron concentration is low compared to Ernakulum district.

Symptoms of boron toxicity and deficiency in *O. sativa* (Pokkali VTL-4)

The first symptom of boron toxicity (19.8 mg/kg) was the appearance of a light brown or yellowish white discoloration at the tips and margins of the leaves about six weeks after planting (Fig-1). Gradually the tips and leaf margins turned yellow. Two to four weeks later, elliptical dark brown blotches appeared at the discolored areas in most of the rice samples (Fig-2). Finally the entire leaf blade turned light brown and withered. Vegetative growth was not markedly depressed until the problem turned severe (Fig-3). Elliptical brown blotches were observed on discolored area along the leaf margins.

Boron toxicity symptoms were observed at the leaf tips of the plants of pot-9 (19.8 mg /kg) initially. Later, symptoms were observed in plants of pots-6 (13.2 mg/kg), 7 (15.4 mg/kg), 8 (17.6 mg/kg), and 10 (22.0 mg/kg). After, 7-8 weeks a progression was observed in the boron toxicity symptoms from leaf tips to the whole plants. In pots 1 (2.2 mg/kg) and 2 (4.4 mg/kg), leaf tips and margins turned yellow, also a spindle like appearance was observed at the leaf tips (Fig-4).

Earlier works of Holloway RE, Alston AM, (1992) stated that in wide variety of plant species, the typical visible symptom of B toxicity is leaf burn – chlorotic or necrotic patches, often at the margins and tips of older leaves. The presence of such necrotic spots in this case confirms that boron toxicity affects the growth and development of Pokkali VTL-4 plants.

Chlorophyll Estimation

In the month of August, 0.7731 mg/g total chlorophyll was obtained as the highest value in pot-6 (13.2 mg/kg) and 0.6011 mg/g total chlorophyll was obtained in pot-8 (17.6 mg/kg) as lowest value. In the month of September, highest value of 0.8735 mg/g total chlorophyll was estimated in pot-5 (11.0 mg/kg), whereas 0.6381 mg/g total chlorophyll was estimated in pot-8 (17.6 mg/kg) as lowest value. In October the peak value was higher compared to previous months, i.e. 0.9734 mg/g total chlorophyll obtained in pot-5 (11.0 mg/kg) and lowest value of 0.7725 mg/g total chlorophyll was estimated in sample-1 (2.2 mg/kg).

Above results indicate that Boron toxicity and deficiency effects photosynthesis. Kastori *et. al*. (1995) and El-Shintinaway (1999) also revealed that boron deficiency and toxicity affects photosynthesis by reducing the photosynthetic oxygen evolution rate and efficiency of photosystem – 2. Hence, the present study reveals that boron toxicity is more drastic condition to *Oryza sativa* (Pokkali VTL-4) plants than boron deficiency.

Boron toxicity and salinity stress

Plant boron concentration before NaCl stress was analyzed for matured *O. sativa* (Pokkali VTL-4) plants. The highest value was estimated at 165 mg/kg fresh weight for sample-10 (22.0 mg/kg) and the lowest value was 9.0 mg/kg FW for sample-1 (2.2 mg/kg). After, 24 hrs of NaCl stress the highest value of 145 mg/kg FW was obtained in sample-9 (19.8 mg/kg); the previous value of sample-9 was 127 mg/kg FW. Again the lowest value obtained was from sample-1 (2.2 mg/kg), 15 mg/kg FW. After 48 hrs of NaCl stress, the rate of boron concentration in sample-9 (19.8 mg/kg) was peaked to 176 mg/kg FW, but the boron concentration in sample-1 (2.2 mg/kg) was only 18 mg/kg FW (Fig – 5).

Sodium (Na) estimated was almost uniform, with negligible difference. Shoot sodium concentration was 125 and 200 µ mol / gm fresh wt after 24 and 48 hrs applications of 150 mM NaCl ; whereas, root sodium concentration was 25 and 50 µ mol / gm fresh weight.

Hence it is confirmed that membrane permeability of pokkali plants was considerably augmented under intense saline condition. The inflow of boron ions was increased under high saline stress. These conditions were earlier observed in tomato and cucumber plants by Alpaslan and Gunes (2001).

Also, Wimmer *et al.* (2003) reported that salinity together with boron toxicity increased soluble boron concentrations in inter and intra cellular compartments of basal leaf sections in wheat when ,compared to either stress alone. Boron transporters play a major role in the transport of boron and Na+ ions through the Boron channels. The transport of sodium ions through boron channels may be facilitated by boron transporters. Boron transporters were not known until, Toru *et al*. (2005) reported BOR1 as the first boron transporter required for efficient xylem loading of boron (B). Also NIP5-1, a protein similar to aquaporin, was identified as a transporter required for efficient B uptake.

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