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Research article

BORON AND ZINC RESPONSE ON GROWTH IN *Vigna radiata* L. *Wilczek* var. Pusa Vishal UNDER SALINITY

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ABSTRACT: The study was undertaken to examine optimum concentration of Boron and Zinc needed to mitigate the harmful effect of salinity at early establishment of seedlings including seed germination. Sterilized seeds of *Vigna radiata L.Wilczek* var.Pusa Vishal were germinated and grown under different levels of salt (10 mM – 200 mM NaCl), B($1\times10^{-3} mM - 5\times10^{-3} mM$) and Zn($1\times10^{-3} mM - 10\times10^{-3} mM$) under controlled conditions. The seed germination decreased with salinity increase as well as there was decrease in no. of leaves, branches, root-shoot length and dry weight of plants. The decrease was reverted with specific optimal concentration of B($3\times10^{-3} mM$) and Zn($4\times10^{-3} mM$). The optimum concentration of B and Zn were thus taken for further sand culture experiments based on maximum germination percentage.

Key words: Salinity, Mungbean, Boron, Zinc.

INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek) is an important traditional legume crop of India characterized by a relative high content of protein [23] and excellent nutritional attributes in terms of methionine and cysteine in adequate quantities otherwise lacking in other food crops (Tsou and Hsu, 2000). Salinity is serious problem for agriculture [14]. The var. Pusa Vishal is preferred for its short growth duration, MYMV (Mungbean Yellow Mosaic Viruses) tolerance, bold seed size, shiny green colour and its ability to fit in various cropping systems (Chandra and Tickoo, 2004). However, the soil salinity is a major limitation to legume production [1]. Salt stress causes decline in seed germination, shoot-root lengths, fresh mass and seedling vigor in mungbean [31, 25]. It is demonstrated that increasing levels of salinity or osmotic stress remarkably decreased seed germination, seedling vigor

and hydrolytic enzymes influencing growth in other legumes also [34, 44, 25, 33] demonstrated salinity caused decrease in mungbean and other plant growth could be mitigated by bioinoculants or hormones. Salt stress is implicated in ionic stress [4] must be culminating into micronutrient imbalances consequently limiting the growth and establishment of plants.

Zn deficiency is one of the most common widespread disorders in plants and soils of different regions of India [39]. Importance of Zn as a micronutrient in crop production has increased in recent years [13], hence considered to be the most yield-limiting micronutrient [12, 10]. The Zn essentially is being employed in functional and structural component of several enzymes (Vallee and Auld, 1990), such as carbonic anhydrase, alcohol dehydrase, alkaline phosphatase, phospholipase, carboxypeptidase [8] and RNA polymerase [36].

Further, plants emerging from seeds with lower Zn could be highly sensitive to biotic and abiotic stresses [28]. Zn enriched seeds performs better with respect to seed germination, seedling growth and yield of crops [5]. The foliar application of Zn modulates the plant growth and production in mungbean including straw yield and crude protein in seeds [19,20] has also indicated that foliar application of Zn, Mn, Fe and Mg significantly increased growth and yield in mungbean plants. Recently, it is suggested that salinity induced decrease in leaf, stem and root dry weights could be diminished with applications of Zn on pistachio seedlings [37].

B, another essential element for all vascular plants, whose deficiency or excess causes impairments in several metabolic and physiological processes [35, 6] including cell wall structure and function [29]. In fact, B has a critical role in growing tissues and any imbalance may inhibit the vegetative and reproductive stages in plants [9]. The Boron toxicity under saline condition is noted [27]. Salinity causes leaf injury due to B deficiency in tomato and cucumber plants [3, 11] have mentioned that increased level of NaCl decreases B in tissues. Further, [43] have proposed that there would be a lower uptake of chloride in the presence of B and vice-versa, forwarded explanation for less toxic effects on growth under combined salinity and B stress. These observations indicate that there must be specific interaction of elements especially micronutrients with in the tissue which must be evaluated in terms of growth consequences.

Therefore, the present study was aimed to analyze the effect of B and Zn individually as well as together on germination and growth in terms of root, shoot length, number of leaves and branches, dry weight and yield in the Pusa Vishal under salinity. Two types of experimental designs i.e., Germination in petridish and Pot culture were selected to find out the optimum concentration of B and Zn to alleviate the salinity stress in this variety.

MATERIALS AND METHODS

Seeds of *Vigna radiata* (L.) Wilczek (var. Pusa Vishal)

were collected from Indian Agricultural Research Institute (IARI), New Delhi. Seeds were surface sterilized with 0.1% $CaOCl_2$ for 3-5 minutes and washed thoroughly with water before sowing.

Hundred seeds in petridishes lined with Whatman's filter paper were allowed to germinate in growth chamber under controlled condition (Temp. 25 ± 2^0C, light 75 Wm^{-2} and RH 70%).

The germinating seeds were watered with half-strength Hoagland nutrient solution (pH 6.5) contained NaCl, B and Zn in the range of 10, 20, 40, 60, 80, 100 and 200 mM NaCl; $1\times10^{-3}, 2\times10^{-3}, 3\times10^{-3}$, 4×10^{-3} and 5×10^{-3} mM B and 1×10^{-3}, 2×10^{-3}, 4×10^{-3}, 5×10^{-3} and 10×10^{-3} mM Zn respectively. Based on observations for optimum response at the germination stage, the NaCl concentration of 100 mM, B: 1×10^{-3} mM and 3×10^{-3} mM; Zn: 1×10^{-3} mM and 4×10^{-3} mM were selected for further experiments.

Daily counts of the number of germinated seeds were recorded for 10 days in three replicates of each petridish.

A sand filled pot experiment was conducted, where the quartz sand of particle size 0.25 to 0.54 mm (obtained by sieving the crude sand) washed with stream of water and used. The water washed sand was treated with a mixture of 1% oxalic acid (W/V) in 17% HCl (V/V) followed by thorough washing with de-ionized water.

The pots were irrigated with Hoagland nutrient solution (500 ml) each day containing various salt combinations as mentioned in case of petridish experiments. There were three replicates for each treatment. Plants were harvested after 60 days.

The data for root length, shoot length, number of branches, number of leaves, dry weight were recorded pre-flowering (20 days), post-flowering (40 days) and maturity of crop (60 days). Data are expressed as Mean ± S.E.

RESULTS AND DISCUSSIONS

The seeds germination was decreased linearly depending on salinity levels (Fig.1). [23] have recorded similar responses in different genotypes of mungbean. This corroborates further with the observation in Phaseolus also [18]. The germination reduction could be due to imbalance in osmotic or ionic concentration rendering ionic toxicity [22], which varies from plant species to species [26]. Germinability indicates the establishment of seedling and crops production.

Application of B and Zn elevated the germinability of seeds under NaCl stress, the germination percentage was maximum at 3×10^{-3} mM of B and 4×10^{-3} mM of Zn (Fig. 2 and 3). The essentiality of B for growth and development of higher plants has been demonstrated earlier also [21, 17].

The B and Zn potentiated the seed germination compared with that of control seeds. The B and Zn supplementation individually or together in pot experiments alleviated the salinity (100 mM NaCl) effect on germination (Fig.4), the optimum response was like as in case of petridish culture at 3×10^{-3} mM B and 4×10^{-3} mM Zn. The B positive effect might be due to water uptake by maintaining integrity of cell wall and cell membrane and thus reduced ionic toxicity arising under salinity (Goldbach and Wimmer, 2007).

The inability of seeds to germinate under salinity could be due to B and Zn deficiency along with other nutrients, and membrane leakage [31, 33] which could be restored optimally at specific concentration.

The result substantiates the understanding that salinity impaired essential nutrients required to activate the embryo axis and enzymes therein could be replenished or modulated by a specific concentration of B and Zn.

Pot experiments revealed that 100 mM NaCl reduced (30-40%) the root length (Fig. 5), shoot length (Fig. 6), number of leaves (Fig. 7), branching (Fig. 8) and dry mass accumulation (Fig. 9) over control. The reductions were over control. Application of B and Zn and their combination checked the decline in growth attributes in stressed plants at all the stages (Fig.5-9).

[30] have also shown that under salinity condition mungbean growth and yield were adversely affected but checked with applications of Zn either pre-treatment of seed or foliar application. The combined effect of B and Zn under salinity has not yet been examined in var. Pusa Vishal of mungbean and the present study indicates the stress amelioration at specific concentration.

Alpaslan and Gunes (2001) have observed that membrane permeability of the tomato and cucumber was significantly augmented by the rising levels of applied B under salinity.

The results suggest that salinity (100mM Nacl) effect could be mitigated by specific concentration of B and Zn in case of this variety.

It is suggested that the negative effect of salt stress on mungbean growth could be mitigated by foliar spray or soil application of Zn [16, 40, 37] or B [2]. It is perceived that optimized B and Zn application from beginning of the plant life could maintain the cell wall plasticity, cell elongation thus integral plasma membrane and related metabolic activities otherwise hampered [3].

The present study is tempted to suggest that B and Zn application under both normal and salinity stress, provides better chance of survival of seeds and seedlings may lead to better growth and yield. Further, B and Zn being critical for growth, their application appears to be an efficient measure to minimize the negative effect of salinity and improvement of growth even under high saline (100 mM NaCl) condition.

Moreover, experiments are in progress to understand the B and Zn response on metabolites implicated in growth.

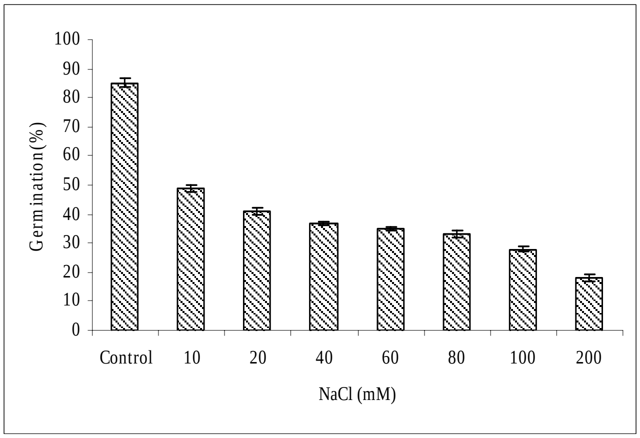

Fig. 1: Effect of salinity on germination in var. Pusa Vishal
Data are Mean ± S.E. of 3 replicates

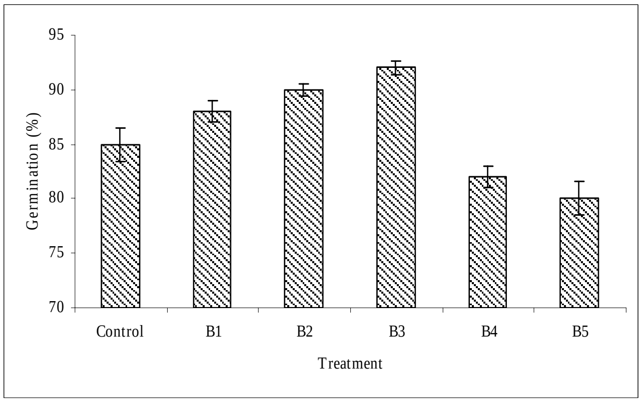

Fig.2: Effect of Boron(B) on germination under salinity (100 mM NaCl)
B1= 1×10^{-3} **mM, B2 =** 2×10^{-3} **mM , B3 =** 3×10^{-3} **mM, B4 =** 4×10^{-3} **mM, B5 =** 5×10^{-3} **mM**

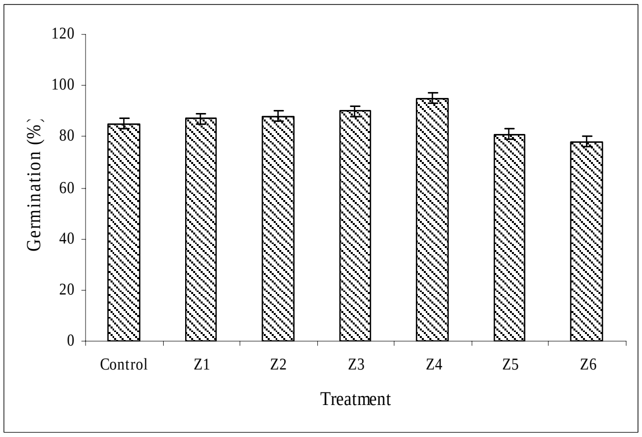

Figure 3: Effect of Zn on germination under salinity (100 mM NaCl)
Z1 = 1×10^{-3} **mM, Z2 =** 2×10^{-3} **mM, Z3 =** 3×10^{-3} **mM, Z4 =** 4×10^{-3} **mM, Z5 =** 5×10^{-3} **mM, Z6 =** 10×10^{-3} **mM**

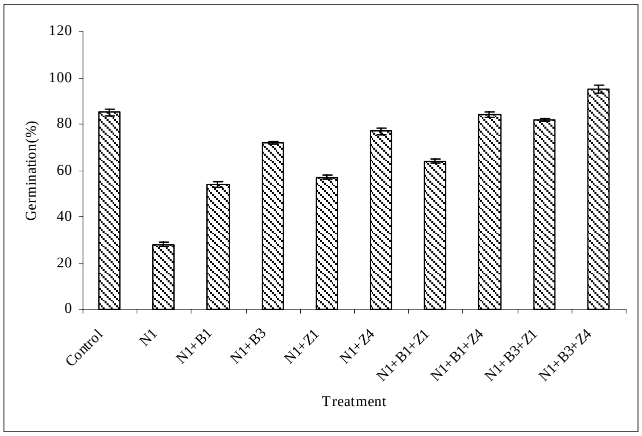

Fig. 4: Effect of different combination of B & Zn on germination of mungbean under salinity
N1 = 100 mM NaCl, B1= 1×10^{-3} **mM, B2 =** 2×10^{-3} **mM , B3 =** 3×10^{-3} **mM, B4 =** 4×10^{-3} **mM, B5 =** 5×10^{-3} **mM, Z1 =** 1×10^{-3} **mM, Z2 =** 2×10^{-3} **mM, Z3 =** 3×10^{-3} **mM, Z4 =** 4×10^{-3} **mM, Z5 =** 5×10^{-3} **mM, Z6 =** 10×10^{-3} **mM**

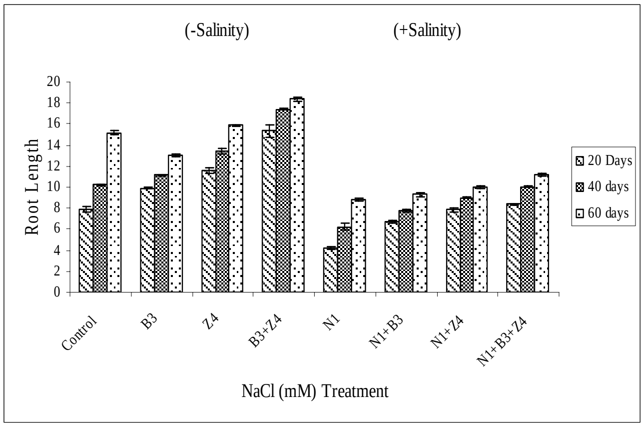

Fig. 5: Effect of salinity(100mM NaCl) on root length (cm) in mungbean

N1 = 100 mM NaCl, B1= 1×10^{-3} **mM, B2 =** 2×10^{-3} **mM , B3 =** 3×10^{-3} **mM, B4 =** 4×10^{-3} **mM, B5 =** 5×10^{-3} **mM, Z1 =** 1×10^{-3} **mM, Z2 =** 2×10^{-3} **mM, Z3 =** 3×10^{-3} **mM, Z4 =** 4×10^{-3} **mM, Z5 =** 5×10^{-3} **mM, Z6 =** 10×10^{-3} **mM**

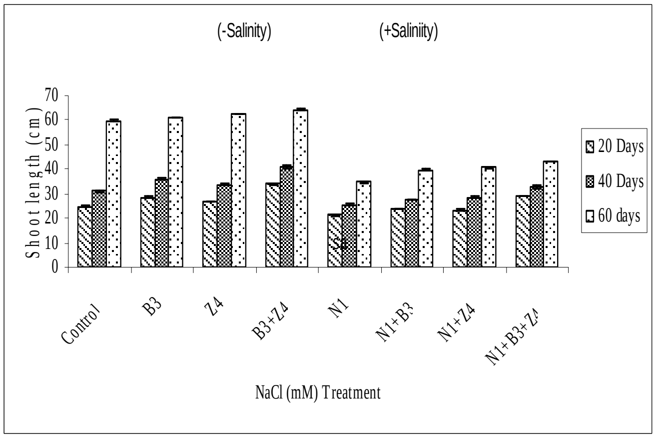

Fig. 6: Effect of salinity on shoot length (cm) in mungbean

N1 = 100 mM NaCl, B1= 1×10^{-3} **mM, B2 =** 2×10^{-3} **mM , B3 =** 3×10^{-3} **mM, B4 =** 4×10^{-3} **mM, B5 =** 5×10^{-3} **mM, Z1 =** 1×10^{-3} **mM, Z2 =** 2×10^{-3} **mM, Z3 =** 3×10^{-3} **mM, Z4 =** 4×10^{-3} **mM, Z5 =** 5×10^{-3} **mM, Z6 =** 10×10^{-3} **mM**

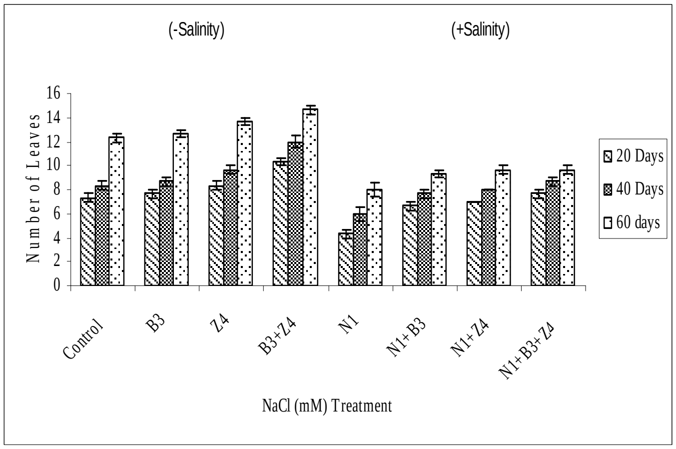

Fig. 7: Effect of salinity on no. of leaves in mungbean

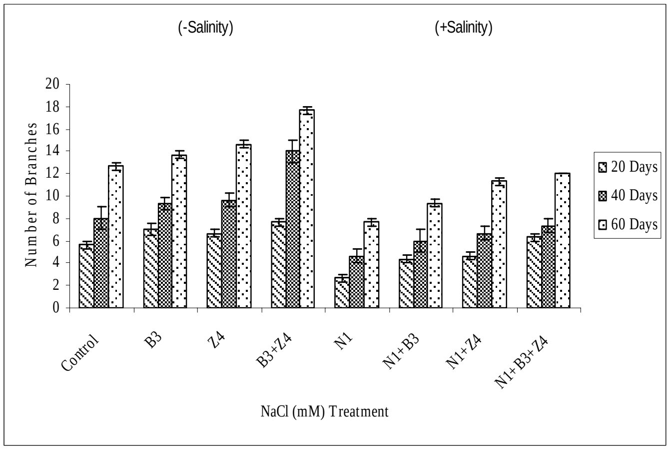

Fig. 8: Effect of salinity on no. of branches in mungbean

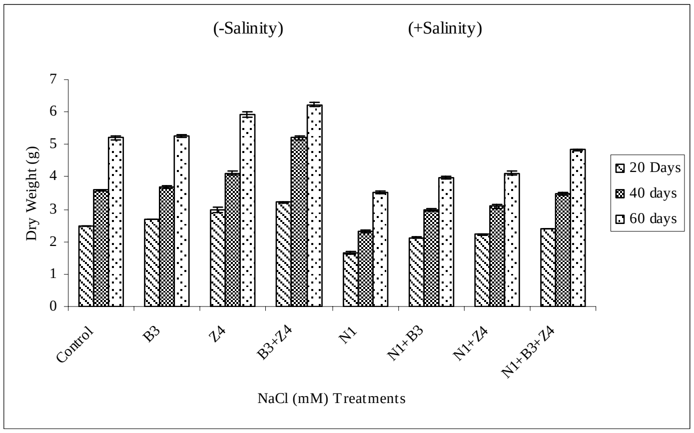

Fig. 9: Effect of salinity on dry weight (g) in mungbean

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