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Nutrition

The impact of foliar boron sprays on reproductive biology and seed quality of black gram

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ABSTRACT

An experiment was conducted under glass house condition to study the effect of foliar application of boron (B) on reproductive biology and seed quality of black gram (*Vigna mungo*). Black gram (*V. mungo* L. var. DPU-88-31) was grown under controlled sand culture condition at deficient and sufficient B levels. After 32 days of sowing B deficient plants were sprayed with three concentrations of B (0.05%, 0.1% and 0.2% borax) at three different stages of reproductive development, *i.e.* prior to flowering, initiation of bud formation and after bud formation. Deficient B supply decreased the anther and pollen size, pollen tube growth, pollen viability as well as stigmatic receptivity which were increased by foliar B application. Foliar spray at all the three concentrations and at all stages increased the yield parameters like number of pods, pod size and number of seeds formed per plant. Foliar B application also improved the seed yield and seed quality in terms of storage seed proteins (albumin, globulin, glutenin and prolamin) and carbohydrates (sugars and starch) in black gram. The foliar application of B in appropriate doses (particularly 0.1%) after bud formation made quantitative and qualitative improvement in seed yield of black gram by supplementing additional/critical B requirements for reproductive development.

Introduction

Boron (B), an essential micronutrient, plays an important role in cell wall structure, membrane stability [1], sugar transportation, and phenol, carbohydrate, nucleic acid and IAA (indole acetic acid) metabolism [2]. In addition to its involvement in numerous metabolic processes, B has a great impact on reproductive structure development especially on microsporogenesis [3], pollen germination [4] and seed development [1]. Studies [5,6] revealed that B deficiency affects the reproductive yield more than biomass yield, even in the absence of any visible sign of deficiency symptoms and therefore the requirement of B for reproductive development appears to be more for reproductive development than for vegetative growth.

Among micronutrients, B deficiency is wide spread throughout India. One of the major reasons of B deficiency is that it is easily leached out from soil. Furthermore high soil pH, coarse (sandy) texture, low organic matter and low moisture reduce B availability to plants. Other major factors that may cause shortfall in B supply for reproductive development are poor translocation of B from leaves and other mature tissues to the floral parts [7] and poor access of the pollen grains and the embryo sacs to the vascular supply [1,8]. In such situation providing B by means of foliar application would be advantageous. Studies on olive and sunflower have reported the significance of the role of foliar B application on the productivity of plants [5,6] but no mention was made of the effect of B on pollen–stigma interaction. The present work was thus carried out to study the role of B on pollen–stigma interaction and its impact on reproductive yield.

In human beings B has been shown to affect the levels of glucose and triglycerides, amino acid and protein, free radicals, bone mineralization [9] and functions of prostrate, mental health, estrogen metabolism and numerous body systems [10]. A sufficient dietary intake of B is thus crucial for human health. In India black gram is widely consumed and is rich source of protein for the vegetarian diet. Since it is sensitive to low B supply [11], it was hypothesized that foliar B application would not only increase seed yield but also fortify the seed quality for improving the yield in terms of protein, carbohydrates and seed B content. The latter would be reflected in improved seedling density, seedling vigor and crop productivity. In the present study an effort was made to study the physiology of the pollen–stigma interaction leading to poor seed set in B deficient plants and to identify the level and stage of supply of B optimum for maximum yield and biofortification of seeds.

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Materials and methods

Plant culture

An experiment was carried out to study the effect of different concentrations of B as a foliar spray on seed yield and quality of black gram (*Vigna mungo* L. var DPU-88-31). To study the importance of B during reproductive stage, plants were raised in purified sand with nutrient solution containing 4 KNO_3, 4 $Ca(NO_3)_2$, 2 $MgSO_4$, 1.33 NaH_2PO_4, 0.1 Fe EDTA (in mM), 10 $MnSO_4$, 1 $CuSO_4$, 1 $ZnSO_4$, 0.1 Na_2MoO_4, 0.1 NaCl, 0.1 $CoSO_4$ and 0.1 $NiSO_4$ (in μM) at normal (0.33 mM) and deficient level (0.033 B mM) of B supplied as H_3BO_3 [12].

After germination plants were separated in two sets. The 1st set was supplied with normal B (0.33 mM) and 2nd set was supplied with deficient B (0.033 mM). Prior to the onset of flowering and after development of B deficiency effects (32 days after sowing), 2nd set of B deficient (BD) plants were further divided into 10 sets. While one lot of B deficient plants continued to receive deficient supply, the remaining set of B deficient plants were sprayed with three concentration of B supplied as 0.05%, 0.1% and 0.2% borax solution at three different stages of reproductive development, *i.e.* prior to flowering, initiation of bud formation and after bud formation. Each foliar spray (250 ml per pot) was done three times and the total treatments were as under:

1st set: B sufficient plants	Control
2nd set: B deficient plants without any foliar spray	BD
3rd set: BD plants given foliar spray of 0.05% prior to flowering	B1 + F1
4th set: BD plants given foliar spray of 0.05% initiation of bud formation	B1 + F2
5th set: BD plants given foliar spray of 0.05% after bud formation	B1 + F3
6th set: BD plants given foliar spray of 0.1% prior to flowering	B2 + F1
7th set: BD plants given foliar spray of 0.1% initiation of bud formation	B2 + F2
8th set: BD plants given foliar spray of 0.1% after bud formation	B2 + F3
9th set: BD plants given foliar spray of 0.2% prior to flowering	B3 + F1
10th set: BD plants given foliar spray of 0.2% initiation of bud formation	B3 + F2
11th set: BD plants given foliar spray of 0.2% after bud formation	B3 + F3

The experiment was conducted in a glass house under controlled conditions in which PAR (photosynthetically active radiation) ranged between 740 and 880 μmol m^{-2} s^{-1} at 12.00 noon, relative humidity (RH) ranged between 68% and 98% at 9.30 A.M. and minimum and maximum temperature ranged between 24–30 °C and 30–42 °C, respectively, during the period of experiment.

Tissue and seed B concentration

Plants were analyzed for concentration of B in vegetative (leaves, stem, root) and reproductive parts (seeds) in HNO_3:$HClO_4$ (10:1) digests by estimating Azomethine-H complex formation [13] spectrophotochemically at 430 nm.

Pollen producing capacity (PPC)

To determine the number of pollen grains per anther, mature anthers per flower was removed from five unopened flowers (bud) of five plants and a homogenous suspension was prepared by gently crushing each anther in 10% glycerol. The number of pollen grains in the suspension was counted under compound microscope.

Pollen viability

Viability of pollen grains was determined by germinating pollen grains by hanging drop method [14]. The pollen grains were considered as germinated when the length of pollen tube was more than the diameter of the pollen grain. Scoring was done of 10 sets of 20 pollen grains each, from each treatment. Acetocarmine staining was also done to study the viable and non-viable pollens.

PAGE and cytochemical localization of enzymes on stigma surface

For study of localization of enzymes on stigma surface, stigma from 10 to 20 flowers was gently excised without injuring the stigma and style. The stigma were placed in a cavity slide so that the styles did not dip into the solution and incubated in the appropriate reaction solution for 10–20 min at 25–35 °C in a humid chamber and stained for localization of peroxidase (POD) and esterase (Est) on pollen and stigma [15]. The poly acrylamide gel electrophoresis (PAGE) was carried out for POD and Est on the pollen grains and stigma exudates by methods described earlier [15].

Pod and seed yield

Plants were tagged before the foliar application and the number of flowers formed thereafter were counted and recorded. When the pods were ready for harvest the number, length and weight of pods and seeds formed were measured.

Seed viability

Seed viability was evaluated by the germination test. Germination tests were performed in dark in plastic petridishes with seed placed on filter paper (Whatman No. 1) with about 10 ml of distilled water. Three replicates of 100 seeds from each treatment were used.

Carbohydrate fractions

Mature and dried seeds were fixed in 50% (v/v) boiling ethanol (1:10) and ground at room temperature for determination of reducing and non-reducing sugars [16] and starch [17].

Seed proteins

Seed proteins were extracted after removal of seed coat [18]. The seeds were ground to a dry powder and then extracted in acetone and centrifuged at 11,500 × *g*. The residue was air dried and the seed flour was extracted with water for albumins, 5% NaCl for globulins, 0.1 N NaOH for glutenins and 70% ethanol with 2 drop of mercaptoethanol for prolamines. Each of the extracts was again centrifuged and supernatant was taken for protein estimation. The protein in the above extracts was estimated by the method of [19].

Statistical analysis

The data have been evaluated by means of ANOVA. The mean values and the standard error (±) have been presented in tables and figures. Significant differences ($P \leq 0.05$) are indicated in figures and tables.

Results

Visual symptoms of B deficiency appeared as necrosis of the apical stem region and cessation of root elongation of black gram. Boron deficient leaves showed reduction in leaf area and thickening of leaves. Young emerging leaves became dark green and brittle after 25 days of deficient B supply. After 28 days, plant growth ceased because of necrosis of the apical growth points of main shoots. This resulted in multiple auxiliary branches arising from the basal region, giving a bush like appearance to the plants. After 60–65 days of growth B deficient plants appeared withered. Foliar applied B helped to recover the B deficiency symptoms in newly