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Enhancing Seedling Growth and Budding Potential in Ber (*Zizyphus mauritiana*) Using Gibberellic Acid and Bacterial Inoculants

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ABSTRACT: Indian jujube (*Zizyphus mauritiana* Lamk.), commonly known as ber, is a commercially significant fruit in arid and semi-arid regions of India due to its high returns, low input costs, and drought tolerance. However, delayed budding in ber rootstock seedlings often results in unbuddable plants, leading to resource wastage. This study aimed to enhance vegetative growth in ber rootstock under nursery conditions using gibberellic acid (GA_3) and liquid bacterial inoculants (LBIs) of *Sphingobacterium* sp. and *Azotobacter* sp. A field experiment was conducted at PAU, Regional Research Station, Bathinda, using a randomized complete block design with five treatments. Seedlings treated with the combined LBIs showed significant improvements in shoot number (27.45%), shoot length (21.04%), collar diameter (29.63%), root length (17.65%), and biomass accumulation compared to control. Enhanced growth is attributed to increased nutrient availability and phytohormone production by inoculated bacteria. Although microbial counts in rhizospheric soil increased numerically in treated plants, the changes were statistically non-significant. The findings suggest that LBIs can effectively improve rootstock growth, making seedlings suitable for timely budding and reducing wastage. Future studies should assess budding success and conduct biosafety evaluations of the microbial strains, alongside soil biofertilizer applications to improve long-term soil health and productivity.

Keywords: Ber seedlings, gibberellic acid (GA_3), liquid bacterial inoculants (LBIs), vegetative growth, budding success

INTRODUCTION

Indian jujube (*Zizyphus mauritiana* Lamk.), a member of the Rhamnaceae family and commonly known as ber, Beri fruit, Indian cherry, or Indian plum, is a prominent commercial fruit crop cultivated extensively in the arid and semi-arid regions of India. Its popularity among farmers is attributed to its high economic returns, minimal cultivation costs, and exceptional drought tolerance [1,2]. Ber also offers higher productivity and superior post-harvest durability, making it highly suitable for commercial cultivation compared to other fruit crops. Typically, ber nurseries are established in summer by sowing seeds of *Z. mauritiana* rootstock in April, with germination initiating within 3 to 4 weeks. Seedlings usually reach buddable maturity by July–August. However, due to variability in growth rates, not all seedlings attain the desired thickness in time, leading to staggered budding in two to three phases. In the subsequent season, overly thickened rootstocks become unsuitable for budding and are often discarded. While some nurserymen attempt to reuse these rootstocks by cutting them to ground level, maintaining them for another year is challenging, and transplant success is poor. Therefore, there is significant potential to enhance seedling growth using plant growth regulators like GA_3 and beneficial bacterial inoculants to promote early buddable maturity [3,4]. This study investigates their combined influence under nursery conditions.

The field experiments were conducted at Punjab Agricultural University, Regional Research Station, Bathinda, Punjab (latitude 30.58°N, longitude 74.18°E; 211m above sea level) The soil texture of experimental site was loamy sand with 81.1% sand, 10.7% silt and 8.2% clay. The experimental soil at 0-15 cm depth was assessed initially for various physico-chemical properties and recorded data showed 8.5 pH, 0.25 dSm^{-1} electrical conductivity, organic carbon content 0.34%, available nitrogen (270 kg ha^{-1}), available phosphorus (16.00 kg ha^{-1}) and available potassium (220 kg ha^{-1}).

The seeds of *ber* rootstock (*Zizyphus mauritiana*) were extracted by cracking the hard shell and sown in the month of April on well-prepared nursery beds raised 15 cm above the ground level, with rows spaced 15 cm apart and plants 30 cm apart within the rows. After germination, three-four weeks old *ber* seedling of uniform vigour and height were treated with liquid bacterial inoculant of respective culture and were transplanted in black polythene bags (size 12" X 17", 250 gauge) filled with sterilized potting mixture consisting of soil and FYM in the ratio of 1:1 to attain buddable maturity. The trial was conducted in RCBD with five treatments i.e., T_1: Control (no treatment), T_2: GA_3, T_3: *Sphingobacterium* sp., T_4: *Azotobacter* sp. and T_5: *Sphingobacterium* sp. and *Azotobacter* sp. with 50 plants per replication The growth parameters (number of shoots, number of leaves, shoot length (cm), collar diameter (cm), shoot weight (g), biomass (above ground), root weight (g), root length (cm) were recorded from five randomly selected plants from each treatment before budding during both the years.

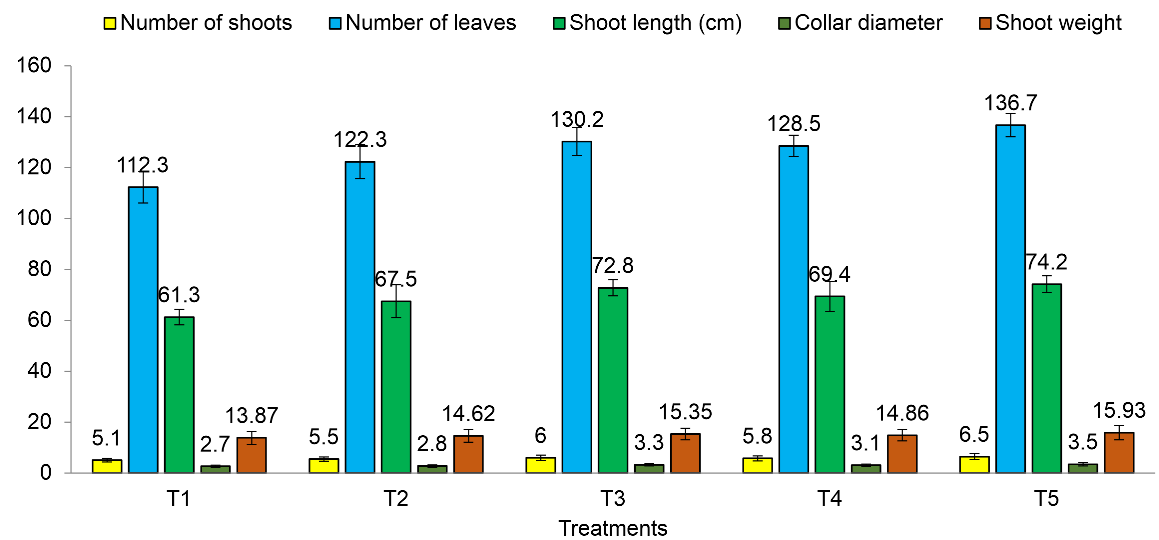
Liquid bacterial inoculants of individual cultures were prepared using the standard method with slight modification [5]. The seedling bacterization was done with liquid bacterial inoculants of respective culture as per treatments. For priming of seedling, respective 10ml of liquid inoculants was dissolved in 10-15L of water followed by dipping of ber seedlings in this solution for one hour and transplanted immediately. Similarly, $GA_{3,}$ a growth hormone, 250ppm was dissolved in 10L of water followed by priming of *ber* seedlings in this solution for 30 minutes and transplant immediately.

The rhizospheric soil sample were collected from the rhizosphere of plant at the time of recording growth parameters from each treatment. The enumeration of bacteria (Nutrient Agar Medium), fungi (Glucose Yeast Extract Medium) and actinomycetes (Ken knights Medium) was done via serial dilution spread plate technique. Serial dilutions were made up to the highest dilution of 10^{-9}. The serial dilutions from 10^{-6} to 10^{-8} were selected for enumeration of bacteria, for fungi 10^{-2} to 10^{-4} serial dilutions and for actinomycetes 10^{-3} to 10^{-5} serial dilutions were used. The petri plates were incubated for 2-6 days at 28 ± 2°C in an inverted position. After incubation, number of colonies appeared on the plates were counted, averaged and multiplied by the dilution factor to find the number of cells per gram of soil sample. To express the count on the basis of soil dry mass, 10 g fresh soil (in triplicate) was dried in an oven to a constant mass and its dry weight was recorded. The percent (%) change in soil weight was determined and this percent change was used to convert CFU per gram wet soil to CFU per gram dry soil.

The experimental data collected on various aspects of investigation was statistically analysed [6]. The comparisons were made with 5% level of significance.

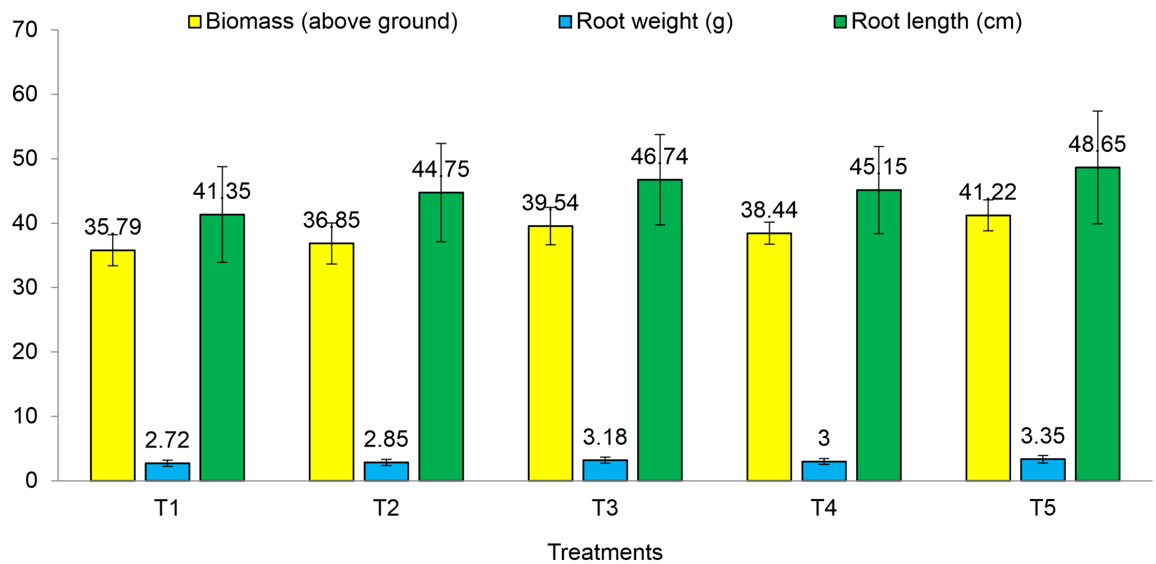
The maximum escalation in vegetative growth parameters, biomass accumulation and root characteristics were observed with the combined application of liquid bacterial inoculants followed by individual application over gibberellic acid and control. The per cent increase in shoot number (27.45), leaf number (21.73), shoot length (21.04), collar diameter (29.63), shoot weight (14.85), root weight (23.16) and root length (17.65) was recorded with the combined application of liquid bacterial inoculants over the control treatment (Figure 1). The per cent increase in above ground biomass (15.17) was also higher over the control with the combined application of *Sphingobacterium* sp. and *Azotobacter* sp. (Figure 2). These results suggest that the liquid bacterial inoculants can play a pertinent role in improving growth attributes of the plants under nursery condition that could further help in successful budding programmes as root stock has great influence on yield and quality parameters of scion.

This might be due to more availability macro and micro nutrients or improved mineralisation in the immediate vicinity of plants owing to the plethora of PGP (plant growth promoting) activities performed by inoculated bacteria. This increase might also be owing to the liberation of plant growth hormones by inoculated bacteria, which get absorbed from the rhizosphere by the roots of treated plants. [7] reported the production of phytohormones (Indole acetic acid and Gibberellic acid) by *Sphingobacterium* sp. over varied temperature conditions and the optimum activity was recorded at 40°C. Strains of *Azotobacter* sp. isolated from the rhizosphere of sugarbeet observed to produce gibberellins [8]. The outcomes are in harmony with those attained. [9] stated an upsurge in growth owing to the simulative result of PSB resulting in higher phosphorus accessibility and uptake by plants. Similarly, [10] reported maximum plant height and number of branches were also observed in *Solanum melongena* with the application of bio-inoculants as compared to control. [11] evaluated how different microbial seed treatments influence wheat seed germination, early growth, and nutrient composition. The findings showed that treating seeds with 10% Mycorrhiza notably enhanced germination rates, seedling and shoot



*Fresh shoot weight was taken

Figure 1. Effect of gibberellic acid and liquid bacterial inoculants on vegetative growth parameters of ber seedlings (rootstock)



*Fresh weight of all the parameters were taken

Figure 2. Effect of gibberellic acid and liquid bacterial inoculants on biomass accumulation and root characteristics of *ber* seedlings (rootstock)

lengths, root development, and protein levels compared to untreated controls. In contrast, *Azotobacter* treatments led to only slight improvements in germination and protein content.

Likewise, [12] conducted an in vitro study on chickpea seeds, applying various combinations of three potent endophytic strains in conjunction with *Rhizobium* GV-2. The results demonstrated statistically significant differences among the treatments. Notably, the combination of *Bacillus thuringiensis*, *B. cereus* strain LPDB2, *B. cereus* strain LPDB5, and *Rhizobium* GV-2 led to a marked improvement in seed quality traits compared to other treatments. [13] conducted a field experiment using a randomized block design with three replications to evaluate the influence of boron and plant growth regulators on hybrid seed yield and quality in ridge gourd. Foliar applications were administered at the two- to three-leaf stage, peak flowering, and fruit initiation. Among the various treatments, the application of boron (1 g/L) combined with GA_3 (50 ppm) (T3) produced

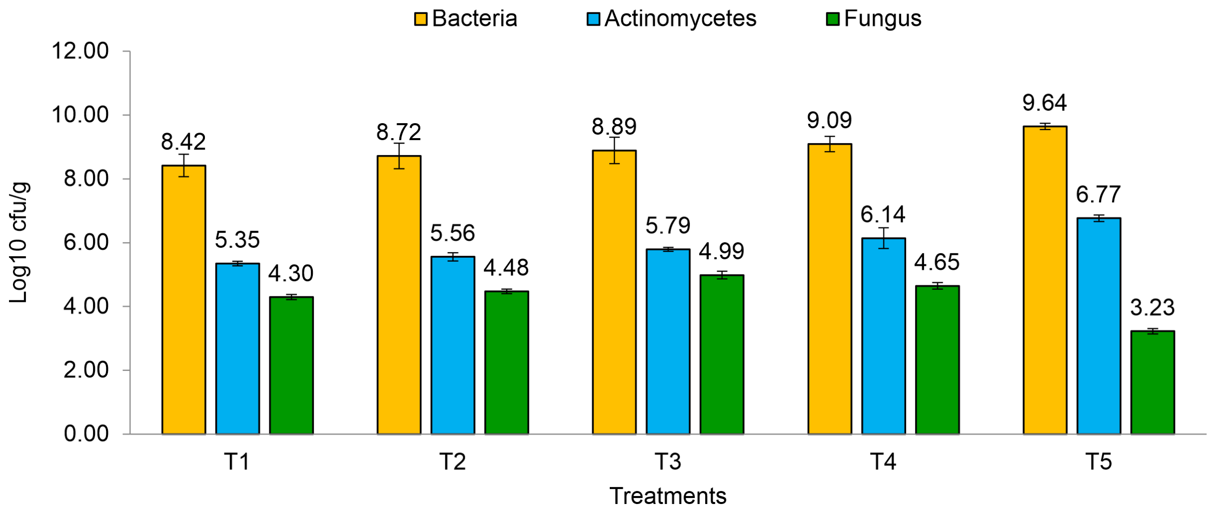


Figure 3. Total viable count (Log_{10} number of CFU/g) of bacteria, fungi and actinomycetes in rhizospheric soil as influenced by GA_3 and liquid bacterial inoculants

significantly superior results. This treatment recorded greater vine length (422.0 cm), number of fruits per plant (5.83), fruit length (26.9 cm), fruit diameter (15.5 cm), fruit weight (27.8 g), number of seeds per fruit (46.7), seed weight per fruit (5.4 g), 100-seed weight (11.60 g), hybrid seed yield (31.5 g/plant; 294.6 kg/ha), seed germination percentage (88.0%), dehydrogenase activity (1.293 OD), shoot length (27.5 cm), root length (15.1 cm), seedling dry weight (942 mg), and seedling vigor index (3749), all significantly outperforming the untreated control.

GA_3 and liquid bacterial inoculants showed non-significant impact on workable count of fungi, bacteria and actinomycetes (Figure 3). However, numeric increase in workable count of bacteria, fungi and actinomycetes was observed in treated seedlings over control. For improving soil health, intermittent inoculations with the extremely competitive strains are desirable for several years [14].

Thus, Liquid bacterial inoculants (LBIs) viz. *Azotobacter* sp. and *Spingobacterium* sp. can play a predominant role in escalating vegetative growth, biomass accumulation and root characteristics of *ber* plants (rootstock) under nursery conditions. The plants (rootstock) thus developed can be used for successful budding ventures. In future, more research work can be carried out to evaluate the success rate of budding on root stock of plants developed from seedlings treated with LBIs in addition to detailed biosafety evaluation of test cultures. For improving soil microbiological properties and to make it viable and live, bulk inoculation of soil with efficient biofertilizers can also be done.

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