Research Article

Seed bio-priming approach to amplify production potential of mungbean (*Vigna radiata* L.) in sandy soils

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Abstract

Seed treatment with living microorganisms is an eco-friendly and effective alternative to chemical seed treatment which improves plant growth and production. To evaluate this approach, three kinds of microbes exclusive and also in combinations were applied to the seeds of two mungbean genotypes and grown under field conditions during the summer season 2016. The bio-primed seeds significantly affected and promoted growth and yield related traits like emergence count (EC), leaf area index (LAI), chlorophyll content (*Chl*), number of nodules plant⁻¹ (NNP), number of pods plant⁻¹ (NPP), number of grains pod⁻¹ (NGP), thousand seed weight (TSW), biomass (BM) and grain yield (GY). The genotype NM-11 under the combined treatment of microbes (MR-1/1 + MR-1/2 + SR-5-1) outperformed genotype NCM by producing higher grain yield 3460 kg ha⁻¹, while untreated seeds observed the lowest grain yield 1969 kg ha⁻¹. Bio-priming proved its supremacy over unprimed seeds by improving growth and yield related traits. Significant and positive correlations were also observed between growth and yield parameters under different treatments of microbes. These results suggest that seed bio-priming by combining different microbes can be used to augment mungbean crop outputs under sandy soils conditions.

Keywords: Bio-priming; Genotypes; Mungbean; Growth; Yield

Introduction

Mungbean (*Vigna radiata* L.) is a short duration pulse crop and considered to be very nutritive due to its protein abundant seed [1]. Mungbean utilizes lesser amount of water for its growth than other summer season crops. Therefore, as a drought resistant crop, it has the tendency to withstand under unfavorable drought

conditions and it can be grown in areas which receives rainfall only for plant growth [2]. When talking about production, mungbean comes second to chickpea among all leguminous crops [3]. Due to its nodule forming nature, it stabilizes and increases fertility of the soil by providing sites for nitrogen fixation process and contributing in sustaining the

agriculture. In Pakistan, with an average yield of 0.72 ton per hectare, mungbean is successfully grown on area of 178.8 thousand hectares and produced 130.1 thousand tons of edible seed during 2016-17 [4].

Poor germination and crop establishment are serious constraints for attaining high yield of mungbean [5]. However, expeditious germination of seedlings could generate deep roots in advance to the drying of upper layer of soil, which may outcome optimum and healthier crop stand and seed yield [6]. Through seed priming, it is possible to achieve better crop stand, growth and yield of leguminous crops from the soils having low fertility status. Seed priming is a physiological approach which allows to begin germination processes without the emergence of radicle [7]. To date, seed priming techniques which have been evolved are halo-priming, osmo-priming, solid matrix priming, hydro-priming and seed bio-priming [8]. In many experiments, seed priming resulted in improving seed germination percentage, plant growth and seed yield of leguminous crops [9]. Besides this, primed seeds outcome healthy plant growth, tillering, early flowering and also promote seed yield, biological yields and harvest index [10]. Using seed bio-priming technique (inoculation of seed with beneficial organism), encouraging results have been obtained especially in salinity stress tolerance experiments. In leguminous crops, the genus *Bacillus* have extensively been used in most of the trials related to abiotic stresses [11]. Seed bio-priming has also been used in numerous crops for controlling different kinds of diseases [12]. The treatment of seeds with *Rhizobium* has found to be effective in promoting nodulation capacity and nitrogenase activity [13] in many crops. Keeping in view the significance of bio-priming approach, we designed this field study to examine the outcome of seed bio-priming on emergence, growth and yield of mungbean genotypes to increase its

production potential in sandy soils of Thal region.

Materials and methods

Experimental site description

A field trials was conducted at Agriculture Research Farm, Bahauddin Zakariya University Bahadur Sub-campus Layyah, Pakistan, during kharif season, 2016. This region is known as “Thal”, with semi-arid climate, consisting of sand dunes and the soils of sandy to sandy loam in texture. The experimental site has sandy loam soil having organic matter <1.0 % and pH 8.4.

Experimental details

Mungbean genotypes NM-11 and NCM used in this study and the seed were obtained from National Agricultural Research Centre (NARC) Islamabad, Pakistan. Prior to bio-priming treatments, the seeds were sterilized with sodium hypochlorite (30%). The pre-soaking of the seeds was done in distilled water for 4 hours according to the pre-determined priming time. Sugar solution was used as a sticking material to inoculate pre-soaked seeds with microbial treatments. Then inoculated seeds were incubated under high humidity for about 48 hours at approximately 25–32 °C [14]. The trial was conducted under randomized complete block design having split plot arrangement with three replications. The main plot treatments include two genotypes V_1 =NM-11 and V_2 =NCM, while the sub-plots include two control treatments as B_1 =dry seed and B_2 =hydro priming, while seven microbial treatments as B_3 =MR-1/1, B_4 =MR-1/2, B_5 =SR-5-1, B_6 = MR-1/1 + MR-1/2, B_7 = MR-1/1 + SR-5-1, B_8 = MR-1/2 + SR-5-1, B_9 = MR-1/1 + MR-1/2 + SR-5-1.

Crop husbandry

In order to make conducive seedbed, a pre-soaking irrigation of 10 cm was applied to the field. At reaching workable conditions, the soil was cultivated with tractor mounted cultivator and planking was done to level the field. The primed seeds were sown on August 5, 2016, according to the treatments in four rows per treatment by keeping row spacing of 30 cm with a net plot size of 1.2

× 3 m. Seeds @ 25 kg ha⁻¹ were sown by single row hand drill and basal dose of N and P fertilizers (30 and 75 Kg ha⁻¹ respectively) was applied in the form of diammonium phosphate (DAP). Manual hoeing was done at 30 days after sowing (DAS) to eradicate weeds. Irrigation was applied at three different intervals according to the demand of the crop, while standard agronomic practices were adopted to keep the field in normal condition.

Observations recorded

Number of emerged plants (emergence count) was recorded from the selected area of 1m² from each treatment until no more seed was germinated. Leaf area index (LAI) was recorded periodically after 15 days interval starting from 30 days after sowing (DAS) until harvest of the crop. For LAI, the leaf area was measured by using leaf area meter (DT Area Meter, Model MK2, Delta T Devices, Cambridge, UK). Later, the LAI was calculated by dividing total leaf area to total ground area used for harvesting for sampling [15].

The chlorophyll (Chl) contents were determined with the help of chlorophyll meter SPAD-502 [16]. The numbers of nodules plant⁻¹ (NNP) were counted and averaged from randomly selected ten plants within a treatment. The number of pods plant⁻¹ (NPP), number of grains pod⁻¹ (NGP) and thousand seed weight (TSW) were calculated from 10 randomly selected plants from each treatment and averaged. For biological yield (BY), each plot was harvested manually, then sun-dried and tied into bundles and finally weighed. After that, bundles were threshed manually and the seed was weighed to record grain yield (GY). BY and GY were then converted into kg ha⁻¹. Harvest index (HI) was calculated as the ratio of GY to the BY and expressed in percentage.

Statistical analysis

The data was statistically analysed on Statistix 8.1 software. The mean values of

all the treatments were separated by LSD test at 5% probability level ($P \leq 0.05$) [17]. Correlation analysis among the parameters was done by using SPSS 16.

Results and discussion

All growth parameters including EC, LAI, NPP and NGP significantly increased in NM-11 genotype, while reduced in NMC genotype. This increase or decrease might be due to genotypic variation in mungbean. However, subjecting seeds with the bio-priming technique especially treating the seed with B₉ = MR-1/1 + MR-1/2 + SR-5-1 microbes significantly increased all the growth and yield contributing parameters of mungbean crop (Table 1). It can be stated that the promoting effect of particular genotypes along with microbes application was very clear. Such improvement in these traits might be due to indirect stimulating role of microbes on photosynthetic pigments and enzyme by optimizing the biological and metabolic activities, which results in promotion vegetative growth of plants [18].

Chlorophyll contents in NM-11 were significantly higher (8.94) than in genotype NMC (8.49). This minor but significant variation could be due to the variation in genetic make-up of the genotypes as both were provided the same environment to grow. Further, the maximum chlorophyll contents (11.49) were found in the plants produced from the seeds treated with the combination of all microbes (B₉). Schtz and Fangmeier [19], reported that the seed treated with microbes accelerate the chlorophyll breakdown and the increase in chlorophyll content could be credited to the useful importance of microbes for photosynthesis, net assimilation and transpiration rates [20]. The interaction effect of genotypes and microbes was found to be non-significant in order to produce any change in chlorophyll contents.

Table 1. Effect of genotype and Seed biopriming on Growth and Yield of Mungbean (*Vigna radiate* L.)

Genotypes = V (Main Plot)	EC	LAI	Chl	NNP	NPP	NGP	TSW	BY	GY	HI
NM-11 (V₁)	58.2 a	1.62 a	8.94 a	12.7 a	19.7 a	8.2 a	3822 a	7529 a	2902 a	38.60 a
NCM (V₂)	57.2 b	1.54 b	8.49 b	12.0 a	18.8 b	7.8 b	3741 b	7289 b	2693 b	35.93 b
LSD 5%	0.6	0.03	0.38	1.12	0.3	0.3	0.0305	0.0162	0.0172	2.263
Bio-priming = B (Sub Plot)										
B₁ (dry seed)	49.7 h	1.29 i	6.18 f	6.2 h	12.0 e	4.2 f	32.147 f	6218 g	1969 f	29.50 d
B₂ (hydro priming)	51.3 g	1.36 h	6.33 f	6.7 h	14.2 d	4.8 ef	33.147 f	6620 fg	2108 f	31.00 d
B₃ (MR-1/1)	60.3 c	1.70 d	10.03 b	14.2 d	21.5 b	9.2 bc	39.480 c	7583 cd	3036 c	38.00 b
B₄ (MR-1/2)	53.7 f	1.41 g	6.98 e	8.2 g	19.2 c	5.8 de	34.682 e	6892 ef	2438 e	35.33 c
B₅ (SR-5-1)	61.7 bc	1.74 c	10.09 b	15.8 c	21.5 b	9.8 b	41.017 b	8020 bc	3307 b	42.00 a
B₆ (MR-1/1+ MR-1/2)	56.0 e	1.45 f	7.71 d	10.0 f	18.2 c	6.8 d	36.360 d	7030 ef	2734 d	38.00 b
B₇ (MR-1/1+ SR-5-1)	63.0 b	1.78 b	11.06 a	17.7 b	22.0 b	11.2 a	42.138 ab	8193 b	3208 b	39.33 b
B₈ (MR-1/2+ SR-5-1)	58.2 d	1.62 e	8.53 c	12.5 e	19.5 c	8.2 c	38.750 c	7373 de	2916 c	39.33 b
B₉ (MR-1/1+ MR-1/2+ SR-5-1)	65.7 a	1.85 a	11.47 a	19.7 a	25.3 a	11.8 a	42650 a	8752 a	3460 a	42.70 a
LSD	1.6	0.04	0.53	1.0	1.4	1.2	1.2141	0.0494	0.0143	0.0202
VxB	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
LSD 5%	2.2	0.05	0.8	1.7	1.8	1.6	1.6	0.07	0.02	0.03

** = Highly significant; * = Significant; NS = Non significant at 5 % probability level

EC (Emergence count), LAI (Leaf area index), *Chl* (Chlorophyll content), NNP (Number of nodules plant⁻¹), NPP (Number of pods plant⁻¹), NGP (Number of grains pod⁻¹), TSW (Thousand seed weight), BY (Biomass), GY (Grain yield), and HI (Harvest index)

Bio-priming treatment B₉ had the greatest stimulatory effect on NNP, NPP, NGP, TSW, BY, GY and HI. On the other hand, a decrease was observed (Table 2) in the control treatments (B₁ and B₂). Dominique *et al.* [21] expressed that, bio-primed seeds of mungbean improved the internodal length and biomass by altering the ability of plants to produce dry matter and pods filling. These results also

suggest that the increased values for growth and yield related traits. These results also suggest that, the pre-sowing inoculation of seeds with microbes could facilitate the germination process and resulted into improved growth parameters and ultimately improved the yield and yield components through mitigating the nutrient demands of mungbean plants.

Table 2. Interaction between genotypes and microbes for the grain yield (kg ha⁻¹) of mungbean

Seed Bio-Priming (B) / Genotype	Grain yield (kg ha ⁻¹)	
	V ₁ = NM-11	V ₂ = NMC
B ₁ = Dry seed	2007.7 n	1929.5 o
B ₂ = Hydro priming	2208.8 m	2006.3 mn
B ₃ = MR-1/1	3194.2 e	2917.5 g
B ₄ = MR-1/2	2486.3 k	2390.0 l
B ₅ = SR-5-1	3351.7 c	3244.9 d
B ₆ = MR-1/1 + MR-1/2	2776.8 i	2692.0 j
B ₇ = MR-1/1 + SR-5-1	3461.8 b	2953.8 f
B ₈ = MR-1/2 + SR-5-1	3193.6 ef	2829.0 h
B ₉ = MR-1/1 + MR-1/2 + SR-5-1	3667.3 a	3243.3 de

Means followed by the same letter are not significantly different at P≤0.05

Significant and positive correlations were observed between growth and yield parameters under different treatments of microbes (Table 3). EC, LAI and *Chl* were positively correlated with each other and also with NNP, NPP, NGP, TSW, BM and GY. The extent of correlation of these three

traits with HI was significant but not as stronger as with the other traits. The biological nitrogen fixation trait NNP was positively and significantly correlation with all growth and yield contributing traits, but it was not correlated with HI. Similarly NPP and NGP also have no correlation with HI.

Table 3. Correlation among growth and yield parameters of mungbean

	EC	LAI	<i>Chl</i>	NNP	NPP	NGP	TSW	BM	GY
LAI	.975**								
<i>Chl</i>	.958**	.955**							
NNP	.950**	.953**	.983**						
NPP	.967**	.926**	.933**	.906**					
NGP	.970**	.944**	.953**	.961**	.969**				
TSW	.983**	.962**	.918**	.903**	.939**	.927**			
BM	.926**	.906**	.881**	.838**	.844**	.824**	.943**		
GY	.964**	.943**	.962**	.929**	.962**	.949**	.934**	.908**	
HI	.782*	.757*	.693*	.647	.656	.625	.842**	.942**	.717*

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)

These findings are in agreement with the observations of Saxena *et al.* [22] and Kumar *et al.* [23]. These correlations suggest that all the microbial treatments contributed towards a better and healthy plant growth which synchronized with the yield attributing traits. The non-significant correlation of HI with NNP and NPP might be due to the fact that HI is a calculated value and dependent upon grain yield and biomass.

Conclusions

Significant increase in growth and yield traits proved the importance of using seed bio-priming in mungbean especially in sandy soils which are considered as less fertile soils. Moreover, it is concluded that seed bio-priming by using combinations of different microbes have maximum potential for better crop stand and better yield in Thal region.

Author's contributions

Conceived and designed the experiments: TA Yasir, A Wasaya, & M Ijaz, Performed the experiments: O Farooq, S Hussain & M Muzammil, Analyzed the data: ZM Bhutta & AW Baloch. Contributed reagents/materials/ analysis tools: HM Aatif & TA Yasir. Wrote the paper: M Muzammil, TA Yasir & Allah Wasaya.

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