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Influence of rock phosphate, phosphorous solubilizing bacteria and lime on phosphorous content and dry matter yield of green gram (*Vigna radiata* (L.) Wilczek)

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Abstract
Rock phosphate (RP) is one of the cheap sources of P but it cannot be used directly as a soil amendment due to its extremely poor solubility in water (0.1%). However, the availability of Phosphorous from RP can be enhanced by applying it with lime and through the specific use of bio-inoculants. A pot experiment was conducted in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Central Agricultural University, Imphal during *the Pre-Kharif* season of 2021 to study the influence of Rock Phosphate, Phosphorous Solubilizing Bacteria and lime on phosphorous content and dry matter yield of Green Gram (var. DGGS-4). Results revealed that all the P treatments showed higher accumulation of plant P concentration and plant dry weight of green gram when compared to control. Comparing among the different treatments, statistically higher accumulation of plant P concentration and dry weight were recorded in soil treated with T_{10} (100% RD of P_2O_5 from RP + PSB_1 +PSB_2 + Lime (18.71t ha^{-1}) followed by T_9 = 100% RD of P_2O_5 from RP + PSB_2+ Lime (18.71t ha^{-1}). Application of rock phosphate in combination with PSB and lime enhanced organic P mineralization thereby increasing plant P concentration and dry matter yield of green gram.

Keywords: Rock phosphate, phosphorous solubilizing bacteria, lime, p content and plant dry weight

Introduction
Soil is the most valuable and widespread natural resource that supports agriculturally based livelihoods. Soil fertility is declining due to lack of replenishment of lost nutrients. Phosphorus (P) deficiency is one of the major limiting nutrients for crop production in most of the soils throughout the world (Hinsinger, 2001) [14]. About 49.3% of Indian soils are in low-P category, 48.8% in medium and only 1.9% are in high-P category (Hasan, 1994) [13]. In Phosphorous deficit soils, Phosphorous may be in an unavailable form or lost through runoff. To make it available to plants, it should be added from an external source (Phosphatic Fertilizers). The use of phosphatic fertilizer in India has increased from 0.0088 million tons (Mt) in 1950–1951 to 7.464 Mt in 2019–2020 (FAI, 2020) [9]. To improve crop yields, large amounts of phosphatic fertilizers are applied which not only cause serious environmental pollution like eutrophication of freshwater bodies, but also a huge drain in terms of foreign exchange. The major problem with P nutrition is not the soil P content but its bioavailability to plants. So, to minimize the harmful effect of phosphatic fertilizer on the environment and to increase its bioavailability to plants, the use of Phosphorous Solubilizing Microbes needs to be encouraged.

It is reported that for the availability of phosphate in acid soil, rock phosphate can be applied with FYM, leaf manure and other organic wastes and are economic to farmers for different crops including legumes (Bhattacharya, 1987; Nayak, 1994; Bhutia *et al.* 2019 and Dev *et al.* 2020) [3, 4, 2]. Rock phosphate is a natural phosphatic fertilizer containing 20-37% P_2O_5 and a good amount of CaO (42%) and traces of several metal ions (Mandal *et al.*, 1997) [17]. However, Soil acidity limits crop production regardless of the application of fertilizer. Soil acidity affects not only the development of rhizobia and nodule formation but also the growth and uptake of nutrients by plants. The use of inoculants can be effective only when applied at the optimal soil pH. Liming is one of the methods of ameliorating soil acidity increasing nutrients available to plant. Bhutia *et al.* (2019) [4] reported that effectiveness of rock phosphate as a P source for crop production is enhanced by the solubility effect of organic manures and lime application.

Crop productivity can be improved by manipulating the rhizospheric microorganisms. Increased yield response of crop plants has been observed following seed or soil inoculation with symbiotic N_2 fixing organisms and phosphate solubilizing bacteria. (Saxena and Tilak, 1994 and Whitelaw, 1999). When inoculated, these organisms colonize the rhizosphere and enhance plant growth by providing it with N and P, respectively. Phosphate solubilizing microorganisms (PSM) render insoluble forms of phosphate available to the plants. Coinoculation effects of two different strains Phosphorous Solubilizing Bacteria, on plant phosphorous concentration and plant dry weight have, however, received little attention. Among grain legumes, green gram (*Vigna radiata* (L.) Wilczek) is the third important conventional crop. Its total P uptake is highest amongst the grain legumes which removes 48.1 kg P_2O_5 per ton of grain produced (FAI, 2011) [8]. It is grown in the tropics around the year on a variety of soils from red laterite soil to black or sandy soil and matures in about 60 to 90 days. It is grown for nitrogen rich easily digestible seeds, which contain about 25% protein. After picking the pods, the crop is also used as green manure and adds about 40–50 kg N ha^{-1} to the soil as residual fertility (Singh, 1996).

P is an integral component of high-energy molecules and biomembranes and is involved in several metabolic reactions and signal transduction pathways (Griffith, 1999) [12]. Therefore, the availability of P has profound consequences on plant growth and P concentration. Sub-optimal P supply in legumes restricts root growth, photosynthesis, translocation of sugars and other processes like P concentration and dry matter accumulation in green gram.

Therefore, it is of great practical importance to study the associative effect of these three groups namely Rock Phosphate, Phosphorous Solubilizing Bacteria and lime on P concentration and dry matter yield of green gram (*Vigna radiata* (L.) Wilczek). The experiment was conducted under controlled conditions in pots.

Materials and Methods

A pot experiment was conducted in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, CAU, Imphal during *Pre-Kharif* season, 2021 to study the effect of applied rock phosphate in the presence or absence of phosphorus solubilizing bacteria and lime on phosphorous concentration and dry matter yield of green gram (*var* DGGS-4). An acid surface soil (0-20 cm depth) was collected from the research farm of the College of Agriculture, CAU, Imphal following the methods as outlined by Jackson (1973) [16]. The physicochemical properties of the soil are presented in table1.

Table 1: Physiochemical properties of the experimental soil

Soil Characteristics	Results
Sand (%)	19.7
Silt (%)	26.9
Clay (%)	53.4
Soil Texture	clayey soil
pH (1:2.5 soil: water ratio)	5.4
EC (1:2.5 soil: water ratio, dSm^{-1})	0.25
CEC [cmol(p+)kg^{-1}]	14.92
Organic carbon (%)	1.87
Available nitrogen (Kg ha^{-1})	320.19
Available P_2O_5 (Kg ha^{-1})	31.47
Available K_2O (Kg ha^{-1})	267.83

Five kg of air dried soil was filled in each of a series of pots. Recommended dose of 20 kg N ha^{-1} in the form of urea and 20 kg K_2O ha^{-1} in the form of muriate of potash were applied in each experimental pots and mixed properly with the soil. According to different sets of treatment rock phosphate and SSP were applied to the pots as phosphorus sources based on the recommended dose (40 kg P_2O_5 ha^{-1}) for the crop green gram (variety DGGS-4). Green gram seeds were treated with two PSBs: PSB_1 (Commercial strain bought from the market) and PSB_2 (*Bacillus megatherium* from the department of Plant Pathology, College of Agriculture, CAU, Imphal). The inoculated seeds were dried under shade and sown immediately after drying. Based on lime requirement determination by SMP buffer method (Shoemaker *et al.*, 1961), liming (18.71 t ha^{-1}) was done two weeks ahead and allowed to react with soil mass according to different sets of treatments. Five seeds of soybean were sown to each pot. After germination, one healthy seedling was maintained throughout the experiment. The soils of each treatment were moistened to 60% of water holding capacity throughout the experiment.

The experiment was conducted in a completely randomized block design replicated thrice. The treatments were as follows

T_1	=	Control
T_2	=	100% RD of P_2O_5 from SSP
T_3	=	100% RD of P_2O_5 from RP
T_4	=	100% RD of P_2O_5 from RP + PSB_1
T_5	=	100% RD of P_2O_5 from RP + PSB_2
T_6	=	100% RD of P_2O_5 from RP + PSB_1 +PSB_2
T_7	=	100% RD of P_2O_5 from RP + Lime
T_8	=	100% RD of P_2O_5 from RP + PSB_1 + Lime
T_9	=	100% RD of P_2O_5 from RP + PSB_2+ Lime
T_{10}	=	100% RD of P_2O_5 from RP + PSB_1 +PSB_2 + Lime

SSP - Single Super Phosphate	PSB - Phosphorus Solubilizing Bacteria
RP - Rock Phosphate	RD- Recommended Dose
PSB_1 - Phosphocare	PSB_2 – *Bacillus megatherium*

The whole plants were collected on 15th, 30th 45th, and 60th days after sowing seeds and at harvest by destructive sampling to estimate phosphorus concentration and dry weight of green gram. Mechanical analysis of the soil for sand, silt, and clay fractions were carried out by hydrometer method (Bouyoucos, 1962). The soil pH was determined using a glass electrode Systronic pH meter in soil: water suspension ratio of 1:2.5 as described by Jackson (1973) [16]. Available nitrogen was determined by the alkaline potassium permanganate method as outlined by Subbiah and Asija (1956). Available phosphorus content was determined spectrophotometrically by Bray and Kurtz No 1 method (Bray and Kurtz, 1945). Available potassium of the soil was extracted by 1N NH_4OAc and determined flame photometrically (Jackson, 1973) [16]. Organic carbon present in the soil samples was determined by Walkley and Black rapid titration method (Walkley and Black, 1934). The plant samples were dried at 65 to 70°C to constant dry weight. Dry weight was recorded at each stage to assess total dry matter production and expressed in grams per plant. For the determination of phosphorus content, plant samples were ground, digested in a tri-acid mixture of nitric acid, H_2SO_4 and perchloric acid in a 10:1:4 ratio and analysed the digested plant materials by Vanadomolybdo phosphoric yellow colour method (Jackson, 1973) [16].

Data obtained from the experiment were statistically analysed through analysis of variance technique for comparing the effects of the treatments (Gomez and Gomez, 1984) [11]. The significance of various effects was tested at 5% level of probability.

Results and Discussion

Total Phosphorus (P) in Plant

Data on changes in P concentration in green gram grown in rock phosphate fertilized soil in the presence or absence of PSB and lime are presented in table 2. Results show that the concentration of P in green gram reduces up to 45th day and increases till harvest in all the treatments. In general, concerning the initial concentration of P in green gram, its content reduces at harvest except in T_3. Presentation of P decline with crop age was also stated earlier by Setia and Sharma (2007). The data signifies that total P concentration was significantly more in green gram grown in soil treated with rock.

phosphate in the presence or absence of lime and PSB over control at different growth stages of the plant. Similar reports on higher P concentration with phosphorus application were also given earlier by Egamberdiyeva *et al*. (2004) [7]; Sarkar *et al.* (2014); Bhutia *et al.* (2019) [4] and Dev *et al.* (2020) [2]. Further study revealed that significantly higher P concentration was accumulated in T_{10} followed by T_9 on 60th day and at harvest. Molla *et al.* (1984) [18]; Gaur (1990) [10] and Adhikari *et al.* (2014) found that the introduction of P solubilizing microorganisms in the soil increase the availability of P from insoluble sources of phosphate, desorption of fixed phosphates and also increases the efficiency of phosphatic fertilizers.

Dry matter yield

The result on changes in the amount of dry matter yield of green gram grown in rock phosphate fertilized soil in presence or absence of PSB and lime are presented in table 3.

Irrespective of different treatments dry matter yield of green gram increased progressively till harvest. All the treatments involving P addition showed statistically better results of dry matter yield when compared to control. This is at par with the findings of Shahzad *et al.* (2008); Sarkar *et al.* (2014); Bhutia *et al.* (2019) [4] and Dev *et al.* (2020) [2]. Higher agronomic effectiveness of rock phosphate was revealed in increased dry matter yield of crop (IKerra *et al*., 1994) [15]. Critical study revealed that significantly higher dry matter accumulation was found in T_{10} followed by T_9 on 60th day and at harvest. There is a significant difference between the treatments that are applied with PSB and lime over without PSB and liming. Irrespective of P and PSB treatment, liming significantly enhances dry matter yield as compared to the unlimed system. Bhutia *et al.* (2019) [4] reported that effectiveness of rock phosphate as a P source for crop production is enhanced by the solubility effect of lime application.

Table 2: Changes in Total-P (ppm in Plant) concentration in green gram grown in rock phosphate fertilized soil applied with phosphorus solubilizing bacteria and lime

Treatments	Days After Sowing				
	15	30	45	60	Harvest
T1	3711.77	3460.71	3066.33	3482.94	3534.98
T2	4098.24	3797.34	3410.97	3894.31	4095.02
T3	4480.59	3859.16	3678.45	4353.32	5143.39
T4	4362.94	4021.64	3872.10	3993.63	4294.75
T5	4836.91	4347.63	4234.45	4409.20	4809.60
T6	5421.77	4898.16	4298.12	5028.23	5235.48
T7	4787.65	4329.20	4325.93	4291.24	4594.04
T8	4921.76	4432.41	4220.98	4507.57	4766.36
T9	5480.59	4875.88	4493.58	5034.51	5243.67
T10	5615.88	5269.69	4540.40	5379.09	5568.79
S.Ed(±)	180.85	160.19	101.49	123.86	108.26
$CD_{0.05}$	377.26	334.17	211.70	258.37	225.84

Table 3: Dry matter yield (g plant^{-1}) of green gram grown in rock phosphate Fertilized soil applied with phosphorus solubilizing bacteria and lime

Treatments	Days After Sowing				
	15	30	45	60	Harvest
T1	0.28	0.89	1.30	3.91	4.05
T2	0.30	1.06	1.90	4.82	4.97
T3	0.33	1.06	1.97	5.08	5.63
T4	0.35	1.32	2.26	5.45	5.97
T5	0.36	1.22	2.44	5.66	5.85
T6	0.36	1.39	2.55	5.74	6.25
T7	0.43	1.53	2.93	6.89	7.34
T8	0.48	1.46	3.06	6.84	7.49
T9	0.55	1.83	3.07	7.45	9.14
T10	0.56	1.85	3.13	7.90	11.74
S.Ed(±)	0.01	0.03	0.12	0.19	0.29
$CD_{0.05}$	0.02	0.07	0.26	0.41	0.60

Conclusion

With the view of above mentioned results it is opined that statistically higher accumulation of plant P concentration and dry matter yield of green gram (*var* DGGS-4) were recorded in soil treated with T_{10} (100% RD of P_2O_5 from RP + PSB_1 +PSB_2 + Lime (18.71t ha^{-1}) followed T_9 (100% RD of P_2O_5 from RP + PSB_2+ Lime (18.71t ha^{-1})). Agronomic efficiency of rock phosphate as P source for crop production is enhanced by the solubility effect of PSB and lime application.

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