extract was used as a nitrogen source cowpea rhizobia showed a heterogenous pattern for acid/alkali production from different sugars (except with mannitol) (Table 1). Of sixteen strains examined the number of strains producing acid from the different sugars were: 13 from ribose, 6 from mannose, 5 from galactose and xylose and 3 from fructose (Table 1). It is interesting to note that the three strains MI-19A, MI-50A and M4-13A which did not produce acid from ribose or any other sugar were isolated from one location in Maradi, Niger[1]. Though different carbon sources are shown to affect pH changes of the medium, yeast extract as nitrogen source clearly influenced acid/alkali production by cowpea rhizobia. Weak alkaline (WK) or weak acid (WA) reactions shown by a few strains turned to alkaline (K) or weak alkaline, respectively, when the concentration of yeast extract was increased from 0.04% to 0.1% (w/v) (Table 1). However, all cowpea rhizobia strains consistently produced alkali on YEMA which holds the validity of the results of Norris[7].

To determine if other nitrogen sources influence pH changes of the culture media by cowpea rhizobia; we examined the effects of different nitrogen sources in combination with different carbon sources on acid/alkali production. We found that: (a) all cow pea rhizobia produced acid when ammonium sulphate was used as nitrogen source and (b) the majority of the strains produced alkali from glutamate, nitrate and urea (Table 2). We have examined two inorganic nitrogen sources (ammonium sulphate and sodium nitrate) and both have shown to produce different effects on acid/alkali production. Similarly, three organic nitrogen sources (glutamate, urea and yeast extract) were examined. Generally speaking, regardless of the carbon sources (except ribose) most of the strains produced alkaline reactions with glutamate, nitrate or urea. Whereas with yeast extract the strains showed a heterogenous pattern for acid/alkali production (Table 1). The pattern of acid/alkali production by the *R. japonicum* strain was similar to that of cowpea rhizobia. However, the fast-growing *R. meliloti* strain produced acid from each nitrogen and carbon source tested. Tan and Broughton[5] suggested that the utilization of nitrogenous substrates by slow-growing rhizobia may cause pH changes of the medium. In this work we have shown that nitrogen sources did affect pH changes of the medium. However, with nitrogenous compounds cowpea rhiozbia generally, but, not necessarily, yielded alkaline reactions (except ammonium sulphate). Some of the strains which produced acid from yeast extract produced alkali when yeast extract was replaced with glutamate or nitrate or urea (Tables 1 and 2).

In summary, we have shown that the utilization of nitrogen sources, like ammonium sulphate, by cowpea rhizobia always caused decline in pH whereas glutamate, nitrate and urea generally caused increase in the pH of the medium. This study indicates that the cowpea rhizobia were as diverse by their pattern of acid/alkali production as they were on the basis of other characteristics[1-3,6,9].

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Effect of presowing seed treatment with molybdenum and cobalt on growth, nitrogen and yield in bean (*Phaseolus vulgaris* L.)

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Summary Bean (*Phaseolus vulgaris* L. Cv. 'Burpees Stringless') seeds were subjected to two cycles of presowing soaking and drying treatments with sodium molybdate and cobalt nitrite at 1 and 5 ppm concentrations used separately and also in combination. Sodium molybdate 2 ppm and cobalt nitrite 1 ppm used singly proved better than the remaining treatments with respect to nodulation, dry matter, nitrogen and yield. Combined treatment with sodium molybdate and cobalt nitrite did not produce additive effect on any parameter studied compared to their usage alone.

Introduction

Molybdenum and cobalt are known to play an important role in nitrogen fixation. Molybdenum forms a part of the enzyme nitrogenase and cobalt has been shown to be essential for normal growth of Rhizobium, the effect being mediated by vit. B_{12} and its coenzyme forms[4,5,6]. Soil or foliar application of molybdenum or cobalt has been shown to increase dry matter, nodule number, leg haemoglobin and nitrogen in many legumes[2,7,8,9]. Though there are some recommendations to exploit the advantage their usage has not been largely adopted. Under different Indian soil conditions very limited trials have been conducted. The published reports also pertain to soil or foliar application which involve lot of wastage and is labour involving. The present trial was undertaken with french bean which is a common legume around Bangalore to try easier methods of administration of these elements and to study their response under field condition.

Materials and methods

French bean (*Phaseolus vulgaris* cv. 'Burpees Stringless') seeds were soaked in 1, 2 and 5 ppm solutions of sodium molybdate and cobalt nitrite separately for 1 hour so as to cause maximum imbibition in them. The solutions were then drained and seeds were dried for 23 h on blotting sheets in the laboratory. Two such soaking and drying treatments were given. Fresh solutions were used during the second soaking. Seeds treated with distilled water under similar conditions and unteated seeds served as controls. During the treatment the minimum and maximum temperature varied between 18–20°C and 27–29°C respectively. The relative humidity ranged between 25–49%.

All the treated seeds and the controls were coated with 3 day old cultures of *Rhizobium phaseoli* grown on yeast extract manitol agar medium and taken in 5 ml of distilled water and mixed with 50 ml of 30% sugar syrup. The seeds were dried under the fan and later stored in polyethylene bags at 10°C until they were taken to the field.

The seeds were sown in 4 × 4 m plots to maintain an interplant and inter row distance of 30 × 50 cm respectively. The treatments were replicated 5 times in a randomised block design. The field soil had a pH of 6.5, 125 ppm available nitrogen, 2.8 ppm phosphorus, 34 ppm potash,

Table 1. Effect of different concentration of molybdenum and cobalt on nodulation, growth, nitrogen content and yield in bean (*Phaseolus vulgaris* cv. 'Burpees Stringless')

Treatment	Nodule number	Nodule fresh weight plant^{-1} (mg)	Leaf area plant^{-1} (sq.cm.)	Shoot dry wt plant^{-1} (g)	Leaf nitrogen (%)	Yield plot^{-1} (g)
Control	8.8	87.0	409.4	3.7	2.17	2876
Water	13.2	136.0	512.2	4.1	2.56	3190
Molybdenum 1 ppm	19.6	195.0	498.6	4.2	2.9	3240
Molybdenum 2 ppm	32.8	341.0	653.6	4.8	3.22	4420
Molybdenum 5 ppm	16.8	160.0	505.6	4.1	1.77	3210
Cobalt 1 ppm	38.2	404.4	631.60	4.84	2.92	4386
Cobalt 2 ppm	21.8	220.0	265.0	4.04	2.88	3021
Cobalt 5 ppm	13.4	220.4	270.6	3.46	2.66	2707
S Em	1.445	15.99	34.67	0.1531	0.1211	215.33
C.D. at 5%	4.189	46.3	100.42	0.443	0.3508	613.59
C.D. at 1%	5.65	62.49	135.48	0.598	0.4732	841.29

0.02–0.05 ppm molybdenum and cobalt. A dose of fertilizer was applied to the seedlings week after emergence at the rate of 50, 70 and 50 kg/ha urea, superphosphate and muriate of potash respectively.

When the plants were 20 and 35 days old after germination five plants were harvested in each plot for studying nodule number, their fresh weight, leaf area (using Licor leaf area meter) and leaf nitrogen (by Micro Kjeldahl method)[1]. Total pod yield was recorded per plot out of the remaining plants. These such trials were conducted from July to September 1982, August to October 1982 and February to April 1983. The maximum and minimum temperature and rainfall range during July to October 1982 were respectively 25–30°C, 18–22°C and 0.8–52 mm. During February to August 1983 the figures were 25–36°C, 15–24°C and 50–80 mm. Results of the trials were analysed statistically using 'F' test.

Based on the results of these trials experiments were conducted to determine if the elements molybdenum and cobalt had any additive effect when administered together. The combinations tried included (a) Molybdenum 2 ppm + cobalt 1 ppm (b) molybdenum 1 ppm + cobalt 1 ppm (c) molybdenum 2 ppm + cobalt 2 ppm and the controls included were molybdenum 2 ppm, cobalt 1 ppm, distilled water and untreated seeds. Molybdenum 1 ppm and cobalt 2 ppm individual treatments were not tested again as they were not found very useful in the previous trial.

Presowing treatments were given to the seeds as mentioned earlier. The field soil conditions and spacings followed, fertilizer application and samplings taken for study were as mentioned for the earlier trial. The experiments were conducted during May–August 1983 and February–August 1984. The field weather conditions during the period did not show significant difference from what was mentioned earlier. Results of the trials were analysed by F test. Results of the 2nd trial have been presented here as the trend in response amongst the trials remained similar.

Results and discussion

Results of the trial revealed that bean seeds treated with molybdenum 2 ppm and cobalt 1 ppm showed statistically significant improvement in nodule number, their fresh weight, leaf area, shoot dry weight, nitrogen and yield over distilled water treatment or untreated controls ($p = 0.01$) (Table1). Molybdenum 2 ppm caused 56 and 38% improvement in yield over untreated and water soaked seeds while with cobalt 1 ppm the improvement was by

Table 2. Combined effect of molybdenum and cobalt on nodulation, growth, nitrogen and yield in bean *Phaseolus vulgaris* Cv. 'Burpees Stringless'

Treatment	Nodule number	Nodule fresh weight (mg)	Leaf area (sq.cm.)	Shoot dry wt (g)	Nitrogen content (%)	Yield (g)
Molybdenum 2 ppm + cobalt 1 ppm	49.5	176.25	414.50	3.13	2.85	3425
Molybdenum 1 ppm + cobalt 1 ppm	106.25	232.5	516.75	3.69	2.94	3875
Molybdenum 2 ppm + cobalt 2 ppm	49.75	101.25	404.00	3.07	2.92	3875
Molybdenum 2 ppm	102.00	293.0	376.25	2.71	3.12	3650
Cobalt 1 ppm	101.5	305.0	389.50	2.67	2.9	4125
Water	108.75	135.5	387.50	2.72	2.7	2825
Control	76	105.0	378.50	3.02	2.4	2750
S Em	14.222	29.707	52.86	0.387	0.249	331.048
C.D. at 5%	42.25	88.26	NS	NS	NS	NS

55 and 37% respectively. In molybdenum 2 ppm treatment nitrogen content improved by 50% and 25% respectively over untreated and distilled water soaked seeds and in cobalt 1 ppm it was 35 and 14% respectively. Combined inoculation did not bring about additive effect on growth (Table 2).

Seed treatment by repeated soaking and drying can provide molybdenum and cobalt required for the bacteria for expressing the activity. As molybdenum forms a part of the enzyme nitrogenase which plays key role in fixing nitrogen its enrichment has obviously increased nitrogen content in the plant. So also is cobalt important for the growth of Rhizobium acting via Vitamin B_{12} and its coenzyme forms. Dilworth *et al.*[3] have reported cobalt dependent nitrogenase activity in *Lupinus angustifolius* L. Hence, the increased number of nodules and enhanced fixation of nitrogen in them have contributed to better dry matter and yield in bean. Combined application of molybdenum and cobalt have however not proved beneficial over the elements supplied individually. The exact interaction of the elements with the enzyme would be interesting to study further. In Indian soils, concentration of molybdenum and cobalt ranges from 0.02–0.08 ppm. And hence molybdenum 2 ppm or cobalt 1 ppm can be safely recommended for seed treatment.

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