THE RESPONSES OF SOYBEAN PLANTS TO MOLYBDENUM TREATMENT

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Abstract: Molybdenum is an essential microelement for the symbiotic growth of soybean plants. With treatments of molybdenum, soybeans became taller, leaves expanded larger and the leaves contained more chlorophyll. In addition, the sizes and numbers of nodules also increased. However, the fluctuations of nitrogen fixing activity of root nodules in the growing season could not entirely be interpreted by these two parameters. For example, plants which were treated with molybdenum at pre-pod filling stage, had heavier nodules than those ones treated with molybdenum at pre-flowering stage, however, the latter plants had higher nitrogen fixing activity than the former ones. The leghaemoglobin content in root nodules did not change as much by the molybdenum treatments as the nitrogen fixing activity did. This means that leghaemoglobin content in root nodules could not be considered as a criteria for judging the activity of nitrogen fixation of plants. Although four components of leghaemoglobin were separated by gel electrophoresis, these electrophoretic components of leghaemoglobin did not vary with the treatments of molybdenum. This suggests that all of these four components of leghaemoglobin may be present simultaneously carrying oxygen to the nitrogen fixing rhizobia.

The nitrogen fixing activity of plants was remarkably stimulated by molybdenum treatments, because molybdenum enhances the nitrogenase activity and promotes the photosynthetic capacity of plants. The increments of nitrogen fixing activity induced the soybeans to form more numerous pods and seeds, consequently, the overall productivity of the plants was increased, and plants receiving molybdenum throught the roots give better yields than those receiving molybdenum through the leaves. The proper time of supplying molybdenum to plants is at the pre-lowering stage.

INTRODUCTION

It has been known that molybdenum is an indispensable micronutrient for nitrogen fixation by a variety of nitrogen fixing plants[6,19,26]. The responses of plants to molybdenum additions depend upon the nitrogen status of the soil or medium. In 1930, Bortels (ref. Anderson) initially observed that a strain of dinitrogen fixing bacteria, *Azotobacter chroococcum*, grew very poorly in a culture medium without combined nitrogen unless traces of molybdenum were added. In 1937, Bortels also reported that molybdenum was highly beneficial in dinitrogen fixation by *Medicago sativa*, *Trifolium pratense*, and *Glycine max*., when these leguminous plants were cultured in sand without combined nitrogen. Bortels's observations were followed by those of the Anderson (1942), who indicated that a few ounces of molybdenum supplied to *Trifolium subterranean* and *Medicago sativa* cultured in relatively acidic soils resulted in significant increases in dry weight[1]. This suggested that molybdenm was unavailable to plants in soils with low pH values, thus, responses of leguminous plants occurred when this microelement was supplied to soil. The experiments of Bortels and Anderson have been confirmed by many workers[3,5,9,11,12,16,17,19~24]. However, most of them apparently were only concerned with the relationships between molybdenum applications and bean harvests, and did not examine the

physiological or biochemical responses of leguminous plants to treatments with molybdenum. This study was undertaken to determine the effects of molybdenum application on the yield of soybean plants mainly by measuring the photosynthesis, nodular leghaemoglobin content, and rhizobial nitrogen fixing activity, because these three parameters predominantly regulate the productivity of leguminous plants.

MATERIALS AND METHODS

Selected seeds of soybean (*Glycine max.* var. Koashiung #3) were sterilized and then sown in germination dishes containing vermiculite. After germination, the young seedlings were transplanted to pots filled with a mixture of sand and vermiculite in a ratio of 1: 1, and then inoculated with *Rhizobium japonicum*. Initially three seedlings were transplanted to each pot, however, after 3 or 4 days following of transplantation, two or three seedlings were removed, thus only one seedling remained in each pot. The combinations of molybdenum (MoO_3) treatments to plants were as are shown in the following table:

Time of MoO_3 application	Way of MoO_3 application	Quantity of MoO_3 applied (gm/ha)
A: MoO_3 applied to seedlings after transplantation	I: MoO_3 added to soils in pots	000, 100, 200, 300
	II: MoO_3 dissolved and sprayed onto leaves	100, 200, 300
B: MoO_3 applied to plants at pre-flowering stage	I: MoO_3 added to soils in pots	000, 100, 200, 300
	II: MoO_3 dissolved and sprayed onto leaves	100, 200, 300
C: MoO_3 applied to plants at pre-pod filling stage	I: MoO_3 added to soils in pots	000, 100, 200, 300
	II: MoO_3 dissolved and sprayed onto leaves	100, 200, 300

There were eight replicates for each quantity of MoO_3 application, therefore, there was a total of 168 pots for this experiment. The plants were irrigated with nitrogen free Hoagland's solution three times a week. The rest of the time plants were supplied with deionized water.

At the end of the 4th week following MoO_3 application (both I and II) each time (A, B, and C, respectively), half of the eight replicates of each treatment were removed and studied. The nitrogen fixing activity of plants was measured by the acetylene reduction technique[16]. The extraction, purification, and determination of leghaemoglobin content in the nodules was done according to the Johnson and Hume's method[8]. The recognition and separation of multiple leghaemoglobin components by gel electrophoresis was achieved by the Appleby's mehtod[2]. After the leaf area of the plants was measured, the total chlorophyll content in the leaves of each plant was determined by the Arnon's method[3].

The plant productivities of each treatment were measured as soon as the color of the pods became brownish black. The pods filled with seeds were harvested and dried in an oven at a constant temperature of 75 C for 36 hours. Then the number of seeds and pods were counted, the dry weight of seeds and pods were weighed separately. Those studies were all carried out in the northern part of Taiwan.

RESULTS

The size and number of nodules changed according to the stage at which the plants were

treated with molybdenum. When plants were treated with molybdenum at the seedling stage, the number of large nodules, which had a diameter more than 2 mm, and those with small nonules, that is with diameters less than 2 mm, were nearly the same, but when treatment with molybdenum was delayed to the pre-flowering stage or pre-pod filling stage, the majority of nodules were large, and only relatively few had small nodules. Although there was no significant difference in the total number of nodules between plants treated with molybdenum at pre-flowering or pre-pod filling stage, the latter had heavier nodules than the former (Table 1 and 2). Besides, plants gave a different response to the molybdenum concentrations used and the way that molybdenum was applied at the different growth stages. Plants receiving molybdenum at the seedling stage (A) from the soil with added molybdenum produced a larger number of nodules and with greater weight. When the concentration of molybdenum was increased, plants treated with molybdenum either at pre-flowering stage (B) or pre-pod filling stage (C), showed that a concentration above 200 gm/ha or 100 gm/ha caused a reduction in

Table 1. Effects of molybdenum on the number and size of soybean nodules. The nodules were harvested and measured 4 weeks after adding molybdenum directly to the soil in pots.

Measurement / MoO_3 (gm/ha)		Nodule diameter >2 mm		Nodule diameter <2 mm		Total	
		number / plant	weight (gm) / plant	number / plant	weight (gm) / plant	number / plant	weight (gm) / plant
A	000	27	0.220	29	0.084	56	0.304
	100	46	0.363	28	0.071	74	0.434
	200	45	0.430	31	0.076	76	0.506
	300	47	0.410	40	0.104	87	0.514
B	000	62	0.767	17	0.043	79	0.810
	100	101	1.384	15	0.032	116	1.416
	200	117	1.480	13	0.025	130	1.505
	300	106	1.120	11	0.024	117	1.144
C	000	92	2.195	8	0.018	100	2.213
	001	131	2.818	4	0.011	135	2.829
	200	111	2.273	2	0.004	113	2.277
	300	112	2.153	2	0.003	114	2.162

The surface area of each pot is 254.47 cm^2.

Table 2. Effects of molybdenum on the number and size of nodules. The nodules were harvested and measured 4 weeks after spraying molybdenum on the leaves of soybeans planted in pots.

Measurement / MoO_3 (gm/ha)		Nodule diameter >2 mm		Nodule diameter <2 mm		Total	
		number / plant	weight (gm) / plant	number / plant	weight (gm) / plant	number / plant	weight (gm) / plant
A	000	27	0.220	29	0.084	56	0.304
	100	55	0.642	21	0.058	76	0.700
	200	48	0.420	35	0.104	83	0.524
	300	42	0.363	52	0.158	94	0.521
B	000	62	0.767	17	0.043	79	0.810
	100	102	1.389	10	0.028	112	1.417
	200	73	1.042	26	0.702	99	1.114
	300	67	1.005	28	0.066	95	1.071
C	000	92	2.195	8	0.018	100	2.213
	100	117	2.125	3	0.000	120	2.175
	200	110	2.100	5	0.010	115	2.060
	300	77	1.920	3	0.008	80	1.928

nodule numbers and nodule weight. However, if molybdenum was supplied to plants at these two stages (B and C) by spraying the microelement onto the foliar parts (Table 2) instead of being applied to the root system (Table 1), a cocentration of molybdenum higher than 100 gm/ha had a depressing effect on nodulation. Apparently, molybdenum has a stimulative effect on the increase of both nodule number and nodule weight, no matter at what growth stage the molybdenum is applied. The treated plants always had more numerous and heavier nodules than untreated ones (Table 1 and 2).

The leghaemoglobin can be separated and recognized in four components, i. e. leghaemoglobin 1, 2, 3, and 4, respectively, by the gel electrophoresis, no matter at what stage the plants are treated with molybdenum. However, 2 or 2-3 components of leghaemoglobin were found only in the snake bean or serredella (ref. Appleby). These four components of soybean leghaemoglobin showed different sensitivities to exposure to air. The component 1 (the slowest electrophoretic component) was much more easily degraded or denatured in the air than the other three components. The reddish-brown color of component 1 gradually faded away at the end of electrophoresis. The R_f value as well as the band-width of each electrophoretic component was not changed significantly by molybdenum treatment (Fig. 1).

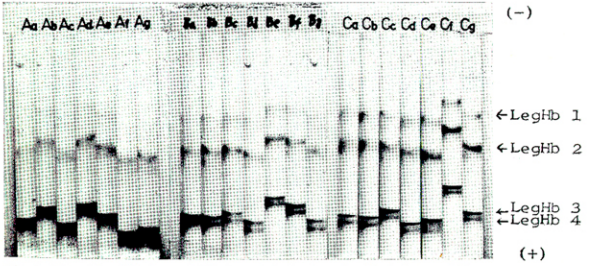

Fig. 1. Separation of leghaemoglobin by gel electrophoresis. The leghaemoglobin were extracted from nodules of soybeans grown in pots. Molybdenum applied to plants at seedling stage (A), pre-flowering stage (B), and pre-pod filling stage (C). The quantities (gm/ha) of molybdenum applied to plants by adding to roots (soils) were 000 (a), 100 (b), 200 (c), and 300 (d), by spraying onto leaves were 100 (e), 200 (f), and 300 (g), respectively. LegHb: leghaemoglobin

Plant appearances were influenced by the treatments with molybdenum. Both plant height and foliar area were obviously promoted by the molybdenum. The treated plants usually were taller than the untreated (Table 5, 6), and the leaf area of treated plants was larger (Table 4). The chlorophyll content in the leaves was changed with the fluctuation of the foliar area of the plants (Table 4). The leaves of plants lacking molybdenum appeared pale green but plants treated with molybdenum had a high level of chlorophyll in their leaves.

The pod weights increased along with the pod numbers (Table 5 and 6). The productivity of the plants could be based on seed weight and numbers of seeds harvested (Table 5 and 6). The relative increments of yields of soybean by molybdenum treatments were from 2% to 69% (I), 0% to 41% (II), depended upon the method and time of molybdenum treatment. Our data

Table 3. Comparative effects of molybdenum on nodule weights, leghaemoglobin content, and nitrogen fixing activity of soybean plants.

Measurement / MoO_3 (gm/ha)		Nodule weight mg/nodule (>2 mm)		Nitrogen fixing activity μmole C_2H_2/plant/hr		Leghaemoglobin mg/gm nodule	
		I	II	I	II	I	II
A	000	8.1	8.1	0.632	0.632	7.302	7.302
	100	7.9	11.7	1.292	1.050	9.566	8.028
	200	9.6	8.8	4.648	4.158	10.069	8.126
	300	8.7	8.6	3.413	3.022	9.058	9.036
B	000	12.4	12.4	7.862	7.862	8.846	8.846
	100	13.7	13.6	8.600	8.967	9.582	8.572
	200	12.6	13.7	11.167	9.695	9.846	9.120
	300	10.6	15.0	9.866	8.418	9.574	9.634
C	000	23.9	23.9	5.320	5.320	9.018	9.018
	100	21.5	18.2	6.578	5.915	9.646	92338
	200	24.5	19.5	6.625	5.954	8.572	8.502
	300	19.2	24.9	5.820	5.816	7.854	6.972

Table 4. Changes of leaf area and chlorophyll content in leaves of soybeans grown in pots 4 weeks after molybdenum treatment.

Measurement / MoO_3 (gm/ha)		Leaf area (cm^2/plant)		Chlorophyll content (mg/g tissue)	
		I	II	I	II
A	000	116.86	116.86	1.812	1.812
	100	167.48	149.65	2.028	2.116
	200	164.04	186.54	2.188	2.028
	300	214.03	176.54	2.188	2.246
B	000	270.59	270.59	3.188	3.188
	100	375.75	408.69	3.203	3.478
	200	552.42	427.46	3.246	2.913
	300	578.67	456.84	3.928	3.435
C	000	223.41	223.41	2.464	2.464
	100	283.74	255.91	2.638	2.478
	200	234.03	208.09	2.333	2.246
	300	238.72	230.91	2.257	2.913

Table 5. Increments of soybean harvests from soybeans grown in pots after molybdenum was added directly to the soil. The following figures are the average value of 4 replicates.

Harvests / MoO_3 (gm/ha)		Seed weight plant (gm)	Seed number plant	Pod weight plant (gm)	Pod number plant	Plant height (cm)
A	000	3.238	18.25	5.143	10.30	20.98
	100	3.307	18.50	5.760	10.50	28.43
	200	4.178	24.25	6.300	13.00	24.98
	300	3.410	20.50	5.840	11.75	28.00
B	000	3.238	18.25	5.143	10.30	20.98
	100	4.765	27.75	6.665	11.75	36.78
	200	5.468	36.50	8.493	19.00	36.38
	300	5.228	32.00	8.058	15.75	31.93
C	000	3.238	18.25	5.143	10.30	20.98
	100	3.638	24.50	6.823	13.25	29.28
	200	3.778	24.75	4.695	9.67	29.88
	300	3.800	21.00	5.708	11.25	32.32

Table 6. Increments of soybean harvests from soybeans grown in pots when molybdenum was sprayed on the leaves. The figures shown below are the average value of 4 replicates.

MoO_3 (gm/ha) \ Harvests	Seed weight plant (gm)	Seed number plant	Pod weight plant (gm)	Pod number plant	Plant height (cm)
A 000	3.238	18.25	5.143	10.30	20.98
100	3.493	19.52	5.295	10.50	26.90
200	3.770	20.25	5.728	11.00	29.78
300	3.765	21.00	5.858	11.75	24.50
B 000	3.238	18.25	5.143	10.30	20.98
100	4.578	24.10	6.993	11.25	36.05
200	4.462	22.70	6.967	12.67	31.25
300	3.498	18.00	5.207	10.50	31.20
C 000	3.238	18.25	5.143	10.30	20.98
100	3.630	21.75	5.970	11.25	28.75
200	3.713	21.25	5.675	11.25	28.53
300	3.713	22.25	5.655	11.00	27.80

Table 7. Comparative length of the life cycle of soybeans after molybdenum treatments. This experiment was carried out from June ro te October 17 outside the room in Taipei

MoO_3 (gm/ha)	Length of life cycle I (days)	II (days)
A 000	109	109
100	104	100
200	103	105
300	102	106
B 000	109	109
100	96	106
200	95	106
300	103	109
C 000	109	109
100	108	102
200	108	103
300	108	106

conclusively demonstrates that the addition of molybdenum to soils rather than being sprayed on leaves causes the plants to grow better (Table 1, 2, 3, 4) and give higher productivity (Table 3, 5, 6), and plants supplied with molybdenum at pre-flowering stage rather than either at seedling stage or pre-pod filling stage usually yield better results (Table 1, 2, 3, 4, 5, 6, 7). The life cycle of untreated plants was between 108 to 110 days but that could be shortened to 95 days by molybdenum treatments (Table 7).

DISCUSSION

In many parts of Pingtung County, the soil pH falls below 6, soybeans cultivated in these acidic areas show light yellow chlorosis of the leaves, the leaf blades fail to expand, and the plants give poor yields. However, these abnormal visual appearances can be corrected by the supply of additional combined nitrogen to the plants (Hong, personal communication). This strongly suggests that plants grown in these acidic soils are suffering from nitrogen starvation. In 1975, Mr. Hong, a senior technician of Koashiung Agricultural Experimental Station, improved

the acidic property of soils by addition of lime, 2000 kg per hectare, which resulted in increased soybean harvests of 25–30%. This indicates that lime can correct the acidic property of the soil and increase the availability of molybdenum in the soils, consequently enhancing the nitrogen fixing activity of soybean plants. It has been reported that the uptake of molybdenum was severely depressed by a decrease in the pH value of soils, because the acidic soils contain ferric or aluminum oxides that strongly react with molybdenum[11,12]. Based upon our observations and measurements in this study, the characteristic symptoms of molybdenum deficiency in symbiotic grown soybean plants were not essentially different from symptoms of nitrogen deficiency, i. e. plants had small leaf area, low chlorophyll content (Table 4), scattered patterns of nodule distributions on the roots, reduction in nodule number and nodule weight (Table 1, 2), decrease in plant productivity (Table 5, 6). Additional supply of molybdenum to plants alleviate deficiency symptoms through enhancement of nitrogen fixing activity of plants (Table 3).

The nitrogen fixing activity of a plant generally parallels the nodule numbers and nodule weights. However, we have found some exceptions in this study, as shown by the data in Table 3, the plants which were treated with molybdenum at the pre-flowering stage had a higher nitrogen fixing activity than those treated at the pre-pod filling stage, but the latter plants had heavier nodules than those receiving the pre-flowering treatment. Apparently, plants treated with molybdenum at the pre-pod filling stage formed many ineffective nodules. It has been reported that there exists a reciprocal relationship between the efficiency of nitrogen fixation of nodules and number of nodules because of a competition for energy among the nodules, but this seems unlikely to be the case as seen in this study. The requirement of molybdenum for the nitrogen fixing process per se can be logically explained in that molybdenum is an essential element for nitrogen fixation (Table 3). The experiments by Jensen and Betty (1943) (quoted by Janssen and Vitosh) showed that legumes contained from five to fifteen fold of molybdenum in their root nodules as compared with that in other parts of the plant. The time of molybdenum application to plants causes differences both in morphological characters and yields. So in order to get the maximum of plant height (Table 5, 6), leaf area and chlorophyll content in the leaves (Table 4), level of nodulation (Table 1, 2, and 3), nitrogen fixing activity (Table 3), and other characteristics of productivity (Table 5, 6), the best time to apply molybdenum is at their pre-flowering stage. Many workers have reported that the climax of the nitrogen fixing activity is found at the blooming stage[13,14]. Therefore, it is logical to believe that a high rate of nitrogen fixing enzyme synthesis may occur at the pre-flowering stage. It may be too early or too later to supply molybdenum at the seedling or at the pre-pod filling stage, since plants may not be in a condition to receive molybdenum for growth and development at a middle or latter growth stage, or at early or middle growth stage, but if plants are treated with molybdenum at the pre-flowering stage the best results are obtained. Our data demonstrates that it was much more harmful to photosynthesis, nodulation, nitrogen fixation, and productivity if plants were under a low or deficient molybdenum condition at the middle and latter growth stage than that at early stage. As to the methods of application, supplying molybdenum to the root system (soils) usually yielded better results (Table 1, 2, 3, 4, 5, 6) than spraying on the leaves. It suggests that uptake of molydenum by the root system is more effeicient and effective than by the foliar absorption. This may be due fact that molybdenum is not retained on the leaf as long as in the soil, and that the total absorptive surface of leaf is not as great as that those of root, in addition, the translocation of molybdenum from the leaf to nodules may not be so readily available as that of molybdenum from root to nodules for the synthesis of the nitrogen fixing enzyme.

The evidence shown in Table 3 and 4 also confirms the fact that the magnitudes of the nitrogen fixation in the root nodules is correlated with photosynthetic activity of plant. The author's previous study[16] showed that, under normal conditions, the photosynthetic activity of

plants regulates the nitrogen fixation of root nodules, because of the need for carbohydrates. But on the contrary, in this study, plant photosynthesis seems to be controlled by the nitrogen fixation. Although the direct involvement of molybdenum in symbiotic nitrogen fixation has been suspected for many years, it has been realized that molybdenum is a component of Franction 1 of nitrogenase[4,6,10,26], which is formed in bacteroids of nodules of leguminous plants supplied with molybdenum. But there have no previous evidence to support the idea that molybdenum had any direct function in chlorophyll synthesis or photosynthesis. Therefore, under an inadequate molybdenum or chemical nitrogen fertilizer condition, plants show chlorosis. This chlorotic appearance of the plants was apparently due to low nitrogen fixing activity of nitrogenase, which consequently resulted in nitrogen starvation, and so entailed a reduction in leaf size, chlorophyll synthesis, and photosynthesis. Besides, there might have been a "source-sink" relationship between photosynthesis and nitrogen fixation. the greater demand for carbohydates by nitrogen fixation could promote vigrous synthesis of carbohydrates by photosynthesis.

It has been reported that leghaemoglobin is located in the bacteroids of the nodule and is an important oxygen diffusive carrier for the nitrogenae[2,7], and so correlates with nodule effectiveness. However we have found that, the leghaemoglobin content in nodules does not change much when plants are treated with molybdenum (Table 3), and neither did the electrophoretic patterns change after treatment with molybdenum. On the other hand the nitrogen fixing activity of the plants does fluctuate with the addition of molybdenum (Table 3). Therefore, the changes of nitrogen fixing activity of plants following molybdenum treatment cannot be interpreted either by the leghaemoglobin content or the electrophoretic patterns.

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