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Effect of Mg, Zn and Mo salts on nitrate reductase activity and soluble protein content in leaves of *Quercus serrata*

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Abstract

Leaves of *Quercus serrata* seedlings were sprayed with solutions of different concentrations of Mg, Zn and Mo-salts and activity of nitrate reductase (NRA) and soluble protein contents were determined in fresh leaves of different ages. The optimum dose for magnesium sulphate and zinc sulphate was 2.5 mM while for ammonium molybdate it was 0.01 mM. Under optimum doses of these nutrients high levels of NRA and protein contents were maintained upto 28 d of spray. Observations on NRA were further confirmed by leaf direct treatment and excised shoot-dipping experiments which showed maximum NRA at comparatively much lower concentrations of ions than those used for spray.

Introduction

In India oaks (*Quercus* sp.) are distributed from Jammu and Kashmir in West to Manipur in North-East all along the temperate and sub-Himalayan belt. The improvement of quality foliage in respect of chemical constituents of leaves seems to be essential part of the present day need. In this respect, the effects of fertilizers applications on the growth and yield parameters were studied. For example, improvement in shoot and root growth of oak seedlings has been achieved by foliar applications of fertilizers (Dixon *et al.* 1981a, 1984). Inoculation of oak seedlings with mycorrhizal fungi has also been reported to improve seedling growth in greenhouse and nursery trials (Dixon *et al.* 1981a, b, Maronek and Hendrix 1979). Most of the studies with fertilizers comprise NH_4NO_3, urea, P_2O_5 and KCl in different doses.

There are little reports in literature concerning the quality of the leaf in respect of its soluble protein content which is an important constituent of the raw material for cocoon production. In the present paper *in vivo* activity of nitrate reductase - the first enzyme of nitrogen assimilation pathway and the soluble protein level in leaves have been determined following foliar application of mineral nutrients (zinc sulphate, magnesium sulphate and amonium molybdate).

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Materials and methods

Seeds of *Quercus serrata* were collected from natural forests of Imphal during the month of October - November. Analytical grade chemicals were used for foliar spray and for biochemical estimations.

Surface sterilized seeds (with 2 % bleaching powder for 5 min) were soaked in tap water for 72 h. The seeds were heaped and covered with a clean wet gunny bag. Germination started after 10 d of soaking. The germinated seeds were transferred in a polythene bag filled with soil media (farm yard manure:soil:sand 1:1:1). Alternate day watering was done.

The seedlings were raised in natural condition during July with average maximum and minimum temperature of 31.4 °C and 22 °C, respectively, relative humidity of 83 % and rainfall 16.50 mm per month.

Solutions of magnesium sulphate and zinc sulphate were prepared in the range of 1.0, 2.5, 5.0 and 10.0 mM while that of ammonium molybdate were in the range of 0.005, 0.01, 0.05 and 0.1 mM in distilled water. Spray treatment of an equal volume of each nutrient (5.0 cm^3 $plant^{-1}$) was given to the foliage of 2 months old plants with the help of a micro-sprayer. The control plants were sprayed with the same volume of distilled water.

NRA was determined *in vivo* in fresh leaves on zero day, *i.e.*, just before spray treatment and continued at weekly interval for 4 weeks. The leaves were harvested from 09.00 to 09.15 on each experiment day. Care was taken in the selection of leaves for enzyme assay. Few leaves (3 to 4) from the middle portion of the shoots were selected for these study as these leaves were neither too young nor too old. The leaves were cut into narrow strips and mixed uniformly, weighed freshly (250 mg sample for each assay) and used immediately for *in vivo* NR assay according to Srivastava *et al.* (1980). For soluble proteins content estimation the leaf harvesting was done as mentioned above and proteins were determined by the method of Lowry *et al.* (1951). All these experiments were conducted in triplicate.

Specific studies on *in vivo* NRA: Two additional experiments were conducted to confirm whether the increase in NRA in sprayed plants was due to the inducible effect of mineral nutrients or influenced by some other factors. These experiments were conducted in triplicate and are described below:

(*A*) Direct treatment experiment - the *in vivo* NRA in leaves was determined in presence of different concentrations of nutrients in the assay mixture for an incubation period of 30 min. The final concentrations of these salts maintained in the assay mixture were: 0, 0.01, 0.05, 0.1, 0.25, 0.50, 1.0, 2.5 and 5.0 mM for magnesium sulphate; 0, 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 mM for zinc sulphate, and 0, 0.001, 0.005, 0.0075, 0.01, 0.05, 0.075 and 0.1 mM for ammonium molybdate. In all cases the control was supplied with distilled water.

(*B*) Excised shoot-dipping treatment - the shoots were cut from the basal portion of 2 months old plants. These shoots were immersed in 20 cm^3 solutions of different concentrations of three inorganic salts in dark vials. The concentrations for this experiment were the same as that of the direct-treatment experiment. The sets were

kept under a 100 W tungsten lamp at a distance of 1 m for 20 h. After 20 h of excised shoot-dipping treatment leaves from these shoots were used for the *in vivo* NR assay as described earlier.

All the colorimetric determination were carried-out by a Bausch and Lombs' *Spectronic-20*. The results were statistically evaluated.

Results

The *in vivo* NRA in control plant increased with age upto 14 d of spray, remained unaltered upto 21 d followed by a decline on day 28, whereas the level of phosphate-

Table 1. *In vivo* nitrate reductase activity (NRA) in fresh leaves of different ages of control *Quercus serrata* plants and of those sprayed with different concentrations of magnesium sulphate, zinc sulphate and ammonium molybdate. Two-months-old seedlings were used for leaf spray treatment. The NRA on zero day was 2.13 ± 0.04 μmol(NO_2^-) h^{-1} g^{-1}(leaf f.m.).

Conc. of nutrients [mM]	NR Activity [μmol(NO_2^-) g^{-1}(leaf f.m.) h^{-1}]			
	Time [d after spray]			
	7	14	21	28
0 (Control)	2.13 ± 0.01*	2.55 ± 0.23	2.55 ± 0.06	1.91 ± 0.09
Mg-salt				
1.0	2.76 ± 0.02	3.19 ± 0.14	3.55 ± 0.15	2.91 ± 0.15
2.5	3.76 ± 0.05	4.11 ± 0.18	4.26 ± 0.22	3.40 ± 0.24
5.0	3.05 ± 0.01	3.40 ± 0.21	3.69 ± 0.24	2.62 ± 0.28
10.0	1.77 ± 0.15	2.05 ± 0.35	2.05 ± O.34	2.34 ± 0.31
C.D. at 5 %	0.048	0.085	0.098	0.145
Zn-salt				
1.0	2.48 ± 0.09	2.98 ± 0.06	3.40 ± 0.04	2.91 ± 0.22
2.5	3.26 ± 0.07	3.97 ± 0.07	4.75 ± 0.06	3.40 ± 0.28
5.0	2.69 ± 0.05	3.33 ± 0.12	3.69 ± 0.14	2.76 ± 0.18
10.0	2.13 ± 0.12	2.55 ± 0.15	2.69 ± 0.21	2.27 ± 0.11
C.D. at 5 %	0.005	0.057	0.082	0.129
Mo-salt				
0.005	3.19 ± 0.03	3.55 ± 0.16	3.97 ± 0.16	2.98 ± 0.24
0.01	4.47 ± 0.08	4.97 ± 0.24	5.32 ± 0.24	3.97 ± 0.41
0.05	2.98 ± 0.18	3.19 ± 0.31	3.40 ± 0.22	2.62 ± 0.39
0.1	1.77 ± 0.12	2.13 ± O.25	2.27 ± 0.16	2.27 ± 0.44
C.D. at 5 %	0.032	0.056	0.067	0.238

*Standard error of the mean (n = 3)

buffer-soluble protein in control plants was unaltered during 7 to 14 d and afterward no significant difference was noticed upto 28 d. With all the inorganic nutrients the

NRA as well as soluble protein were enhanced with increase in the concentration to a certain level (Tables 1 and 2). However, in case of 10.0 mM magnesium sulphate and 0.1 mM ammonium molybdate inhibition was noticed. The optimum concentrations for magnesium sulphate and zinc sulphate were noticed at 2.5 mM while for ammonium molybdate it was obtained at 0.01 mM (Tables 1 and 2). Thus, the NRA

Table 2. Phosphate-buffer-soluble proteins in fresh leaves of different ages of control *Quercus serrata* plants and of those sprayed with different concentrations of magnesium sulphate, zinc sulphate, and ammonium molybdate. Two-months-old seedlings were used for leaf spray treatment. The soluble protein on zero day was 49.23 ± 0.23 mg g^{-1}(leaf f.m.).

Conc. of nutrients [mM]	Phosphate-buffer-soluble protein [mg g^{-1}(leaf f.m.)]			
	Time [d after spray]			
	7	14	21	28
0 (Control)	53.71 ± 0.16*	53.71 ± 0.21	52.22 ± 0.08	53.22 ± 0.19
Mg-salt				
1.0	67.14 ± 0.03	74.60 ± 0.25	92.50 ± 0.15	67.14 ± 0.15
2.5	120.85 ± 0.15	140.24 ± 0.22	170.08 ± 0.21	149.20 ± 0.09
5.0	93.99 ± 0.42	98.47 ± 0.15	111.90 ± 0.07	92.50 ± 0.02
10.0	56.69 ± 0.13	64.15 ± 0.08	70.12 ± 0.04	62.66 ± 0.05
C.D. at 5 %	1.108	2.679	2.987	4.862
Zn-salt				
1.0	64.15 ± 0.01	71.61 ± 0.16	80.56 ± 0.24	62.66 ± 0.15
2.5	92.50 ± 0.15	113.39 ± 0.31	122.34 ± 0.19	80.56 ± 0.14
5.0	58.18 ± 0.21	76.09 ± 0.25	85.04 ± 0.42	56.69 ± 0.09
10.0	53.71 ± 0.25	59.68 ± 0.28	62.66 ± 0.22	53.71 ± 0.04
C.D. at 5 %	2.025	2.732	3.058	3.721
Mo-salt				
0.005	83.55 ± 0.21	95.48 ± 0.09	105.93 ± 0.21	80.56 ± 0.19
0.01	119.36 ± 0.15	185.00 ± 0.08	259.60 ± 0.32	167.10 ± 0.21
0.05	70.12 ± 0.18	83.55 ± 0.14	92.50 ± 0.34	64.15 ± 0.25
0.1	38.79 ± 0.11	56.69 ± 0.13	67.14 ± 0.42	58.18 ± 0.28
C.D. at 5 %	1.879	2.219	3.135	4.029

*Standard error of the mean (n = 3).

and soluble protein were dose dependent with all the three nutrients. With optimum dose of magnesium sulphate, zinc sulphate and ammonium molybdate the levels of NRA and soluble protein were higher by 67.05 and 225.69, 86.2 and 134.27, and 108.62 and 397.12 %, respectively. Among the three nutrients ammonium molybdate proved to be most effective.

The regression analyses and correlation coefficient between soluble protein and *in vivo* NRA in fresh leaves on day 21 with all the three mineral nutrients were

analysed and the values of regression coefficient in case of magnesium sulphate, zinc sulphate and ammonium molybdate were 0.85, 0.91 and 0.99 respectively and all the values were significant at 1 % level (Fig. 2).

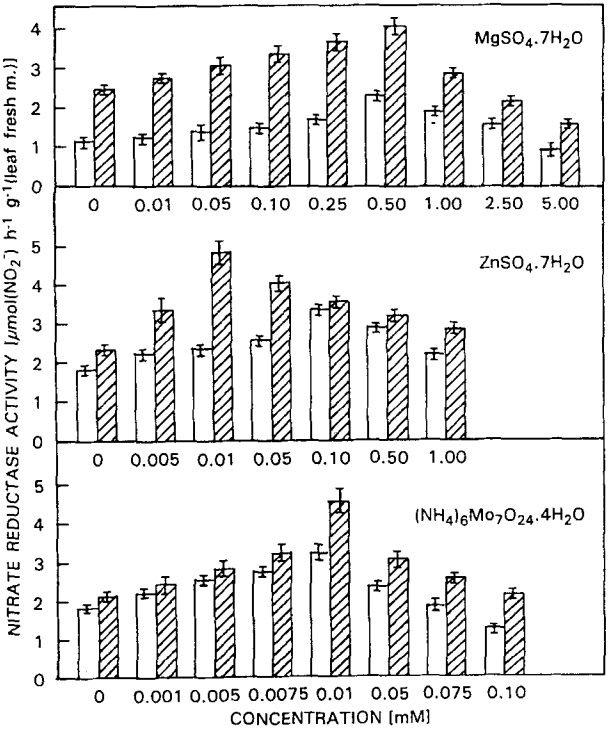

Fig. 1. *In vivo* NRA in leaves of *Quercus serrata* in two ways with different concentrations of mineral nutriens. Direct treatment (*open columns*) during NR assay (30 min incubation) and excised shoot-dipping treatment (*full columns*) for 20 h followed by NR assay (30 min incubation).

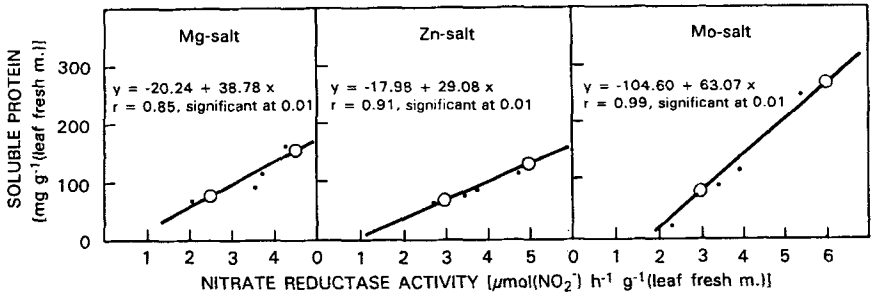

Fig. 2. Regression analyses and correlation coefficient of the data on day 21 given in Tables 1 and 2 for three different mineral nutrients. Open circles show the points used for drawing the line.

In case of direct treatment by nutrients on the leaves all the three salts increased the NRA. The optimum doses were: 0.5 mM magnesium sulphate, 0.1 mM zinc

sulphate and 0.01 mM ammonium molybdate (Fig. 1). The observations recorded in the excised shoot-dipping experiments were similar to that of the direct-treatment experiment for all the three nutrients. The optimum concentrations of magnesium sulphate and ammonium molybdate coincided with the optima as observed for direct treatments, but in case of zinc sulphate the optimum dose was recorded at very low concentration (0.01 mM) instead of 0.1 mM as observed in the direct treatment experiment (Fig. 1). Data on NRA from the above two types of experiments also revealed that NRA was higher in the excised shoot-dipping experiments and lower in the direct-treatment experiment.

Discussion

The observed enhancement of NRA and soluble protein levels in leaves of the oaks sprayed with different doses of various nutrients can be of interest because of their physiological significance as well as utilitarian value (Broyer and Stout 1959, Evans and Sorger 1966, Epstein 1972, Hewitt *et al.* 1976, Rains 1976, Oaks *et al.* 1977). Therefore, the stimulation observed in NRA and soluble protein level would have been caused through general stimulatory effect of these ions on the cell.

The results presented here clearly demonstrated that the spraying of leaves by ammonium molybdate was the most effective amongst the three nutrients which is possibly due to its action as a co-factor for nitrate reductase. Further verification of these results by two additional experiments (direct-treatment and excised shoot-dipping experiment) revealed that the enhancement in NRA by these nutrients were due to their effect on induced synthesis of the enzyme.

From the above observations it is clearly evident that high level of soluble protein was found only in those treatments of nutrients where the NRA was also higher and *vice versa*. Thus the *in vivo* NRA can be taken as an index of higher protein level in the leaves. Also, it has been established by Johnson *et al.* (1976) that NRA is such a parameter which ultimately determines future of the actively growing plant particularly in terms of yield or leaf biomass. Thus, NRA may be considered as an index of soluble protein. Moreover, the coefficient of correlation between soluble protein and NRA analysed for day 21 indicated a positive correlation for all the nutrients (Fig. 2).

Therefore, spray of optimum doses of these nutrients which enhance NRA is useful as that treatment increased protein level in the leaves and that protein enriched leaves are preferred by the oak-tasar larvae. From the view point of silkworm nutrition, it has been observed that leaves obtained from plants sprayed with minerals containing Mg^{2+}, Mn^{2+}, Fe^{2+},Fe^{3+}, Mo^{5+}, Mo^{6+} and Zn^{2+}, have a potentiality to enhance the larval development and cocoon yields (Horie *et al.* 1967, Vishwanath 1979). This is in agreement with our earlier observation where foliar treatment of optimum doses of Mg, Zn and Mo salts improved quality of oak-tasar cocoons (Ghosh and Srivastava 1993).

The over all discussion emphasizes the need for use of these mineral nutrients at optimum level, for, ortherwise it would not only be a waste, in economic sence, but may also prove counterproductive.

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