# Leaf Analysis as a Guide to Sulfur Fertilization of Legumes

W.Z. Huang, J.J. Schoenau and K. Elmy
Department of Soil Science, University of Saskatchewan
Saskatoon, Saskatchewan, CANADA S7N 0W0

## ABSTRACT

Tissue analysis is a diagnostic tool which can be used in identifying S deficiencies and predicting S fertilizer requirements of crops. A water extraction procedure for removal and measurement of inorganic sulfate in plant leaves was developed and assessed as a measure of S availability in four legume crops. Relationships between sulfate concentration and yield were determined for alfalfa (*Medicago sativa* L.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba*) and lentil (*Lens culinaris*) grown on two Saskatchewan soils, with five rates of S fertilizer supply, in a growth chamber experiment. The sulfate concentration in the leaf tissue was measured at the time of seventh leaf and early flowering stages to estimate the current S status of the plants. The results showed significant relationships between the water extractable sulfate in the leaf and the supply of available S in the soils. The water extraction procedure is recommended for routine analyses because of its simplicity and sensitivity.

## INTRODUCTION

Plant tissue analysis is being used throughout the world to diagnose nutrient deficiencies and excesses for a wide variety of crop species. Plant growth will be restricted when the concentration of an essential element is lower than a critical level. Plant analyses may be grouped into two general classes: (1) tissue analysis which measures only one form of the nutrient, and (2) total analysis which measures total nutrient concentration. Plant analysis in the first category usually is quicker and simpler than total analysis and often a specific nutrient fraction in tissue is more sensitive to availability than the total nutrient concentration.

Legumes have a high requirement for S and frequently respond to S fertilization. Total S and $SO_4$-S, both expressed as percentages of the dry matter, have been used as indexes of S sufficiency in alfalfa. Critical concentrations of total S in alfalfa (*Medicago sativa* L.) are dependent upon stage of development (Pumphrey and Moore, 1965), but

generally group around 0.20 to 0.25% S in whole tops (Mertz and Matsumato, 1956; Ensminger and Freney, 1966; Martin and Walker, 1966; Martin and Matocha, 1973; Cornforth and Sinclair, 1982). Approximately 150 μg/g $SO_4$-S in the second to fourth mature leaf, and 40 μg/g $SO_4$-S in the midstems at the bloom stage are considered critical concentrations of $SO_4$-S under greenhouse conditions for alfalfa (Ulrich et al. 1967). However, S-deficient alfalfa contained 250 to 300 μg/g $SO_4$-S, and S-fertilized plants contained over 700 μg/g $SO_4$-S in a similar field study, suggesting a critical level of whole plant tops at early bloom near 500 μg/g $SO_4$-S (Martin and Walker, 1966). Nitrogen to S ratios in plant tissue have also been considered to predict the S requirements of legumes and other plants (Dijkshoorn et al. 1960; Leggett et al. 1966; Dijkshoorn and Van wijk, 1967; Stewart and Porter, 1969; Aulakh et al., 1976; Janzen and Bettany, 1984). Nuttall (1985) has suggested that the N/S ratio could be used as an index of S availability for alfalfa. However, little information exists on S critical concentrations for grain legumes such as chickpea, faba bean and lentil.

A plant tissue analysis method suitable for estimating the S status of plants should be rapid and simple. Fresh sample extraction has shown to be a good index of K deficiency (Huang et al., 1992). Grunau and Swiader (1986) have successfully used a Dionex ion chromatograph to measure the anion content of plant leaf tissues. The use of inorganic sulfate concentrations in plant leaf tissues is attractive for determining S status if $SO_4$-S can be determined rapidly and accurately and if the $SO_4$-S is sensitive to S availability in soil.

The objective of this study was to develop and evaluate a simple tissue test to assess S availability in forage and grain legumes, involving the use of water extraction to remove inorganic sulfate in legume leaf tissues. This paper presents the relationships found between %$SO_4$-S in plant leaf tissue and the supply of available S in soil and yields of alfalfa (*Beaver*), chickpea (*Desi*), faba bean (*Tick*) and lentil (*Laird*) grown on two Saskatchewan soils.

## MATERIALS AND METHODS

*Laboratory study:*

To optimize the extraction parameters in the proposed water extraction procedure, the effect of tissue sample size and extracting time were evaluated.

Samples of plant tissue of approximately 2 g were removed from growing plants using a hole punch. The 2 g samples were then cut uniformly into smaller pieces of 0.1, 0.5 and 1.0 $cm^2$ and shaken on a gyratory shaker at 300 r.p.m. for different times with 50

mL deionized water at room temperature. Three extraction times (0.5, 1 and 2 hr) were compared. Following the shaking, the extractions were filtered through a filter paper, and inorganic sulfate in the extracts measured using a Dionex ion chromatograph (Grunau and Swiader, 1986). After extraction, the residual leaf tissue was dried at 60°C, weighed and analyzed for total S using a Fisher S analyzer.

*Growth chamber experiment:*

A growth chamber experiment was conducted with two Gray Luvisol soils, a Sylvania loamy sand of low $SO_4$-S status (soil 1) and a Waitville sandy loam of high $SO_4$-S status (soil 2) (Table 1). Sulphur was applied at the rate of 0, 5, 15, 30, and 45 mg S/kg soil.

Plastic pots were filled with 1000 g air dry soil. To each pot a nutrient solution containing nitrogen, phosphorus and potassium was added plus an additional 10 mL of a basal micronutrient solution. Approximately 15 seeds of alfalfa, 12 seeds of chickpea, 12 seeds of faba bean, or 12 seeds of lentil were sown into each pot. After germination, the pots were thinned to 5 alfalfa plants, 4 chickpea plants, 5 faba bean plants, or 5 lentil plants per pot. The pots were transferred to a growth chamber with 16 hour day length, kept at 25°C during the day and 12°C at night. The soil moisture was maintained at 90% of field capacity by daily watering with deionized water. The pots were completely randomized and re-positioned every week to minimize any effects of uneven environmental factors such as light and temperature. After 4 weeks, additional N in solution was added to each of the pots at rates of 50 mg N/kg soil, to ensure that N deficiency did not limit growth.

TABLE 1. Properties of the 2 Saskatchewan soils used in the growth chamber experiment.

| Soil no. | Soil type | pH | Conductivity $mS \cdot cm^{-1}$ | Organic C% | $NO_3$-N | P | K | $SO_4$-S |
|---|---|---|---|---|---|---|---|---|
| | | | | | --------- µg/g soil --------- | | | |
| 1 | Sylvania loamy sand | 7.3 | 0.2 | 1.8 | 4.2 | 26.0 | 88 | 7.0 |
| 2 | Waitville sandy loam | 7.2 | 2.0 | 3.3 | 10.2 | 24.0 | 306 | 42.0 |

Two leaf samples were taken for tissue testing: one at seventh leaf stage (four weeks after seeding) and one at early flowering stage (six weeks after seeding). The

second to fourth mature leaves of each plant were sampled at the seventh leaf and early flowering growth stages. Tissue samples of approximately 2 g were obtained using a hole punch and were collected in 150 mL Erlenmeyer flasks. The flasks then were transferred to the laboratory where the samples were extracted with water and analyzed for inorganic sulfate.

## RESULTS AND DISCUSSION

*Effect of sample size and shaking time:*

Results of the analysis indicated that the amounts of inorganic sulfate extracted were mainly affected by the size of tissue pieces used. Extracting time longer than 0.5 hr did not appreciably increase the inorganic sulfate extracted (Table 2). The largest size (1.0 $cm^2$) gave the lowest amounts of inorganic sulfate extracted from the leaf tissues. When larger tissue pieces are used, there is less opportunity for the extracting solution to penetrate into the succulent interior of the leaf and remove sulfate. Damage to cells permits the contents of the cells to be more easily extracted. The overall extent of tissue and cell damage is lower when larger tissue fragments are obtained such as by using a larger punch. These observations point to the need for ensuring uniformity in sample piece size when water or other mild extractions are used as tissue tests. The extraction conditions which gave the highest efficiency of inorganic S extraction from the leaf tissue of the legumes, and was the most convenient to use, were 0.5 $cm^2$ and 0.5 hr extraction. Therefore, these extracting conditions were adopted in the study.

Table 2. Effect of extraction conditions on water extractable inorganic sulfate†.

| Extraction condition | | $\%SO_4$-S§ |
|---|---|---|
| Tissue sample size | time | |
| 0.1 $cm^2$ | 0.5 hr | 0.170 ± 0.03 |
| 0.5 $cm^2$ | 0.5 hr | 0.167 ± 0.02 |
| 1.0 $cm^2$ | 0.5 hr | 0.101 ± 0.02 |
| 0.5 $cm^2$ | 1 hr | 0.160 ± 0.02 |
| 0.5 $cm^2$ | 2 hr | 0.168 ± 0.02 |

† leaf samples were taken at the seventh leaf stage of alfalfa grown on the Sylvania soil.
§ as per cent of dry matter.

*Relationship between inorganic S in leaf tissue and S availability in soil:*

Table 3 presents means and standard deviations for triplicate tissue water extraction analyses for the four legumes grown on the Sylvania loamy sand soil (soil 1). The water extraction procedure was quite reproducible with coefficients of variation (C.V.) of around 10% in most cases.

TABLE 3. Water extractable inorganic sulfate values for the four legumes grown on the Sylvania soil†

| S levels | Alfalfa | | Chickpea | | Faba bean | | Lentil | |
|---|---|---|---|---|---|---|---|---|
| mg/kg | %S†† | Stdev | %S | Stdev | %S | Stdev | %S | Stdev |
| | | | | Seventh leaf growth stage | | | | |
| 0 | 0.11 | 0.02 | 0.13 | 0.01 | 0.030 | 0.004 | 0.32 | 0.07 |
| 5 | 0.14 | 0.01 | 0.16 | 0.01 | 0.034 | 0.002 | 0.35 | 0.05 |
| 15 | 0.15 | 0.01 | 0.16 | 0.01 | 0.035 | 0.002 | 0.39 | 0.03 |
| 30 | 0.17 | 0.02 | 0.19 | 0.01 | 0.035 | 0.002 | 0.40 | 0.08 |
| 45 | 0.17 | 0.02 | 0.20 | 0.03 | 0.042 | 0.009 | 0.40 | 0.31 |
| | | | | Early flowering growth stage | | | | |
| 0 | 0.021 | 0.001 | 0.10 | 0.01 | 0.020 | 0.001 | 0.10 | 0.03 |
| 5 | 0.022 | 0.002 | 0.12 | 0.01 | 0.020 | 0.003 | 0.10 | 0.01 |
| 15 | 0.031 | 0.001 | 0.12 | 0.02 | 0.022 | 0.002 | 0.14 | 0.04 |
| 30 | 0.030 | 0.003 | 0.14 | 0.01 | 0.027 | 0.003 | 0.14 | 0.02 |
| 45 | 0.034 | 0.006 | 0.14 | 0.02 | 0.024 | 0.005 | 0.14 | 0.01 |

† means of triplicate analyses.
†† as per cent of dry matter.

Regression analyses were used to describe the relationships between $SO_4$-S in leaf and S fertilizer rate applied. The correlation coefficients (r) and linear equation coefficients (a) are presented in Table 4. The results indicated significant relationships between inorganic sulfate concentrations in the legume leaves and the levels of S supply.

TABLE 4. Regression coefficients determined for the relationship between S supply and inorganic sulfate concentration (%) in four legume leaf tissues.

| Soil | | Alfalfa | Chickpea | Faba bean | Lentil |
|---|---|---|---|---|---|
| | | | Seventh leaf stage | | |
| 1 | Correlation coefficient (r) | 0.899 | 0.944 | 0.909 | 0.857 |
| | Slope (a)† | 0.00125 | 0.00142 | 0.00021 | 0.00165 |
| | | | Early flowering stage | | |
| | Correlation coefficient (r) | 0.897 | 0.898 | 0.773 | 0.847 |
| | Slope (a)† | 0.00028 | 0.00076 | 0.00012 | 0.00111 |
| 2 | | | Seventh leaf stage | | |
| | Correlation coefficient (r) | 0.951 | 0.759 | 0.866 | 0.708 |
| | Slope (a)† | 0.00184 | 0.00179 | 0.00021 | 0.00237 |
| | | | Early flowering stage | | |
| | Correlation coefficient (r) | 0.924 | 0.764 | 0.707 | 0.831 |
| | Slope (a)† | 0.00057 | 0.00094 | 0.00035 | 0.00079 |

† Linear equation $y = ax + b$, $n = 5$,
where a = slope of inorganic S in plant tissue response curve to S fertilizer supply.

The relationships between the percent $SO_4$-S and the S supply for the four legumes for the two soils are shown graphically in Figure 1. The concentration of inorganic sulfate increased in the legume leaf tissues as the levels of S supply in the soil increased, indicating that the $SO_4$-S concentration in plant tissue reflects the S nutritional status of the plants, with greater concentration of "free" sulfate associated with greater S availability. The results showed that both sampling times (seventh leaf and early flowering stages) are suitable for diagnosing S nutritional status of the legumes. However, as can be seen from Table 3 and 4, $SO_4$-S concentration in the seventh leaf stage (early growth stage) is more sensitive to differences in S availability than at the early flowering stage. Therefore, sampling at the seventh leaf growth stage or earlier is preferred. As well, this makes correction of the S deficiency in the current growing season more feasible. The results showed that $SO_4$-S in faba bean leaf was not as sensitive as for other crops. Also, the $SO_4$-S concentration was lower in faba bean leaf than that in other crop leaves. This might be attributed to more rapid conversion of

inorganic sulfate into organic forms in faba bean leaf tissues compared to the other legumes.

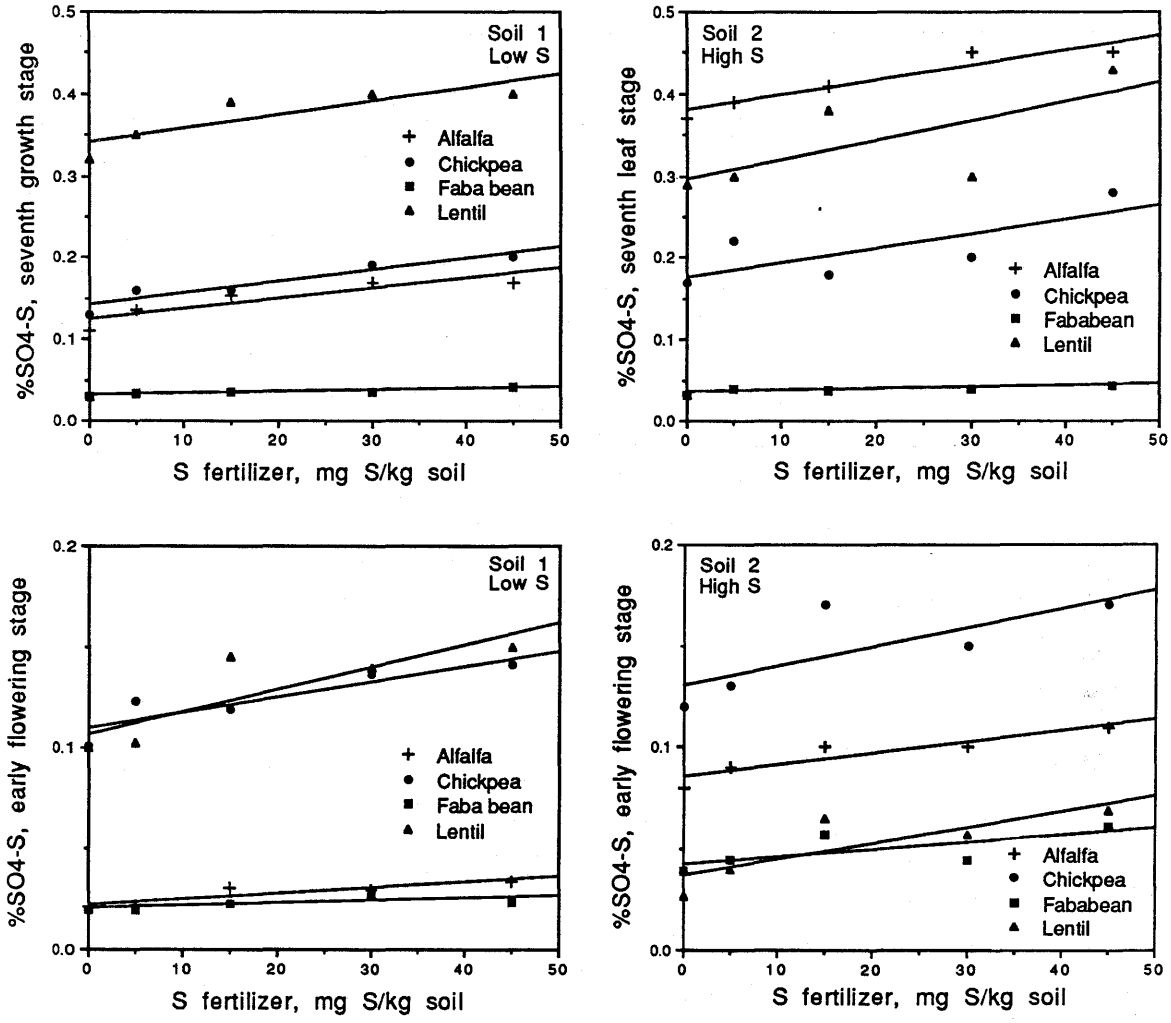


Figure 1. Relationships between S fertilizer supply and $\%SO_4$-S in leaf tissue at the seventh leaf and early flowering stages.

*Relationship between inorganic sulfate in leaf tissue and plant yields:*

Forage and seed yield of the crops significantly increased as inorganic sulfate concentration increased in the crop leaf tissues, reflecting the greater availability of soil S as fertilizer S rate was increased. Figure 2 shows the relationships between inorganic sulfate concentrations in the four legume leaves and the plant dry matter yields. Similar to the dry matter yields, good relationships were found between inorganic sulfate concentrations in the four legume leaves and the plant seed yields. Figure 3 shows the

relationships between the seed yields and inorganic S concentrations in the plant leaf tissues.

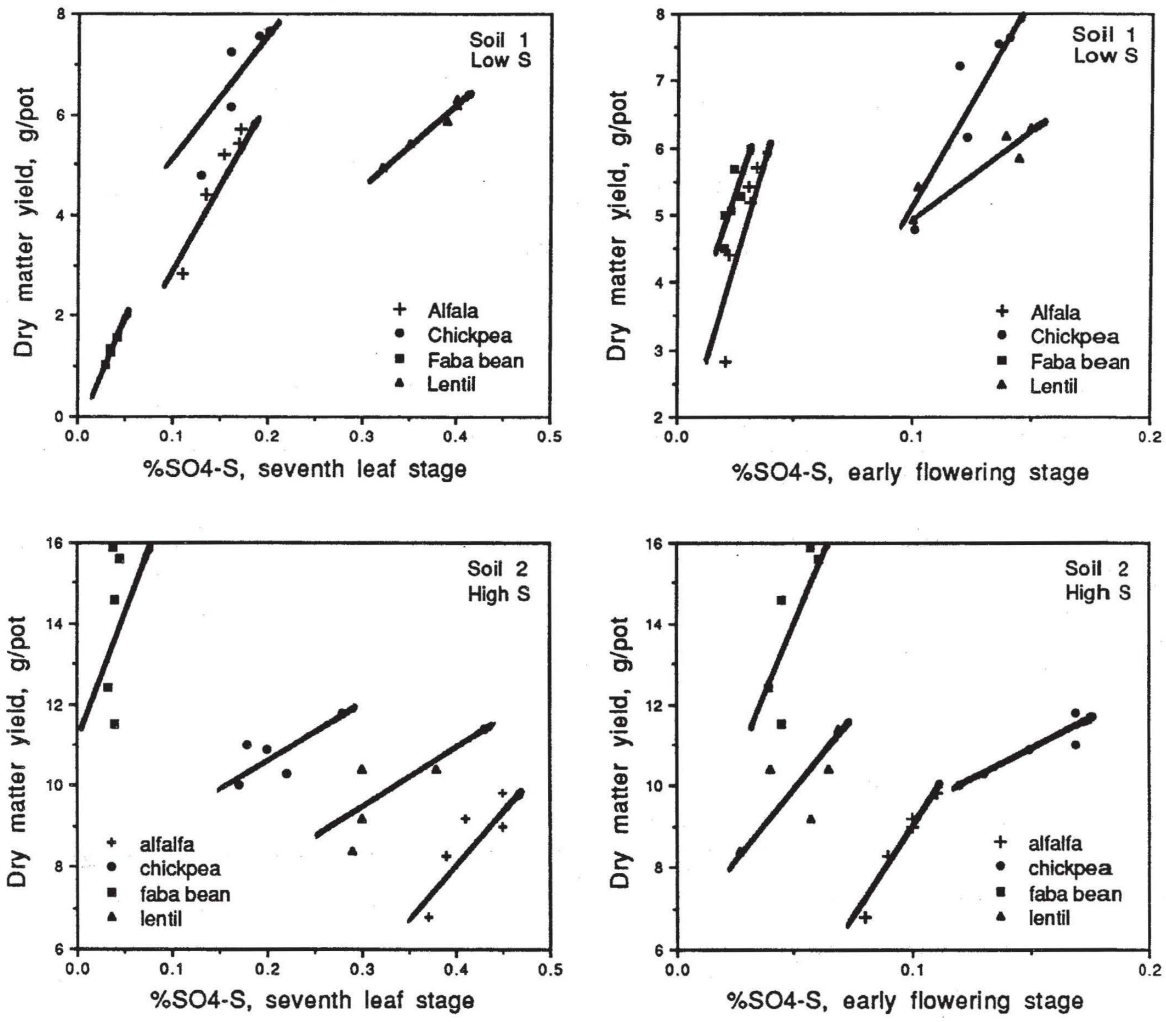


Figure 2. Relationships between plant dry matter yields and %$SO_4$-S in leaf.

Similar trends were found for different species and different times of sampling. Both forage and seed yields increased as inorganic sulfate concentration increased in the legume leaf tissues, indicating that the percent $SO_4$-S in the leaf is sensitive to the S nutritional status of the plants as it affects yields. The critical concentration has been suggested as that which produces 80 to 90 per cent of the maximum yield (Ulrich and Hills, 1967; Martin and Matocha, 1973). Ninety per cent of the maximum yield was obtained when the leaf contained 0.39% $SO_4$-S at the seventh leaf stage or 0.10% $SO_4$-S at early flowering stage for alfalfa (Figure 2). The yield of alfalfa significantly decreased

when the leaf contained lower than 0.39% $SO_4$-S at the seventh leaf stage. For chickpea, 90 per cent of the maximum yields were obtained when the leaf contained 0.18% $SO_4$-S at the seventh leaf stage or 0.17% $SO_4$-S at early flowering stage (Figure 2 and 3). For faba bean, 90 per cent of the maximum yields were obtained when the leaf contained 0.038% $SO_4$-S at the seventh leaf stage or 0.057% $SO_4$-S at early flowering stage (Figure 2 and 3). For lentil, 90 per cent of the maximum yields were obtained when the leaf contained 0.29% $SO_4$-S at the seventh leaf stage or 0.045% $SO_4$-S at early flowering stage (Figure 2 and 3).

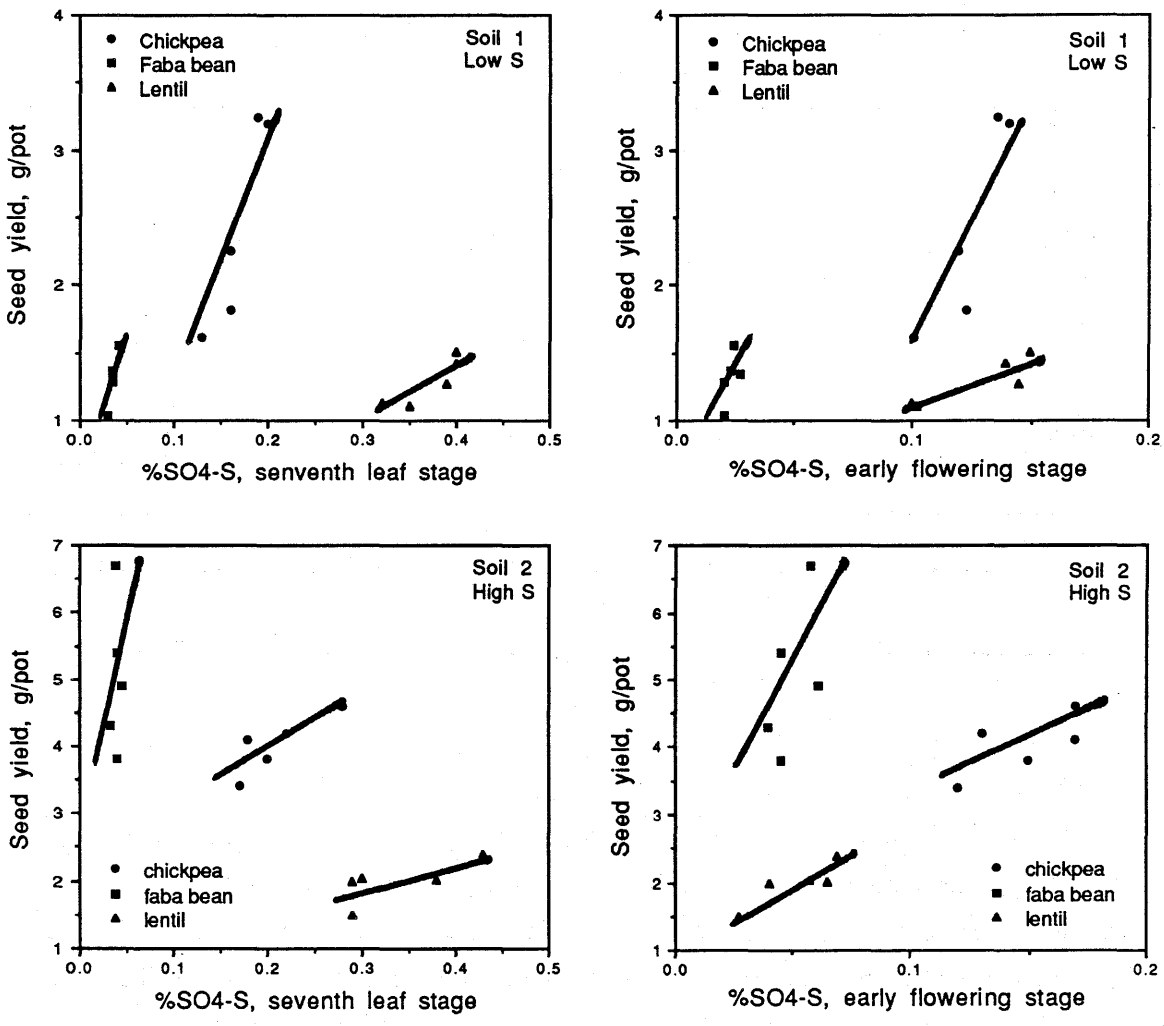


Figure 3. Relationships between plant seed yields and %$SO_4$-S in leaf.

## CONCLUSIONS

Water extractable inorganic sulfate from fresh legume tissue provides a simple and rapid measuring of S nutritional status of forage and grain legumes and could provide a useful guide to S fertilization of legumes. Inorganic sulfate concentrations increased in leaf tissue as the available S supply increased. Inorganic sulfate concentration (%) in the leaves of four legumes at both seventh leaf and early flowering stages was found to be satisfactory index of S deficiency in alfalfa, chickpea, faba bean and lentil. The water extraction procedure is recommended for routine analyses because it is simple and sensitive. The results of this study suggest that the critical sulfate concentrations of the second to fourth leaf for the four crops were 0.39% for alfalfa, 0.18% for chickpea, 0.038% for faba bean and 0.29% for lentil at the seventh leaf growth stage. Further investigation of the use of water extraction method to diagnose other nutrient deficiencies, such as P and K, in legumes is encouraged.

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