PLANT TISSUE ANALYSIS FOR NITRATE AND PHOSPHATE USING ANION EXCHANGE MEMBRANE

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ABSTRACT

An extraction procedure for removal and measurement of inorganic nitrate and phosphate in plant leaves using anion exchange membrane was developed and assessed as a tissue test for N and P availability to sunola and wheat. Relationships between tissue nitrate and phosphate concentrations and yields were determined for sunola grown on a Saskatchewan soil, with five rates of N and P fertilizer supply, in a growth chamber experiment. Relationships between tissue nitrate concentration and yield were determined for wheat in a field trial, with six rates of N fertilizer supply. The nitrate and phosphate concentrations in the leaf tissue were measured at the time of seventh leaf stage to estimate the current N and P status of the plants. The results showed significant relationships between the membrane extractable nitrate and phosphate in the leaf and the supply of available N and P in the soils.

INTRODUCTION

The use of ion exchange membranes to extract plant nutrient ions from soils has recently been reported (Saggar et al., 1990; Schoenau and Huang, 1991; Schoenau et al., 1992; Abrams and Jarrel, 1992). The ion exchange membrane extraction procedure is recommended for routine soil analyses because of its simplicity and sensitivity. However, there is no reported information on the use of the ion exchange membranes for plant tissue analysis.

Leaf tissue analysis for nitrogen (N) and phosphorus (P) is used to provide an estimate of the current N and P status of plants at the time of sampling. Various methods, with different plant parts, are used for tissue analysis for N and P. Total nutrient concentration in the tissue may be used as the diagnostic criteria, or else just a fraction such as water extractable ion (Huang et al., 1992a). Total N concentration has been most widely used in the past. The first fully expanded leaf is considered to be one of the most suitable parts for tissue analysis (Thenabadu, 1966; Thompson et al., 1976; Hafez and Mikkelsen, 1981; McLachlan, 1982; Oosterhuis and Bate, 1983; McLachlan et al. 1987; Ulrich and Hills, 1990). A good method of leaf tissue analysis should be simple and sensitive. Potassium removed by simple HCl solution and sulfate by water extractions of fresh plant tissue have shown to be good indexes of potassium and sulfur deficiencies (Huang et al. 1992a, b). The use of an ion exchange membrane in the extraction procedure for fresh plant tissue analysis is attractive because the need for filtration of the extract can be eliminated. As well the ions are retained on the strip such that only a strip need be transported for analysis. This is an important consideration if the tissue extraction is to be carried out in the field.

The intent of this study was to develop and evaluate an anion exchange membrane (AEM) extraction procedure as a simple tissue test to assess N and P availability in sunola (*Helianthus*) and spring wheat (*Triticum*). Relationships between soil and plant nutrient status and yield are examined.

MATERIALS AND METHODS

Growth chamber experiment:

A growth chamber experiment was conducted using a Luvisol soil (Waiteville sandy loam) of low NO_3-N and phosphate status (Table 1).

Table 1. Properties of the soils used in the experiments.

Soil	pH	Conductivity $mS \cdot cm^{-1}$	Organic C%	NO_3-N	P	K	SO_4-S
				--------- µg/g soil ---------			
Waiteville sandy loam†	7.2	0.1	2.3	4.4	15.0	276	8.9
Sutherland clay††	7.9	0.6	n.d.	23.0	34.0	680	16.0

† Soil used in the growth chamber experiment.
†† Soil used in the field experiment.
n.d. denotes not determined.

Plastic pots were filled with 1200 g of air dry soil. For the nitrogen treatment, nitrogen was applied at the rate of 0, 50, 100, 150, and 200 mg N/kg soil. For the phosphate treatment, phosphorus was applied at the rate of 0, 40, 80, 120, and 160 mg P/kg soil. To each pot a minus N (for the nitrogen treatment) or a minus P (for the phosphorus treatment) nutrient solution was added plus an additional 10 mL of a basal micronutrient solution, to ensure that availability of other nutrients did not restrict growth. Approximately 12 seeds of sunola (*Helianthus*) were sown into each pot. After germination, the pots were thinned to 3 sunola 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.

The youngest fully expanded to second leaves were sampled at sixth leaf stage (20 days after seeding) from each plant on each pot. The plants were harvested at 85 days after seeding. The tops and heads of the plants were removed and the roots separated from the soil by sieving and washing. The plant materials were then dried at 60°C and weighed.

Field experiment:

Field plots were located at the Kernen Farm, Saskatoon, Saskatchewan. Properties of the Sutherland clay soil at the site are presented in Table 1.

The experiment was a split-plot design, with six main plot treatments and five sub-plot treatments, five varieties of spring wheat and three replications. The five varieties were *AC Taber*, *AC Reed*, *BW 90*, *Katepwa* and *Roblin*. Nitrogen fertilizer was applied as broadcast ammonium nitrate at 0, 40, 80, 120, 160 and 240 kg N/ha to provide the six main plot treatments for each variety and each replicate. An additional 20 kg P/ha was broadcast on each plot. Each sub-plot was 5.7' x 20', with a total area of 60' x 215' for three blocks. Plants were seeded at 80 g seed/sub-plot on May 6, 1992, and harvested at mature stage on September 11, 1992.

Forty to fifty plants were sampled from each sub-plot at the tillering stage (Westfall, 1990). The samples were collected in plastic bags. Tissue samples were taken using a hole punch device from the first fully expanded leaves and the tissues analyzed for nitrate using AEM and water extraction procedures. The remainder of the samples were analyzed for total N by sulfuric acid-peroxide digestion (Thomas et al. 1967).

Tissue analyses procedure:

AEM procedure: Plant leaf tissue was obtained from the desired plant part using a hole puncher. The hole puncher produced about 200 (about 2 g) small and uniform tissue pieces. The tissue pieces were placed in 100 mL plastic bottles and one strip of anion exchange membrane (AEM, size 6 x 2 cm) was placed in each bottle. Then, 50 mL of

deionized water was added. The bottles containing the leaf tissues + membrane + water were then capped and shaken on a gyratory shaker at 200 r.p.m. for 2 hours at room temperature. Following the shaking, the membranes were removed from the bottles and rinsed free of leaf tissue. The next step involved removal (elution) of the inorganic nitrate and phosphate absorbed to the membrane for measurement. The washed membranes were transferred to clean 50 mL centrifuge tubes and 20 mL of 0.5 M HCl was added as eluent. The membranes were shaken with the 0.5 M HCl for 30 minutes on the gyratory shaker. The membranes then were removed from the tubes and the eluents were determined for nitrate and phosphate using an Autoanalyzer. The residual leaf tissue was dried at 60°C and weighed.

Water extraction procedure: The procedure used in this study was similar to that outlined by Huang et al. (1992a). Samples of plant leaf tissue obtained using hole puncher were weighed (about 2 g) and shaken on a gyratory shaker (200 r.p.m.) with 50 mL of deionized water for 2 hours at room temperature. The extractions were filtered through a filter paper and nitrate and phosphate in the extracts measured using an Autoanalyzer.

Total tissue N analysis: 0.250 g of oven dry 20-mesh plant sample was weighed into a digesting tube. Five mL of sulfuric acid was added into the tube. The rack of tubes was placed onto a digestion block at 360°C and allowed to heat for 30 minutes. After 30 minutes, the rack of tubes were removed to cool. Once the tubes were cool, 0.5 mL of H_2O_2 (30% hydrogen peroxide) was added and the rack was returned to the block to heat for 30 minutes. This procedure was continued (at least 6 times) until the solution had turned completely clear. Then, the tubes were brought to volume with deionized water. Each tube was shaken and the sample was poured into vials for analysis using an Autoanalyzer (Thomas et al. 1967).

RESULTS AND DISCUSSION

Reproducibility of the AEM procedure

Means and coefficients of variation (C.V.) for triplicate tissue AEM extractions for the sunola are presented in Table 2. The AEM extraction procedure showed good reproducibility with coefficients of variation lower than 10% in all cases.

Similarly, the AEM procedure showed good reproducibility for the nitrogen tissue analyses of spring wheat in the field experiment.

Table 2. AEM extractable nitrate and phosphate values for the sunola†

N level	%NO_3-N††	C.V. (%)	P level	%P††	C.V. (%)
(mg/kg)			(mg/kg)		
0	0.024	8.5	0	0.010	8.2
50	0.034	7.4	40	0.010	5.8
100	0.032	6.4	80	0.018	9.8
150	0.039	2.8	120	0.016	9.4
200	0.051	4.9	160	0.025	8.5

† means of triplicate analyses.
†† as per cent of dry weight basis.

Relationships between test methods

Both AEM and water extraction procedures gave similar test values for nitrate and phosphate in the leaf tissues of sunola and wheat. This is not surprising since the

membrane simply acts to adsorb and accumulate the nitrate and phosphate that is removed from the leaf tissue by the water.

Linear regressions were used to evaluate the relationships between AEM and water extractable N, AEM extractable N and total N, and water extractable N and total N (Table 3). AEM extractable N in wheat leaf tissue was significantly correlated with total N ($r^2 > 0.81$) and water extractable N ($r^2 > 0.61$). Water extractable N was also significantly correlated with total N. These findings are consistent with the similarity in mechanism by which AEM and water extractions act to remove nitrate from leaf tissue. The results also indicate that both AEM and water extractable N are good indicators of total N status in sunola and wheat plants.

Both AEM and water extraction methods offer advantages in terms of simplicity. Water volume during shaking is not highly critical in the AEM extraction, so that accurate and precise dispensing of deionized water is not required. The AEM strips are highly durable and do not appear to lose efficacy after prolonged usage. The only expendable chemical is the 0.5 M HCl used in the elution procedure. Another important consideration is the possibility of using a single extraction as an index for two or more elements.

Table 3. Regression equations and correlation coefficients (r^2) for relationships between the methods.

Plant species	Regression Equation			r^2
				n = 5
Sunola	N_{AEM}†	=	0.002477 + 0.8059 N_W††	0.79**
	P_{AEM}†	=	0.007825 + 1.1250 P_W††	0.84**
Wheat				n = 6
AC Taber	N_{AEM}	=	0.004529 + 0.1263 N_W	0.84***
AC Reed	N_{AEM}	=	- 0.03676 + 0.5763 N_W	0.75**
BW 90	N_{AEM}	=	- 0.001632 + 0.5150 N_W	0.81***
Roblin	N_{AEM}	=	0.007739 + 0.6253 N_W	0.61**
Katepwa	N_{AEM}	=	0.009424 + 0.8216 N_W	0.92***
AC Taber	N_{AEM}	=	- 0.004041 + 0.05996 N_t†††	0.87***
AC Reed	N_{AEM}	=	- 0.03791 + 0.02485 N_t	0.93***
BW 90	N_{AEM}	=	- 0.01824 + 0.01292 N_t	0.81***
Roblin	N_{AEM}	=	- 0.09059 + 0.1072 N_t	0.93***
Katepwa	N_{AEM}	=	- 0.1716 + 0.1934 N_t	0.91***
AC Taber	N_W	=	- 0.05498 + 0.04198 N_t†††	0.81***
AC Reed	N_W	=	0.01386 + 0.03624 N_t	0.88***
BW 90	N_W	=	- 0.03199 + 0.02496 N_t	0.98***
Roblin	N_W	=	- 0.02688 + 0.1068 N_t	0.60**
Katepwa	N_W	=	- 0.1816 + 0.2201 N_t	0.87***

† Anion exchange membrane extractable N or P.
†† Water extractable N or P.
††† Total N.
, * Significant at the 0.05 and 0.01 probability levels respectively.

Relationship between N in leaf tissue and N availability in soil:

Regression analyses were used to describe the relationships between N in the leaf and N fertilizer rate applied. The results indicate strong direct relationships between nitrate concentrations in the plant leaves and the levels of N supply in the soil.

The relationship between the percent NO_3-N in the tissue and the soil N supply for the sunola and wheat are shown in Figure 1. The concentration of nitrate increased in the sunola and wheat leaves as the levels of N supply in the soil increased, indicating that the NO_3-N concentration in plant tissue reflects the N nutritional status of the plants, with greater concentration of AEM extractable nitrate associated with greater N availability. The results showed that the sampling time is suitable for diagnosing N nutritional status of the sunola (at sixth leaf stage) and wheat (at tillering stage). Sampling at an early growth stage may allow time for correction of nitrogen deficiencies in the current crop.

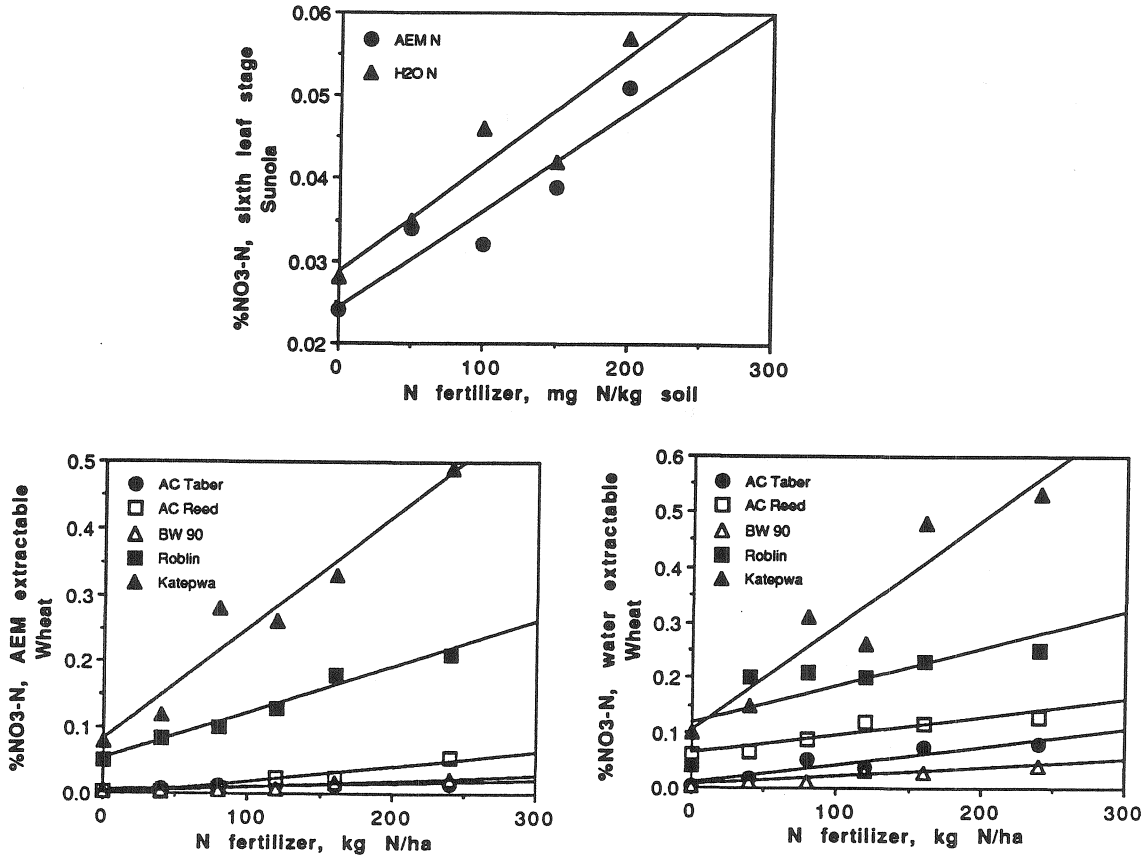

Figure 1. Relationship between N fertilizer supply and %NO_3-N in leaf tissue.

Relationship between nitrate in leaf tissue and plant yields:

As the dry matter and head yields of sunola significantly increased in response to greater N availability in soil, so did the nitrate concentration increase in the plant leaf tissues, reflecting the greater availability of soil N as fertilizer N rate was increased. Figure 2 shows the relationships between nitrate concentrations in the sunola leaves and the plant dry matter yields. Similar to the dry matter yields, good relationships were found between nitrate concentrations in the sunola leaves and the plant head yields.

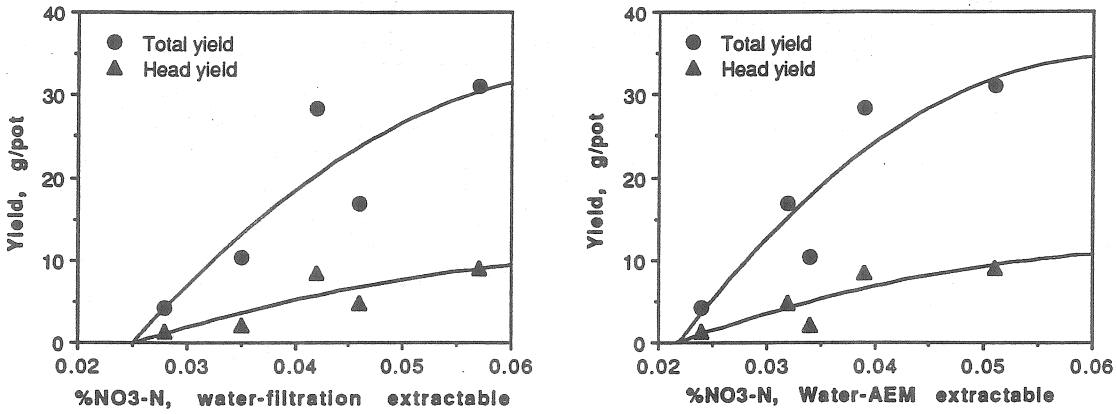

Figure 2. Relationship between plant yields and %NO_3-N in sunola leaf.

Figure 3 shows the relationship between nitrate concentrations in the plant leaves and the final seed yields of *Katepwa* and *Roblin*. Similar trends were also found for the other varieties of wheat. Seed yields increased as nitrate concentration increased in the leaf tissues, indicating that the percent NO_3-N in the leaf is sensitive to the N nutritional status of the plants as it affects yields.

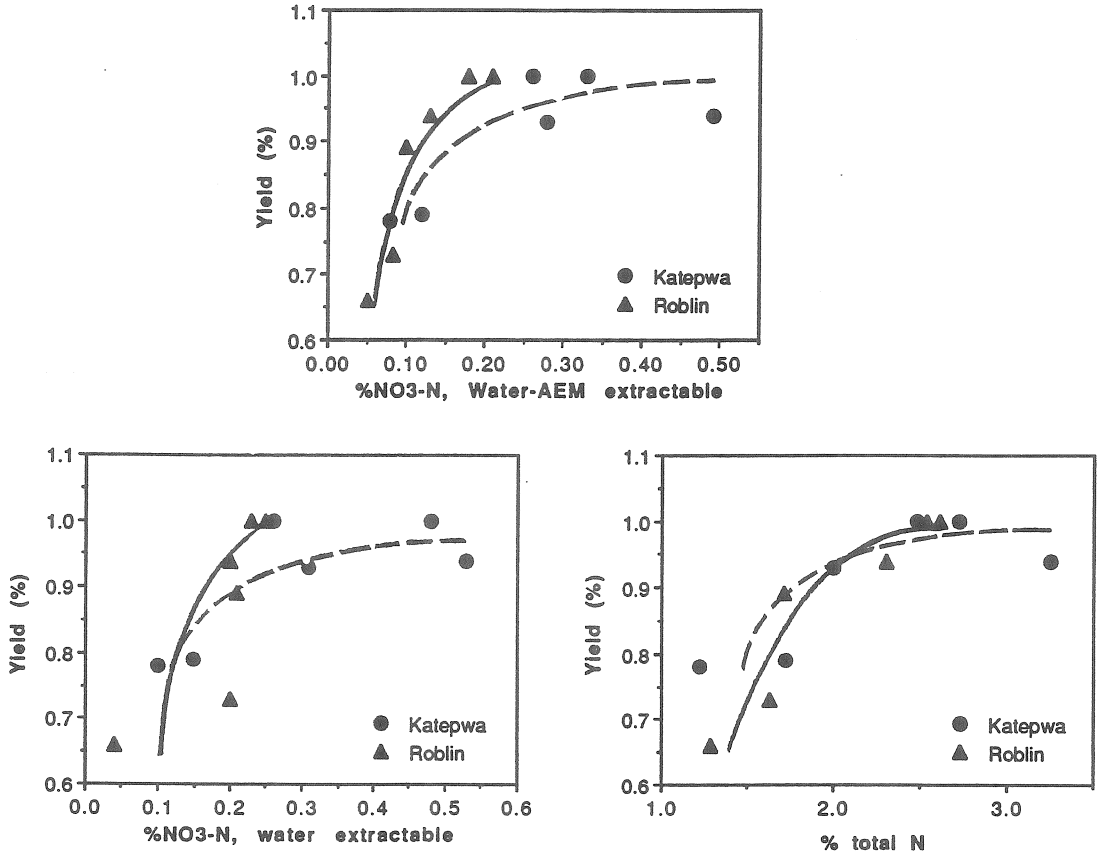

Figure 3. Relationship between plant seed yields expressed as % of maximum yield and %NO_3-N in wheat leaf.

The critical tissue concentration of a nutrient has been suggested as that associated with 80 to 90 per cent of the maximum yield (Martin and Matocha, 1973; Ulrich and Hills, 1990). Most workers identify a critical nutrient range (CNR). Tissue concentrations in the critical nutrient range are considered to represent a nutrient supply adequate for maximum growth and development. Tissue concentrations below the CNR indicate deficiency. At the sixth leaf stage for sunola, the CNR for AEM extractable NO_3-N is from 0.04% NO_3-N to 0.05% NO_3-N (Figure 2). The head yield of sunola significantly decreased when the leaf contained lower than 0.030% NO_3-N at the sixth leaf stage. The critical nutrient range for AEM extractable nitrate ranged from 0.20 to 0.30% NO_3-N for *Roblin* and *Katepwa* wheat (Figure 3). For total N the CNR appears to range from 2.0 to 2.5% N.

AEM and water extractable inorganic P in leaf tissue and yields of sunola:

Table 4 reports the AEM and water extractable inorganic phosphate values, and dry matter yields of sunola. The concentration of inorganic phosphate increased in the sunola leaf tissues as the levels of P supply increased in the soil, indicating that the inorganic phosphate concentration in plant tissue reflects the P nutritional status of the plants, with greater concentration of AEM extractable phosphate associated with greater P availability. The results also showed that the plant dry matter yields increased as inorganic phosphate concentration increased in the plant leaf tissues, reflecting the greater availability of soil P as fertilizer P rate was increased. Ninety percent of the maximum yield was obtained when the leaf contained 0.018% AEM extractable phosphate.

Table 4. Inorganic phosphate and yields of the sunola†

P level (mg/kg)	P (%)†† AEM	P (%)†† H_2O	Yield (g/pot) Dry weight	Yield (g/pot) Head weight
0	0.010	0.016	15.5	3.1
40	0.010	0.015	17.9	4.4
80	0.018	0.024	17.8	4.6
120	0.016	0.024	20.5	4.6
160	0.025	0.026	21.5	4.7

† means of triplicate analyses.
†† as per cent of dry weight basis.

CONCLUSIONS

The use of AEM and water to extract nitrate from fresh sunola and wheat leaf tissues provides a simple index of N nutritional status of plants and could provide a useful guide to N fertilization. Nitrate concentrations increased in leaf tissue as the available N supply in the soil was increased through fertilization. Nitrate concentration (%) in the leaves of sunola and wheat was found to be satisfactory indicator of N deficiency or sufficiency in sunola and wheat. The AEM and water extraction procedures could be used to provide a simple simultaneous index of plant N, P and S status.

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