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Prediction of grain protein in wheat and barley in a subtropical environment from available water and nitrogen in Vertisols at sowing

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Summary. In many subtropical environments, cereal crops develop and mature largely on residual water in the soil. This research involves evaluation of the impact of plant available nitrogen (N) and water in soil at sowing on grain protein in wheat and barley in such a subtropical environment.

Estimates of grain protein concentration of wheat (cv. Hartog) were made using plant available water and available N (soil nitrate-N and fertiliser N, where applied) at sowing using data obtained from an experiment conducted at Warra, Queensland, from 1987 to 1995. Treatments included: grass + legume leys of 4-year duration followed by continuous wheat with 0 or 50 kg N/ha.year applied as urea at sowing; 2-year rotation of lucerne and wheat; 2-year rotation of annual medics and wheat; 2-year rotation of chickpea and wheat, no-tillage wheat; and conventional tillage wheat. Fertiliser N as urea was applied to both no-tillage wheat and conventional tillage wheat at 0, 25 and 75 kg N/ha.year. The conventional tillage wheat also received N at 12.5 and 50 kg N/ha.year. Estimates of wheat grain yield required both rainfall during the fallow period or plant available water in the soil profile at sowing and rainfall from sowing to anthesis and, therefore, it could not be predicted precisely at sowing. Increasing plant available water (mm) in soil at sowing linearly reduced grain protein. In comparison, available N at sowing increased grain protein curvilinearly from 10.0% at 50 kg N/ha to 14.5% at 200 kg N/ha (0–120 cm depth). Variation in grain protein concentration was best accounted for by the available water : available N ratio at 0–90, 0–120 or 0–150 cm depths. The protein concentrations of wheat (cv. Hartog) grown in 1996 at Warra and Nindigully, and wheat (cv. Cunningham) grown from 1991 to 1995 at Billa Billa, and barley (cv. Tallon) grown in 1996 at Nindigully and Formartin, Queensland, were successfully predicted using the relationship between the available water : available N ratio and wheat grain protein concentration developed using data from Warra during 1987–95. Thus, available water should be matched by N supply at sowing to ensure the production of Prime Hard grade wheat and malting grade barley in the subtropical environment. As a ‘rule of thumb’, for 0–120 cm depth of soil sampling, each millilitre of available water matched with each kilogram of N per hectare of available N, at sowing, would produce about 13% protein wheat in this semi-arid region. It requires only 0.5 kg of N/ha for each millilitre of available water in 0–120 cm depth of soil to produce malting grade barley of about 10.5% protein concentration. Available water in soil at sowing can be approximated with rainfall during the fallow period, with rainfall (mm) : available N (kg/ha for 0–120 cm depth) ratios of 3.7 and 7.4 for respective 13 and 10.5% grain protein concentrations for both wheat and barley.

Introduction

Soil fertility decline, especially decreasing nitrogen (N) supply from continuous cereal cropping of north-eastern Australian soils, has been associated with decreases in grain protein concentration of wheat (*Triticum aestivum* L.) in recent years (Dalal *et al.* 1991). The premium paid for high protein wheat, such as ‘Prime Hard’ (PH) grade (>13% protein), and the export demand for PH grade wheat, has highlighted the need for management practices that can lead to the production of PH grade wheat.

Climatic factors that affect the N nutrition of the wheat crop cause differences in grain protein concentration of winter wheat (Sosulski *et al.* 1966; Smika and Greb 1973; Campbell *et al.* 1981; Benzian and Lane 1986; Rao *et al.* 1993). Although high temperatures (>35°C) during the grain filling period have been positively correlated with grain protein concentration (Blumenthal *et al.* 1991), this was at least partly because the grain yield was adversely affected (Taylor and Gilmour 1971; Campbell *et al.* 1981; Blumenthal *et al.* 1991). Also, deficit moisture stress

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before flowering increased protein concentration (Campbell *et al.* 1981) but the effects of temperature and moisture on protein concentration were mainly due to effects on yield (Sosulski *et al.* 1966).

Nitrogen supply, from soil and fertiliser, influences grain protein directly (Campbell *et al.* 1981). Nitrogen fertiliser application up to and during anthesis can often increase grain protein concentration given adequate water supply. However, in unreliable rainfall regions, in-crop N fertilisation is a less certain option. Even in semi-arid subtropical environments where rainfall from sowing to anthesis is extremely variable (Angus *et al*. 1980), N fertiliser applied at sowing frequently increases grain protein concentration (Doyle and Holford 1993). Since Vertisols can store a substantial amount of soil water, the crop can be grown with little rainfall during crop growth. When this occurs, fertiliser N application after sowing does not always increase grain protein concentration because of isolation of applied N in a dry zone near the soil surface and consequently, a yield and hence financial loss is likely to occur.

The relationship between available water and available N at sowing was examined to estimate grain protein concentration of wheat using data from an experiment conducted at Warra, Queensland, from 1987 to 1995. Depending on the amount of stored plant available water and plant available N, a fertiliser N management strategy can be used at sowing to target the production of PH grade wheat (>13% protein) and malting grade barley (about 10.5% protein). The applicability of such a relationship was tested by predicting grain protein concentrations of 2 cultivars of wheat grown at 3 sites and 1 cultivar of barley (*Hordeum vulgare* L.) grown at 2 sites in southern Queensland.

Materials and methods

Field experiment to develop relationships between available water and nitrogen, and grain protein

The data on plant available water, plant available N in soil (>98% nitrate-N and < 2% ammonium-N), N fertiliser applied at sowing, grain yield and grain protein concentration (at 12% moisture) were collected from a long-term field experiment at Warra (25°47'S, 150°53'E), Queensland, from 1987 to 1995. Complete experimental details are provided by Dalal *et al.* (1995). Briefly, the soil at the experimental site is a Vertisol containing 55% clay, 0.7% organic carbon, 0.07% total N and pH 8.5 in the top 0.1 m layer. Long-term (1890–1987) mean annual rainfall for Warra, about 15 km away from the site, is 685 mm.

Seventeen treatments were established in a randomised block design with 4 replications in plots each 25.0 m long by 6.75 m wide. Treatments were grouped into 5 major crop and soil management practices: grass + legume ley–wheat rotation, legume ley–wheat rotation, grain legume–wheat rotation, and conventional tillage (CT) and no-tillage (NT) wheat cropping without and with fertiliser application. The wheat cultivar Hartog was used in all rotations and was sown at the rate of 40 kg/ha in May and June or 50 kg/ha in July (1990 and 1993).

Treatments 1, 2 and 3 were mixed grass + legume ley pastures established in 1986, 1987 and 1988, respectively, and consisted of purple pigeon grass (*Setaria incrassata*), Rhodes grass (*Chloris gayana*), lucerne (*Medicago sativa*) and annual medics (*M. scutellata* and *M. truncatula*). These were grown for 3.75 years followed by continuous wheat. Treatments 4 and 5 were 2-year rotations of wheat and lucerne, and treatments 6 and 7 were 2-year rotations of wheat and annual medics. Treatments 8 and 9 consisted of a 2-year rotation of chickpea (*Cicer arietinum*) and wheat.

In treatments 10, 11, 12, 13 and 14, wheat was grown under CT practice, and fertiliser N as urea was applied at sowing at rates of 0, 12.5, 25, 50 and 75 kg N/ha.year, respectively. Wheat was grown under NT practice in treatments 15, 16 and 17, with fertiliser N as urea applied at rates of 0, 25 and 75 kg N/ha.year, respectively.

Field experiments for validation of the relationship between available water and nitrogen, and grain protein

For the purpose of predicting grain protein concentrations in wheat and barley at different sites, measurements were made in 1996 for wheat (cv. Hartog) grown at Warra, and Nindigully and wheat (cv. Cunningham) grown from 1991 to 1995 at Billa Billa, and for barley (cv. Tallon) grown at Formartin and Nindigully, Queensland. The soil type at the Nindigully site (28°29'S, 148°45'E) is Thallon clay (Dalal and Mayer 1986) containing 50% clay and 0.05% total N. Plant available water and available N in 0–120 cm depth of soil at sowing of wheat and barley were 203 mm and 60 kg N/ha. Fertiliser N as urea was applied to wheat at sowing at rates of 0, 30, 60 and 90 kg N/ha, and 0 and 40 kg N/ha to barley (cv. Tallon). The soil type at the Formartin site (27°20'S, 151°23'E) is a variant of Waco clay containing 55% clay and 0.08% total N. The ranges in plant available water and N in 0–120 cm depth of soil at sowing of barley after various phases of crop rotations (sorghum–barley, chickpea–barley, mungbean–barley, continuous barley) were from 180 to 236 mm and from 31 to 92 kg N/ha. Fertiliser N as urea was applied to barley at sowing, at rates of 0, 50, 100 and 150 kg N/ha.

Wheat was grown from 1991 to 1995 at Billa Billa (28°10'S, 150°15'E) on a red-brown earth both under CT and NT practice, each without and with 60 kg N/ha.year fertiliser, nematicide application and soil fumigation. Further experimental details are provided by Thomas *et al.* (1996). Plant available water ranged from 100 to 197 mm and available N ranged from 102 to 545 kg N/ha in 0–120 cm depth of soil.

Table 1. Range and mean (± s.d.) crop total rainfall and rainfall to anthesis, fallow rainfall, fallow rainfall efficiency, plant available water (0–120 cm) at sowing, soil nitrate-N (0–120 cm) at sowing, soil total N (0–10 cm), grain yield, grain protein concentration and total grain N from 1987 to 1995 at Warra, Queensland

	Range	Mean
In-crop total rainfall (mm)	97–217	146 ± 42
In-crop rainfall to anthesis (mm)	11–169	76 ± 42
Fallow rainfall (mm)	317–790	553 ± 148
Fallow rainfall efficiency (%)	13–46	28 ± 8
Plant available water (mm)	58–209	147 ± 37
Soil nitrate-N (kg N/ha)	32–324	111 ± 64
Soil total N (%)	0.07–0.10	0.08 ± 0.01
Grain yield (t/ha)	0.5–4.8	2.5 ± 1.1
Grain protein concentration (%)	7.8–16.4	11.9 ± 2.3
Total grain N (kg N/ha)	13–105	52 ± 24

Analytical techniques

Wheat and barley grain yields were measured from 1.75 by 23 m of the central areas of all plots. After determination of grain water content, grain yields were adjusted to 12% water content. Wheat and barley grains were analysed for N concentrations in Kjeldahl digests using automated ammonium-N analysis (Crooke and Simpson 1971). Grain protein concentration was calculated by multiplying grain N concentration by 5.7 for wheat and 6.25 for barley and adjusted to 12% water content.

Soil water and nitrate-N were measured from soil cores (2–5) collected annually from each plot in May, using a 50 mm diameter tube sampler, down to 150 cm depth. The soil core was sectioned into 0–10, 10–20, 20–30, 30–60, 60–90, 90–120 and 120–150 cm depth intervals. Samples were bulked, sealed in plastic bags, and stored at 4°C until analysis. Soil was dried at 35 ± 5°C in a forced draught oven, moisture loss recorded and the air-dried samples were ground to <2 mm for colorimetric determination of nitrate-N (Best 1976) after extraction of 10 g of soil in 100 mL of 2 mol KCl/L. Total soil moisture content was calculated from soil samples dried at 105°C for 48 h. Plant available soil water was calculated by subtracting soil water content at the estimated lower limit of plant availability for each soil layer from the measured volumetric water content. At the Warra site, the lower limits of plant available water were 21.8, 23.5, 25.0, 78.8, 85.5 and 94.5 mm for soil layers 0–10, 10–20, 20–30, 30–60, 60–90 and 90–120 cm, respectively. Similar measurements were made at the Nindigully, Formartin and Billa Billa sites.

Results and discussion

The range and mean ± s.d. of total rainfall through the cropping season, rainfall to anthesis, rainfall during fallow periods, available soil water and nitrate-N at sowing, grain yield, grain protein content and total grain N yield at Warra from 1987 to 1995 are given in Table 1.

Although total rainfall during crop growth ranged by less than 3-fold, from 97 to 217 mm, rainfall to anthesis ranged by more than 15-fold, from 11 to 169 mm. Fallow rainfall efficiency ranged from 13 to 46%, with a mean value of 28%. The range in soil nitrate-N was 10-fold; a much smaller range in soil total N was observed. During 1987 and 1995, wheat grain yield ranged from 0.5 to 4.8 t/ha, while grain protein concentration ranged from 7.8 to 16.4%. Thus, during the 9-year period, a wide range in rainfall, nitrate-N, wheat grain yield and grain protein concentrations were observed.

Formulation of the relationship between available water and nitrogen, and grain protein

Relationships between plant available water, available N (nitrate-N and fertiliser N) and grain protein concentration were established from linear and non-linear regression analysis using the pooled data of all 4 replicates (n = 116).

Grain yield was positively correlated with crop rainfall to anthesis, fallow rainfall and available water at sowing (Table 2). Almost 77% of the variation (R^2 = 0.766) in grain yield was accounted for by fallow rainfall (x_1) and in-crop rainfall to anthesis (x_2):

$$\text{Grain yield (kg/ha)} = -1627 + 5.7x_1 + 13.5x_2 \quad (1)$$

However, by substituting plant available water at sowing (x_1) with fallow rainfall in equation 1, only 43% of the variation in wheat grain yield was accounted for:

$$\text{Grain yield (kg/ha)} = -530 + 13.9x_1 + 13.5x_2 \quad (2)$$

These observations confirm the predominant effect of moisture on grain yield in this environment, similar to that observed in temperate (Sosulski *et al.* 1966; Campbell *et al.* 1981) and mediterranean environments

Table 2. Correlation coefficients between crop total rainfall and rainfall to anthesis, fallow rainfall, plant available water (0–120 cm) at sowing, available N [nitrate-N (0–120 cm) and fertiliser N applied at sowing], and wheat grain yield, grain protein and total grain N at Warra, Queensland

Values significant at P = 0.05 and P = 0.01 are 0.18 and 0.24, respectively (n = 116)

	Grain yield	Grain protein	Total grain N
In-crop total rainfall	n.s.	n.s.	–0.21
In-crop rainfall to anthesis	0.49	–0.20	0.35
Fallow rainfall	0.72	–0.22	0.70
Available water	0.43	–0.60	0.24
Available N (nitrate + fertiliser N)	n.s.	0.68	0.21
Fertiliser N	n.s.	0.29	0.20

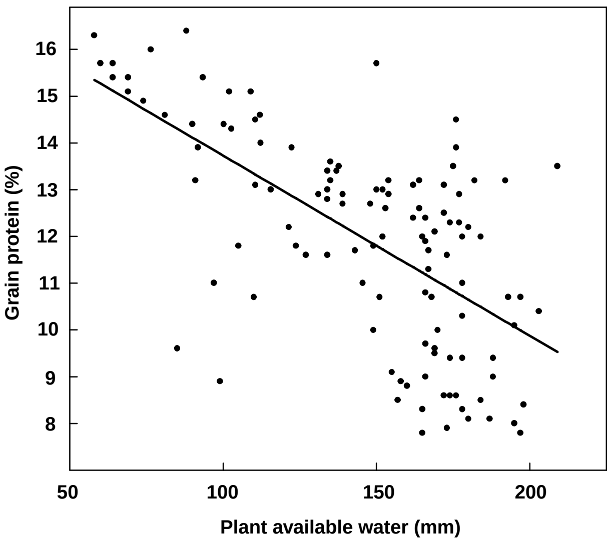

Figure 1. Relationship between available water (0–120 cm depth) at sowing (x) and grain protein concentration (y) from 1987 to 1995. The equation of the line is:

$$y = 17.5 - 0.04x \quad (R^2 = 0.36, P<0.01)$$

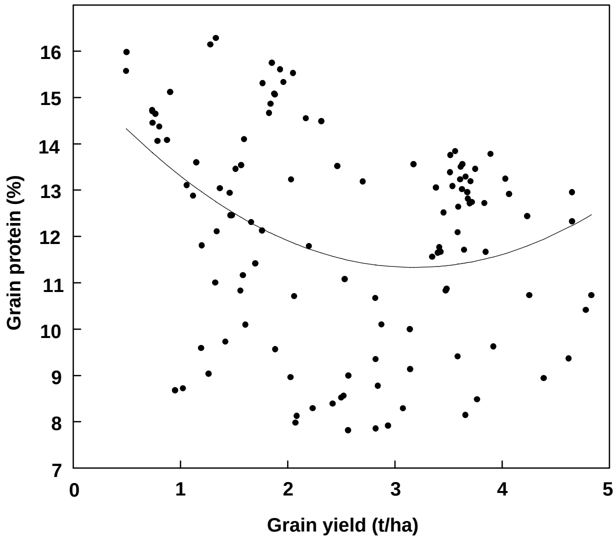

Figure 2. Relationship between wheat yield (x) and protein concentration (y) at Warra from 1987 to 1995. The equation of the line is:

$$y = 15.5 - 2.6x + 0.4x^2 \quad (r = 0.36, P<0.01)$$

(French and Schultz 1984). The in-crop rainfall to anthesis was more than twice as effective as fallow rainfall in increasing grain yield although plant available water at sowing was equally effective as fallow rainfall. Since in-crop rainfall is highly variable, grain yield cannot be estimated precisely at sowing.

Grain protein concentration was negatively correlated with fallow rainfall and available water at sowing (Table 2). Available water at sowing accounted for 40% of the variation in grain protein concentration (Fig. 1). The increase in available soil water from 100 to 200 mm decreased the protein concentration from 13.7 to 9.9%; a decrease of 3.8% in protein concentration for each 100 mm increase in available water at sowing. Smika and Greb (1973) also found that wheat grain protein concentration decreased with increasing amounts of plant available water at sowing, although the decreases in grain protein concentration were from 2.1 to 3.2%/100 mm of available soil water (0–120 cm depth). Since total grain N yield was positively associated with plant available water at sowing ($r = 0.24$, $P<0.05$, Table 2), the negative effect of plant available water on grain protein concentration was partly due to the dilution of grain N through increased grain yield from 0.5 to 3 t/ha (Fig. 2). A similar trend was observed by Doyle and Holford (1993). Sosulski *et al.* (1966) also found that soil moisture was the most important factor in increasing grain yield.

Grain protein concentration increased curvilinearly with increasing amounts of available N (nitrate-N and fertiliser N, if applied) (Fig. 3). It increased from 9.9% protein concentration with 50 kg N/ha of available N to 13.0% protein concentration (PH grade) with 125 kg N/ha of available N. Smika and Greb (1973) reported a linear increase in grain protein concentration with increasing amounts of soil nitrate-N (0–120 cm depth), from 30 to 150 kg N/ha, in the semi-arid central

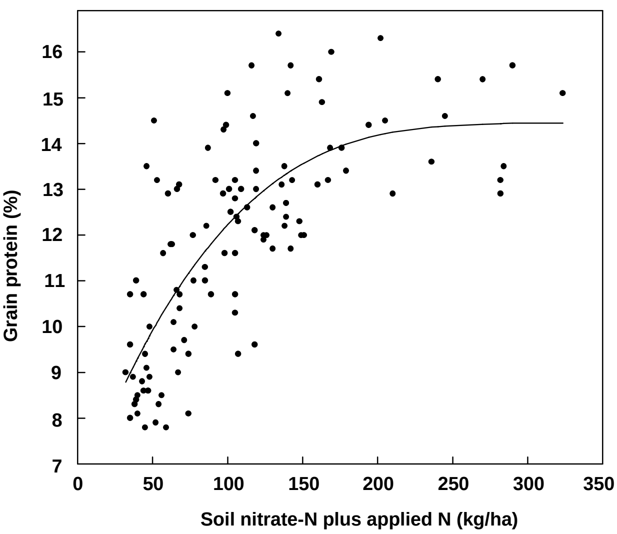

Figure 3. Relationship between available N (soil nitrate-N, 0–120 cm depth, and fertiliser N, where applied) at sowing (x) and grain protein concentration (y) from 1987 to 1995. The equation of the line is:

$$y = 14.8 - 9.3 \text{ x } \exp(-0.013x) \quad (R^2 = 0.56, P<0.01)$$

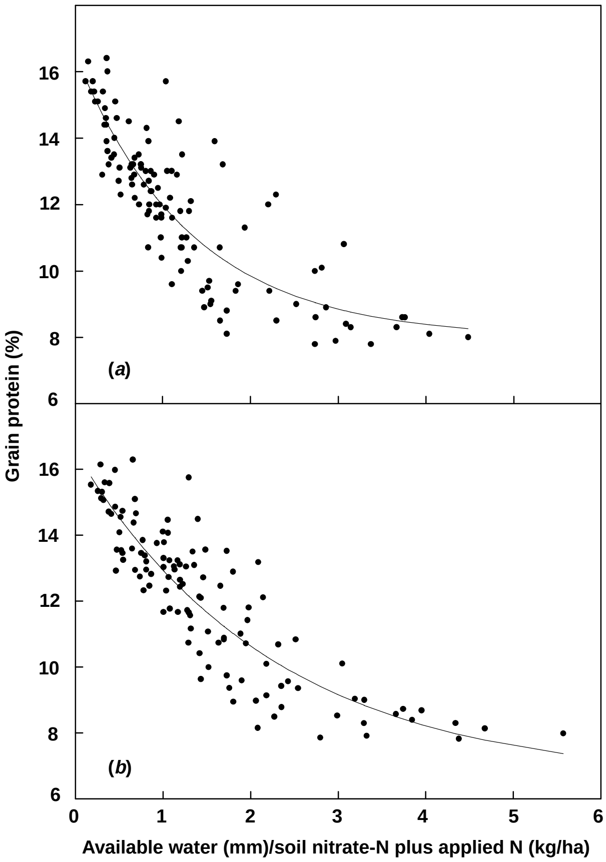

Figure 4. Relationship between available water (mm)/available N (nitrate-N and fertiliser N; kg N/ha) (x) at different depths (a) 0–60 cm depth and (b) 0–120 cm depth, at sowing and grain protein concentration (%) (y). The equations of the lines are:

0–60 cm depth: $y = 7.77 + 8.75 \times \exp(-0.69x)$ ($R^2 = 0.74$)
0–120 cm depth: $y = 6.52 + 10.0 \times \exp(-0.44x)$ ($R^2 = 0.78$)

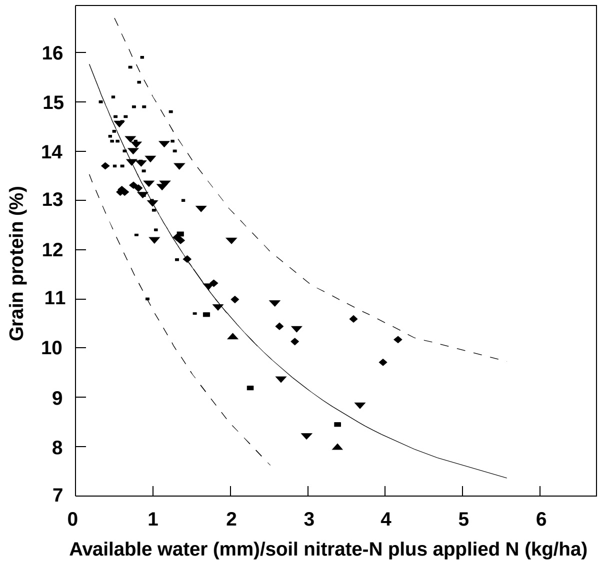

Figure 5. A plot of measured grain protein concentrations of barley (cv. Tallon) and wheat (cv. Hartog) grown in 1996, shown in Figure 6, and wheat (cv. Cunningham) grown from 1991 to 1995, within 95% confidence limits of the line in Figure 4*b*. Barley: Formartin (▼) and Nindigully (▲); and wheat: Warra (◆), Nindigully (■) and Billa Billa (▪). The solid line was drawn using equation 3 in the text. The broken lines show 95% confidence intervals about the solid line.

Great Plains of Nebraska and Colorado. Increased grain protein concentration at high N supply has been reported previously (Sosulski *et al.* 1966; Campbell *et al.* 1981; Doyle and Holford 1993; Strong and Holford 1997).

Grain protein concentration decreased as the amount of available water at sowing increased (Fig. 1), whereas grain protein concentration increased as the amount of available N at sowing increased (Fig. 3), thus demonstrating the interaction of available water and available N on grain protein concentration of wheat. The available water : available N ratio, as a single index, appropriately reflected this interactive effect (Fig. 4). Grain protein concentration decreased exponentially as the available water : available N ratio increased for all depths studied (0–60, 0–90, 0–120 and 0–150 cm). However, for a given protein concentration, the available water : available N ratio varied depending on the soil depth considered. For example, to produce wheat of 13% grain protein concentration (PH grade) the plant available water : available N ratio was 0.75 for 0–60 cm, 0.87 for 0–90 cm, 0.99 for 0–120 cm and 1.15 for 0–150 cm soil depth. The increasing plant available water : available N ratios in soil with increasing depth may be due to: (i) the nitrate-N distribution in the soil profile; (ii) depth of available soil water stored; (iii) soil water-use pattern of wheat (Smika and Greb 1973); and (iv) the time and method of fertiliser N application. There was an increase from 74 to 78% in the variation in grain protein concentration accounted for by the available water : available N ratio as the soil sampling depth increased from 0–60 cm (Fig. 4*a*) to 0–90 cm depth (data not shown), but essentially no further increase was observed for 0–120 cm (Fig. 4*b*) and 0–150 cm depths (data not shown). Ideally, the soil would be sampled to the crop's rooting depth which is unknown at sowing time. It can be sampled down to 90 cm or deeper for measuring plant available water and nitrate-N just before sowing. From these measurements, required amounts of N fertiliser can be applied to achieve desired grain protein concentrations in wheat and barley in the north-eastern grain belt.

The standard error of estimate of grain protein

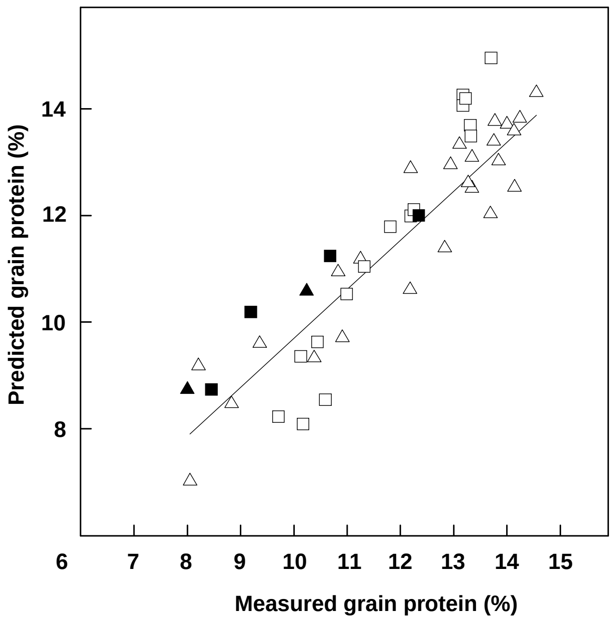

Figure 6. Relationship between measured grain protein concentrations (x) of barley grown at Formartin (△) and Nindigully (▲) and wheat grown at Warra (□) and Nindigully (■) in 1996 and grain protein predicted (y) from available water : available N ratio using the equation for 0–120 cm depth (equation 3 in the text). The equation of the line is:

$$y = -0.12 + 0.99x \quad (R^2 = 0.83)$$

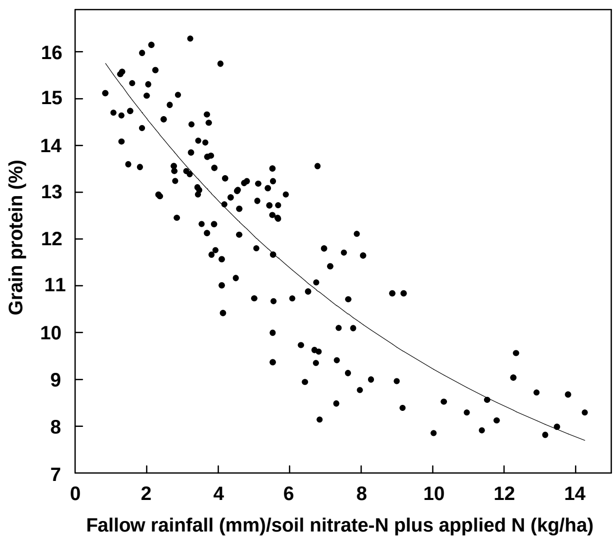

Figure 7. Relationship between fallow rainfall : available N ratio (x) and wheat grain protein concentration (y) at Warra from 1987 to 1995. The equation of the line is:

$$y = 4.76 + 11.95 \times \exp(-0.01x) \quad (R^2 = 0.74)$$

concentration using available water and available N (0–120 cm depth) was 1% protein although confidence limits (95%) were about ± 2% protein concentration for grain protein concentration range of 10–16% (Fig. 5). For cropping seasons with above-average rainfall to anthesis, such as in 1988, standard error of estimate was only 0.5% protein although the range in available water : available N and protein concentration was narrower than the pooled data. However, for the range of crop growing conditions encountered from 1987 to 1995, resulting in a wide range in the available water : available N ratio, a standard error of 1% for grain protein concentration is expected. Moreover, it makes this relationship widely applicable in different cropping systems and varied growing conditions.

Validation of the relationship between available water and nitrogen, and grain protein

Grain protein concentrations of wheat and barley measured at Warra, Formartin and Nindigully in 1996 were plotted against the predicted values (Fig. 6) using the equation:

$$\text{Grain protein (\%)} = 6.52 + 10.0 \times \exp(-0.44 \text{ available water/available N}) \quad (3)$$

where plant available water and available N in 0–120 cm depth of soil were measured at sowing of wheat and barley. For all sites and crops, the relationship between measured grain protein and predicted values was essentially 1:1 (regression coefficient 0.99 ± 0.06) (Fig. 6), although for individual sites slight differences were observed. However, all the measured grain protein concentrations of wheat and barley were within 95% confidence limits of equation 3 (Fig. 5). Even the grain protein concentrations of wheat (cv. Cunningham) grown at the Billa Billa site from 1991 to 1995, which was adversely affected by crown rot (*Fusarium graminearum*) and, therefore, not included in Figure 6, were within 95% confidence limits. These observations confirm the impact of both plant available water and available N on grain protein concentrations of wheat and barley. It also shows that grain protein concentration of cereal crops is a good integrator of available water and available N in soil. Further research is required to establish these relationships for other non-legume crops in a semi-arid subtropical environment, especially in crop growing conditions where N losses through leaching and gaseous emissions are likely to be small.

Conclusions

Our work shows that if the soil is routinely sampled to 120 cm depth, then the amount of plant available water (mm) should be equally matched by available N (kg N/ha) to produce 13% protein concentration PH grade wheat. Malt grade (10.5% protein concentration) barley would require about half of the available N needed for PH grade wheat. The deficit in available N from nitrate-N present in the soil profile can be met by fertiliser N application at sowing.

As a further approximation, rainfall during the fallow period can be used instead of plant available water at sowing. The relationship between fallow rainfall : available N ratio and grain protein (Fig. 7) shows that ratios of 3.7 and 7.4 are required to achieve 13 and 10.5% grain protein concentrations, respectively. Also, available N (almost entirely as nitrate-N) in Vertisols used for winter cropping can be measured in April or May, at least 1 month before sowing so that fertiliser N requirements can be determined and applied just before or at sowing of winter crops. Alternatively, available N in soil can be estimated from mineralisable N, total N and age of cultivation (Dalal and Mayer 1990) or N yields (grain yield by grain N concentration) from the previous cereal crop.

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