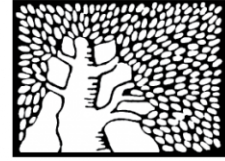


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## Genotypic and fertilization effect on grain protein content in wild and cultivated tetraploid wheats

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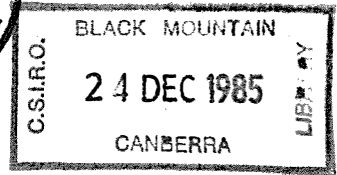
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GENOTYPIC AND FERTILIZATION EFFECTS ON GRAIN  
PROTEIN CONTENT IN WILD AND CULTIVATED  
TETRAPLOID WHEATS

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ABSTRACT. — Five lines of tetraploid wheat were tested for their grain protein content at 10 levels of fertilization ranging from 90 to 2610 mg pure nitrogen per plant. The low levels yielded, in all genotypes, the protein percentage normally obtained under agricultural practice or in the natural habitat. The five lines included: two high protein accessions of the wild wheat, *Triticum turgidum* var. *dicoccoides*, one *durum* cultivar (Inbar), and the  $F_1$  and  $F_6$  derivatives of a cross between one of the var. *dicoccoides* accessions and Inbar. Protein percentage of all genotypes was strongly affected by fertilization, although to a different degree; a significant genotype  $\times$  fertilization interaction was observed. As a result of that interaction the genetic estimate of dominance (“d”) for protein percentage was found to be significantly affected by the fertilization level: at low levels of fertilization the low protein parent (Inbar) was partially dominant, whereas at high levels — the high protein parent (var. *dicoccoides*). At the low levels of fertilization, the differences between genotypes were more pronounced than at high levels. Hence, the commonly applied agricultural levels are recommended for any genotypic evaluation of germplasm for protein percentage. Heterosis was observed in protein weight per grain and grain weight. Protein weight per grain was almost unaffected by the level of fertilization and is therefore suggested as a good parameter for breeding wheat with high protein content.

## INTRODUCTION

Wheat grain protein content (percentage or weight per grain) is known to be strongly affected by genetic and environmental factors. JOHNSON *et al.* (1983) analyzed a collection of 20000 cultivated wheats and found that grain protein content varied from 8 to 20% — which is partially explainable by environmental variation, including the available level of nitrogen in the soil. Indeed, nitrogenous fertilizers were reported to affect grain protein content in wheat (KRAMER, 1979).

AVIVI (1978, 1979) found that several Israeli collections of the wild tetraploid wheat *Triticum turgidum* var. *dicoccoides* — the progenitor of most cultivated wheats — had an exceptionally high percentage of grain protein, ranging from 24 to 29% when grown in their natural habitat, and up to 43% under greenhouse conditions.

In any screening for lines with a genetic potential for increased grain protein content, environmental factors, particularly nitrogenous fertilizers and genotype  $\times$  environment interaction may mask genotypic evaluations. Considering the high protein content of several wild tetraploid genotypes, it seemed of importance to compare the response of a cultivated line and wild high protein lines to nitrogen fertilization, in terms of grain protein content. Such a study was carried out with two accessions of var. *dicoccoides*, one durum cultivar as well as their  $F_1$  and  $F_6$  hybrid derivatives at 10 levels of nitrogen fertilization.

## MATERIALS AND METHODS

Plants were sown in November 1983 and grown in pots under a nethouse, in Rehovot, (coastal plain region), Israel. The following five genotypes were included: two high protein accessions of *Triticum turgidum* var. *dicoccoides*, TTD12 and TTD09; the low protein cultivar 'Inbar' of *T. turgidum* var. *durum*; the  $F_1$  hybrid of the cross between Inbar and TTD12 (215  $F_1$ ) and the high-protein  $F_6$  line of the same cross (1243). The latter was selected for the non-fragility of its spike, free-threshing and high protein percentage (AVIVI *et al.*, 1983). Plants were grown in three liter pots filled with a mixture of 50% volcanic gravel, 33% peat and 17% vermiculite (v/v). A single plant was grown in each pot.

The experiment was conducted at 10 levels of fertilization, using a slow release fertilizer ("Osmocot") applied at rates of zero to nine grams per pot. The fertilizer was given to each pot twice, and at equal amounts: at sowing time and at heading — about three months later. Considering that the release of nutrients lasts about three months, this procedure ensured the availability of nitrogen throughout the whole growth period. The peat was originally enriched with a basic fertilizer containing 10% pure nitrogen, supplying about 90 mg per pot. Since the slow release fertilizer contains 14% pure nitrogen, the total amounts of pure nitrogen for each of the 10 levels were the following: 90, 370, 650, 930, 1210, 1490, 1770, 2050, 2330 and 2610 mg of pure nitrogen per pot for the fertilization levels zero to nine, respectively.

The experimental design consisted of three blocks; each replicate consisted of a single pot. In each block all the combinations genotype  $\times$  fertilizer were present, except for the combination 215 F<sub>1</sub>  $\times$  zero level of fertilizer which was not grown because of lack of F<sub>1</sub> seeds; i.e., a total of  $(5 \times 10) - 1 = 49$  treatments were included in this experiment.

The following parameters were considered: grain protein percentage determined by near-infrared reflectance using a neotec G<sub>0</sub>A, grain weight (mg) and protein weight per grain (mg) obtained by multiplying the protein percentage and grain weight.

## RESULTS

### *Protein percentage*

The interaction fertilization  $\times$  genotype, for protein percentage, was significant at a 5% level (Table 1), i.e., different genotypes had a different pattern of response to the fertilizer (Fig. 1): a linear increase (Inbar), an optimum curve (TTD 09) and a plateau response at fertilization levels between five and six (TTD 12, 1243 and 215 F<sub>1</sub>). The magnitude of the response (the highest value of protein percentage minus the lowest value) was different for each genotype—relatively low for the two accessions of var. *dicoccoides* TTD09 and TTD12 (5 to 6%), medium for the cultivated variety Inbar (7%), and high for the two F<sub>1</sub> and F<sub>6</sub> hybrids, 215 F<sub>1</sub> and 1243 (about 10-11%). At most levels of fertilization, the performance in protein percentage of the five genotypes was according to

TABLE 1

*Analysis of variance for grain protein percentage, protein weight per grain (mg) and grain weight (mg)*

Source of variation	df	Protein percentage		Protein weight per grain		Grain weight	
		Mean square	P(F)	Mean square	P(F)	Mean square	P(F)
Block	2	11.9	0.0026	20.5	0.0060	110.5	0.1035
Fertilization	9	69.7	0.0001	26.4	0.0001	108.0	0.0258
Genotype	4	666.1	0.0001	86.0	0.0001	2033.2	0.001
Fertilization × Genotype	35	3.3	0.0205	3.6	0.5293	71.8	0.0711
Error	74	1.8		3.7		47.1	

the following order: TTD 12, > TTD09, > 1243, > 215 F<sub>1</sub> > Inbar, but the differences between genotypes depended on the fertilization level (Table 2 and Fig. 1). As a result of the interaction genotype × fertilization, the genetic estimate for dominance ("d") of protein percentage was found to be significantly influenced by the fertilization level. This phenomenon is expressed in Fig. 2. The value of "d" was calculated according to the formula: "d" = (protein percentage of 215 F<sub>1</sub> — protein percentage of the midparent) when the protein percentage of the midparent = 1/2 (protein percentage of TTD12 + protein percentage of Inbar). These "d" values varied from — 2.5 to + 2.0 with a standard error SE ("d") = 1.05. Furthermore, a non-random deviation of the F<sub>1</sub> from the midparent was apparent: the low protein parent was dominant at the low levels of fertilization, whereas the high protein parent was dominant at the high levels of fertilization.

#### *Protein weight per grain*

The interaction fertilization × genotype for protein weight per grain was not significant (see Table 1). As seen from Table 2, 215 F<sub>1</sub> had invariably a higher protein weight per grain than both parents (TTD 12 and Inbar), and higher than all other genotypes;

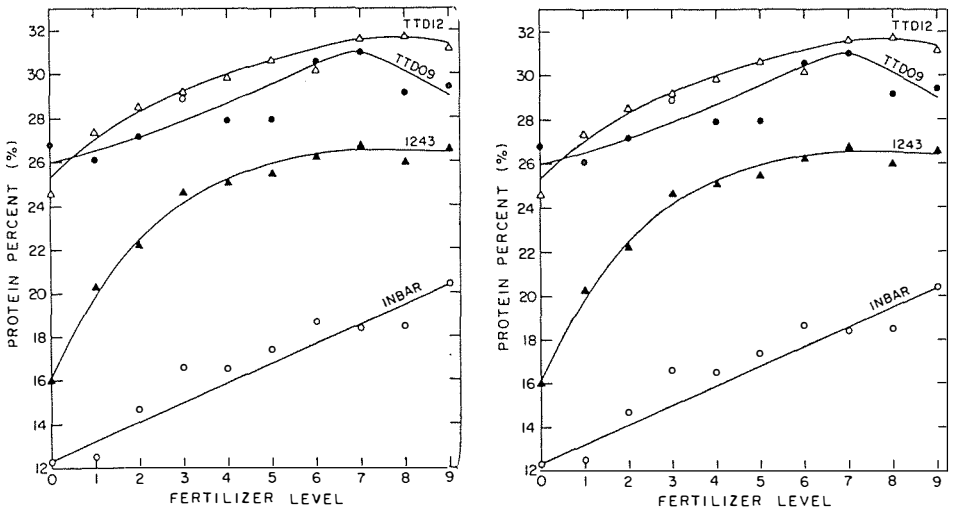


Fig. 1 - Protein percentage of four tested genotypes at 10 levels of nitrogen fertilizer: var. *dicoccoides* {TTD 12 ( $\Delta$ ) and TTD09 ( $\bullet$ ), the *durum* cultivar Inbar ( $\circ$ ) and the  $F_6$  derivative (1243) of the cross between Inbar and TTD12 ( $\blacktriangle$ ).

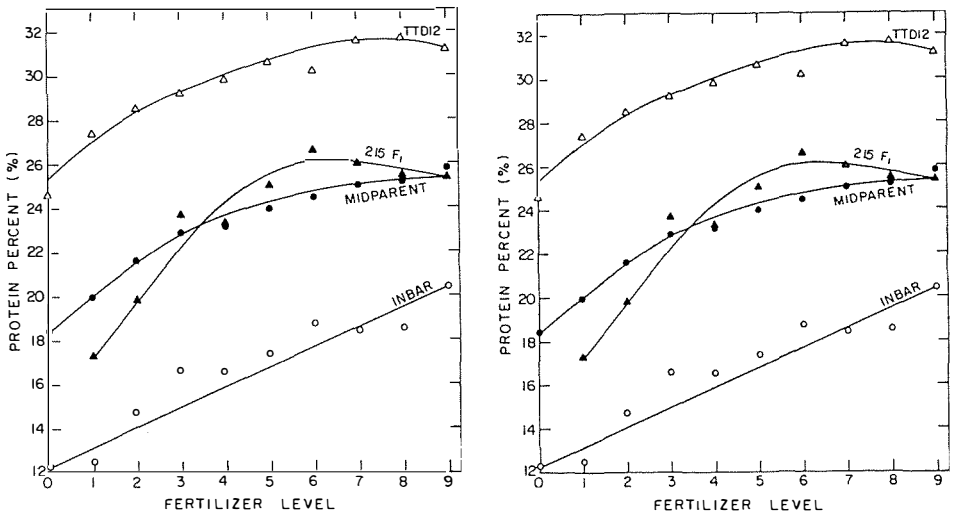


Fig. 2 - Protein percentage of three tested genotypes at 10 levels of fertilizer: var. *dicoccoides* TTD12 ( $\Delta$ ), the *durum* cultivar Inbar ( $\circ$ ), and the  $F_1$  hybrid (215F<sub>1</sub>) of the cross between TTD12 and Inbar ( $\blacktriangle$ ). The midparent values of the above cross are also presented ( $\bullet$ ).

Due to typographical misunderstanding the figures 1 and 2 of page 297 have been reproduced twice.



TABLE 2  
*Means of protein percent, protein weight per grain (mg), grain weight (mg) in the five genotypes at 10 levels of fertilization and their SE (\*)*

Fertilization level	Protein percent (SE = 0.86)				Protein weight per grain (SE = 1.20)				Grain weight (SE = 4.2)						
	TTD09	TTD12	215F <sub>1</sub>	Inbar	1243	TTD09	TTD12	215F <sub>1</sub>	Inbar	1243	TTD09	TTD12	215F <sub>1</sub>	Inbar	1243
0	26.8	24.6	—	12.3	16.0	11.16	8.53	—	8.15	7.21	41.55	35.32	—	66.07	44.89
1	26.1	27.4	17.3	12.5	20.3	8.44	8.78	11.25	8.17	9.34	32.02	32.17	65.11	64.40	46.03
2	27.2	28.5	19.8	14.7	22.2	9.37	11.80	13.39	8.17	10.31	34.01	41.67	67.83	55.67	46.40
3	28.9	29.2	23.7	16.6	24.6	11.67	11.88	15.08	8.15	12.01	40.30	40.27	63.72	51.36	48.66
4	27.9	29.8	23.3	16.5	25.0	12.23	11.96	16.18	8.54	13.21	43.58	40.13	69.19	53.16	52.90
5	27.9	30.6	25.0	17.4	25.5	10.15	10.59	15.41	8.80	12.89	37.78	34.79	61.65	48.81	50.56
6	30.6	30.2	26.6	18.7	26.3	12.82	12.50	14.75	8.46	13.99	41.87	41.67	55.15	51.41	53.17
7	31.0	31.6	26.0	18.4	26.8	15.82	14.64	16.51	9.65	12.80	52.40	46.25	63.65	51.68	46.56
8	29.1	31.7	25.5	18.5	26.0	12.43	12.71	14.37	9.50	12.90	42.80	40.15	56.42	48.40	49.20
9	29.4	31.2	25.4	20.4	26.6	11.65	13.56	12.37	10.99	11.16	39.53	43.33	48.78	53.70	41.80

(\*) SE was calculated using the mean square of the error in Table 1.

Inbar had invariably the lowest protein weight per grain, in spite of its high grain weight, and TTD 09, TTD 12 and 1243 had a similar medium protein weight per grain. Protein weight per grain was a stable character, usually not affected by the level of fertilization, except at the very low levels (0 and 1).

### *Grain weight*

The interaction fertilization  $\times$  genotype for grain weight was not significant at a 5% level of significance ( $P(F) = 7\%$ , see Table 1), but not negligible. Table 2 shows that at most levels of fertilization, grain weight was according to the following order: 215 F<sub>1</sub> > Inbar > 1243 > var. *dicoccoides*. Both TTD09 and TTD 12 were similar for this character. Grain weight was a stable character only lightly affected by fertilization. No general trend was found for the effect of fertilization on grain weight.

### DISCUSSION

Although the nitrogenous fertilizer strongly influenced protein percentage in all tested lines the effect was strongly dependent on the genotype. Interestingly, both var. *dicoccoides* accessions had a high protein percentage at low fertilization levels. This phenomenon may reflect a selective pressure to guarantee minimal, though high protein percentage, as reported for seeds of various wild Gramineae in their wild habitat: var. *dicoccoides* (AVIVI, 1978, 1979); *T. timopheevii* var. *araraticum*, *Aegilops longissima* (LEVY *et al.*, 1985), wild barley (AHOKAS, 1982) and wild oats (LADIZINSKY and FAINSTEIN, 1977). The protein percentage of Inbar increased linearly in response to nitrogen but probably could not reach a higher value at higher nitrogen levels (unpublished data). The interaction genotype  $\times$  fertilizer may mask genetic differences between genotypes: the difference between genotypes was relatively smaller at the high levels of fertilization (7 to 9), and more pronounced at the fertilization levels between 2 and 3; at these low levels, the grain protein percentage corresponded to that attained under normal agricultural practices, or in the natural habitat.

The genetic parameter of dominance for protein percentage ("d") was strongly affected by the interaction genotype  $\times$  environ-

ment: the low protein parent was dominant at low levels of fertilization while the high protein parent — at the high ones. Since heterozygosity is composed of “high” and “low” protein alleles it is speculated that at low levels of fertilization the low protein alleles were preferentially expressed whereas at high levels of fertilization — the high protein alleles. This differential expression may explain some controversial cases in the literature concerning the dominance of protein percentage. AVIVI *et al.* (1983) found partial dominance of low protein percentage in  $F_2$  population between var. *dicoccoides* and Inbar, while KRALJEVIC BALALIC *et al.* (1982) found cases of dominance of the high protein parent.

Goos *et al.* (1982) suggested that grain protein percentage in the harvested seeds of winter wheat could serve as an indicator of nitrogen availability in the soil. They determined a critical protein percentage of 11.5%, below which there was a nitrogen deficiency in the soil. This criterion was regardless of genotypic differences. We suggest that a line such as 1243, whose protein percentage is strongly affected by the commonly used levels of nitrogen fertilization (0-3), could be a good tester for indicating the nitrogen level in the soil.

There was an impressive heterosis in grain weight and protein weight per grain at all levels of fertilization. Since high grain weight is known to be a dominant character (MILET and PINTHUS, 1980), this heterosis shows that the var. *dicoccoides* accession contained genes for increased grain weight — not present in the cultivar Inbar. The observed heterosis for protein weight per grain, is mostly a result of the heterosis in grain weight. Protein weight per grain has been shown in this work to be a very stable character, not affected by environmental changes but showing strong genotypic dependence and therefore, is a suitable character to breed for. It has also been shown to be positively correlated both to protein percentage and grain weight (LOFFLER and BUSCH, 1982). The heterosis for this character could be exploited in hybrid wheat production.

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