EFFECT OF SEED SIZE AND PROTEIN CONTENT AND N APPLICATION TIMING ON SEEDLING VIGOR AND GRAIN YIELD OF BARLEY[1]

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ABSTRACT - The objective of this study was to evaluate the effect of seed size, protein content and N application timing on barley seedling vigor and grain yield. Two seed sizes, three protein levels and two genotypes (Hazen and Hazen semi-dwarf, SD, isotype pair) were used in a laboratory and in a field study. In a greenhouse study with Hazen only, N application timing (at sowing and 20 days after emergence) treatments were added to the other treatments. High seed protein content and large seed positively affected coleoptile length, root dry weight and seedling dry weight at early stages of plant development, but had no effect on emergence rate index and plant and root dry weights at 40 days after emergence. Both the standard height and semi-dwarf isotypes responded similarly. The delay of N supply until 20 days after emergence negatively affected the initial seedling growth, but had no effect on plant dry weight 40 days after emergence in the greenhouse. Grain yield and yield components were not affected by seed protein content nor seed size under field conditions.

Index terms: seed protein, seed vigor, nitrogen, grain yield.

EFEITO DO TAMANHO DA SEMENTE, DA CONCENTRAÇÃO DE PROTEÍNA E DA ÉPOCA DE APLICAÇÃO DE NITROGÊNIO NO VIGOR E RENDIMENTO DE GRÃOS DE CEVADA

RESUMO - O objetivo deste trabalho foi avaliar o efeito do tamanho da semente, seu conteúdo de proteína, e época de aplicação do nitrogênio, no vigor das plântulas e no rendimento de sementes de cevada. Dois tamanhos de sementes, três níveis de proteína e dois genótipos (Hazen e um mutante Hazen semi-anão induzido) foram utilizados em um estudo de laboratório e de campo. Em estudo de casa de vegetação, somente um genótipo foi utilizado, com épocas de aplicação de N, acrescido aos outros tratamentos. Proteína alta e sementes maiores afetaram de forma positiva o tamanho do coleóptilo e o peso seco das raízes e das plântulas nas primeiras fases de desenvolvimento. Contudo, não houve influência destes fatores na velocidade de emergência e no peso seco da parte aérea e das raízes das plantas 40 dias após a emergência. Ambos os genótipos apresentaram resposta similar. O retardamento da aplicação de N teve efeito negativo no desenvolvimento inicial das plântulas, porém não afetou o peso seco das plantas 40 dias após a emergência. O rendimento de sementes não foi afetado pelo conteúdo protéico ou pelo tamanho da semente.

Termos para indexação: proteína na semente, tamanho de semente, vigor, rendimento de sementes.

INTRODUCTION

Seedling establishment in the field is intensively competitive, especially under stress conditions, such as temperature and moisture extremes, high weed populations and low soil fertility levels. Under these situations, factors related to seed quality may have profound effects on the rate of seedling growth and establishment. It has been reported that seed source, seed size and seed protein content are positively related to early growth and sometimes with economical yield (McFadden, 1963; Lowe & Ries, 1972; Evans & Batt, 1977; Bulisani & Warner, 1980; Torres & Paulsen, 1982; Linhares & Nedel, 1984). It has also been shown that with high soil N availability, seed protein content has no effect on seedling vigor (Lopes & Grabe, 1973; Welch, 1977; Konesky et al.,

[1] Accepted for publication on November 22, 1995.

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1989). Greenhouse studies using sand, vermiculite or hydroponic solutions, have indicated that withholding N negatively affected seedling growth and dry weight (Dale, 1972; Bulisani & Warner, 1980; Perby & Jensen, 1986). However, in most of these studies, a relatively constant and high temperature was used (20°C) and no initial N was present. These conditions are generally not found during seed germination and seedling growth and establishment in field situations.

The objectives of this study were to evaluate: 1) the effect of seed size and seed protein content on seedling vigor and grain yield under laboratory and field conditions with a standard height-semi-dwarf isotype pair of barley; and 2) the effect of seed protein content and timing of nitrogen application on barley seedling vigor under relatively low soil temperatures under greenhouse conditions.

MATERIALS AND METHODS

Seed of the 6-row cultivar Hazen and an induced semi-dwarf (SD) Hazen mutant (Aydin, 1985; Ullrich & Aydin, 1988) used for the experiments were obtained from a fertilizer experiment conducted in the field in 1988 (Table 1).

Seed were sized based on four different width categories with screens with slotted holes. The screen sizes were: 2.78 mm x 19.0 mm; 2.58 mm x 19.0 mm; 2.38 mm x 19.0 mm. The separation was repeated four times. Seeds that remained on top of the 2.58 mm and 2.38 mm sieves were used in the experiments. Kernel weights were determined from four samples of 100 seeds from each isotype of the two width categories.

TABLE 1. Seed protein content and seed size of the two barley isotype pair used in the seedling vigor experiment.

Isotype	Sieve size class[1]	Kernel wt. (mg)	Field N treatments (kg/ha) 0	60	120
			----- Protein content (%) -----		
Hazen	small	35.3	7.4	9.4	11.7
Hazen	large	40.3	7.8	10.0	11.8
Hazen SD	small	24.7	9.5	10.8	12.9
Hazen SD	large	32.7	9.4	11.2	13.2

[1] Small and large = seeds that remained on 2.38 and 2.58 x 19.0 mm sieves, respectively.

Seed nitrogen concentration was determined by the Micro-Kjeldahl method (Association of Official Agricultural Chemists, 1965). Percentage of protein was determined by multiplying % N by 6.25.

Laboratory experiment

Four replications of ten seeds of each isotype from each seed protein content class and seed size class were placed in moistened paper towels and held for ten days in a dark germinator at 15°C. The experiment was set up as a completely randomized factorial design.

The following determinations were carried out on the seedlings developed under the conditions mentioned above: coleoptile length, shoot length, number of seminal roots, length of the primary seminal root, total root length, shoot dry weight and root dry weight.

Greenhouse experiment

Seeds from the large size class (on 2.58 x 19 mm sieve) of three seed protein contents of the standard height genotype Hazen were used. Each seed was individually weighed. The seed weight ranged from 40 to 42 mg.

The light/dark regime and air temperature were not controlled. However, the growing medium (soil) temperature was controlled to simulate the average soil field temperature during the first stages of barley development under Rio Grande do Sul, Brazil, conditions.

The maximum soil temperature during the day 5 cm deep, was approximately 17°C and at night approximately 12°C until ten days after planting. After ten days the soil temperature control was shut off during the day and was only activated if the temperature reached 23°C. The soil used was a mixture of Palouse silt loam (fine-silty, mixed mesic Pachic Ultic Haploxeroll) and Shano silt loam (coarse, silty, mixed mesic, Xerollic Camborthid). The nitrate concentration of the mixture was 17 ppm. Ammonium nitrate was the fertilizer formulation used at a rate of 126 kg/ha of N (121.5 mg of N/pot with 2.1 kg of soil). Two N application timings and three seed protein contents were the treatments. The two N application timings were: 1) 26 kg N/ha applied at sowing time 5 cm below the seed and the remaining 100 kg/ha of N applied to the soil surface 20 days after emergence (Nd); 2) 126 kg N/ha applied to the soil surface 20 days after emergence (Nt). To supply phosphorus and sulphur, 100 mg/pot of triple superphosphate and gypsum ($CaSO_4$), respectively, were applied at planting time. In each pot seven seeds were planted 3 cm deep. Immediately after emergence the seedlings were thinned to four/pot. The experiment was set up as a completely randomized factorial design with four replications.

Plants were sampled at three times: 10, 20 and 40 days after emergence. At each sampling time the following determinations were made: shoot dry weight, root dry weight and shoot N content. Emergence rate index (ERI) was calculated as described by Allan et al. (1962).

Field experiment

The effect of seed protein content and seed size (Table 1) on yielding ability of the Hazen standard-Hazen SD isotype pair was studied in 1989 at Spillman Agronomy Farm, Pullman, WA in a Palouse silt loam soil. Nitrogen was applied in a liquid form as ammonium nitrate at a rate or 70 kg/ha during drilling. Phosphorus and sulphur were pre-plant broadcast and incorporated into the soil as triple superphosphate and calcium sulphate at a rate of 45 kg P/ha and 20 kg S/ha, respectively.

Seedling dry weight was determined from ten seedlings sampled randomly twenty days after emergence from within segments of the six internal rows where a uniform stand was observed.

Plot size was 2.0 x 1.20 m (eight rows spaced at 0.15 m). The design was a randomized complete block arranged in split-plots with six replications. Seed sizes were the main plots, and protein contents the sub-plots.

RESULTS AND DISCUSSION

Laboratory experiment

All seedling vigor variables were significantly affected by seed size and seed protein content, except seed size had no effect on coleoptile length and shoot length. Two-way and three-way interactions were not significant. There was a significant positive effect of increasing seed protein percentage on number of seminal roots, coleoptile length, shoot length, root length, shoot dry weight and root dry weight (Table 2). There was in general a significant increase in the means of these same variables by increasing seed size (Table 2). Although the differences in the number of seminal roots were significant, they were numerically very small, consequently there is little or no biological significance. The primary seminal root was present in all seedlings and its length was not significantly affected by seed size or protein content. Consequently, the differences observed in root length and root dry weight due to seed size and protein content may be attributed to the secondary seminal roots. The non-significant association between number of secondary seminal roots and root length (r=0.25), indicated that the size of the secondary seminal roots was responsible for the increase in total length and dry weight of the seminal root system.

Although semi-dwarf barleys do not seem to have the field emergence problems to the extent that semi--dwarf wheats have (Allan, 1980; Bacaltchuk & Ullrich, 1983), the positive effect observed on coleoptile length by increasing seed size and protein content (Table 2) may be of importance to good stand establishment in situations where deep planting is necessary. Torres & Paulsen (1982) also reported that wheat seeds with the highest protein content had greater emergence under deep planting conditions. The positive effect of seed size and protein content on shoot and root length and weight indicated in this experiment (Table 2) has also been reported by Kaufmann & McFadden (1962), Lopes & Grabe (1973) and Bulisani & Warner (1980).

Nitrogen content per seed, more than seed size and percent protein, appeared to affect shoot dry weight (Table 3). The same trend was observed in both genotypes. Within and across seed size categories higher seed N concentration was reflected consistently in greater shoot dry weight. Therefore, higher seed N concentration overcame any small seed size disadvantage. Bulisani & Warner (1980) reported similar results in wheat from a greenhouse experiment. The other parameters used to evaluate seed vigor did not show the same consistency as shoot dry weight, which indicates that other factors besides N content may be limiting seedling emergence. The shoot/root dry weight ratio was significantly higher for the highest protein seeds (1.07 vs 0.98), indicating a higher rate of growth of the shoot in relation to the root. This response is similar to that observed in other N fertilization experiments (Bulisani & Warner, 1980; Marschner, 1986).

The semi-dwarf isotype had lower means than the standard height isotypes for all seedling vigor traits evaluated except number of seminal roots (Table 2).

TABLE 2. Seedling vigor parameters as affected by seed size, seed protein content and genotype under laboratory conditions[1].

Factors	Seminal roots	Coleoptile length	Shoot length	Root length	Shoot dry wt.	Root dry wt.
	------ no. ------		------ cm ------		------ mg ------	
Large seed	5.2 a	5.9 a	6.9 a	61.3 a	6.30 a	6.34 a
Small seed	5.0 b	5.7 a	6.7 a	57.5 b	5.82 b	5.76 b
High protein	5.2 a	5.9 a	7.3 a	62.6 a	6.61 a	6.31 a
Medium protein	5.2 a	5.7 ab	6.7 b	59.2 b	5.97 b	6.02 b
Low protein	5.0 b	5.6 b	6.3 c	56.5 c	5.60 c	5.82 b
Hazen	5.2 a	5.9 a	7.2 a	65.9 a	6.25 a	6.80 a
Hazen SD	5.1 a	5.6 b	6.3 b	52.9 b	5.88 b	5.29 b

[1] Column means, within a factor, followed by the same letter do not differ at P=0.05 by F test where two means were compared, and LSD test where three means were compared.

TABLE 3. Dry matter accumulation of ten-day old seedlings under laboratory conditions as affected by seed N concentration and seed size[1].

Genotype	Small seed			Large seed		
	P1[2]	P2	P3	P1	P2	P3
Hazen	------- μg N/seed -------			-------- μg N/seed --------		
	674	528	416	777	640	499
Shoot dry wt. (mg/pl)	6.63 a	6.09 b	5.49 c	7.08 a	6.42 b	5.79 c
Hazen SD	------- μg N/seed -------			-------- μg N/seed --------		
	514	432	365	697	590	480
Shoot dry wt. (mg/pl)	6.18 a	5.37 b	5.21 b	6.58 a	6.03 b	5.93 b

[1] Means in a row followed by the same letter within the same seed size category do not differ at P = 0.05 by LSD test.

[2] P1, P2, P3 = High, medium and low seed protein content, respectively.

Greenhouse experiment

Protein content in the seed significantly affected the shoot and root dry matter production and total N in the shoot at the first two sampling times. Shoot and root dry weight and N content in the shoots from seed with the lowest seed protein content were significantly lower than those from seed with medium and high seed protein levels, at these first two sampling times (Table 4). Although not significant, the highest protein seed showed a consistent tendency to produce greater seedling shoot and root dry weights than the seed with medium protein content. The differences observed in total N content at the two first sampling times was due to the differences in dry matter production rather than in N concentration in the tissue. There was a reduction in the N concentration in the shoot tissue with increased seed protein level (1st sampling: low protein = 4.9%, and high protein = 4.8% of N in the shoot tissue; 2nd sampling: low protein = 4.1%, and high protein = 3.9% of N in the shoot tissue) probably due to a dilution effect.

The differences in parameters measured at the first two sampling times among seeds with three protein levels disappeared by the third sampling time (Table 4). There was a proportional reduction in the differences among seed protein contents for shoot and root dry weight and total N in the shoot from the first to the second sampling culminating in no differences at all by the third sampling time (Table 4). This indicates that the N supplied by the soil was enough to overcome the initial differences in seed protein content. Konesky et al. (1989) did not find an association between barley seed protein and seedling growth after four weeks and they attributed this to the constant and high supply of N to the plant in the hydroponic system was used. There was no significant difference in the emergence rate index among seed protein content levels. Similar results were reported by Bulisani & Warner (1980).

TABLE 4. Hazen isotype seedling vigor parameters as affected by seed protein content and N application time under greenhouse conditions[1].

Factor[2]	10 DAE[3]			20 DAE			40 DAE			Emergence rate index
	Shoot dry wt.	Total N	Root dry wt.	Shoot dry wt.	Total N	Root dry wt.	Shoot dry wt.	Total N	Root dry wt.	
	mg									-- index --
P1	55.9 a	2.7 a	28.7 a	182.8 a	7.2 a	120.6 a	892.8 a	29.1 a	526.6 a	78.8 a
P2	54.6 a	2.7 a	27.1 a	176.6 a	6.9 b	118.1 a	875.9 a	28.9 a	525.6 a	79.3 a
P3	47.6 b	2.4 b	21.7 b	161.8 b	6.6 b	99.9 b	878.4 a	29.9 a	483.8 a	73.8 a
Nt	52.1 a	2.5 b	26.0 a	165.1 b	6.1 b	114.4 a	887.7 a	30.0 a	498.5 a	76.1 a
Nd	53.3 a	2.7 a	25.7 a	182.4 a	7.7 a	111.4 a	877.1 a	28.5 b	526.6 a	78.5 a

[1] Column means, within a factor, followed by the same letter do not differ at P = 0.05 by LSD test.
[2] P1, P2, P3, = High, medium and low seed protein content, respectively. Nt = All N (126 kg/ha) applied 20 days after seedling emergence; Nd = Part of the N (26 kg/ha) applied at sowing, and the rest (100 kg/ha) applied 20 days after seedling emergence.
[3] DAE = Days after seedling emergence.

The supply of N 5 cm below the seed with sowing vs delayed application significantly affected shoot dry weight at only the second sampling time (Table 4). The absence of a significant effect at the first sampling time could be explained by the earliness of the sampling time (ten days after emergence). The non-significant effect at the third sampling was probably due to the N supplied by the soil which was adequate to overcome the differences observed at the second sampling time. These results suggest that N application can be delayed up to 20 days after emergence in soils with similar N and water availability as in this experiment. These results agreed with those obtained by Wietholter et al. (1989) with triticale under field conditions. The main factor that determined shoot dry weight in the present study at the first sampling time was seed protein content rather than N application timing (Table 5). At the second sampling time both factors, N application timing and seed protein content affected shoot dry weight (Table 5). The N added at sowing had a greater effect on the dry weight of shoots from seeds with the medium and high protein content than that of the shoots from seed with low protein content at the second sampling time (Table 5). That is, the more developed seedlings which originated from seed with higher protein content were able to respond better to the initial application of N.

Under experimental conditions the delay in N application did not affect root growth (Table 4). This indicated that enough carbon was assimilated and transferred to the roots to allow their development. At the second sampling when shoot dry weight was negatively affected by delay in N supply, the lack of effect on root dry weight may indicate that the root system had a larger sink capacity and the assimilate was transferred preferentially to the roots rather than being reatined in the shoot. The significantly higher concentration of N in the tissue from the plants that received N at sowing, probably accounted for difference in the shoot N content in the first sampling time (Table 4). At the second and third sampling time, the difference in N content in the shoot was mainly due to the difference in the shoot dry weight. Emergence rate index was not affected by N fertilizer treatments (Table 4).

Field experiment

Seedling vigor, as measured by seedling dry weight was significantly affected by seed size and seed protein. Seedlings originating from the larger seed had significantly higher dry weights. Seed with medium and high protein contents produced seedlings with significantly higher dry weights than the low protein seed (Table 6). Grain yield and yield

TABLE 5. Hazen isotype seedling dry weight as affected by seed protein content and N application timing under greenhouse conditions[1].

Seed protein[2]	Seedling dry weight					
	10 DAE[3]		20 DAE		40 DAE	
	Nt[4]	Nd	Nt	Nd	Nt	Nd
	mg					
P1	56.4 aA	55.6 aA	170.9 aB	195.0 aA	882.5 aA	903.1 aA
P2	52.9 bB	56.2 aA	167.2 aB	185.9 aA	887.5 aA	864.4 aA
P3	46.9 cA	48.1 bA	157.3 bB	166.3 bA	893.1 aB	863.8 aA

[1] Means followed by the same letter do not differ at P = 0.05 by LSD test for columns (lower case) and t-test for rows (upper case) within a given sampling time.
[2] P1, P2 and P3 = High, medium and low seed protein content, respectively.
[3] DAE = Days after emergence.
[4] Nt = All N (126 kg/ha) applied 20 days after seedling emergence; Nd = Part of N (26 kg/ha) applied at sowing, and the rest (100 kg/ha) applied 20 days after seedling emergence.

TABLE 6. Seedling growth parameters, grain yield and yield components, as affected by seed size, seed protein content and genotypes under field conditions[1].

Factors	Seedling shoot		Yield	Spikes/m²	Kernels/spike	Thousand kernel wt.
	dry wt.	N				
	mg/pl		kg/ha	no.		g
Large seed	97.2 a	5.0 a	4807.7 a	296 a	46.2 a	36.4 a
Small seed	86.6 b	4.5 b	4787.7 a	296 a	46.4 a	35.3 a
High protein	96.1 a	5.0 a	4837.7 a	301 a	46.5 a	36.1 a
Medium protein	95.1 a	5.0 a	4779.1 a	297 a	46.0 a	36.0 a
Low protein	84.6 b	4.4 b	4776.3 a	289 a	46.3 a	35.4 a
Hazen	90.2 a	4.8 a	5763.6 a	321 a	48.5 a	40.0 a
Hazen SD	93.6 a	4.8 a	3831.8 b	271 b	44.1 b	31.7 b

[1] Column means, within a factor, followed by the same letter do not differ at P = 0.05 by F test where two means were compared, and by LSD where three means were compared.

components were not affected by seed protein content and seed size (Table 6). These data coincide with the greenhouse experiment results to some extent in that significant effects detected early tend to disappear later. The two isotypes had similar seedling development patterns, but, Hazen had higher yield and yield components than Hazen SD. The lack of effect of seed protein on grain yield agreed with the results of Lopes & Grabe (1973), Welch (1977) and Bulisani & Warner (1980) but disagreed with results of Ries et al. (1970) and Ries (1971). In concerning seed size, the results were similar to those of Linhares & Nedel (1984).

CONCLUSIONS

1. Both the standard height and the semi-dwarf isotypes respond in a similar way to protein content and seed size.

2. Nitrogen can be delay up to 20 days after emergence without affecting plant productivity.

3. Grain yield is not affected by seed size and seed protein content.

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