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Original Contribution

VARIABILITY OF SEEDLING TRAITS IN SEMI-DWARF AND NON-DWARF SPRING BARLEY CULTIVARS

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ABSTRACT
The aim of the present study was to study some seed and seedling characteristics and their relationship with plant height in semi-dwarf and non-dwarf spring barley varieties. The study was conducted at the Institute of Agriculture - Karnobat, Southeastern Bulgaria during the 2019 and 2020 growing seasons. Ten semi-dwarf and ten non-dwarf cultivars of spring barley from the USA and Canada were studied. The semi-dwarf cultivars showed significantly lower mean values for the length of coleoptile and seedling and seminal root number compared to non-dwarf cultivars. However, there were semi-dwarf cultivars with a coleoptile length similar to that of non-dwarf cultivars. Correlation analysis showed a significant association of plant height with seedling length and seminal root number. The rest of the seedling characteristics were uncorrelated with plant height and, hence allowing breeding for that trait without compromising high seedling vigour.

Key words: *Hordeum vulgare*, germination, seedling characteristics, plant height

INTRODUCTION

Early vigour has been identified as a desirable trait for cereal crops including barley (1). The rapid development of leaf area during early growth reduces evaporative water loss by shading the soil surface from direct solar radiation, allowing more water to be available for crop and improve water-use efficiency and enable drought avoidance (2). Early vigour also provides other agronomic benefits, such as improved nitrogen and phosphorus uptake (3, 4) and enhanced weed competitiveness (5). Variation of crop establishment and early vigour is related to a number of seed and seedling characteristics (6).

Genes responsible for the semi-dwarf growth habit in barley have a significant effect on the most traits associated with the initial growth.

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Semi-dwarfism has been considered a valuable trait in cereal breeding since short-stature cultivars show increased lodging resistance and improved harvest index (7).

In barley, different types of semi-dwarf mutants as *brachytic* (*brh*), *breviaristatum* (*ari*), *slenderdwarf* (*sld*), *erectoides* (*ert*), *semidwarf (s dw)*, *semi-brachytic (uzu*) and *dense spike* (*dsp*) have been identified but only few of semi-dwarf genes have been used in barley breeding (8). Short-culm genes *uzu 1* (*semi-brachytic 1*), *ari-e* (*breviaristatum-e*), and *sdw1*(*semi-dwarf 1*) are the most extensively used in barley breeding programs (9).

The semi-dwarf cultivars with *uzu1* gene are commonly cultivated in East Asia. The *uzu1* gene has pleiotropic effects on the coleoptile, leaf, rachis internode, awn, glume, and grain which are frequently reduced in length and increased in width (10). The plant architecture is more erect with acute leaf blade angles. Spikes are compact, short-awned with dense basal spikelets, often with opposite spikelets in the tip due to the irregular length of rachis internodes (11 Dockter *et al.*, 2014). Heat and drought stress have an effect on the phenotypic expression of some of the traits of the *uzu1* mutants (11-13).

The *sdw1/denso* gene has been used in many modern barley cultivars in European countries, the USA, Canada, and Australia (14). The spread of the *sdw1/denso* is likely the result of less pleiotropic effects of this gene. Nevertheless, some negative effects of *sdw1* mutant alleles as delayed maturity, smaller grain size, and reduced malt quality have been reported (15, 16).

The *ari-e* gene from Golden Promise is a widely used semi-dwarf gene in spring barley in Europe. This gene significantly affects some agronomic traits such as grain size, grain composition, malting quality and yield (17). Mutants with *ari-e* showed improved salt tolerance (18).

The pleiotropic effect and environment-specific phenotypic expressions of some of the traits of semi-dwarf cultivars suggest that additional information is needed for these cultivars before their inclusion in a particular barley breeding program. Therefore, the aim of the present investigation was to study some seed and seedling characteristics and their relationship with plant height in semi-dwarf and non-dwarf spring barley varieties.

MATERIAL AND METHODS

Plant material and evaluation of plant height and 1000-grain weight

Ten semi-dwarf and ten non-dwarf cultivars of spring barley from the USA and Canada were included in this study **(Table 1).**

The study was conducted at the Institute of Agriculture - Karnobat (42°39' N, 26°59' E) situated in the Southeastern Bulgaria during 2019 and 2020 growing seasons under the same growing technology. The weather conditions during the growing season 2020 were adverse due to the limited precipitation, whereas season 2019 was more favourable for the formation of optimal plant height. The cultivars tested were sown at a rate of 4.0 million viable, untreated seeds per ha in 2.2 m^2 plots in three replications.

Plant height was measured on 25 plants of each replication from the soil surface to the spike tip excluding awns at full maturity. The 1000-grain weight (g) was determined from the grain weight of 200 randomly taken grains multiplied by 5.

Evaluation of germination characteristics

The experiment was started 5 months after harvesting to ensure a high seed germination rate. One hundred uniform seeds of each cultivar were placed in a Petri dish (diameter 12 cm) with double-layer filter paper in three replications. The number of germinated seeds was recorded daily for 7 days. Germination energy was determined on the 3rt day and the germination on the 7th day as a percent of the germinated seed of the total number of seeds. The germination index was calculated by the following equation: Germination index = (No. of germinating seeds/Days of first count)+….+(No. of germinating seeds/Days of final count) (18).

Evaluation of seedling characteristics

Twenty-five seeds with similar seed size per cultivar were put 2 cm from the top of a germination towel made from filter paper. The total height of the towel was 25 cm. Each seed was placed on the germination towel with its embryo down. The towels were rolled loosely and fastened with a rubber band. The wrapped towels were moistened and ran¬domly arranged vertically in plastic containers with about 3-4 cm water in the bottom. On the 7th day, 20 plants from each cultivar and each replication were measured: coleoptile length (CL, cm), seedling length (SL, cm), seminal root length (RL, cm), seminal root number (RN), seedling fresh weight (SFW, g), seedling dry weight (SDW, g), seminal root fresh weight (RFW, g), seminal root dry weight (RDY, g).

Both experiments for evaluation of germination and seedling characteristics seed samples were conducted in a growth chamber under 16 h/8 h light-dark photoperiod at 22°C.

Statistical analyses

The *Duncan's Multiple Range Test* with a significance level $p \leq 0.05$ was used to reveal differences among cultivarss. Additionally, to test differences in germination and seedling characteristics and plant height across semi-dwarf and non-dwarf cultivars, the t-test ($p \leq 0.05$) was used. Correlation analysis with a significance level $p \leq 0.05$ and 0.01 was done to compare the relationships between studied traits.

Table 1. *Origin of studied cultivars*

Semi-dwarf cultivars			Non-dwarf cultivars		
Name	Year of release	Country of release	Name	Year of release	Country of release
Bold	1978	USA	AC Hamilton	1994	Canada
Ishi	2005	USA	AC Lacombe	1991	Canada
Jackson	1985	Canada	AC Malone	1999	Canada
Mahigan	1998	Canada	AC Nadia	1993	Canada
Maranna	1993	USA	AC Stephan	1992	Canada
Niska	1999	Canada	Creel	2005	USA
Tukwa	1996	Canada	Creme	1988	Canada
UC828	1995	USA	Husky	1953	Canada
Vivar	2000	Canada	Nord	1958	Canada
WestBred Barcott	1985	USA	Sophie	1980	Canada

RESULTS

Mean values of germination characteristics, seedling and root traits, plant height, and 1000-grain weight of semi-dwarf and non-dwarf cultivars are presented in **Table 2** and **Table 3,** respectively. There were no significant differences between studied non-dwarf cultivars for germination energy and germination. In this set of cultivars, germination energy varied between 94.67% (Nord) and 100.00% (AC Stephen and AC Hamilton), and germination was between 96.67% (AC Nadia) and 100.00% (Creme, AC Stephen and AC Hamilton). In semi-dwarf cultivars, significantly lower germination energy and germination were observed in cultivars Niska (86.00% and 92.67%) and Maranna (86.00% and 91.33%). The highest germination energy was found in Bold and UC828 and the highest germination in Vivar, Bold, and UC828. Values of germination index varied from 34.37% (Niska) to 49.07% (UC828). Three cultivars – two non-dwarf (Sophie - 4.20 cm and AC Hamilton - 4.30 cm) and one semi-dwarf (Bold - 4.21 cm) had significantly longer coleoptile compared to the rest studied genotypes. The shortest coleoptile was measured in W. Barcott (2.35 cm) and Maranna (2.17 cm). The seedling length raged from 8.44 cm (AC Nadia) to 11.07 cm (Nord) in non-dwarf varieties and from 5.70 cm (Maranna) to 8.86 cm (Jackson) in short stature cultivars. Shorter seminal roots were observed in Maranna (7.35 cm) while cultivar Bold (11.72 cm) had the longest seminal roots. The average number of seminal roots in most cultivars was between 5.00 and 6.00 roots per germinated seed. The lower number of seminal roots was recorded in W. Barcott, Mahigan, and Ishi. Cultivar Bold had the highest values of seedling and root fresh and dry weight. The height of the plant was between 94.61 cm (Creel) and104.20 cm (Nord) in non-dwarf and between 54.23 cm (Bold) and 81.66 cm (Vivar) in semi-dwarf varieties.The highest 1000-grain weight was found in Creme (46.43 g), followed by Bold (46.18 g), while Tukwa had the lowest value for the 1000-grain weight (30.90 g).

Table 2. *Mean values of germination characteristics, seedling and root traits, plant height, and 1000-grain weight of semi-dwarf spring barley cultivars (2019-2000)*

Cultivar	GE	G	GI	CL	SL	RL	RN	SFW	SDW	RFW	RDY	PH	TGW
Bold	100.00a*	100.00a	48.83ab	4.21a	8.33h	11.72a	5.10ef	2.27a	0.20a	1.63a	0.23a	54.23k	46.18a
Ishi	98.67a	99.33a	40.17ef	3.78b	7.55i	9.44j	4.70g	1.91b-d	0.17b-d	1.33b-g	0.18de	73.02hg	43.28c
Jackson	99.33a	99.33a	46.67a-d	3.69bc	8.86fg	11.19bc	5.13ef	1.95bc	0.19ab	1.06gh	0.17e	80.80ef	40.22ef
Mahigan	94.67a	96.67a	44.07a-e	3.04h	7.51i	11.04c	4.82g	1.51ij	0.14d-f	1.58ab	0.17e	79.32f	33.92j
Maranna	86.00b	91.33b	35.90fg	2.17j	5.70k	7.35l	5.28de	1.21k	0.12f	1.08gh	0.18de	72.19h	37.92gh
Niska	86.00b	92.67b	34.37g	3.08gh	6.53j	8.48k	4.94fg	1.17k	0.12ef	0.72i	0.13f	73.83g	36.03i
Tukwa	96.67a	98.00a	42.53de	3.03h	7.70i	10.04g-i	5.30de	1.43j	0.14d-f	1.36a-f	0.22a-c	80.59ef	30.90k
Vivar	96.67a	100.00a	43.33b-e	3.14gh	8.41h	10.37d-f	5.12ef	1.57h-j	0.16cd	1.08gh	0.19b-e	81.66e	44.67b
UC828	100.00a	100.00a	49.07a	3.67bc	8.38h	10.23e-h	5.10ef	1.75d-g	0.16cd	1.50a-c	0.22a-d	63.78i	40.98de
W. Barcott	96.67a	98.00a	41.57de	2.35i	6.43j	9.45j	3.98h	1.53h-j	0.15c-e	1.46a-d	0.24a	58.63j	36.57hi

GE - germination energy, G - germination, GI - germination index, CL - coleoptile length, SL - seedling length, RL - seminal root length, RN - seminal root number, SFW - seedling fresh weight, SDW - seedling dry weight, RFW - seminal root fresh weight, RDY - seminal root dry weight, PH – plant height, TGW - 1000-grain weight; * means in columns followed by the same letter are not significantly different at $p \leq 0.05$ the according to Duncan's Multiple Range Test

Table 3. *Mean values of germination characteristics, seedling and root traits, plant height, and 1000-grain weight of non-dwarf spring barley cultivars (2019-2000)*

Cultivar	GE	G	GI	CL	SL	RL	RN	SFW	SDW	RFW	RDY	PH	TGW
AC Hamilton	100.00a	100.00a	48.50ab	4.30a	9.77cd	10.44de	5.71b	1.95bc	0.20a	1.19d-h	0.19b-e	96.92c	42.12cd
AC Lacombe	98.00a	98.00a	45.37a-e	3.82b	11.00a	11.23bc	6.03a	2.05b	0.19ab	1.56a-c	0.23ab	95.76cd	41.12de
AC Malone	98.00a	99.33a	44.63a-e	3.45de	9.18e	9.52j	5.61bc	1.66f-i	0.15c-e	1.28c-g	0.17e	102.59b	39.05fg
AC Nadia	96.00a	96.67a	42.80c-e	3.59cd	8.44h	9.88i	5.63bc	1.62g-i	0.15c-e	1.09f-h	0.18c-e	101.99b	37.35hi
AC Stephen	100.00a	100.00a	47.03a-d	3.80b	9.12ef	9.93hi	5.43cd	1.79c-f	0.17b-d	1.31b-g	0.18de	95.69cd	38.98fg
Creel	96.00a	97.33a	44.13a-e	3.69bc	9.54d	10.51de	5.06ef	1.77d-g	0.18a-c	1.29c-g	0.19b-e	94.61d	41.38de
Creme	99.33a	100.00a	48.20a-c	3.23fg	8.71g	10.34d-g	5.46cd	1.78d-g	0.18a-c	1.15e-h	0.20a-e	96.99c	46.43a
Husky	97.33a	98.00a	40.43ef	3.59cd	10.20b	10.08f-i	5.85ab	1.69e-h	0.16cd	0.99h	0.17e	95.68cd	34.72j
Nord	94.67a	97.33a	43.83a-e	3.33ef	11.07a	11.35b	5.80ab	1.56h-j	0.15c-e	1.43a-d	0.19b-e	104.20a	37.29hi
Sophie	98.67a	99.33a	45.67a-e	4.20a	9.97bc	10.65d	5.14ef	1.85c-e	0.20ab	1.37a-e	0.21a-e	101.27b	39.23fg

GE - germination energy, G - germination, GI - germination index, CL - coleoptile length, SL - seedling length, RL - seminal root length, RN - seminal root number, SFW - seedling fresh weight, SDW - seedling dry weight, RFW - seminal root fresh weight, RDY - seminal root dry weight, PH – plant height, TGW - 1000-grain weight; * means in columns followed by the same letter are not significantly different at $p \leq 0.05$ the according to Duncan's Multiple Range Test

Table 4 summarizes the seed and seedling characteristics of the 10 semi-dwarf and10 non-dwarf spring barley cultivars used in the study. A comparison of means showed that there were significant differences ($p \leq 0.05$) between two groups of barley cultivars for coleoptile length, seedling length, seminal root number, and plant height. Coefficients of variation for all studied traits of semi-dwarf cultivars were higher compared to those of non-dwarf cultivars. Moreover for most of the traits (germination energy, germination, germination index, coleoptile length, seminal root length, seminal root number, and plant height) values of coefficient of variation were over two times higher for semi-dwarf cultivars than for non-dwarf cultivars indicating the presence of considerable variation between short stature cultivars for those traits.

Table 4. *Comparison of germination characteristics, seedling and root traits, plant height, and 1000-grain weight of semi-dwarf and non-dwarf cultivars spring barley cultivars*

Traits	Semi-dwarf cultivars					Non-dwarf cultivars				
	Mean	Min	Max	SD	CV	Mean	SD	CV	Min	Max
GE	95.47a*	86.00	100.00	5.28	5.53	97.80a	1.81	1.85	94.67	100.00
G	97.53a	91.33	100.00	3.13	3.21	98.60a	1.27	1.29	96.67	100.00
GI	42.65a	34.37	49.07	4.94	11.58	45.06a	2.47	5.49	40.43	48.50
CL	3.21a	2.17	4.21	0.64	19.82	3.70b	0.34	9.30	3.23	4.30
SL	7.54a	5.70	8.86	1.03	13.61	9.70b	0.89	9.16	8.44	11.07
RL	9.93a	7.35	11.72	1.31	13.23	10.39a	0.58	5.58	9.52	11.35
RN	4.95a	3.98	5.30	0.39	7.86	5.57b	0.31	5.50	5.06	6.03
SFW	1.63a	1.17	2.27	0.34	20.96	1.77a	0.15	8.52	1.56	2.05
SDW	0.16a	0.12	0.20	0.03	16.85	0.17a	0.02	11.35	0.15	0.20
RFW	1.28a	0.72	1.63	0.29	22.45	1.27a	0.17	13.19	0.99	1.56
RDY	0.19a	0.13	0.24	0.03	17.98	0.19a	0.02	9.32	0.17	0.23
PH	71.81a	54.23	81.66	9.80	13.65	98.57b	3.53	3.58	94.61	104.20
TGW	39.07a	30.90	46.18	4.89	11.61	39.77a	3.22	7.53	34.72	46.43

GE - germination energy, G - germination, GI - germination index, CL - coleoptile length, SL - seedling length, RL - seminal root length, RN - seminal root number, SFW - seedling fresh weight, SDW - seedling dry weight, RFW - seminal root fresh weight, RDY - seminal root dry weight, PH – plant height, TGW - 1000-grain weight; *means in rows followed by the same letter are not significantly different at $p \leq 0.05$ the according to t-test

A significant positive correlation was found between germination energy, germination, and germination index (**Table 5**). Association between germination energy and traits coleoptile length, seedling length, seminal root length, seedling fresh weight, seedling dry weight, seminal root fresh weight, seminal root dry weight was also observed. A positive correlation of germination with coleoptile length, seedling length, seminal root length, seedling fresh weight, seedling dry weight, seminal root dry weight, 1000-grain weight was detected. Germination index positively correlated with all studied traits except seminal root number and plant height. Coleoptile length was related to germination energy, germination, germination index, seedling length, seminal root length, and seedling fresh and dry weight. The seedling length was associated with germination energy, germination, germination index, coleoptile length, seminal root length and number, seedling fresh and dry weight, and plant height. Seminal root length correlated with all investigated traits except seminal root number and dry weight, plant height, 1000-grain weight. Seminal root number had a positive correlation with seedling length and plant height. Seedling fresh weight was associated with most of the studied traits - germination energy, germination, germination index, coleoptile and seedling length, seminal root length, seedling fresh and dry weight, seminal root fresh and dry weight, and 1000-grain weight. Seedling dry weight was positively correlated with germination energy, germination, germination index, coleoptile length, seedling length, seminal root length, seedling fresh weight, 1000-grain weight. There was a relationship between seminal root fresh weight and germination energy and index, coleoptile and seedling length, seminal root length and dry weight, and seedling fresh weight. The correlation of seminal root dry weight with germination energy, germination, germination index, seedling fresh weight, and seminal root fresh weight was significant. Seedling length and seminal root number showed a positive correlation with plant height. Germination, germination index, and seedling fresh and dry weight significantly correlated with 1000-grain weight.

Table 5. *Correlations among of germination characteristics, seedling and root traits, plant height, and 1000-grain weight of spring barley cultivars*

Traits	G	GI	CL	SL	RL	RN	SFW	SDW	RFW	RDY	PH	TGW
GE	0.97**	0.86**	0.67**	0.55*	0.68**	0.12	0.82**	0.76**	0.49*	0.52*	0.16	0.42
G	1	0.83**	0.63**	0.51*	0.66**	0.07	0.75**	0.70**	0.41	0.45*	0.13	0.49*
GI		1	0.63**	0.54*	0.75**	0.23	0.76**	0.75**	0.55*	0.52*	0.16	0.49*
CL			1	0.68**	0.61**	0.40	0.81**	0.81**	0.21	0.10	0.33	0.43
SL				1	0.68**	0.70**	0.56*	0.62**	0.22	0.15	0.71**	0.19
RL					1	0.29	0.70**	0.70**	0.55*	0.41	0.18	0.27
RN						1	0.20	0.22	-0.13	-0.12	0.72**	0.03
SFW							1	0.92**	0.48*	0.46*	0.05	0.63**
SDW								1	0.32	0.42	0.21	0.60**
RFW									1	0.74**	-0.20	0.10
RDY										1	-0.26	0.22
PH											1	-0.08

GE - germination energy, G - germination, GI - germination index, CL - coleoptile length, SL - seedling length, RL - seminal root length, RN - seminal root number, SFW - seedling fresh weight, SDW - seedling dry weight, RFW - seminal root fresh weight, RDY - seminal root dry weight, PH – plant height, TGW - 1000-grain weight; * correlation is significant at the 0.05 level (2-tailed), ** correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Previous studies had found that most of the semi-dwarf genes used in barley breeding affect some seedling and root traits (19-20). Out of 10 semi-dwarf cultivars used in the present study information for dwarfing factors was available only for cultivar Bold, possessing *uzu1* gene and for cultivars, Ishi and UC828, possessing *sdw1* gene. It has been observed that genotypes with *uzu* gene had reduced coleoptile and seedling length especially under high temperature (21). Our results showed that cultivar Bold had indeed shorter seedlings but not shorter coleoptiles compared to non-dwarf cultivars. A possible explanation for this result is the finding of Saisho *et al.* (10) that *uzu* gene has a specific suppressive effect on elongation on coleoptile under dark conditions. Additional studies of coleoptile length under specific field conditions are needed especially for semi-dwarf cultivars due to modifying effects of different factors on the phenotypic expression of the trait.

According to Zhang and Zhang (22), the *sdw1* semi-dwarf gene itself is not associated with short coleoptile length. In our study, we also observed that the coleoptile length of the cultivars bearing *sdw1* gene (Ishi and UC828) did not differ significantly from those of non-dwarf cultivars.

Some of the studied tall cultivars had coleoptile lengths that were shorter than the average. Similar findings were reported by Paynter and Clarke (23) who screen 44 spring barley cultivars for coleoptile length. In the present study, two old Canadian cultivars (Creme and Nord) had shorter coleoptiles. Kaufmann (25) also found short coleoptiles in older Canadian cultivars. Including short-coleoptile cultivars in barley breeding program may cause producing of breeding lines with short coleoptiles which will be intolerant to deep sowing. The deep seeding tolerance of barley becoming an important adaptive trait as climate change increases the variability of precipitation at the beginning of the vegetation period. Longer coleoptiles are also beneficial when stubble retention is practiced and when chemicals for disease and weed control reducing coleoptile length are used (23).

Ellis *et al.* (19) reported that the semi-dwarfing genes *sdw1* and *ari-e.GP* affects root length, root weight, and nitrogen isotope discrimination but their effects on root traits were influenced greatly by the environment. The *sdw1* mutation was associated with the increased size of seminal and adventitious root systems, particularly during the early stages of plant development (20, 24). While the *ari-e.GP* mutation was related with fewer roots, shorter roots, and lesser root spread in 10-day-old seedlings (24), lower root weight of six-week-old seedlings, and reduced size of the root system of field-grown plants at grain filling (20). The results of the present study showed that most of the semi-dwarf cultivars had a lower number of seminal roots while the root length was not reduced compared to studied tall varieties. Considerable reduction in both number and length of the seminal roots was observed in semi-dwarf cultivars W. Barcott, Niska, and Ishi while tall cultivars Nord and AC Lacombe had the highest values for the number and length of the roots.

A decrease in grain size and weight of genotypes carrying *sdw1* and *uzu* was found (10, 16). However, *sdw1*-containing cultivars, such as UC 828, which are characterized by large grain and low screening have been reported (26). In the current study, considerable variation between semi-dwarf cultivars for 1000-grain weight was observed. There were short stature cultivars as Bold, Ishi, and Vivar which showed a very high weight of 1000-grain for feed barley (over 42 g). The grain weight is not only a seed quality indicators but also an important trait related to grain yield and quality in barley so the absence of correlation between 1000-grain weight and plant height is beneficial because indicates the possibility for breeding short stature varieties with high grain weight.

CONCLUSION

The semi-dwarf cultivars showed significantly lower values of mean length of coleoptile and seedling and seminal root number compared to non-dwarf cultivars. Nevertheless among short stem cultivates there were cultivars with coleoptile length similar to those of non-dwarf cultivars. If those results confirmed under particular field conditions semi-dwarf cultivars without reduction of coleoptile can be used in the development of new varieties with desirable height and coleoptile length. Correlation analysis showed a significant association of plant height with seedling length and seminal root number. The rest of the seedling characteristics were uncorrelated with plant height and, hence allowing breeding for that trait without compromising high seedling vigour.

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