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ISSN 1479-2621

Plant Genetic Resources: Characterization and Utilization **7**(1); 88–93
doi:10.1017/S1479262108994247

Short Communication

Variation in south Asian wheat germplasm for seedling drought tolerance traits

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Received 19 December 2007; Accepted 20 April 2008 – First published online 13 June 2008

Abstract

Higher seedling vigour and greater coleoptile length are important for early establishment of wheat crops and subsequently higher grain yield in many dry environments. Seedling vigour includes those seed properties that determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions. Genotypes with the widely used gibberellic acid (GA)-insensitive dwarfing genes *Rht-B1b* and *Rht-D1b* have good partitioning and grain yield under optimal conditions, but may perform poorly under stressed conditions due to poor crop establishment. Breeding programmes are in search of GA-sensitive dwarfing genes that do not affect seedling vigour under dry conditions. This study evaluated 40 genotypes currently used in wheat breeding programmes of south Asia for seedling vigour-related traits in greenhouse and field experiments during 2006–2007 at IAAS, Rampur, Nepal. Wide variation in coleoptile length, seedling vigour, as well as sensitivity to GA was observed. Among the genotypes studied, there were positive correlations among coleoptile length, leaf width and plant height. Genotypes, SW89-5193, SW89-5422/NL251 and SW89-5422, were found to have longer coleoptile, higher seedling vigour and response to GA application. This shows a promise for their further applications in the breeding programmes.

Keywords: coleoptile length; gibberellic acid response; seedling vigour

Introduction

Long coleoptile, rapid seedling growth and establishment have been suggested as useful traits for improving yield under dry conditions. Limited genetic variation has been reported for these traits in wheat (Richards and Lukacs, 2001; Matsui *et al.*, 2002). Shorter coleoptiles and poor emergence have commonly been associated with presence of the *Rht-B1b* and *Rht-D1b* dwarfing genes (Matsui *et al.*, 2002). Although these alleles result in a reduced plant height that maximizes yield in favourable environments, they limit the length of coleoptiles and may cause poor establishment in drought-prone environments (Rebetzke *et al.*, 2007). Both these genes decrease sensitivity of vegetative tissues to endogenous gibberellins to slow cell elongation and reduce cell size (Keyes *et al.*, 1989). This reduces emergence as well as overall seedling vigour. In order to overcome the negative effects of these genes, wheat researchers have sought to identify gibberellin-sensitive dwarfing genes that will reduce plant height without affecting coleoptile length (Ellis *et al.*, 2004). The potential value of such genes has been suggested in other studies (Rebetzke *et al.*, 1999; Rebetzke and Richards, 2000;

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Rebetzke *et al.*, 2007). Large wheat-growing areas in south Asia are rainfed or partially irrigated. Breeding suitable genotypes in this region requires a search for adapted genotypes with high seedling vigour and long coleoptile length may prove very useful. Thus, the objective of this study was to evaluate variation in seedling vigour-related traits, coleoptile length and responsiveness of coleoptiles to gibberellic acid (GA) treatment in south Asian germplasm.

Materials and methods

Forty genetically diverse wheat genotypes were obtained from CIMMYT's South Asian Regional Office. The genotypes included were released cultivars, lines commonly used as parents and newly introduced genotypes targetting other specific breeding objectives for south Asia (Table 1).

Table 1. Variation in coleoptile length, leaf width at two-leaf stage, plant height at maturity and effect of application of gibberellic acid (GA) on coleoptile length of south Asian wheat genotypes

					Coleoptile length (mm)	
S. No.	Genotype	Type[a]	Plant height (cm)	Leaf width (mm)	GA non-treated	Increase[b]
1	Annapurna-1	CV	70	3	47.9	0.48
2	Siddhartha	CV	76	3.3	57.2	−0.6
3	Bhrikuti	CV	77	3.6	48	1.1
4	SW89-5193	EL	86	4.2	70.8	6.6
5	UP262	CV	87	3.3	42.2	0.8
6	SW89-5422/NL251	EL	87	4.2	68.3	6.1
7	SW89-5422	EL	89	3.9	68.8	5.3
8	BL2806	EL	96	4.2	58	3.8
9	Vaskar	CV	98	3.4	62.6	5.2
10	Triveni	CV	98	4	61.9	2.6
11	K-7	EL	98	3.9	59.4	7.2
12	BL2737	EL	98	4	62.1	9.3
13	Pashang Lahmu	CV	98	3.5	66.7	1.9
14	RR21	CV	99	4	52.2	8.5
15	Rohini	CV	99	3.7	70.8	2.7
16	NL297	CV	100	3.7	69.8	−1.5
17	BL1067	EL	100	4.1	59.6	10.5
18	Chirya-3	EL	101	4	62.7	7.6
19	Ciano79	EL	102	3.7	58.6	6
20	BL2498	EL	102	4.2	59.5	7.1
21	Chirya-1	EL	102	4.1	60.2	3.3
22	WK1123	EL	102	3.9	59.4	9
23	Nepal-251	CV	102	3.6	60.4	4.2
24	Gautam	CV	102	4.1	64.2	7
25	Chirya-7	EL	103	4.2	63.7	2.6
26	NL750	EL	103	4.2	59.9	5.8
27	BL1135	EL	104	3.8	76.2	2.3
28	BL1022	CV	105	4.2	75.3	−0.9
29	Kanchan	CV	105	3.9	63	3.2
30	Kanti	CV	105	3.9	59.3	3.5
31	Sabuf	EL	106	4.1	68.2	9
32	BL2710	EL	106	3.9	73.3	5.5
33	WK1204	EL	106	4.2	60.4	5.6
34	NL971	CV	106	4.4	68	6.8
35	Achyut	CV	108	4	61	4.3
36	PBW-343	CV	108	4.4	63.1	5
37	Annapurna-4	CV	108	4.5	63.9	3.4
38	BL2715	EL	108	3.6	74.2	5.3
39	Yangmai-6	EL	116	4.8	84.1	5
40	Longmai-10	EL	120	4.6	84.9	7.6
		lsd (5%)	8.2	0.8	6.5	2.9

[a] CV, released cultivar; EL, experimental line/genotype.
[b] Increase in coleoptile length due to GA treatment.

Greenhouse experiments

Coleoptile length and GA responsiveness

The genotypes were grown in a four replicate, two-factor, randomized complete block design arranged in split plots. The number of samples from each plot was ten. GA treatment was the main plot and genotypes subplot factors. For GA treatment, seeds were soaked for 4 days at 2°C in 10 mL of GA, [500 mg/l of potassium gibberellin A_3 ($C_{19}H_{21}O_6K$)] and 0.2 ml/l of Vitavax-200 fungicide (Pereira *et al.*, 2002).

The seeds of uniform size were shown at a uniform depth (10 mm) in deep wooden trays containing a fertile potting mixture watered to field capacity. The trays were covered with an opaque plastic sheet to exclude light and placed in an air-conditioned room with temperature maintained between of 10 and 20°C. After 10 days, the plastic sheet was removed and coleoptile length was measured in eight randomly selected seedlings per plot. The vigour assessment experiment was repeated twice during 2006–2007.

After initial screening of the entire set of genotypes, a subset was selected for further testing. In the second assay, the selected genotypes were screened for coleoptile length with inclusion of known checks and two more precisely controlled temperature regimes at 5 and 18°C. The three check genotypes included were Tandem, Crimson and Alliance. Tandem and Crimson are known as long coleoptile genotypes, whereas Alliance is a short coleoptile dwarf genotype (Hakizimana *et al.*, 2000). After GA treatment as discussed above, coleoptile length was measured using a blotter-paper germination protocol (Hakizimana *et al.*, 2000). The samples were placed vertically in plastic trays in a dark incubator at 4°C for 4 days to reduce dormancy. After 4 days, the samples were placed in a dark incubator at either 5°C for 30 days or 18°C for 16 days before coleoptile length was measured. The coleoptile length was measured after reaching a maximum length as indicated by the emergence of the primary leaf from the coleoptile tip.

Vigour assessment

For measurement of seedling vigour, a separate greenhouse experiment was carried out. Seeds of uniform size were planted at uniform depth in a deep tray containing a fertile potting mixture and then watered. Width of the first two leaves was measured with a ruler upon full expansion of the second leaf as an indicator of seedling vigour (Richards and Lukacs, 2001). In addition to leaf width, other traits evaluated include time of emergence of seedlings, non-destructive measurement of chlorophyll content of leaves with Minolta chlorophyll meter (SPAD-502), leaf numbers and number of coleoptile tillers. The vigour assessment experiment was repeated twice during 2006–2007.

Field evaluation of plant height

Genotypes were evaluated in four replicate randomized complete block design for measurement of plant height in plots of 2 m^2. Plant height was measured at maturity in ten random plants per plot to obtain an average.

Data analysis

Normality of data was tested using Anderson–Darling test (Anderson and Darling, 1952). After confirming normality of distribution, the analysis of variance was carried out with the general linear model procedure of the MINITAB (1996) statistical software. Means were compared using least significant differences (Steel and Torrie, 1980).

Results

Wide variation was observed among genotypes for coleoptile length, seedling vigour, plant height and GA treatment response of coleoptiles (Table 1). Differences among genotypes were significant for coleoptile length, plant height and leaf width ($P < 0.05$) (ANOVA not shown). For chlorophyll content, seedling emergence and leaf number, no significant differences between genotypes were found. Plant height measurements showed a positive correlation with coleoptile length, GA response to coleoptile length and leaf width (Fig. 1). There were, however, exceptions to this strong linear relationship. Coleoptile length and leaf width were also found to be positively correlated (Fig. 1). Response to GA application was variable among both tall and dwarf plants (Table 1). In general, tall plants have a longer coleoptile, wider leaves and their coleoptile shows a GA response (Table 1).

In contrast to other dwarf genotypes, SW89-5193, SW89-5422/NL251 and SW89-5422 have the longest coleoptiles, the widest leaves and the greatest increase in coleoptile length with GA treatment. This indicates that they might carry GA_3-responsive dwarfing genes, although further genetic studies would be necessary to prove this. As expected, four dwarf genotypes, Annapurna-1, Bhrikuti, UP262 and Siddhartha, did not respond to GA treatment and had a relatively short coleoptile and narrower leaves. Generally, genotypes with GA-responsive coleoptiles have broader leaves (Table 1). The dwarf genotypes with GA-responsive coleoptiles, i.e. SW89-5193, SW89-5422/NL251 and SW89-5422, also had broader leaves than GA-insensitive dwarfs, indicating higher seedling vigour in these genotypes. GA responsiveness

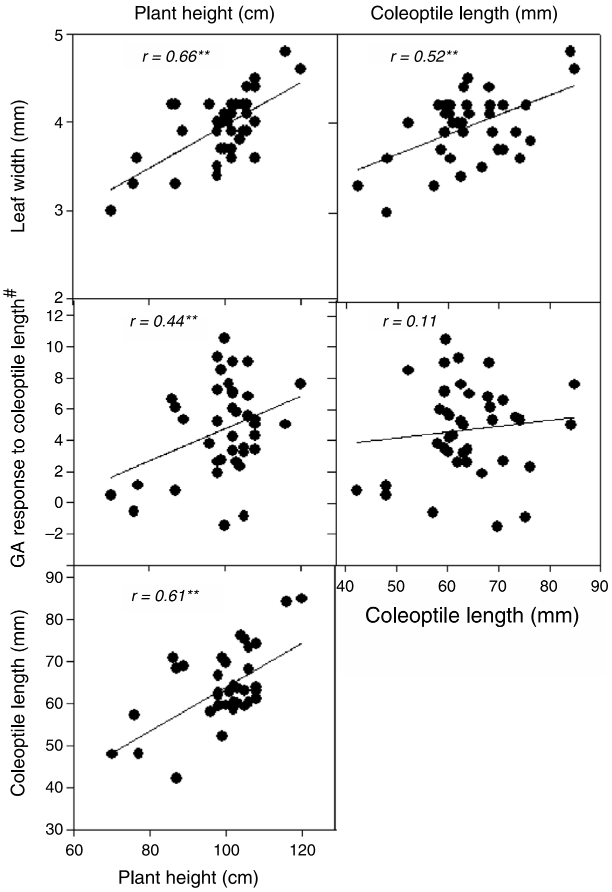

Fig. 1. Pearson's correlation matrix among plant height and seedling vigour-associated traits in south Asian wheat genotypes. #Gibberellic acid (GA) response to coleoptile length (mm) was measured as increase in coleoptile length after application.

and coleoptile length did not have a predictable relationship (Fig. 1).

In the first set of experiments at 10–20°C incubation conditions, the range of coleoptile length and response to GA treatment was lower than expected. The result of the second experiment (including selected genotypes under two temperature regimes and with known checks) further confirms the results (Table 2). The results clearly show that the overall low value for coleoptile length was related to higher temperature during screening with average 17°C (variation 10–20°C). Reducing temperature to 5°C did not change the overall classification, although both coleoptile length and GA response were proportionally reduced. Interestingly, some tall genotypes that were GA non-responsive at 18°C became GA responsive at 5°C (Table 2). All three GA-responsive dwarfs were more responsive to GA at 5°C. Coleoptile length and GA response was comparatively lower than tall check genotypes (Table 2).

Discussion

Genotypic increases in coleoptile length have been shown to improve stand establishment, vigour and grain yield of deep-sown wheat (Rebetzke *et al.*, 2007). Among different traits associated with seedling vigour, leaf width has been found promising with high heritability (Rebetzke and Richards, 1999). Most recently recommended south Asian wheat genotypes are GA-insensitive semi-dwarfs, which are inherently low in their early vigour (Table 1). The experimental lines showed higher vigour than released cultivars. A suitable selection methodology needs to be developed to transfer genes for high early vigour from these exotic, unadapted wheats to commercial south Asian wheat varieties.

Significant effect of GA is due its promotion of amylase production for endosperm starch hydrolysis during seed germination and cell elongation in different organs and tissues throughout plant growth and development (Karssen *et al.*, 1989). In our experiments, total increase in coleoptile length was significantly lower than expected (Hakizimana *et al.*, 2000; Table 1). The relatively low response to GA application may have been due to higher temperature at germination (Pinthus and Abraham, 1996; Pereira *et al.*, 2002). This was further evident while assaying genotypes at 5°C. Interestingly, for unknown reason some tall or medium height genotypes showed insensitivity to GA applications at 18°C. However, these genotypes become sensitive at 5°C. Similar temperature-dependent response was observed by Pinthus and Abraham (1996).

GA sensitivity has been reported previously to be temperature as well as allele dependent (Pereira *et al.*, 2002). Above 15°C, coleoptile length is reduced and GA response is lower. Also, *Rht-B1b* has wider temperature response range than *Rht-D1b*. In our first assay, the temperature ranged from 10 to 20°C (with an average of 17°C), which could explain the shorter coleoptile length observed in all genotypes. This was shown clearly when the same genotypes assayed at 5°C had longer coleoptiles and GA response than at 18°C (Table 2). While coleoptile length was greater at 5°C, we were interested in the higher temperature as it is more representative of the likely soil temperature range during germination in many wheat-growing areas of south Asia.

Most of the dwarf and semi-dwarf wheat lines from Nepal have the Japanese variety Akakomugi among their ancestors (Rosyara and Joshi, 2005). This variety was the donor of the reduced height gene *Rht8* and the daylength-insensitive gene *Ppd-D1* in Italian wheat varieties bred by N. Strampelli in the 1920s, which were widely used as parents by breeding programmes in many countries. These two genes are closely linked and

Table 2. Coleoptile length and gibberellic acid response on selected genotypes south Asian wheat genotypes at two different temperatures of incubation (5 and 18°C)

			18°C		5°C	
S. No.	Genotype	Plant height (cm)	GA non-treated (mm)	Increase[a] (mm)	GA non-treated (mm)	Increase[a] (mm)
Dwarf, non-sensitive to GA at first experiment[b]						
1	Annapurna-1	70	48	0.48	68	3.0
2	Siddhartha	76	57	−0.6	70	1.0
3	Bhrikuti	77	48	1.1	59	−1.8
Dwarf, sensitive to GA at first experiment						
4	SW89-5422/NL251	87	68	6.1	90	14.1
5	SW89-5422	89	69	5.3	86	15.3
6	SW89-5193	86	71	6.6	89	16.6
Tall, sensitive to GA at first experiment						
7	BL1067	100	60	10.5	99	25.5
8	Sabuf	106	68	9.0	100	29.0
9	Longmai-10	120	85	7.6	105	30.6
Tall, non-sensitive to GA at first experiment						
10	BL1022	105	75	−0.9	98	12.2
11	BL1135	104	76	2.3	97	12.3
12	Annapurna-4	108	64	3.4	96	18.4
Checks						
13	Tandem	Tall	69	9.1	104	32.2
14	Crimson	Tall	71	10.5	102	28.1
15	Alliance	Dwarf	48	3.2	70	7.2
	lsd (5%)	–	5.2	2.1	7.2	4.8

[a] Increase in coleoptile length (in mm) due to GA treatment.
[b] GA, gibberellic acid.

located on chromosome 2D. By contrast, Akakomugi is way back in the pedigree of the varieties, and there are fairly high chances of not finding *Rht8* gene. Besides this there are other GA-sensitive dwarfing genes than *Rht8*, which reduce plant height (Rebetzke and Richards, 2000). For confirmation of results, genotyping with known dwarfing gene-associated molecular markers may be very useful. One important point to consider while screening for *Rht8* is the presence of marker *Xgwm261*$_{192}$ is only indicative of *Rht8* in wheat cultivars that have inherited this allele from Akakomugi or a Strampelli wheat ancestor (Ellis *et al.*, 2007). The old Japanese cultivar Norin 10, used by Norman Borlaug to introduce *Rht-B1b* and *Rht-D1b* into Mexican wheats, also has a 192 bp allele at the *Xgwm261* locus, and the sequence of the amplified product is identical to that of Akakomugi. Thus, it has been suggested that the widespread use of Norin 10-derived germplasm during and after the green revolution introduced a second haplotype into international germplasm, in which *Xgwm261*$_{192}$ has no association with *Rht8*.

In many of the breeding programmes in south Asia, however, marker screening capabilities have not been well developed and a quick, simple and cheap GA assay may be a very useful screening tool in the identification of GA-insensitive dwarf genotypes. The GA-responsive dwarf genotypes identified in this study have practical application as parents in the breeding programmes.

Acknowledgements

The seeds of genotypes for this study were obtained from CIMMYT South Asia Regional Programme. The study was partly supported by Eiselen Foundation Ulm, Germany, IFS Sweden and University Grants Commission of Nepal. We would like to thank anonymous reviewers for their valuable suggestions that improved the manuscript.

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