Dwarfing Gene Effects on Coleoptile Length in Pearl Millet

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ABSTRACT

Dwarfing genes have been reported to affect either or both the length of the coleoptile and mesocotyl in several cereal species, which can reduce seedling emergence. Such an effect of dwarfing genes has not been reported for pearl millet [*Pennisetum glaucum* (L.) R. Br.]. The objective of this study was to assess the effect of the d_2 dwarfing gene in pearl millet on the length of the coleoptile, mesocotyl, and plumule in 12 pairs of isogenic tall/dwarf (d_2) inbred lines and 16 pairs of isogenic tall/dwarf hybrids. Culm lengths were measured in replicated field trials in two seasons and the lengths of coleoptiles and mesocotyls in seedlings germinated in incubators in the dark at 35 °C. Culm length differed significantly between tall and dwarf entries of both inbreds and hybrids but the length of both coleoptile and mesocotyl were similar in both height classes. There were significant differences in coleoptile and mesocotyl lengths among individual entries tested but these were due to parental line or hybrid parent effects. The d_2 gene in pearl millet did not affect coleoptile or mesocotyl length as reported for dwarfing genes in other cereals.

DWARFING GENES used in the breeding of the well known semidwarf cultivars of rice, (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* J.) have been widely reported to reduce coleoptile length (Allan et al., 1961; Fick and Qualset, 1976; Ceccarelli et al., 1980; and Turner et al., 1982). This has resulted in reduced emergence and plant stands in semidwarf cultivars in certain environments (Allan et al., 1962; Whan, 1976; Turner et al., 1982). Emergence from deep seeding of rice, however, has been shown to depend upon both the mesocotyl and coleoptile lengths (Turner et al., 1982). The effect of dwarfing genes on mesocotyl length has not been studied in any of the above-quoted wheat and barley references; whether mesocotyl and coleoptile were considered together and labelled as "coleoptile" or whether the mesocotyl was not considered in these studies is not clear. As a result it is not clear whether dwarfing genes affect coleoptile, mesocotyl, or both.

Most studies comparing tall with dwarf or semidwarf cultivars report that despite overall relationships of culm length and coleoptile length, there is considerable variation in coleoptile length of both tall and dwarf cultivars; including several reports of dwarf or semidwarf cultivars with coleoptile lengths equal to or greater than those of tall cultivars (Allan et al., 1961; Hoff et al., 1973; Ceccarelli et al., 1980). In addition, most of the wheat studies do not report which of several dwarfing genes (Gale and Youssefian, 1985) are represented in the materials tested. Different dwarfing genes may affect the relationship of culm length and coleoptile length. In fact, at least one of the known *Rht* genes in wheat does not affect coleoptile length at all (Konzak, 1976; quoted in Gale and Youssefian, 1985).

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Dwarfing genes in pearl millet were first reported by Burton and Fortson (1966) and subsequently by Appa Rao et al. (1986). These reports and reports on the use of dwarfing genes in breeding programs (Bakshi et al., 1966; Thakare and Murty, 1972; Chantereau and Arnaud, 1981) fail to mention an effect of dwarfing gene(s) on coleoptile length or resulting emergence. In a study of the response of two dwarf and one tall pearl millet lines to gibberellic acid, Rao et al., (1981) reported a slightly shorter coleoptile length in only one of the dwarf lines used, although the within population variation in all these lines was far larger than the differences in population means.

This study was carried out to determine the effects of the d_2 dwarfing gene on the lengths of the coleoptile, mesocotyl, and plumule of pearl millet.

MATERIALS AND METHODS

We used a known dwarfing gene (d_2, Burton and Fortson, 1986) and isogenic pairs of tall and dwarf lines and hybrids to remove background genetic effects. Experiment 1 compared 12 pairs of isogenic lines produced from progenies derived from a backcrossing program to produce dwarf versions of seven standard height breeding composites. The pairs used were derived by selfing progenies segregating for the d_2 gene, from the BC_3F_1 to BC_3F_9 generations (Gale and Youssefian, 1985) from the Early, Medium, and Nigerian Composities. Phenotypically similar, tall and dwarf pairs were selected in the F_{10} generation and seed produced by an additional generation of selfing.

Experiment 2 compared 16 pairs of tall and dwarf isogenic hybrids. These were made by crossing four pairs of tall and dwarf isogenic lines (selected from the set used in Exp. 1) onto four dwarf cytoplasmic male-sterile lines. Because the d_2 dwarfing gene is recessive, the dwarf isolines produced dwarf hybrids (homozygous at the d_2 locus) and their tall counterparts produced tall hybrids (heterozygous at the d_2 locus). These hybrids allowed a comparison of the effects of the d_2 gene with male sterile (A-line) and pollinator effects, as well as overcoming the loss of vigor associated with inbreeding in Exp. 1.

Seeds were germinated on blotter paper holders made by placing a 1-mm thick piece of blotter paper between two thinner pieces of filter paper. These were placed in a vertical position in slots in plexiglass rack in 230 × 120 × 100 mm plastic boxes, which were filled to a depth of 75 mm with distilled water. The filter paper extended 3 mm above the upper edge of the blotter paper and 4 mm below the blotter paper to provide a wick to conduct water to the seeds. Seeds were placed at the upper edge of the blotter paper between the filter papers. Each box held 24 such blotter paper holders.

The inbreds and hybrids were tested separately. Twenty five seeds per entry (from three adjacent blotter paper holders in a single box) were used per plot. The order of entries in the boxes was randomized. Three plastic boxes were required per replicate for the inbreds and four for the hybrids. Both sets of materials were tested in three replicates, with seeds of each replicate germinated in a separate incubator maintained at 35 °C.

On the sixth day the lengths of plumule, mesocotyl, and

coleoptile were measured. In this study, coleoptile is the modified leaf structure surrounding the apical meristem and leaf primordia and mesocotyl refers to the internode between the scutellar node and coleoptile node (Fahn, 1974). The entire axis, including mesocotyl and coleoptile, is the plumule.

Culm lengths of the 12 pairs of tall and dwarf inbred isolines were determined in replicated field trials conducted at ICRISAT Center, Patancheru (17 °N) in the rainy seasons of 1984 and 1985 and at Bhavanisagar (11 °N), India, during the rainy seasons of 1985 and 1986. Trials were established as a split-plot design with four-row plots and four replicates. Inbred pairs were main plots and tall and dwarf lines within a pair were subplots. Row spacing was 500 mm at Bhavanisagar and 600 mm at Patancheru with 100-mm spacing between plants at both locations. Culm lengths were measured from the base of the plants to the base of the panicle on five random plants from the central two rows of each plot, excluding end plants. Analysis was conducted on the plot means.

Culm lengths of the tall/dwarf hybrid pairs were measured for replicated field plots in a randomized-block design during the rainy seasons of 1985 (six-row plots) and 1986 (four-row plots) at ICRISAT center, Patancheru. Row spacing was 750 mm with 100 to 150 mm spacing between plants. Mean culm length was measured as the distance from the soil surface to a rod held horizontal to the soil surface at the base (visual estimate) of the average panicle in the two center rows of plants. Data are means of five replications in 1985 and four in 1986.

Data from all experiments were analysed to partition differences in culm, coleoptile, and plumule lengths between the dwarfing gene and genetic background. The latter was the effect of pair in the inbred lines and the effects of pollinator (=pair), male sterile line and their interaction in the hybrids. Culm length data from the 2-yr field trials were combined because there were no significant interactions of genotypes with years or locations (Exp. 1) or of genotypes with years (Exp. 2).

RESULTS AND DISCUSSION

Mean culm length for the inbreds (Exp. 1) was 1.4 m for the tall and 0.7 m for the dwarf isolines (Table 1). For the hybrids (Exp. 2), culm length was 1.8 m for the tall and 1.3 m for the dwarf lines. Culm length differences between the two height groups were highly significant ($P< 0.001$), for both sets of materials. Differences among lines were primarily due to the d_2 dwarfing gene, but there were also significant contributions of genetic background, i.e. the pair or A-line and pollinator, and evidence for interaction of the dwarfing gene and background (Table 2).

The range in coleoptile length among entries of both experiments was small (Table 1) and differences were not significant (Table 2). Hybrids had longer coleoptiles than inbreds (20 vs. 15 mm), but within each genetic background tall and dwarf isolines had identical coleoptile length.

The mesocotyl length, however, was significantly different among lines and hybrids. Hybrids, in general,

Table 1. Means and ranges of the coleoptile, mesocotyl, plumule, and culm lengths for tall and dwarf pairs of isogenic inbreds and hybrids.

		Inbreds			Hybrids		
	Length	Tall	Dwarf	SE†	Tall	Dwarf	SE
Coleoptile							
	Mean (mm)	15	15	±0.1	20	20	±0.2
	Range	13–17	11–18		18–21	17–22	
Mesocotyl							
	Mean (mm)	13	12	±0.3	22	21	±0.3
	Range	7–22	7–21		17–28	14–28	
Plumule							
	Mean (mm)	28	27	±0.3	42	41	±0.5
	Range	19–39	18–31		36–49	32–49	
Culm							
	Mean (m)	1.41	0.72	±0.028	1.83	1.26	±0.021
	Range	1.11–1.68	0.55–0.90		1.58–2.05	1.05–1.47	

† SE = Standard error of the mean values in the range.

Table 2. Mean squares for coleoptile, mesocotyl, plumule, and culm lengths for the tall and dwarf pairs of isogenic inbreds and hybrids.

Pairs	df	Coleoptile length	Mesocotyl length	Plumule length	Culm length
Inbreds (12)					
Lines	23	6.56	36.21*	56.43**	23676***
Tall vs. dwarf	1		11.0	12.0	451347***
Pairs	11		67.43**	104.68***	6870***
Tall vs. dwarf × pairs	11		7.39	12.23	1603***
Error	23	3.32	16.23	21.38	147
Hybrids (16)					
Hybrids	31	4.30	47.50***	65.33***	9884***
Tall vs. dwarf	1		68.34**	52.50*	255791***
Pollinators	3		112.29***	212.90***	10430***
A-lines	3		318.26***	407.62***	4309***
A lines × pollinators	9		1.77	1.30	231
Tall vs. dwarf × pollinators	3		22.59*	24.37	224
Tall vs. dwarf × A-lines	3		2.78	1.37	781*
Tall vs. dwarf × pollinators × A lines	9		2.26	2.49	146
Error	62	2.78	5.57	11.57	218

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

had longer mesocotyls than inbreds (Table 1). Most of the variation for mesocotyl length in both sets of materials was due to the genetic background effects (Table 2). There was no effect of the dwarfing gene on mesocotyl length of the inbreds (Table 2). Although there was a significant difference in mesocotyl length between tall and dwarf hybrids, the mean difference was only 1 mm (Table 1), which is not agronomically meaningful.

As expected, variation for plumule length followed variation for combined coleoptile and mesocotyl length (Table 1). Most of the variation for plumule length was due to genetic background (Table 2). The small, but significant, effect of the dwarfing gene in the hybrids was due to the effect on mesocotyl length. The hybrids possessed a longer average plumule length (40 mm) than inbreds (28 mm). This difference, in turn, is reflected both in coleoptile and mesocotyl lengths, though it is greater for mesocotyl (Table 1). The differences may arise as an expression of heterosis for seedling axis length.

The correlations between culm lengths and lengths of coleoptile, mesocotyl, or plumule were small ($r < 0.20$) and nonsignificant for both experiments. The dwarfing gene, which reduces culm length in pearl millet, does not appear to influence the length of the plumule, coleoptile, or mesocotyl. This observation differs from results reported for wheat, barley, and rice (Allan et al., 1961; Fick and Qualset, 1976; Ceccarelli et al., 1980; Turner et al., 1982). The similar plumule lengths of tall and dwarf pearl millets probably explains why problems of establishment of dwarf millets have not been reported.

The major factor affecting plumule length of pearl millet is background genetic differences for mesocotyl length. The effects of the specific pair of isogenic inbreds and of both pollinator and A-line in the isogenic hybrids were highly significant (Table 2). Thus selection for longer plumule or mesocotyl lengths in pearl millet should be possible. Greater variability for mesocotyl, rather than coleoptile, length was also reported in rice by Turner et al., 1982. Whether or not a similar case exists in wheat is not known because studies of dwarf wheats report coleoptile lengths only.

The coleoptile of pearl millet is generally shorter than for dwarf wheat and barley. The average length is 50 mm for dwarf wheat (Allan et al., 1961) and 25 mm for dwarf barley (Ceccarelli et al., 1980). Similarly, mesocotyl length of rice ranged from 20 to 50 mm (Turner et al., 1982) longer than most pearl millet inbreds (Table 1). Our data, however, agree with those of Rao et al. (1981) for pearl millet; a coleoptile length of 16 mm reported in their studies for the dwarf, Tift 23DB, closely agrees with our results for isogenic inbreds in Table 1. Lengths of coleoptile or mesocotyl may be related to seed size of cereal crops. Pearl millet possesses the smallest seed among the cereals studied.

Our results are specific to the d_2 gene (Burton and Fortson, 1966), the only one presently in widespread use for pearl millet. Other dwarfing sources have been reported in pearl millet (Appa Rao et al., 1986), some of which are nonallelic to the d_2 source. The effects of other non-d_2 sources on coleoptile and mesocotyl lengths are not known, but in wheat different *Rht* genes have different effects on coleoptile length (Gale and Youssefin, 1985). Consequently our results for d_2 gene effects on coleoptile length should not be extrapolated to other dwarfing genes.

REFERENCES

Allan, R.E., O.A. Vogel, J.R. Burleigh, and C.J. Peterson, Jr. 1961. Inheritance of coleoptile length and its association with culm length in four winter wheat crosses. Crop Sci. 1:328–332.

——, ——, and C.J. Peterson, Jr. 1962. Seedling emergence rate of fall-sown wheat and its association with plant height and coleoptile length. Agron. J. 54:347–350.

Appa Rao, S., M.H. Mengesha, and C. Rajagopal Reddy. 1986. New sources of dwarfing genes in pearl millet (*Pennisetum americanum*). Theor. Appl. Genet. 73:170–174.

Bakshi, J.S., K.O. Rachie, and A. Singh. 1966. Development of dwarf strains of pearl millet and an assessment of their yield potential. Curr. Sci. 35:355–356.

Burton, G.W., and J.C. Fortson. 1966. Inheritance and utilization of five dwarfs in pearl millet (*Pennisetum typhoides*) breeding. Crop Sci. 6:69–72.

Ceccarelli, S., M.T. Pegiati, and F. Simeoni. 1980. Relationship between coleoptile length and culm length in barley. Can. J. Plant Sci. 60:687–693.

Chantereau, J., and M. Arnaud, 1981. Recherche des effects du gene nanisme D2 du petit mil par l'interpretation statistique multivariee de croisements entre populations naines et grandes. Agron. Trop. (Paris) 36:142–150.

Fahn, A. 1974. Plant anatomy, 2nd ed. Pergamon Press, New York.

Fick, G.N., and C.O. Qualset. 1976. Seedling emergence, coleoptile length, and plant height relationships in crosses of dwarf and standard height wheats. Euphytica. 25:679–684.

Gale, M.D., S. Youssefian. 1985. Dwarfing genes in wheat. p. 1–35. *In* G.E. Russell (ed.) Progress in plant breeding. Butterworths, London.

Hoff, J.C., B.J. Kolp, and K.E. Bohnenblust. 1973. Inheritance of coleoptile length and culm length in crosses involving Olsen's dwarf spring wheat. Crop Sci. 13:181–184.

Konzak, C.F. 1976. A review of semi-dwarfing gene sources and a description of some new mutants useful for breeding short stature wheats. p. 79–93. *In* Induced mutations in crop breeding. Proc. of an advisory group, Vienna, Austria. 13-17 Oct. 1975. IAEA, Vienna, Austria.

Rao, M.K., U. Koduru, and R.V.K. Rao. 1981. Dwarfism in pearl millet: its relationship to gibberellic acid and coleoptile length. Beitr. Biol. Pflanz. 55:195–202.

Thakare, R.B., and B.R. Murty. 1972. Incorporation of dwarfing loci in populations of pearl millet. Indian J. Genet. Plant Breed. 32:445–450.

Turner, F.T., C.C. Chen, C.N. Bollich. 1982. Coleoptile and mesocotyl lengths in semi-dwarf rice seedlings. Crop Sci. 22:43–46.

Whan, B.R. 1976. The emergence of semidwarf and standard wheats, and its association with coleoptile length. Aust. J. Exp. Agric. Anim. Husb. 16:411–416.