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# Genetic variation in grain-filling ability in dwarf pearl millet [*Pennisetum glaucum* (L.) R. Br.] restorer lines

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**Abstract** The *d2* dwarfing gene in pearl millet [*Pennisetum glaucum* (L.) R. Br.] carries a yield penalty due to an associated reduction in individual grain mass. This reduction, however, varies with genetic background, indicating that it may be possible to select against poor grain filling in *d2* dwarf backgrounds, given an effective measure of grain filling. This study was conducted to assess genetic variability for grain-filling ability (in contrast to simply grain size), and its relationship to grain yield, in dwarf pearl millet restorer (R) lines. The grain-filling ability (GFA) of an individual R line was defined as the least squares estimate of its effect on individual grain mass in the analysis of variance, following a linear covariance adjustment for grain number. The study was based on 93 dwarf hybrids involving 31 *d2* dwarf R-lines, evaluated over 3 years. Half of the variation in individual grain mass in the 93 hybrids was related to variation in grain number. Covariance adjustment in individual grain mass for grain number resulted in highly significant differences among hybrids and R lines in GFA. The R-line combining ability for GFA accounted for 26% of the variation in the R-line combining ability for yield, compared to 46% for the combining ability for grain number, and just 8% for the combining ability of individual grain mass. The combining ability for GFA was independent of the combining ability for various pre-flowering effects, including grain number, but was related to the combining ability for individual grain mass and harvest index. Improvement in individual grain mass achieved through selection for GFA should translate directly into yield improvement, whereas improvement by direct selection for individual grain mass is less-likely to do so.

**Keywords** Pearl millet · Grain-filling ability · Grain yield · Restorer lines

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## Introduction

The *d2* dwarfing gene in pearl millet was first identified 35 years ago (Burton and Fortson 1966), but very few dwarf hybrids have reached farmers' fields, despite the wide use of *d2* genetic material in breeding programs (Bidinger and Raju 1990). Comparisons of near-isogenic tall/dwarf inbred lines, and of near-isogenic hybrids made with them, have suggested a yield penalty associated with the *d2* gene, in comparison to the tall *D2* allele, usually due to a smaller individual grain mass (Bidinger and Raju 1990; Rai and Rao 1991).

A similar association of dwarfing genes and smaller grain size has been reported in bread wheat, *Triticum aestivum* L. (Gale and Youssefian 1985); barley, *Hordeum vulgare* L. (Ali et al. 1978); and sorghum, *Sorghum bicolor* (L.) Moench (Casady 1965). Reduced grain mass in dwarf wheat genotypes is frequently a consequence of the positive effect of the *Rht* series of dwarfing genes on grain numbers per ear (Flintham and Gale 1983; Fischer and Stockman 1986). However, in crops where grain numbers per inflorescence (or unit area) do not differ between tall and dwarf isolines, a reduced grain mass in dwarf lines is apparently due to a negative effect of the dwarfing gene(s) on grain filling (Casady 1965; Pinthus and Levy 1983; Bidinger and Raju 1990).

Many studies of dwarfing-gene effects indicate that the magnitude of the negative effect of dwarfing genes on grain filling is highly influenced by the genetic background of the experimental material (Casady 1965; Ali et al. 1978; Bush and Evans 1988). This is also the case in pearl millet (Bidinger and Raju 1990; Rai and Rao 1991). It should thus be possible to produce dwarf lines with well-filled grains by careful choice of parents and specific selection for superior grain-filling ability.

The determination of individual grain mass in pearl millet, as in other cereals, is complex, as it includes both the direct effects of the genotype (G), the environment (E) and their interaction (G×E), as well as an indirect (and inverse) effect of the numbers of grains per panicle or unit area on the actual grain mass. Because of the effect of grain numbers on grain size, individual grain mass provides a measure of genetic differences in grain-filling ability *only* where grain numbers do not differ among the genotypes under comparison, which seldom if ever is the case. A true estimate of grain-filling ability must therefore account for both the direct effects of E and G×E on individual grain mass, which are largely post-flowering occurrences, as well as the indirect effects of grain number, which are largely pre-flowing occurrences.

The experiment reported in this paper was carried out: (1) to evaluate a method of quantifying true genetic differences in grain-filling ability (GFA), (2) to assess genetic variability in the average combining ability for GFA in the full ICRISAT collection of *d2* dwarf restorer (R) lines, and (3) to quantify the effects of variation in the R-line combining ability for GFA on the combining ability for grain yield and grain size. The R-line combining ability for GFA was defined in this study as the least-squares estimate of the R-line effect for individual grain mass, obtained by linear-covariance adjustment for grain number, using a general linear additive model of hybrid individual grain mass, which accounted for the effects of year, male-sterile (A) and R lines, and their interactions.

## Materials and Methods

### Field experiments

The experiment evaluated 93 hybrids that were developed from the factorial combination of the complete collection of 31 *d2* R-lines available in the ICRISAT pearl millet breeding program, at the time that the work was initiated, and three phenotypically and genetically diverse *d2* male-sterile lines – ICMA 1 (81 A), 833 A, and ICMA 2 (843 A). Eighteen of the R-lines originated from a pedigree breeding program designed specifically for producing dwarf R-lines, and have diverse pedigrees (B.S. Talukdar 1986, personal communication). The remainder originated as single plant selections from several *d2* dwarf breeding populations (Rai 1990).

These 93 hybrids were evaluated in a randomized complete block design (RCBD) with three replications in the rainy seasons of 1987 1988 and 1989 at ICRISAT, Patancheru, near Hyderabad, India (17.45°N, 78.49°E). Individual plots were 2 rows×0.75 m ×4.0 m long ; all observations were made on the center 3.0 m of both rows (4.5 $m^2$). Plots were machine-sown and hand-thinned at approximately 15 days after emergence to 12 plants per $m^2$. The crop was well-fertilized (85 kg N $ha^{-1}$ and 18 kg P $ha^{-1}$) and weeds were controlled by machine cultivation and one-hand weeding. There were no significant disease or pest problems. Heavy rains at the beginning of the flowering period in 1988 resulted in low pollen availability and poor seed set in several of the earliest flowering hybrids (nearly all made on ICMA 2). As a result, 25 out of a total of 279 plots in 1988 were treated as missing plots in the analysis.

Data were recorded on days to 50% flowering, plant height, panicle number, oven-dry panicle, grain and stover (straw) yields per plot, and individual grain mass (determined by weighing duplicate 100-grain samples from the bulk grain harvest for each plot). Biomass and harvest index, grain number $m^{-2}$ (grain yield $m^{-2}$ divided by individual grain mass) and per panicle, and grain-filling index (see below) were calculated from these data. Data were analyzed according to the field design, with the hybrid (92 degrees of freedom, *df*) sums of squares partitioned into R-line (30 *df*), A-line (2 *df*) and A×R-line interaction (60 *df*) effects. Error variances for the 3 individual years were homogeneous, so data were analyzed across years, with blocks considered as nested within a year.

### Statistical methodology for estimation of grain-filling ability

The following linear additive model was assumed to represent observations $Y_{ikl}$ on individual grain mass corresponding to hybrid k in block l of year i. It was derived as an extension of the RCBD ANOVA model, by including the grain number ($X_{ikl}$) as a covariate

$$Y_{ikl}=\mu+y_i+b_{(i)l}+G_k+(Gy)_{ik}+\beta(X_{ikl}-\mu_X)+\varepsilon_{ikl} \quad (1)$$

where:

i=1,..,3; k=1,..,93; l=1,..,3,
$\mu$ is the general mean,
$y_i$ is the effect of year i,
$b_{(i)l}$ is the effect of block l within year i,
$G_k$ is the effect of hybrid k,
$(Gy)_{ik}$ is the effect of interaction between hybrid k and year i,
$\beta$ is the linear regression coefficient of Y on X,
$X_{ikl}$ is the number of grains in hybrid k in block l in year i,
$\mu_X$ is the average number of grains in the whole experiment over 3 years, and
$\varepsilon_{ikl,}$ is the random error term, assumed to be a normally distributed random variable with a mean of zero and a constant variance $\sigma_\varepsilon^2$.

Since the major objective of the study was to investigate differences in combining ability for grain filling in the R-line collection, the hybrid effect $G_k$ in model (1) was expanded to reflect its factorial structure as $G_k=R_j+A_m+(RA)_{jm}$, where $R_j$ is the effect of R-line j (j=1,.., 31) and $A_m$ is the effect of male-sterile line m (m=1,..,3), and $(RA)_{jm}$ represents the effect of their interaction. Model (1), after substitution for $G_k$, becomes

$$Y_{ikl}=\mu+y_i+b_{(i)l}+R_j+A_m+(RA)_{jm}+(Ry)_{ij}+(Ay)_{im}+(RAy)_{ijm} +\beta(X_{ijlm}-\mu_X)+\varepsilon_{ijml}, \quad (2)$$

where i=1,..,3; j=1,..,31; m=1,..,3; and l=1,..,3

The least squares (LS) estimates of the effects in both models and the analysis of variance (adjusted for the covariate X) were computed using the statistical package Genstat release 5.4.1 (Genstat 5 Release Committee 1993). The LS estimate of $R_j$, corrected for the number of grains X as covariate, and denoted here as $R_j$*, was interpreted as the estimate of the average combining ability for GFA for R-line j. Genstat release 5.41 provides estimates of $R_j$* and their standard errors which, using a single-sample *t*-test, were used to identify significant $R_j$* effects. Significant estimates of $R_j$* with negative values were interpreted as indicative of poor GFA, and with positive values as indicative of a superior GFA. The importance of GFA to grain yield was assessed on an R-line combining-ability basis by correlation of the R-line effect (average combining ability) for GFA with the R-line effect for grain yield and yield components.

## Results and Discussion

### Definition of grain-filling ability

There was considerable variability in both grain number per unit area and in individual grain mass in the 93 test crosses. The mean test-cross grain numbers (over the 3 years) ranged from 20000 to nearly 80000 $m^{-2}$ and the mean individual grain mass from 5.2 mg to 11.8 mg (Fig. 1). As expected, there was a strong ($P$<0.001) negative relationship between the two, with variation in grain number accounting for 50% of the variation in individual grain mass. However, as is evident from Fig. 1,

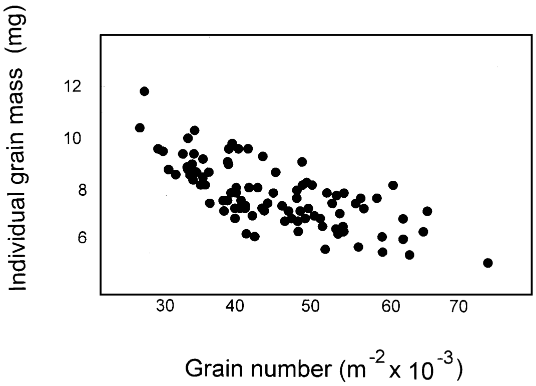


**Fig. 1** Dependence ($r$=–0.71) of individual grain mass on grain number per unit area for 93 dwarf pearl millet hybrids made by crossing 31 *d2* dwarf pollinators on three dwarf male-sterile lines. Data points are the means of 3-year replicated trials at ICRISAT, Patancheru

there was also considerable variability in individual grain mass at any given grain number, suggesting that there were differences in the ability to fill grains among hybrids which affected realized individual grain mass, in addition to the obvious effects of grain number on individual grain mass.

The analysis of variance of individual grain mass adjusted by covariance for grain number (Table 1) indicated a highly significant ($P$<0.001) effect of grain number on grain mass (penultimate row in Table 1) as expected from Fig. 1. There was also a highly significant ($P$<0.001) effect of hybrids on grain mass following the covariance adjustment, indicating significant differences in GFA, as we defined it, among the hybrids (Table 1). The year (environment) effect was of considerably greater magnitude than the hybrid (genotype) effects, but the hybrid by year (gentoype×environment) effect, although significant, was of much lesser importance than the genotype effect.

For the analysis of covariance, i.e. the analysis of variance (adjusted for covariate X), to be valid, the covariate X (grain number) should not be affected by (i.e. should be orthogonal to) the corresponding treatment term. Also, this term should be highly linearly correlated with X for the analysis of covariance to be useful in providing increased precision and accuracy. This latter requirement was met by our data as there was a highly significant negative linear relationship ($\beta$=–6.18×10$^{-6}$, $P$<0.001) between grain mass and grain number. The fact that, in our data, the R-line component in the analysis of covariance had a covariance efficiency factor of 0.93 (Table 1) allowed us to treat the covariate X as orthogonal to the R-line treatment term (Genstat 5 Release 3 Reference Manual 1993; Payne and Tobias 1992). Also, as required for the validity of covariance analysis, the analysis of covariance residuals exhibited a normal distribution. The plot of residuals against fitted values (data not shown) indicated that the assumption of homoscedasticity was met in the data.

## R-line differences in combining ability for GFA

To make effective use of any trait in a hybrid breeding program, it is necessary to identify parental lines with the ability to produce hybrids which strongly express the target trait. Thus to improve grain filling, and thereby grain yield, in dwarf millet hybrids, it will be necessary to identify parental lines with good general combining ability for GFA. To this end, we partitioned the *df* for hybrids into effects of A-line (2 *df*), R-line (30 *df*), and A×R-line interaction (60 *df*), and repeated the analysis of variance of the covariance-adjusted individual grain mass. The adjusted A-, R-, and A×R-line effects were highly significant, indicating strong parental effects for grain-filling ability. The R-line component accounted for 70% of the hybrid sums of squares for GFA. (Table 1). Twelve R-lines had significant ($P$<0.001) positive values for GFA (>+0.033), and ten R-lines had significant ($P$<0.001) negative values for GFA (<–0.033).

**Table 1** Combined analysis of variance of hybrid and hybrid-parent effects on individual grain mass (adjusted for grain number per square meter, X). Data are plot means of 3-year replicated trials of 93 dwarf pearl millet hybrids made by crossing 31*d2* dwarf pollinators onto three *d2* dwarf male-sterile lines grown at ICRISAT, Patancheru

| Source of variation | *df*[a] | Sums of squares | Mean square | *F* statistic | Covariance efficiency | *P* value |
|---|---|---|---|---|---|---|
| Year | 2 | 2.5493 | 1.2746 | 71.18 | 0.06 | <0.001 |
| Covariance X | 1 | 0.0027 | 0.0022 | 0.15 | | >0.50 |
| Rep (Year) | 5 | 0.0895 | 0.0179 | 5.82 | 0.86 | |
| Hybrid | 91(1) | 5.8126 | 0.0639 | 20.74 | | <0.001 |
| A-line | 2 | 1.2546 | 0.6273 | 203.89 | 0.46 | <0.001 |
| R-line | 30 | 4.0595 | 0.1353 | 43.98 | 0.93 | <0.001 |
| A x R-line | 60 | 0.4985 | 0.0083 | 2.68 | 0.99 | <0.001 |
| Hybrid×year | 184(4) | 1.3021 | 0.0072 | 2.32 | | <0.001 |
| *A-line*×year | 4 | 0.2260 | 0.0565 | 18.37 | 0.97 | <0.001 |
| R-line×year | 59(1) | 0.5931 | 0.0101 | 3.27 | 0.99 | <0.001 |
| A x R-line×year | 116(4) | 0.483 | 0.0042 | 1.35 | 1.00 | <0.015 |
| Covariance X | 1 | 0.6403 | 0.6403 | 208.2 | | <0.001 |
| Residual | 525(26) | 1.6152 | 0.0031 | | 1.39 | |

[a] Degrees of freedom; figures in brackets indicate missing

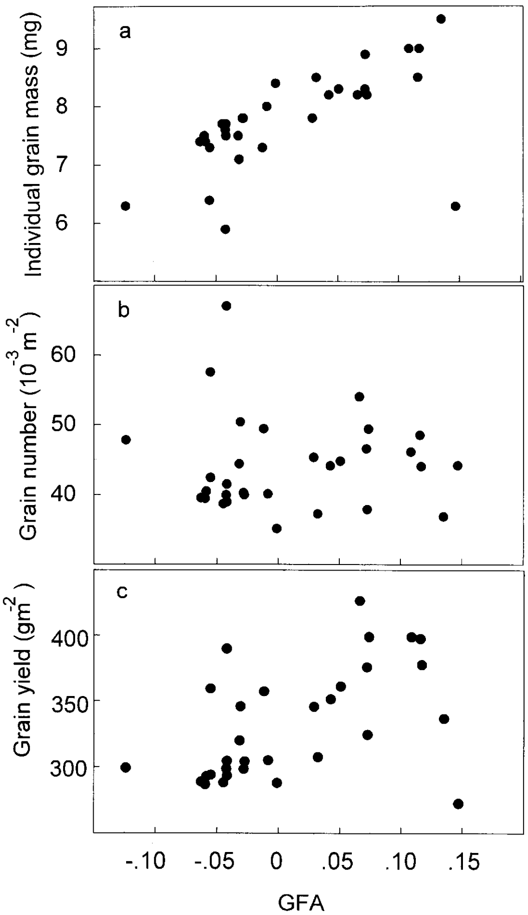


**Fig. 2** Relationship of R-line combining ability for grain-filling ability (GFA) to: **a** the R-line combining ability for individual grain mass, **b** the R-line combining ability for grain number per unit area, and **c** the R-line combining ability for grain yield Data are means of 3-year replicated trials at ICRISAT, Patancheru, of 93 dwarf pearl millet hybrids made by crossing 31 *d2* dwarf pollinators on three dwarf male-sterile lines

The R-line combining ability for GFA was closely and positively linked to the R-line combining ability for individual grain mass, confirming the intended effect of improving the combining ability for GFA (Fig. 2a). The R-line combining ability for GFA was independent of the R-line combining ability for grain numbers per unit area, as expected from the definition of GFA (Fig. 2b). Although the two R-lines with the greatest combining ability for grain number had negative GFA values, it was possible to identify several R-lines with a significant positive combining ability for GFA, plus a greater than average combining ability for grain number per unit area (Fig. 2b).

## Combining ability for GFA as a determinant of combining ability for yield

The mean R-line combining ability for grain yield was positively correlated with the mean R-line combining ability for the following: time to flowering, height, stover yield, grain numbers and GFA (Table 2). These correlations (apart from that with GFA) represent the promotive effects of greater pre-flowering growth on grain yield in short-duration hybrids. These were partly a reflection of variation in time to flowering among R-lines, as the R-line combining ability for stover yield and grain number was significantly and positively correlated to R-line combining ability for time to flowering (data not presented). The R-line combining ability for grain number per unit area was the primary determinant of the R-line combining ability for grain yield, accounting for 46% of the R-line effect on grain yield. The R-line combining ability for individual grain mass was not correlated with the R-line combining ability for grain yield.

The mean R-line combining ability for GFA was correlated with the mean R-line combining ability for individual grain mass (as expected), and for grain yield and harvest index, all primarily post-flowering events (Table 2) There was generally no relationship of the R-line combining ability for GFA and for various aspects of pre-flowering growth (time to flowering, plant height and stover mass, grain number, and grain number components). Forty percent of the variability in the R-line combining ability for individual grain mass and 32% of the R-line combining ability for harvest index were accounted for by the R-line combining ability for GFA. The relationship between com-

**Table 2** Range in the R-line combining ability ($n$=31) and phenotypic correlations of the R-line combining ability for grain yield and GFA, to the R-line combining ability for a range of crop-growth and yield-component variables. Data are based on the means of 3-year replicated trials of 93 dwarf pearl millet hybrids made by crossing 31 *d2* dwarf pollinators onto three *d2* dwarf male-sterile lines, grown at ICRISAT, Patancheru

| Variable | Range in R-line combining ability | Correlation (r) to | |
|---|---|---|---|
| | | Grain yield | GFA |
| Flowering (days) | 43–50 | 0.51**[a] | 0.11 |
| Plant height (cm) | 68–107 | 0.76*** | 0.36* |
| Grain yield (g $m^{-2}$) | 272–426 | – | 0.51** |
| Stover yield (g $m^{-2}$) | 327–557 | 0.76*** | 0.16 |
| Biomass yield (g $m^{-2}$) | 716–1067 | 0.91*** | 0.32* |
| Harvest index (%) | 35–43 | 0.50** | 0.57*** |
| Panicle number ($m^{-2}$) | 20–31 | 0.27 | 0.06 |
| Grain no. $panicle^{-1}$ | 1450–2395 | 0.59*** | –0.05 |
| Grain no. $m^{-2}$ | 35200–67000 | 0.68*** | –0.02 |
| Grain mass (mg $grain^{-1}$) | 0.59–0.95 | 0.28 | 0.63*** |
| Grain filling ability | –0.124–+0.147 | 0.51** | – |

[a], *, **, *** Significant at the 0.05, 0.01, 0.001 levels of probability, respectively

bining ability for GFA and for yield was subject to considerable scatter, but the majority of the R-lines with a significant positive combining ability for GFA had an above-average combining ability for grain yield (Fig. 2c). Differences in the R-line combining ability for GFA made a much greater contribution to explaining differences in the R-line combining ability for grain yield (26%, $P<0.01$) than did differences in the R-line combining ability for individual grain mass (8%, $P>0.10$).

These results thus confirm the independence of GFA as a genetic trait, not only from the influence of grain number, but also from the influences of differences in pre-flowering growth rate and/or growth duration, which are linked to grain number. The linkages of GFA to individual grain mass, and consequently to harvest index, confirm that GFA represents primarily genetic differences acting during the grain-filling phase itself. Although differences in combining ability for the pre-flowering genetic differences were the main determinants of grain yield in the hybrids used in this experiment, the combining ability for GFA was also a significant, if lesser, determinant of grain yield.

### Use of GFA in hybrid parent breeding

Grain-filling ability, as defined in this study, has several potentially useful aspects for the genetic improvement of dwarf pearl millet.

(1) Because of the independence of grain number and GFA, it is possible to manipulate both grain number and GFA simultaneously in a breeding program, without having to balance gains in one against losses in the other, as in the case of grain number and individual grain mass. It is thus equally possible to have well-filled (but small) grains in a genetic background which produces high-grain numbers, as it is to have well-filled (but large) grains in a low-grain number genetic background (Fig. 1).
(2) Combining ability for GFA, in contrast to combining ability for grain mass, was a useful determinant of grain yield, accounting for a three fold greater percentage of the R-line combining ability for grain yield than did the R-line combining ability for individual grain mass (Table 2). The latter is seldom indicated as a major determinant of yield in pearl millet (e.g. Jindla and Gill 1984) due to its inverse relationship with grain number.
(3) Measuring GFA does not require any additional measurements to those required to assess individual grain mass, and the additional calculations required are trivial with a modern statistical package.

The apparent ability of GFA to improve both grain yield and grain mass simultaneously is its most intriguing aspect as a selection criterion. Yield components, in general, show negative relationships among themselves because of the sequential nature of their development (Adams 1967), so that the ultimate positive effect on grain yield of selection for any one is usually diluted by a negative relationship with a subsequent component (e.g. Rassmussen and Cannell 1970; Knott and Talukdar 1971). This was the case with combining ability for individual grain mass in this experiment, as it had a negative correlation with combining ability for grain number ($r=-0.50$, $P<0.01$), which was the major determinant of combining ability for grain yield ($r=0.68$, $P<0.001$). Consequently, the relationship of combining ability for individual grain mass itself with combining ability for grain yield was negligible ($r=0.28$, $P>0.10$). GFA, because of the way in which it was defined, is unrelated to grain number, so that selection for improved combining ability for GFA will not necessarily result in a decrease in grain number, and therefore in little effect on grain yield. The increase in individual grain mass achieved through selection for combining ability for GFA, rather than by direct selection for combining ability for individual grain mass, should translate directly to an increase in grain yield.

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