

Path coefficient and cluster analyses of yield and morphological traits in *Pennisetum purpureum*

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Abstract

In order to develop and select improved populations and germplasm of elephant grass for forage and bioenergy production, important morphological traits including dry matter yield, leaf:stem ratio, plant height, tillers/plant, leaf length, leaf width and stem width were investigated in 16 lines of elephant grass (*Pennisetum purpureum*), one purple elephant grass and hybrid pennisetum (pearl millet × elephant grass). Data were examined using correlation analysis, path analysis and cluster analysis. Plant height and tillers/plant were positively ($P < 0.05$) correlated with yield, while leaf length and leaf:stem ratio were negatively ($P < 0.05$) correlated with DM yield. The factors with greatest effect on DM yield were plant height and leaf:stem ratio. The elephant grasses and hybrid pennisetum were divided into 4 groups by cluster analysis based on DM yield, leaf:stem ratio, plant height etc. The 4 groups were: Group 1 - dwarf type with numerous tillers, lowest DM yield and highest leaf:stem ratio; Group 2 - semi-dwarf types with plentiful tillers, plus higher DM yields and plant heights and fewer tillers/plant than Group 1; Group 3 - intermediate height types with moderate tillering, but higher DM yields than Groups 1 and 2; Group 4 - tall types with limited tillering but highest DM yields and lowest leaf:stem ratios.

Introduction

Elephant grass (*Pennisetum purpureum*), also known as napier grass, is a warm-season perennial grass, which is widely planted in tropical and subtropical regions of the world (Wang *et al.* 2005). It is known throughout much of the wet tropics for its prolific growth and usage as forage for ruminants (Rusland *et al.* 1993; Yasin *et al.* 2003a, 2003b). Introduced to Guangdong and Sichuan provinces, China from Burma and India in the 1940s, it has been planted in many provinces, including Guangdong, Guangxi, Hunan, Hubei, Sichuan, Guizhou, Yunnan, Fujian, Jiangxi and Taiwan provinces in south China (Lai *et al.* 1998; Zhong *et al.* 2002; Bai and Yang 2002; Zhou *et al.* 2007), and is one of the most promising summer forages in the region.

Owing to their tall growth habit and thick stems, tall elephant grass types have undesirable leaf:stem ratios and unacceptable nutritive value (low crude protein and digestibility) for stock, particularly as they mature (Williams and Hanna 1995), which limits their widespread use. In order to improve the quality of elephant grass as livestock feed, a hybrid pennisetum (pearl millet × elephant grass) was bred in the 1940s, which possessed both the high production potential of elephant grass and the good nutritive value of pearl millet (*Pennisetum glaucum*) (Osgood *et al.* 1997). This hybrid was introduced to Jiangsu Academy of Agricultural Sciences in the 1980s. Inter-specific crosses between male-sterile pearl millet and elephant grass were achieved after solving the key problem of making elephant grass flower at high latitudes. This was achieved by simulating a short-day environment using a black plastic cloth at the Jiangsu Academy of Agricultural Sciences (Gu *et al.* 1994). Questions still remain about the persistence of these hybrids. In recent years, some hybrid pennisetums have displayed yellow leaves and poor tillers at the seedling stage, resulting in stunted plants with low biomass yield. The reason, in part, was that the

elephant grass N51, the male parent, was a highly hybrid line.

Seeds from selfed progeny of elephant grass N51 were collected and used in the present study. The objectives of the study were to: (1) study the variation within the selfed progeny of elephant grass N51; (2) evaluate the importance of different morphological traits to dry matter yield in elephant grass; (3) determine the direct and indirect effects of these morphological traits on dry matter yield; (4) develop selection criteria for higher biomass yield of elephant grass through the use of path-coefficient analysis; and (5) develop and select improved populations and germplasm of elephant grass for forage and bioenergy production.

Materials and methods

The trial was conducted during 2007 at Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu province (118°48'E, 32°32'N), China. The area has a humid subtropical monsoon climate, with cold winters and hot summers, and a long-term average temperature of 15.7°C and annual precipitation of 1000 mm. The soil was a yellow brown earth, known as magan clay loam in China. The field was fallowed during winter and was ploughed before planting.

Experimental design

The plant material used in this experiment consisted of 16 elephant grass lines derived from the selfed progeny of elephant grass N51 and with apparently contrasting characteristics, one purple elephant grass and hybrid pennisetum (pearl millet × elephant grass).

The experimental design was a randomised block with 3 replications. Plot size was 4 m × 1.4 m. Each plot was comprised of 2 rows (0.7 m apart) of 6 plants, spaced at 0.6 m within the row. Planting was carried out on May 23 using root divisions. Urea was broadcast at the rate of 40 kg/ha N (20 kg/ha on June 15 and 10 kg/ha on July 25 and September 7). All plants within each plot were hand-harvested on October 18. Fresh material was weighed, and a subsample was taken from each plot and hand-separated into leaf and stem + leaf sheath fractions. These samples were oven-dried at 70°C to constant weight.

Dry matter (DM) production per hectare and leaf:stem ratio were calculated. The sorted fractions were ground to pass a 0.1 mm screen and used for chemical analyses. Before harvest, the number of tillers per plant was counted, and plant height, leaf blade length and width (the first fully expanded leaf from the top) and stem width (the third internode from the base) were measured.

Statistical analyses

Data were treated in Excel and analysed in SAS (Hu and Wang 2001). Plot mean values for all traits were used in GLM to test line differences. The LSD test was used to compare mean differences among lines at the 5% probability level. A linear correlation analysis was applied pair-wise to all the parameters studied. Multiple regression analysis was carried out to calculate the partial regression coefficients necessary for path analysis (Diz *et al.* 1994; Hu and Wang 2001; Li *et al.* 2005). The causal relationships for the path coefficient analysis involved the 6 biomass yield components as predictor (cause) variables and biomass yield as the response (effect) variable (Figure 1). Path coefficients were obtained by the simultaneous solution of the following equations:

$$P_{1y} + r_{12}P_{2y} + r_{13}P_{3y} + r_{14}P_{4y} + r_{15}P_{5y} + r_{16}P_{6y} = r_{1y}$$

$$r_{12}P_{1y} + P_{2y} + r_{23}P_{3y} + r_{24}P_{4y} + r_{25}P_{5y} + r_{26}P_{6y} = r_{2y}$$

$$r_{13}P_{1y} + r_{23}P_{2y} + P_{3y} + r_{34}P_{4y} + r_{35}P_{5y} + r_{36}P_{6y} = r_{3y}$$

$$r_{14}P_{1y} + r_{24}P_{2y} + r_{34}P_{3y} + P_{4y} + r_{45}P_{5y} + r_{46}P_{6y} = r_{4y}$$

$$r_{15}P_{1y} + r_{25}P_{2y} + r_{35}P_{3y} + r_{45}P_{4y} + P_{5y} + r_{56}P_{6y} = r_{5y}$$

$$r_{16}P_{1y} + r_{26}P_{2y} + r_{36}P_{3y} + r_{46}P_{4y} + r_{56}P_{5y} + P_{6y} = r_{6y}$$

where P_{iy} is the direct effect of Character i on y (the path coefficient), the ' r_{ij} 's denote correlation coefficients between Characters i and j , and indirect path coefficients were calculated as $r_{ij} \times p_{iy}$.

Results

Yield and morphological traits in elephant grass and hybrid pennisetum

Variation analyses of yield and morphological traits in elephant grass and hybrid pennisetum (pearl millet × elephant grass) were conducted. Means for the traits involved in the path analyses are shown in Table 1. Differences were found among elephant grass lines for DM

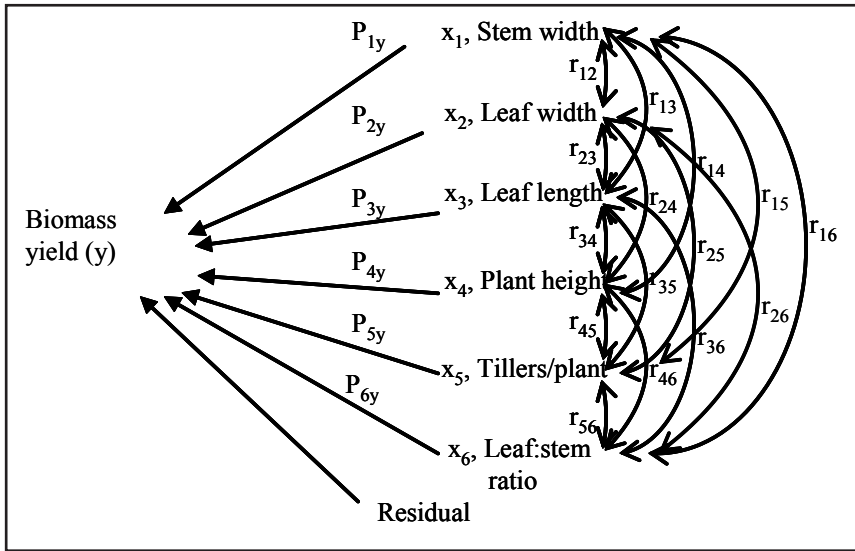


Figure 1. Path diagram showing direct and indirect effects for 6 morphological traits in elephant grass. Unidirectional arrows represent path coefficients (direct effects), while bidirectional arrows represent correlation coefficients between morphological traits.

yield, leaf:stem ratio, stem width, leaf width, leaf length, plant height and tillers per plant ($P < 0.05$). The CVs for all traits were high, especially for tillers per plant.

Correlation between yield and morphological traits

Simple and partial correlation coefficients for yield and morphological traits are presented in Table 2. Simple correlation coefficients indicated that plant height, stem diameter and leaf width were positively ($P < 0.05$) associated with yield, while leaf:stem ratio was negatively ($P < 0.05$) associated with yield. There were negative ($P < 0.05$) correlations between leaf:stem ratio and stem width, leaf width, leaf length and plant height, but a positive ($P < 0.05$) correlation between leaf:stem ratio and tillers per plant. Partial correlation coefficients indicated that plant height and tillers per plant were positively ($P < 0.05$) correlated with DM yield, and leaf length and leaf:stem ratio were negatively ($P < 0.05$) correlated with DM yield.

Path analysis of DM yield and morphological traits

Path-coefficient analysis was performed to obtain further information on the inter-relationships among morphological traits and their effects on DM yield (Table 3). This table shows the correlation matrix, with direct effects (path coefficients) on the main diagonal and indirect effects on both off-diagonal portions, corresponding with their positions in the equations (Gao 1986; Hu and Wang 2001). The matrix is asymmetrical. For example, the correlation between x_1 and y is 0.5420, consisting of 6 components, the direct effect (*italic*) of x_1 on y (0.1358) and 5 indirect effects through its relationship with the other 5 morphological traits.

The direct effects of x_1 (stem diameter), x_2 (leaf width), x_4 (plant height) and x_5 (tillers/plant) on y (DM yield) were positive, and those of x_3 (leaf length) and x_6 (leaf:stem ratio) were negative. The largest positive direct effect was that of x_4 (plant height; 0.6614) and the largest negative direct effect was that of x_6 (leaf:stem ratio; -0.5561). As a result, the direct effects of x_4 (plant height) and x_6 (leaf:stem ratio) had the greatest impact on DM yield. The indirect effects of x_i via x_1 (stem diameter), x_2 (leaf width) and

Table 1. Variation in yield and morphological traits of 16 elephant grass lines, one purple elephant grass and hybrid pennisetum (pearl millet x elephant grass).

Line	DM yield (t/ha)	Leaf :stem ratio	Stem width (cm)	Leaf width (cm)	Leaf length (cm)	Plant height (cm)	Tillers/plant
001	50.2±2.00	0.54±0.02	1.43±0.24	3.3±0.2	102.5±4.2	288±23	21±12
023	17.3±2.03	0.80±0.04	1.35±0.18	2.7±0.3	79.7±7.1	191±9	18±12
033	51.1±3.00	0.32±0.02	1.54±0.30	3.6±0.1	96.5±8.8	300±57	12±4
048	8.3±1.02	1.43±0.13	0.96±0.14	1.3±0.1	44.2±3.8	107±15	48±8
058	45.8±2.01	0.38±0.01	1.26±0.09	3.5±0.4	108.5±9.7	287±38	23±8
071	28.8±2.05	0.52±0.03	1.15±0.12	3.1±0.3	90.0±4.6	231±9	29±7
073	46.5±1.78	0.58±0.04	1.29±0.10	3.1±0.1	96.3±7.6	264±11	29±12
081	15.5±2.02	0.92±0.11	1.14±0.15	2.6±0.2	67.5±2.9	171±18	28±9
088	33.5±2.02	0.45±0.01	1.44±0.13	2.7±0.2	105.0±7.4	276±24	17±10
094	33.4±2.03	0.42±0.01	1.31±0.07	3.2±0.2	97.7±3.1	267±27	17±9
095	24.8±2.05	0.66±0.04	1.56±0.11	3.2±0.1	88.3±1.6	250±22	10±3
097	17.8±2.00	0.76±0.03	1.23±0.09	2.3±0.2	61.7±3.7	174±12	27±10
106	39.6±2.01	0.48±0.02	1.36±0.14	3.7±0.3	92.3±2.7	315±10	15±7
112	61.4±2.03	0.35±0.01	1.49±0.16	4.2±0.3	99.3±4.5	374±36	23±13
114	15.2±2.05	0.53±0.02	1.35±0.14	4.4±0.3	143.2±8.8	334±27	12±3
115	38.5±2.02	0.43±0.01	1.44±0.11	3.9±0.2	105.8±8.7	334±13	15±4
121	56.0±2.00	0.44±0.02	1.51±0.16	4.6±0.2	103.5±3.5	378±13	18±5
Hybrid pennisetum	74.0±2.00	0.32±0.01	1.47±0.17	4.6±0.2	88.7±13.5	353±40	19±9
Mean	36.5	0.57	1.35	3.3	92.8	275	21
CV (%)	48.9	46.8	15.5	25.6	23.2	26.5	53.9
LSD _{0.05}	3.50	0.06	0.172	0.26	7.7	29.8	10.7

Table 2. Simple and partial correlations between DM yields and morphological traits¹.

	Stem diameter	Leaf width	Leaf length	Plant height	Tillers/plant	Leaf:stem ratio
Leaf width	0.6039** 0.0471					
Leaf length	0.4892* -0.0080	0.7587** 0.1649				
Plant height	0.6327** 0.0088	0.9163** 0.5893**	0.7439** 0.3778**			
Tillers/plant	-0.5878** -0.4055*	-0.5217** 0.0014	-0.5750** 0.0916	-0.5385** -0.2243		
Leaf:stem ratio	-0.6420** -0.0424	-0.8013** -0.0575	-0.7089** -0.4555**	-0.8378** 0.0388	0.5793** 0.3737**	
DM yield	0.5420** 0.2109	0.6880** 0.0281	0.3289* -0.5666**	0.7605** 0.4959**	-0.2035 0.4972**	-0.7388** -0.5643**

¹ Upper and lower correlation values are simple and partial, respectively.

Table 3. Path analysis of DM yield and morphological traits. Direct (*italicised*) and indirect effects on DM yield are shown for each morphological trait. x₁: Stem diameter; x₂: Leaf width; x₃: Leaf length; x₄: Plant height; x₅: Tillers/plant; x₆: Leaf:stem ratio; y: DM yield; r²: correlation coefficient between x_i and y.

Traits	Path coefficient (P _{i→j→y})						r ²
	x ₁ →y	x ₂ →y	x ₃ →y	x ₄ →y	x ₅ →y	x ₆ →y	
x ₁ →	<i>0.1358</i>	0.0733	-0.2663	0.4184	-0.1827	0.3634	0.5420**
x ₂ →	0.0820	<i>0.1214</i>	-0.4129	0.6060	-0.1621	0.4536	0.6880**
x ₃ →	0.0665	0.0921	<i>-0.5443</i>	0.4920	-0.1787	0.4013	0.3289*
x ₄ →	0.0859	0.1112	-0.4049	<i>0.6614</i>	-0.1673	0.4743	0.7605**
x ₅ →	-0.0799	-0.0633	0.3130	-0.3561	<i>0.3108</i>	-0.3279	-0.2035
x ₆ →	-0.0872	-0.0973	0.3858	-0.5541	0.1800	<i>-0.5661</i>	-0.7388**

x_5 (tillers/plant) were small, and those of x_1 via x_4 (plant height) and x_6 (leaf:stem ratio) were high.

Cluster analysis of morphological traits and DM yield

A dendrogram was constructed using the WARD clustering method. This approach successfully

discriminated all the elephant grass lines tested and hybrid pennisetum. Cluster analysis based on DM yield, leaf:stem ratio, plant height etc. resulted in 4 cluster groups (Groups 1–4; Figure 2). Yield and morphological differences among the 4 cluster groups are illustrated in Table 4.

Group 1 included only line 048, a dwarf type with numerous tillers. This group had lower DM

Table 4. Mean yields and morphological traits of 4 groups formed by Ward's clustering analyses. CK represents hybrid pennisetum (pearl millet \times elephant grass hybrid).

Group	Line	DM yield (t/ha)	Leaf:stem ratio	Stem diameter (cm)	Leaf width (cm)	Leaf length (cm)	Plant height (cm)	Tiller number
1	048	8.3	1.43	0.96	1.3	44.2	107	47.7
2	023, 071, 081, 097	19.9	0.72	1.22	2.7	74.7	192	25.4
3	001, 058, 073, 088, 094, 095, 106, 114, 115	36.4	0.48	1.38	3.4	104.4	291	17.6
4	033, 112, 121, CK	60.6	0.35	1.50	4.2	97.0	351	17.9

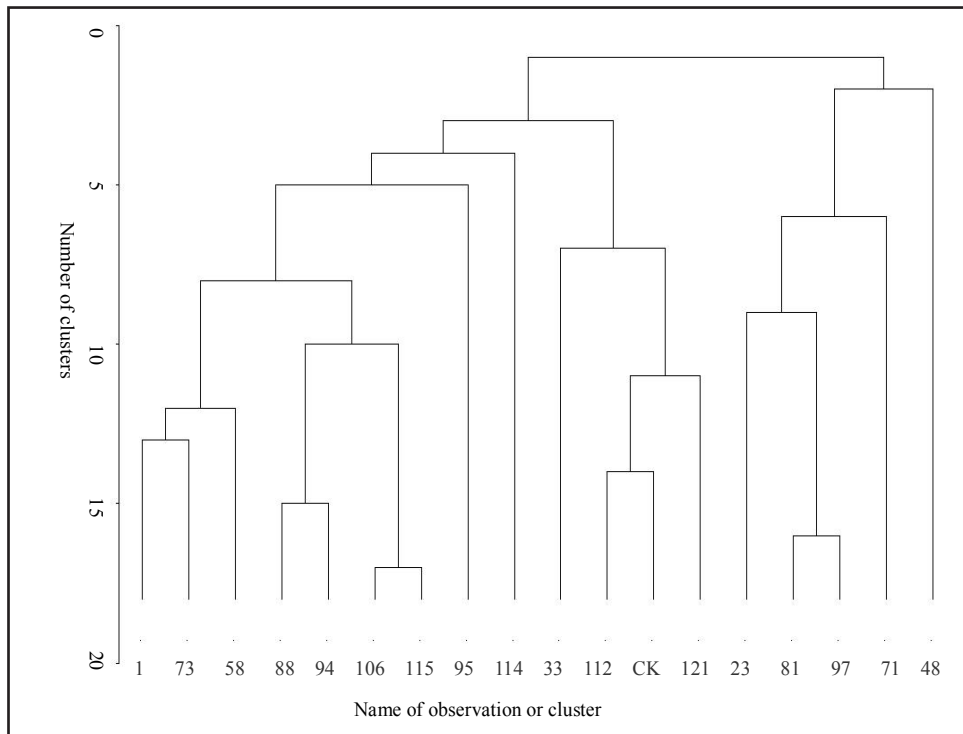


Figure 2. Cluster dendrogram of 16 elephant grass lines, one purple elephant grass and hybrid pennisetum clustered by the WARD clustering method on the basis of DM yield and 7 morphological traits. CK represents hybrid pennisetum (pearl millet \times elephant grass hybrid).

yield, higher leaf:stem ratio (1.43) and more tillers per plant than Groups 2, 3 and 4. It was leafy and the stems were short and tender. Its forage has very high nutritive value (Zhang *et al.* 2009) because of the abundance of leaves available to animals.

Group 2 was semi-dwarf types with plentiful tillers, including lines 023, 071, 081 and 097. Mean DM yield was 19.87 t/ha, leaf:stem ratio was 0.72 and plant height was greater than for Group 1.

Group 3 showed intermediate height and moderate tillering and included lines 001, 058, 073, 088, 094, 095, 106, 114 and 115. DM yields and plant heights of lines in this group were greater than those for Groups 1 and 2, but leaf:stem ratios were lower. Mean leaf length was longer than for lines in Group 4 owing to line 114 (purple elephant grass), which had very long leaves.

Group 4 contained very tall plants with few tillers, and included lines 033, 112, 121 and the pearl millet × elephant grass hybrid. This group showed the greatest DM yield, plant height, stem width and leaf width, but the lowest leaf:stem ratio.

Discussion

Correlation coefficient analysis

The simple correlation coefficient between 2 variables describes their joint behaviour with other factors interacting, while partial correlation describes the relationship between 2 variables, with the effects of one or more other variables removed (Li *et al.* 2005). Therefore, a partial correlation coefficient and a simple correlation coefficient for the relationship between 2 variables can differ substantially as seen in Table 2, and sign reversals are even possible. For example, the simple correlation between yield and tillers per plant was -0.2035 ($P > 0.05$), while the partial correlation was 0.4972 ($P < 0.01$). Eliminating the influences of other variables on DM yield by using partial correlation analysis has revealed the true relationship between yield and tillers per plant.

Path-coefficient analysis

Path-coefficient analysis is a straight-forward extension of multiple regression, which has been

used in determining selection criteria in a number of crops, including wheat (García del Moral *et al.* 2003), maize (Mohammadi *et al.* 2003), soybeans (Ball *et al.* 2001) and grasses (Diz *et al.* 1994; Das *et al.* 2004; Maman *et al.* 2004). This method of analysis measures the direct influence of one variable on another and also separates this correlation coefficient into components of direct and indirect effects. For the path analyses, indirect effects played a more important role than direct effects and sometimes masked the direct effects. For example, the direct effect of x_2 (leaf width) was small (0.1214), but its indirect effects through x_4 (plant height) and x_6 (leaf:stem ratio) were high (0.6060 and 0.4536, respectively), explaining why x_2 (leaf width) was highly positively correlated with y (DM yield; 0.6880, $P < 0.01$). The direct effect of x_3 (leaf length) had a negative direct effect of -0.5443 , but this was confounded by the larger indirect effects through x_4 (plant height; 0.4920) and x_6 (leaf:stem ratio; 0.4013), explaining why there was a positive correlation between x_3 (leaf length) and y (DM yield; 0.3289, $P < 0.05$).

The direct effects of x_4 (plant height) and x_6 (leaf:stem ratio) were most closely related to DM yield, indicating that taller plants with high proportions of stem produced highest DM yields in elephant grass. Degree of tillering also had an important effect on DM yield. While simple correlations suggested that tillers had little effect on DM yield, partial correlations showed the importance of a high level of tillers in obtaining high DM yields in elephant grass. The study suggests that the partial correlation method and path-coefficients are helpful tools for investigating the correlation between yield and morphological parameters.

The use of elephant grass germplasm

This study has highlighted the wide variation in yield and quality attributes within the lines of elephant grasses that we tested. Using cluster analysis, it has been possible to group the tested lines into groups with similar characteristics, but with significant differences in mean DM yields and morphological traits between the groups. This information will be of significant benefit in determining how various lines can be used and their potential value in breeding programs.

While the only line in Group 1 had some excellent characteristics in having very high numbers

of tillers, thin stems and the highest leaf:stem ratio of all lines tested, unfortunately its DM yields were very low. The high labor requirement and cost associated with establishment because of vegetative propagation, have limited the widespread use of elephant grass and low-yielding lines have little application. This line produced more tillers per plant and a lower plant height than Mott elephant grass reported by Yasin *et al.* (2003a; 2003b). It was not available for grazing in south China owing to its low DM yield of 8.3 t/ha. Some of the positive attributes could possibly be transferred to other lines through appropriate crossing programs.

As one advances from Group 1 to Group 4, plants become taller, with fewer tillers, thicker stems and higher yields, but the leaf:stem ratio declines dramatically. Leaf is the most nutritious component of forage. While lines 001, 073, 121 and the hybrid pennisetum varied in total DM yield from 46.5 t/ha (073) to 74 t/ha (hybrid), the yields of leaf DM ranged only from 17.1 to 17.9 t/ha. The lines in Group 4 were very tall plants with few tillers, thick stems, low leaf:stem ratios and high DM yields. As a C₄ species, they showed considerable promise as energy crops with high rates of photosynthesis and high DM yields (Rahmani *et al.* 2000; Baldwin and Cossar 2005; Xie *et al.* 2008; Zhang *et al.* 2008), but showed lower forage quality (such as IVDMD) for livestock feeding than those in Groups 1, 2, and 3 (Ding *et al.* 2008; Zhang *et al.* 2009).

Yields of grasses decrease significantly (Chaparro *et al.* 1995; Bayble *et al.* 2007), while forage quality increases significantly (Townsend *et al.* 1978; Chaparro and Sollenberger 1997; Hsu *et al.* 2004; Bayble *et al.* 2007), as cutting frequency increases. Bayble *et al.* (2007) reported that CP concentrations in elephant grass at cutting intervals of 60, 90 and 120 days were 14.1, 10.4 and 7.8%, respectively, with IVOMD levels of 71.9, 68.0 and 63.5%. Since the optimal time to harvest a crop or pasture is determined on the basis of a combination of yield and quality, some of the lines with high stem levels and high yields could be more suitable for livestock feed if harvested at younger ages. For example, it was interesting that the hybrid pennisetum (pearl millet × elephant grass) had the highest yields of all lines tested and the lowest leaf:stem ratio. While it would not be good quality stock feed at this age of harvesting (150 days), options exist to harvest it at an earlier growth stage to improve quality. Bai and Yang (2002) concluded that hybrid pen-

nisetum (pearl millet × elephant grass) was one of the most promising summer forages, providing excellent material for grazing or hay-making if harvested at about 28–30 days in south China, where it could not survive the winter. However, Lin *et al.* (2006) reported that frequent defoliation (30 days) reduced above-ground biomass production and depleted the root system, while harvesting at 45-day intervals was more appropriate for providing livestock feed in areas where the hybrid pennisetum could survive the winter.

Lines 112 and 121, which also had high yields and a lower leaf:stem ratio than the hybrid pennisetum (pearl millet × elephant grass), could be used as energy crops (EECI 1998–2000; Rahmani *et al.* 2000; Baldwin and Cossar 2005; Xie *et al.* 2008). They would be useful as forage crops in areas where elephant grass can survive the winter, if harvested at an early growth stage to improve forage quality. They could also be used as parents to cross with pearl millet, since the DM yield of pearl millet × line 121 was significantly higher ($P < 0.01$) than that of hybrid pennisetum (pearl millet × elephant grass) (Ding *et al.* 2008). Gu *et al.* (1994) solved the key problem of making elephant grass flower at high latitudes and achieved inter-specific crosses of pearl millet and elephant grass by simulating a short-day environment using a black plastic cloth. This enabled the production of the hybrid of pearl millet × line 121, which was more productive as a forage than line 121 in south China.

Lines in Groups 2 and 3 could also be used as parents for crossing with pearl millet, although Ding *et al.* (2008) reported that DM yields of hybrids from pearl millet × lines from Groups 2 and 3 were significantly lower than that of hybrid pennisetum (pearl millet × elephant grass).

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