Relationships among Three Non-Destructive Seedling Vigour Traits in Maize

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ABSTRACT

Three non-destructive methods of seedling vigour assessment were compared in a pot experiment and two soil fertility environments in a field experiment. Ten and 23 maize (Zea mays L.) genotypes were evaluated in the pot and field experiments, respectively. The experimental design was a randomized complete block with three replicates. The seedling vigour traits investigated were seedling height, seedling length and vigour score on a scale of 1 to 9 (where 1 = excellent vigour; 9 = very poor vigour). Data were collected at 2 and 4 weeks after planting (WAP) in pots, and 4 WAP in the field. The maize genotypes showed genetic variation for the three seedling vigour traits studied. Seedling height, seedling length and vigour score were significantly ($P < 0.01$) correlated in pots and in the field. Phenotypic correlation ranged from -0.76 to 0.89 in pots and -0.51 to 0.96 in the field while genotypic correlation ranged from -0.91 to -1.04 in pots, and -0.57 to 0.96 in the field. Heritability for the three traits was moderate to high (0.38-0.67) in pots and high in the field (0.71-0.88). Any one of the three traits could, therefore, be used for the assessment of seedling vigour in maize. Vigour score integrates qualitative and quantitative aspects of seedling performance. The ease and rapid nature of its determination place it at advantage over the other two seedling vigour traits.

INTRODUCTION

Seed and seedling vigour have implications for stand establishment. In contrast to seed vigour that is often measured by the speed and uniformity of germination/emergence, germination/emergence percentage, germination/emergence index and emergence rate index (ERI) in several crops including maize (Perry 1976; Fakorede and Ojo 1981), Lucerne (Wang et al. 1996) and watermelon (Al-Maskri et al. 2004), seedling height, dry matter and relative growth rate have been used as indices of seedling vigour in normal endosperm maize (Knittle and Burris 1976; Fakorede and Mock 1980), sweetcorn (Douglass et al. 1993) and rice (Biswas et al. 2000). In general, better seed production practices such as timely planting and harvest, improved processing, packaging and handling are known to improve seed vigour considerably (Gusta et al. 2004), often resulting in comparable seed vigour among genotypes that would otherwise exhibit differences for these traits. Genotypes with similar seed vigour may, however, show differences in seedling vigour indices. In sorghum, Cisse and Ejeta (2003) found non-significant relationships between germination and emergence percentages on one hand, and seedling height (determined at 1, 2 and 3 weeks after planting) on the other. Their results suggest that the genetic control of seed vigour is different from that of seedling vigour.

Breeding for early vigour is particularly desirable in semi-arid areas and regions where rainfall at the onset of the growing season is often erratic. Genotypes with high early seedling vigour are expected to establish faster by maximizing the use of available water, nutrients and solar energy. While the determination of height as an index of seedling vigour is non-destructive, dry matter determination and growth analysis based on the latter require that plants be destructively sampled. Indices of seedling vigour that would be most useful in a breeding programme are those that can be determined rapidly and are non-destructive in nature.

In maize, seedling height, one of these indices, is determined from the base of the plant to the collar of the upper most fully expanded leaf. This measurement suffers from the limitation that it does not take into consideration possible differences in the size of the leaf blade. Thus, genotypes with similar height but which show differences in the size of the leaf blade are not differentiated. A non-destructive method of seedling vigour assessment that takes into consideration the development of the leaf blade is required. One such possibility is seedling length, measured as the distance from the base of the plant to the tip of the leaf blade when the latter is held in an upright position. It is also possible to score for vigour based on a visual assessment of the height of the seedling, and the extent of development and colour of the leaf blade (Adetimirin et al. 2006). Cisse and Ejeta (2003) reported the use of a visual scoring system in a genetic study of vigour in sorghum. The visual scoring system is based, in part, on the range of variation for seedling size in the population under study. The objectives of this study were to assess the variability in tropical maize for three non-destructive seedling vigour traits, and to determine the relationships among them.

MATERIALS AND METHODS

The study involved pot and field experiments. The pot experiment was carried out in the greenhouse premises of the Department of Agronomy, University of Ibadan, Ibadan, Nigeria while the field experiment was carried out at the Teaching and Research Farm extension of the Faculty of Agriculture, University of Ibadan (lat. 7°24’N and long. 34°48’E) at Aijibode. Ibadan is in the derived savanna and has a bimodal rainfall pattern with approximately 1280 mm rainfall per year. For the pot study (February to March 2001 – the end of the 2000/2001 dry season), polythene bags measuring 10.5 × 18.3 × 14.5 cm were each filled with 3.2 kg top soil. Ten maize genotypes, consisting of five single cross hybrids and one open pollinated variety, were sown in pots.
five lines at the C4 stage of inbreeding, were used. The pots were
arranged in a randomized complete block design, with three re-
petitions. There were five pots per genotype per replicate. Two
seeds of each of the ten genotypes were planted per pot on the 9th
February 2001. The pots were watered regularly and thinned to
one plant at one week after planting (WAP). Weeds were hand-
picked throughout the duration of the experiment. Data were col-
clected at 2 and 4 WAP, and these include seedling height (deter-
mined as the distance from the soil surface to the uppermost visi-
table collar), seedling length (determined as the distance from the
soil surface to the tip of the leaf blade when the latter was held in
an upright position), and vigour score. Vigour was scored on a
scale of 1 to 9, where 1 = excellent vigour, and 9 = very poor
vigour (Cisse and Ejeta 2003; Adetimirin et al. 2006). A definition
of the 1 to 9 scale is provided in Table 1 while plants representa-
tive of each score in the rating scale are indicated in Fig. 1. In ad-
dition to these, seedling shoot dry matter at 4 WAP was deter-
mained. Freshly harvested shoots were dried at 70°C to constant
weight. For shoot dry matter determination, three seedlings per
genotype were used. The three non-destructive indices of seedling
vigour were related to one another and to shoot dry weight using
rank correlation analysis.

For the field experiment, land was cleared and ridged. The
soil was loamy-sand. Soil test values were 8.1 g/kg organic carbon,
1.2 g/kg total nitrogen and 26.3 mg/kg available P (Bray-1). Ex-
changeable cations (cmol/kg) were as follows: Ca = 3.1, K = 0.20
and Mg = 1.20. A total of 23 maize genotypes were evaluated at
two rates of nitrogen viz. 15 and 30 kg per hectare. The recom-
mended nitrogen application for a full-season maize crop in the
derived savanna is 60 kg per ha, with half applied at 2 WAP and
the other half applied at 6 WAP. However, farmers apply less than
half of this rate or no fertilizer at all. Thus, the 30 kg N/ha applica-
tion in the present study correspond to the first dose of the recom-
mended rate for a full-season maize crop. Phosphorus and potas-
sium were applied at planting at the elemental rate of 30 kg per
hectare. The genotypes, which consisted of 16 single cross hybrids
and seven open-pollinated varieties, were planted in three repli-
cates. The experiment was a split-plot with nitrogen rate as the
main plot factor and genotype as the sub-plot. Sub-plots consisted
of single row ridges, each 5 m long and spaced 0.75 m apart. Two
seeds were planted per hill on 7 May, 2001 at 0.25 m apart on rid-
ges. Plots were thinned to one plant per hill at 10 days after plan-
ting. Similar data collected in the pot experiment were also collec-
ted, except shoot dry matter which was not determined in the field.
All data were collected at 4 WAP.

Data were analyzed using SAS software (SAS Institute., Cary,
NC). A single degree of freedom orthogonal contrast was used to
compare the two groups of genotypes in the pot (hybrids vs. inbred
lines) and field (hybrids vs. open-pollinated varieties) experiments.
Mean squares and F values of the single degree of freedom con-

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very tall seedlings, very large and green leaf blades, excellent vigour</td>
</tr>
<tr>
<td>2</td>
<td>Very tall seedlings, large and green leaf blades, very high vigour</td>
</tr>
<tr>
<td>3</td>
<td>Tall seedlings, moderately large and green leaf blades, high vigour</td>
</tr>
<tr>
<td>4</td>
<td>Tall seedlings, moderately large and slightly pale leaf blades, fairly high vigour</td>
</tr>
<tr>
<td>5</td>
<td>Moderately tall seedlings, moderately large and green leaf blades, moderate vigour</td>
</tr>
<tr>
<td>6</td>
<td>Short seedlings, green and moderate leaf blades, fairly low vigour</td>
</tr>
<tr>
<td>7</td>
<td>Short seedlings, pale green and moderate leaf blades, moderately low vigour</td>
</tr>
<tr>
<td>8</td>
<td>Very short seedlings, small yellowish leaf blades, poor vigour</td>
</tr>
<tr>
<td>9</td>
<td>Very short seedlings, very small yellowish leaf blades, very poor vigour</td>
</tr>
</tbody>
</table>

Fig. 1 Maize plants with vigour scores of 1 to 9. Scores measured at 4 weeks after planting are described in Table 1 (scale × 0.06).
RESULTS

Seedling vigour of 10 genotypes in pots

Seedling height

Differences in seedling height among the 10 genotypes evaluated in pots were not significant at 2 weeks after planting. By 4 WAP, differences among the genotypes were significant at p <0.05. Hybrids 34C2251 and 34C2239 had the highest seedling height at 2 and 4 WAP, although ranks of the two hybrids at 2 WAP were reversed at 4 WAP (Table 2). The reversal in relative ranking of these two hybrids from 2 to 4 WAP observed for seedling height and seedling length were also observed for vigour score. Inbred line K1414 SR/SR had the highest vigour score at 2 and 4 WAP. Mean vigour score at 2 WAP was 3.7 for the hybrids and 5.7 for the inbreds. At 4 WAP, vigour score averaged 3.9 and 5.5 for the hybrids and inbreds, respectively. A single degree of freedom test indicated the hybrids to have significantly (p <0.05) lower vigour scores (i.e. higher vigour) than the inbred lines, only at 2 WAP. Thus, the superior vigour of the hybrids over the inbreds was limited to 2 WAP.

The CVs for vigour score was 22.3% at 2 WAP and 25.2% at 4 WAP.

Shoot dry matter

Shoot dry matter at 4 WAP was highest for 34C2251, followed by 4C2-15 – an inbred line, and then hybrid 34C2239. Differences in shoot dry matter among the genotypes were significant at p <0.05. Mean shoot dry matter averaged 7.8 g per plant for the hybrids and 6.5 g per plant for the inbred lines. The difference between the two groups of genotypes was not significant. The CV was 25.0%.

Correlation of rank order of genotypes for the various indices of seedling vigour in pots

Ranks of genotypes for seedling height at 2 WAP were significantly correlated to ranks of genotypes for seedling length (p <0.01) and vigour score (p <0.01) at 2 WAP, as well as ranks of genotypes for seedling height and seedling length at 4 WAP (Table 3). However, ranks of genotypes for height at 2 WAP were not significantly correlated to genotype ranks for vigour score and shoot dry matter at 4 WAP. Genotype ranks for seedling length determined at 2 WAP were significantly correlated (r = 0.83 to 0.96) to ranks of genotypes for all the other indices of vigour determined at 2 and 4 WAP. The significant correlation between genotype

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Table 2 Vignour indices of 10 maize genotypes at 2 and 4 weeks after planting.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Seedling height (cm)</th>
<th>Seedling length (cm)</th>
<th>Vigour score (1–9)</th>
<th>Shoot dry matter (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 WAP</td>
<td>4 WAP</td>
<td>2 WAP</td>
<td>4 WAP</td>
</tr>
<tr>
<td>Hybrid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9044-29 STR</td>
<td>10.9</td>
<td>16.1</td>
<td>45.3</td>
<td>62.7</td>
</tr>
<tr>
<td>9021-18</td>
<td>11.4</td>
<td>17.2</td>
<td>51.9</td>
<td>74.8</td>
</tr>
<tr>
<td>34C2239</td>
<td>13.6</td>
<td>22.5</td>
<td>60.5</td>
<td>77.6</td>
</tr>
<tr>
<td>34C2251</td>
<td>13.1</td>
<td>24.7</td>
<td>56.2</td>
<td>90.9</td>
</tr>
<tr>
<td>34C2049</td>
<td>11.4</td>
<td>20.5</td>
<td>48.9</td>
<td>74.4</td>
</tr>
<tr>
<td>Inbred</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9071 STR</td>
<td>10.9</td>
<td>18.1</td>
<td>47.0</td>
<td>72.5</td>
</tr>
<tr>
<td>KU1414 SR/SR</td>
<td>9.6</td>
<td>16.0</td>
<td>39.7</td>
<td>61.0</td>
</tr>
<tr>
<td>3C2-3</td>
<td>7.4</td>
<td>14.8</td>
<td>37.2</td>
<td>66.2</td>
</tr>
<tr>
<td>4C2-15</td>
<td>10.2</td>
<td>18.6</td>
<td>46.0</td>
<td>72.0</td>
</tr>
<tr>
<td>3C2-44</td>
<td>10.7</td>
<td>20.1</td>
<td>44.2</td>
<td>72.7</td>
</tr>
<tr>
<td>Mean</td>
<td>10.89</td>
<td>18.87</td>
<td>47.69</td>
<td>72.48</td>
</tr>
<tr>
<td>SE (D.F. = 18)</td>
<td>1.37</td>
<td>1.06</td>
<td>4.33</td>
<td>12.01</td>
</tr>
<tr>
<td>CV (%)</td>
<td>18.60</td>
<td>10.62</td>
<td>15.72</td>
<td>12.01</td>
</tr>
</tbody>
</table>
ranks for seedling length at 2 and 4 WAP indicate that seedling length at 2 WAP has the potential for use in predicting seedling vigour at 4 WAP.

Vigour score at 2 WAP was not significantly correlated to vigour score and shoot dry matter at 4 WAP. These results indicate that vigour score at 2 WAP would not be reliably indicative of vigour at a later date. However, genotype ranks for vigour score was significantly correlated to ranks for seedling length, seedling height and shoot dry matter, all determined at 4 WAP.

With the exception of seedling length, correlation coefficients of genotype ranks of seedling vigour indices were higher within each time of vigour determination than between the two times of vigour assessment.

Seedling vigour of 23 maize genotypes in the field

Seedling height at two levels of applied nitrogen

There were significant differences (p <0.01) in seedling height among the 23 genotypes studied. The rate of nitrogen applied significantly influenced seedling height (p <0.01). Averaged over the 23 genotypes, seedling height at 30 kg N per hectare was 26% higher than the seedling height at 15 kg N per hectare. The genotypes differed in their linear responses to the applied nitrogen. This was evidenced by the significant genotype × N rate interaction.

Seedling height at 15 kg N/ha was lowest for hybrid 9022-13 STR and highest for hybrid 9114-12 (Table 4). At 30 kg N/ha, seedling height ranged from 17.1 cm for hybrid 9114-4 to 24.4 cm for hybrid 9114-12. Under the lower nitrogen rate (15 kg N per hectare), mean seedling height of the hybrids (48.3 cm) was not significantly different from the mean seedling height of the open-pollinated varieties (50.9 cm). At 30 kg N per hectare, however, mean seedling height of the open-pollinated varieties was significantly higher than the mean seedling height of the hybrids. A number of hybrids had comparable seedling height as the open-pollinated varieties, while several hybrids had comparably lower seedling height than the latter group. The CV for seedling height was 14.8% at 15 kg N per hectare and 11.0% at 30 kg N per hectare.

Seedling length at two levels of applied nitrogen

Genotype and N rate effects were significant at p <0.01. At 15 kg N/ha seedling length was lowest for hybrid 8522-2 and highest for hybrid 9114-12. As with seedling height at 30 kg N/ha, seedling length ranged from 17.1 cm for hybrid 9114-4 to 24.4 cm for hybrid 9114-12. Under the lower nitrogen rate (15 kg N per hectare), mean seedling length of the hybrids (19.7 cm) was not significantly different from the mean seedling length of the open-pollinated varieties (21.0 cm). At 30 kg N per hectare, however, mean seedling length of the open-pollinated varieties was significantly higher than the mean seedling length of the hybrids. A number of hybrids had comparable seedling length as the open-pollinated varieties, while several hybrids had comparably lower seedling length than the latter group. The CV for seedling length was 15.3% at 15 kg N per hectare and 11.0% at 30 kg N per hectare.

Comparison of the hybrid and open-pollinated groups
indicated that the two groups were not significantly different at 15 kg N/ha but mean seedling length of the open-pollinated varieties (85.6 cm) was significantly (p < 0.01) higher than the mean seedling length of the hybrids (79.2 cm) at 30 kg N/ha. The CV for seedling length at 15 and 30 kg N/ha were 11.4 and 9.1%, respectively.

**Vigour score at two levels of applied nitrogen**

As with seedling height and seedling length, differences in mean vigour score among the genotypes and between the two nitrogen rates were significant at p < 0.01. Genotype × environment interaction was also significant at p < 0.01. Seven genotypes (4 hybrids and 3 open-pollinated varieties) had vigour scores equal to or less than 4.0 (Table 4). Nitrogen significantly improved vigour. This was evidenced by the mean reduction of 1.47 (on a 9 point scale) – representing 31.3%, at 30 kg N/ha compared to 15 kg N/ha.

Differences between the hybrid and open-pollinated variety groups were not significant at 15 and 30 kg N/ha. The CV for vigour score was 12.9% at 15 kg N/ha and 16.5% at 60 kg N/ha.

**Phenotypic and genotypic correlation coefficients and heritability of seedling vigour traits in pots and in the field**

Phenotypic correlation coefficients among seedling height, seedling length and vigour score were more than twice their respective standard errors, and therefore significant (p < 0.05) in pots and in the field at both 15 and 30 kg N/ha (Table 5). The highest phenotypic correlation coefficients were obtained between seedling height and seedling length. Correlation coefficients between vigour score on one hand, and seedling height and seedling length on the other were negative. Genotypic correlation coefficients among these traits were also significant. In the field, genotypic correlation coefficients among the three traits were higher at 15 kg N/ha than at 30 kg N/ha. In the field study, heritability for the three traits was high and ranged from 0.73 to 0.88 at 15 kg N/ha and 0.71 to 0.72 at 30 kg N/ha (Table 6). Heritability for the traits was lower in pots (where the maize lines were grown) than at 30 kg N/ha. In the field study, heritability for the traits was lower in pots (where the maize lines were grown) than at 30 kg N/ha.

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**Discussion**

Genetic variation for seedling vigour was present among the maize genotypes evaluated in the study, and this was evidenced by all three non-destructive seedling vigour indices considered, viz. seedling height, seedling length and vigour score. Seedling vigour is commonly assessed at about 4 WAP in maize. At this time, the three indices of vigour were related to one another in the pot and field studies, indicating that a significant aspect of vigour captured by any one of the traits is embodied in the other two traits. In effect, any one of the three traits could be reliably used as an index of vigour in a breeding programme, especially since the three traits have moderate to high heritability. Heritability is one of the factors influencing response to selection, with traits of moderate to high heritability being more easily improved than those of lower heritability (Hallauer and Fo 1981). In addition, the results of the present study indicate that screening for early vigour could be reliably done in pots. This would be particularly useful in the dry season in tropical areas where irrigation is not available for water supply to field-grown crops. At 2 and 4 WAP in maize, endosperm reserves are exhausted and the differences in canopy size, colour, and general appearance of the young plants are the result of differences in ability to produce assimilates (Revilla et al. 1999). This contrasts pre-emergence seedling growth that is seed reserve-dependent (Oaks 1997; Betty et al. 2000). The genetic correlation coefficients between vigour score and other indices of vigour determined in this study were higher than the values between seedling height and vigour score in sorghum (r = -0.35 to -0.54) reported by Cisse and Ejeta (2003). The choice of an appropriate trait to use as an index for seedling vigour would be determined by the nature and objective of the investigation as well as the resources available for data collection. In breeding programmes, where a large number of genotypes are usually evaluated and the identification of a few outstanding genotypes are of importance rather than the relative ranking of all genotypes, assessment of seedling vigour by scoring offers the advantage of being more rapid and less laborious over precise measurements of seedling height and seedling length. Few studies have reported the use of visual scoring for seedling vigour, among which are those of Shapital and Witcombe (1998) in rice, Revilla et al. (1999) in maize, and Cisse and Ejeta in sorghum (2003). In the three cases, the scores in the scales were not fully described. The present paper provides full description of the scores in a 1 to 9 visual vigour scale.

Although seedling vigour could be assessed at any time during the juvenile growth stage, the development of vigour is dynamic and the relative vigour of genotypes could change between any two intervals during the juvenile growth stage. This was evident from the results of the pot study, in which comparatively lower correlation coefficients were obtained between seedling height and vigour score at 2 WAP on one hand, and the same traits at 4 WAP on the other. Seedling length at 2 WAP was the only trait with ability to predict vigour at the later juvenile stage of 4 WAP (r = 0.87 to 0.96). The difference between seedling height and seedling length measurements is that the latter integrates the extent of development of the leaf blade with the plant height. The implications of these results are that (i) seedling length could be used as a reliable index of vigour between 2 and 4 WAP, and (ii) genotypes that demonstrate superior capability for the development of their leaf blades by 2 WAP are likely to retain their superior vigour at the later juvenile stage of 4 WAP. Although seedling vigour was influenced by the macro-environment as evidenced by the higher seedling vigour at 30 kg N/ha, the high broad-sense heritability values (0.71-0.88) obtained in the field for the three traits indicated a high level of genetic control for the traits.
The heritability estimates from the field study are similar to those of Cisse and Ejeta (2003), who reported broad-sense heritability estimates of 0.78 for visual vigour score, and 0.71, 0.61 and 0.37 for sorghum seedling height determined at 1, 2 and 3 weeks after planting, respectively. In rice, Redona and Mackill (1996) obtained a heritability estimate of 0.80 for seedling length in a cross between two rice lines. The high heritability estimates obtained in the present study, together with the genetic variation observed for seedling vigour, indicates the possibility of improving seedling vigour in tropical maize through breeding. The comparatively lower heritability estimates from the pot study could be attributed to the segregating nature of the lines used.

The superior vigour of the hybrid group over the inbred group was expressed only in the visual score – the composite indicator of vigour, and this was at both 2 and 4 WAP in the pot study. Thus, heterosis, often reported for mature plant traits such as grain yield (Betran et al., 2003; Ahmadzadeh et al., 2004; Tollenaar et al., 2004), is also manifested in seedlings, although a number of inbred lines showed vigour comparable to those of the vigorous hybrids. The inability of seedling height and seedling length to detect these differences may be due to the fact that the qualitative aspect of vigour (in particular, the colour of the leaves) that is integrated into vigour score is not reflected in seedling height and seedling length. In the field study, seedling height and seedling length indicated superior vigour of the open pollinated group over the hybrid group at 2 WAP, although genotypes with the highest vigour were found among the hybrids. These results are due to the comparatively lower vigour of some of the large number of hybrids tested, and indicate that seedling vigour is genotype-specific and not the attribute of a group. The higher vigour obtained for the open-pollinated varieties at 30 Kg N suggests that the recently developed open-pollinated varieties evaluated in this study are responsive to nitrogen, and may serve as sources of genes for high seedling vigour under optimum fertilizer input system.

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