Ife J. of Agric. 22 (1) 2007

OPTIMUM SAMPLE SIZE FOR MAIZE SEEDLING EVALUATION

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ABSTRACT

Linear measurements of seedling parts are laborious and time-consuming, thereby limiting the number of treatments that can be handled at any time. A possible solution is to collect data only on the minimum number of seedlings that is sufficiently representative of a population. From investigations involving different maize genotypes × soil compaction, maturity stages × duration of storage, seed testing substrata × sterilization treatments, individual measurements of root and shoot length as well as number of primary roots of 8,300 seedlings were used to estimate the optimum sample size for maize seedling evaluation. Using data on each seedling only once, the data were randomly grouped into different sample sizes ranging from 5 to 20 plants in increments of five. Mean, standard deviation and skewness were calculated for each sample size and analysis of variance was done. Sample size had no significant effect ($P>0.05$) on mean of all traits in the combined analysis and its interaction with treatment effects was also negligible ($P>0.05$). But despite significant effect of sample size on deviations and skewness, its contribution to observed variability was less than 1%. A similar pattern was observed when data were analyzed experiment by experiment. In studies like those considered here, there is no statistical and economic advantage in collecting data on more than 15 seedlings per replication when the mean value is the criterion for comparison and decision-making.

Keywords: sample size, mean, seedling analysis, maize

INTRODUCTION

Seedling (linear) growth tests are recommended vigour tests because unlike germination test, they allow detection of differences in rate of germination and subsequent growth (Hampton and TeKrony, 1995). Seedling length or the combined lengths of both root and shoot is directly related to development and growth in the field and is a widely used measure for vigour (Kruse, 1994; Ajayi *et al.*, 2001). Linear measurements on maize seedlings under stress conditions in laboratory tests are good estimators of final plant stand under adverse field conditions (Venter, 1988) and for ranking maize genotypes for drought tolerance (Elemery *et al.*, 1995). Seedling vigour index (SVI), defined as the product of root and hypocotyl length divided by germination percentage, is a good indicator of seed maturity and genotypic differences associated with plant stand and yield differences in cultivars of groundnut, *Arachis hypogeal* L. (Dayal *et al.*, 1999). Feil *et al.* (1990) suggested that root traits of maize, including number, length and diameter of seminal and adventitious roots, might be indicators of the efficiency of N utilization at later growth stages. Root growth measurement is also used extensively in physiological investigations (Venter, 1988; Feil *et al.*, 1991; Bingham *et al.*, 1994; Liang *et al.*, 1996; Bingham and Merritt, 1999) and in investigations on soil-root relationships (Schortemeyer *et al.*, 1993; Liedgens *et al.*, 2000) especially in compaction studies (Asady *et al.*, 1985; Bushamuka and Zobel, 1998; Soyelu *et al.*, 2001).

A major limitation of seedling linear measurements is that it is laborious and time-consuming; data have to be recorded on sheets of paper and then transferred to a personal computer for analysis (Kruse, 1994). This limits the number of treatments that can be handled in any one investigation when detailed evaluation is involved and number of seedling evaluated per replication and treatment is high. Despite the simplicity of the semi-automated device for quick measurement of liner dimensions developed by Kruse (1994), the number of primary roots still has to be counted manually for each seedling evaluated. A more efficient strategy for increasing the number of treatments within an investigation could be to limit the number of seedlings on which data are collected per replication and treatment. The accuracy, reliability and repeatability of seed vigour test results depend largely on adherence to recommended procedures and methods, including sample size for various tests (Coster, 1993; International Seed Testing Association, 1999; Bányai and Barabás, 2000). The recommended procedure for maize seedling growth test (Hampton and TeKrony, 1995) utilizes only shoot length and the method is rarely used when seedling analysis is for other purposes than assessment of vigour. In such situation, there exists a wide variability on number of seedlings on which data are collected, mostly ranging from 5 to 50. The decision on number of seedlings to be used is at the discretion of the investigators depending on the available time.

In order to ensure that sample size or number of seedlings on which data are collected represents the lot from which the sample is drawn, extensive data collected from our previous investigations were analyzed to determine the optimum sample size for maize seedling evaluation.

MATERIALS AND METHODS

Fifty-two secondary data sets comprising individual measurements on 8,300 maize seedlings from three different experiments were pooled for this study. The experiments which were conducted in the laboratory and had been earlier reported were seed maturity stages × seed testing interaction before and during cold storage of a maize hybrid seeds (Ajayi and Fakorede, 2000); substrata for seed testing with or without sterilization (Ajayi *et al*., 2000); and genotypes evaluated under several soil compaction levels (Soyelu *et al*., 2001). 10 of the 52 data sets were replicated four times and the rest three times. The seed testing and seedling analysis in the above studies were carried out at the Seed Science Laboratory, Obafemi Awolowo University, Ile-Ife and the procedures followed had been reported in the publications cited above. Briefly, 100 seeds per replication were tested in all the studies and at the end of each experiment, the maize seedlings were classified into normal and abnormal according to rules for seedling evaluation (Gutormson, 1991). In each replication, 50 normal seedlings were randomly selected, the number of seminal roots were counted, the length of seedling primary root and shoot were measured individually on the selected fifty normal seedlings using a ruler.

In order to estimate the optimum sample size for maize seedling evaluation, the 52 data sets of seedling measurements from all the studies above were pooled. From each data set, data collected on the 50 seedlings from each replication were randomly divided into sub-samples (herein after referred to as sample size) comprising individual measurements of 5, 10, 15 or 20 seedlings such that the data on any single seedling were used only once. Thereafter, mean, standard deviation and skewness were calculated for each sample size and replication in each data set. Combined analysis of variance was done and the proportion of the total variability attributable to each source of variation was calculated. Treatment means were compared with Duncan multiple range test. Pearson correlation coefficients were calculated for the relationship between sample size and the different statistical parameters for each seedling trait. Similarly, data sets for all treatments within an experiment were analyzed separately to investigate whether the optimum sample size for seedling evaluation would be influenced by the different experimental treatments. All statistical analyses were done with SAS software version 8.1 (SAS Institute, 1999a, b).

RESULTS

The combined analysis of variance across experiments revealed significant ($P<0.05$) treatment effects (genotypes, maturity stages, substrata, sterilization of substrata, different levels of soil compaction) for all statistical parameters and traits (Table 1). Significant mean square due to sample size was detected for mean root and shoot lengths and for skewness of root number. The interaction effect between sample size and treatments on all statistical parameters and traits was negligible ($P>0.05$). Averagely, the R^2 associated with the model for analysis of variance were in the order mean>standard deviation>skewness. Accordingly, the different treatments accounted for most of the variability, up to 90%, in mean values for the traits investigated while the contribution of sample size, despite its significant effect on some traits and statistical parameters, was in the range 0.10-0.52% (Figure 1). On the average, the interaction effect and error or unaccounted sources of variability contributed 0.89 and 8.6%, respectively to mean root and shoot length; for root number the percentage of the total variability explained by these two sources were comparatively higher. The range of the contributions of unaccountable sources of variation to deviations of individual measurements as well as skewness or distribution of the whole data around the mean was 30-69% and 63-68%, respectively. Similarly, sample size explained between 6.61 and 0.29 % of the total variability in standard deviations and skewness.

Mean and standard deviations of the three seedling traits tended to increase with sample size but the magnitude of increase tended to decrease as sample size increased (Figure 2). But t-test revealed that differences between the different sample sizes were generally negligible ($P<0.05$). In the only two significant ($P<0.05$) parameters- mean shoot length and standard deviation of root number, the significant differences were between sample size 5 and 20. The skewness of both shoot and root lengths were directly opposite, between sample size

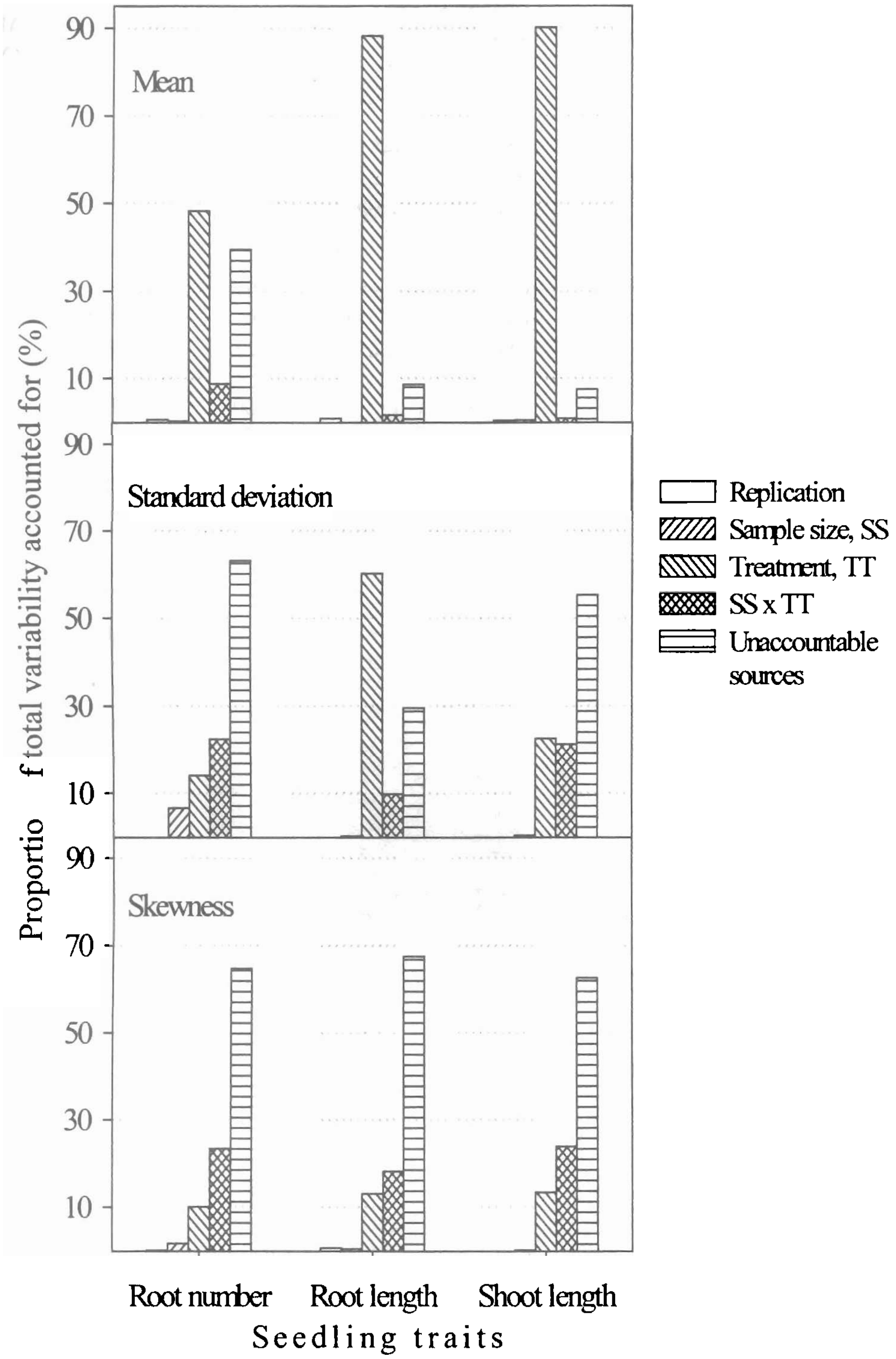

Figure 1: Proportion of variability explained by sources of variation in the combined analysis of variance.

Table 1: Analysis of variance combined for sample sizes and treatments.

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

Statistic	Trait	Mean Square				R^2	Mean (N=664)
		Replication (df=3)	Sample size, SS, (df=9)	Treatment TT, (df=51)	SSxTT (df=459)		
Mean	Root number	0.44	0.20	1.94***	0.12	60.43***	5.16
	Root length (cm)	41.24***	15.24**	237.22***	1.50	91.40***	16.28
	Shoot length (cm)	40.02***	47.77***	491.09***	1.62	92.50***	14.39
Standard deviation	Root number	0.003	0.06	0.13***	0.07	36.83*	1.06
	Root length (cm)	0.22	0.93	11.23***	0.61	70.38**	2.89
	Shoot length (cm)	0.77	9.19	26.20***	8.23	44.68***	2.55
Skewness	Root number	0.22	2.34**	0.78*	0.60	35.28	0.13
	Root length (cm)	0.84	0.61	0.92**	0.43	32.45	-0.03
	Shoot length (cm)	0.15	0.39	0.15***	0.68	37.42*	0.03

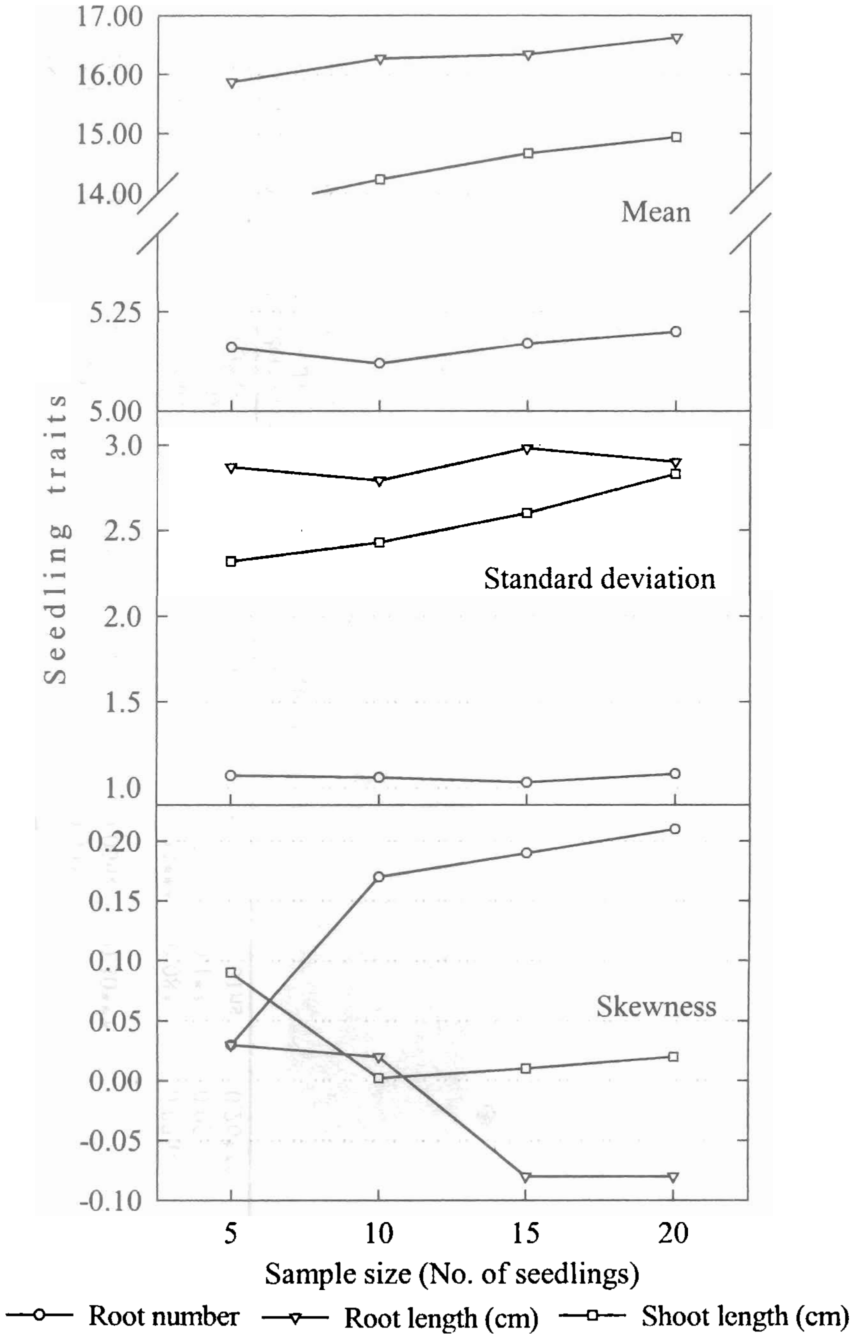

Figure 2: Effect of number seedlings on which data were collected on some statistical parameters

5 and 10, root lengths tended to be negative but positive when sample size was above 10. Generally, pattern of changes in all the statistical parameters were less dramatic as from sample size 15. But despite statistical significant relationships revealed by the correlation analysis ($P<0.01$), the strength of these associations was rather weak, r <0.30 (Table 2). The mean of root and shoot lengths on the one hand and standard deviations on the other were positively correlated ($P<0.001$) but neither was correlated with sample size in any direction.

As it was observed in the combined analysis of variance, R^2 values associated with the mean values of the three traits in each of the separate experiments were generally greater than for standard deviation and skewness. Most of the main factor and interaction effects in the separate experiments were significant ($P<0.05$-0.001) for the different statistical parameters of all the traits (Table 3). But out of 108 different main and interaction effect combinations involving sample size across the three different experiments, mean square attributable to sample size was significant ($P>0.05$) in only 10 combinations. In two of the

Table 2: Pearson correlation coefficients (N=664) between sample size and the statistics associated with seedling traits

	Trait[1]	Sample size	Mean			Standard deviation (S.D)			Skewness (Skew.)	
			RNO	RLT	SLT	RNO	RLT	SLT	RNO	RLT
Mean	RNO	0.03ns		0.13**						
	RLT	0.06ns	0.13**							
	SLT	0.07ns	0.13***	0.70***						
S.D	RNO	-0.004ns	0.26***	0.05ns	-0.01ns					
	RLT	0.03ns	0.08ns	0.56***	0.66***	0.09*				
	SLT	0.06ns	0.05ns	0.19***	0.43***	0.06ns	0.30***			
Skew	RNO	0.11**	-0.06ns	0.17***	0.09*	0.23***	0.08*	0.07ns		
	RLT	-0.07ns	-0.04ns	-0.15***	-0.02ns	-0.01	0.1**	0.05	-0.08*	
	SLT	-0.03ns	0.07ns	-0.17***	-0.05	0.08*	0.01ns	0.26***	-0.05ns	0.03ns

[1] RNO- Root number, RLT-shoot length (cm) and SLT- shoot length (cm)

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

ns- r not significant at the 0.05 probability level.

Table 3: Analysis of variance for individual experiments

A. Effect of maturity × seed test before and after four months in cold storage

Statistic	Trait[1]	Mean Square								R^2	Mean N=160
		Replication df=3	Size, SS df=3	Storage, SG df=1	Maturity, M df=4	SS×SG df=3	SS× MT df=12	SG×M df=4	SS×SG ×M df=12		
Mean	RNO.	0.48*	0.07	0.002	0.99***	0.44*	0.18	2.53***	0.09	53.03***	4.93
	RLT.	5.86	10.15	1.08	49.85***	0.49	2.51	432.49***	2.54	81.17***	17.41
	SLT.	0.33	7.80*	23.03**	22.01***	0.92	0.55	691.79***	1.88	92.24***	12.58
Standard	RNO.	0.07	0.23*	0.08	0.15	0.07	0.04	0.06	0.03	26.59	1.08
Deviation	RLT.	0.54	0.99	0.70	2.31***	0.81	1.02	5.75***	0.65	44.76***	2.86
	SLT.	01.18	0.04	0.88	5.68***	0.58	0.27	4.43***	0.24	61.52***	1.83
Skewness	RNO.	0.42	0.04	1.30	0.49	0.05	0.43	0.84	0.41	23.29	
	RLT.	0.77	0.22	0.86	0.30	0.31	0.41	0.69	0.52	28.80	-0.0005
	SLT.	0.47	0.56	0.05	1.25	0.51	0.23	2.44**	0.19	28.97	-0.07

B. Effect of substrata for seed testing × sterilization

Statistic	Trait	Mean Square								R^2	Mean N= 288
		Replication df=2	Size, SS df=3	Sterilization, ST df=1	SubstrataSB df=2	SS×ST df=3	SS×SB df=6	ST×SB df=2	SS×ST×SB df=6		
Mean	RNO.	0.20	0.06	0.58	0.65	0.16	0.07	0.57	0.07	5.13	5.18
	RLT.	5.89	5.55	5.66	2268.31***	0.66	1.10	13.52	0.83	74.16***	13.65
	SLT.	28.78**	17.84**	71.86***	304.82***	0.48	0.31	23.14**	0.56	43.89***	9.72
Standard	RNO.	0.07	0.05	0.03	0.10	0.26*	0.08	0.01	0.08	9.90	1.04
Deviation	RLT.	0.21	0.90	1.37	20.90***	0.18	0.15	0.32	0.12	26.83	2.11
	SLT.	3.03	2.07	1.88	3.70*	0.48	1.64	0.34	0.58	11.52	1.76
Skewness	RNO.	0.04	2.97**	0.002	2.16*	0.81	0.67	1.14	1.80*	15.75**	0.10
	RLT.	0.66	1.86*	0.48	6.97***	0.36	0.63	0.64	0.28	14.67*	-0.07

C. Effect of level of soil compaction × variety

Statistic	Trait[1]	Mean Square								R^2	Mean N=216
		Replication df=2	Size, SS df=3	Compaction Level, CP df=3	Variety, V df=4	SS× CP df=9	SS×V df=12	CP×V df=10	SS×CP×V df=30		
Mean	RNO.	0.97*	0.11	0.42	2.78***	0.14	0.05	0.72***	0.12	49.92***	5.31
	RLT.	161.00***	5.21	45.26***	38.82***	2.65	2.32	8.86***	2.45	69.95***	18.96
	SLT.	369.98***	25.47*	258.97***	79.20***	3.26	4.40	26.07***	2.79	69.14***	21.96
Standard Deviation	RNO.	0.17	0.06	0.03	0.11	0.03	0.11	0.04	0.06	36.60	1.07
	RLT.	0.58	0.04	2.55*	3.15*	1.26	1.02	3.16***	0.63	42.66*	3.93
	SLT.	0.13	17.49	45.90	18.54	20.64	22.48	27.63	22.75	25.66	4.12
Skewness	RNO.	0.54	0.20	0.42	0.30	0.30	0.58	0.42	0.53	30.68	0.14
	RLT.	0.25	0.65	1.06	0.20	0.13	0.25	0.35	0.30	26.26	0.004
	SLT.	1.28	0.99	1.17	0.15	0.42	0.63	0.83	0.67	35.43	0.08

[1] RNO- Root number, RLT-shoot length (cm) and SLT- shoot length (cm)

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

experiments where seedling growth was not directly restricted by treatment (Table 3a and b), the standard deviation for root length>shoot length>root number. In the soil compaction experiment where substrata used for seed testing restricted seedling root growth, however, the models for the analysis of variance for deviations and skewness of the data and the R^2 associated with them were not significant ($P<0.05$). In each experiment, the proportion of total variability explained by sample size for each trait was minimal. Contributions of other main treatments to variability in seedling root and shoot linear measurements were reported previously (Ajayi and Fakorede, 2000; Ajayi *et al.*, 2000; Soyelu *et al.*, 2001) and the range was 85-97.4%. Contribution of replication was also less than 1% when differences in seedling growth are due only to inherent quality or Vigour differences (Table 3a; Ajayi and Fakorede, 2000). But when seedling analysis involved investigation of effect of factors external to the seed, for example, substrata and soil compaction, replication alone accounted for up to 30% of observed variability (Table 3b and 3c; Ajayi *et al.*, 2000; Soyelu *et al.*, 2001).

DISCUSSION

In all of the analyses reported herein, sample size effect on the mean, standard deviation and skewness of root number, root length and shoot length, were minimal. Where significant mean squares were detected, the relative contribution of sample size to total observed variability was generally less than 1%. Correlation analysis revealed no relationships between sample size and seedling linear measurements implying that sample size has little direct consequence on the expected value of a seedling trait. This notwithstanding, a sufficient number of seedlings need be selected to give enough representative information about the population from which it is drawn. Root and shoot lengths are often measured separately or together as seedling length. But the strong positive relationship between the mean of these two traits suggest that when time and resources are limited, either of the traits could be used.

Despite the fact that the different treatments in the three experiments were wide enough to affect significantly seedling size, as high as 40% of variability in mean root number of maize seedlings was not accounted for by the sources of variation in the combined variance analysis. This has been a consistent observation in all our previous works aimed at partitioning variations in maize seedling germination and subsequent growth (Ajayi and Fakorede, 2000; Ajayi *et al.*, 2000; Ajayi and Fakorede, 2001; Soyelu *et al.*, 2001). Root number is largely pre-determined in the embryo (Kiesselbach, 1999). Varietal differences alone have been reported to account for about 27% of observed variability in this trait (Soyelu *et al.*, 2001). We have found from earlier studies that root number is also highly influenced by seed storage condition and duration of equilibration before testing seed stored in cold storage conditions (Ajayi and Fakorede, 2001). The number of seminal roots is a polygenic trait and highly variable between different maize lines (Feix *et al.*, 2002). The factors associated with seedling root number early in the life of the plant, that is during germination, deserve further detailed investigations because it is still unclear which role the seminal roots play for a good performance of the seedling (Feix *et al.*, 2002). But it has been implicated as one of the seedling traits controlling the efficiency with which maize plants utilize nitrogen at maturity (Feil *et al.*, 1990).

The implications of significant treatment and interaction effects on maize seed testing and seedling evaluation were discussed in the earlier report of each experiment (Ajayi and Fakorede, 2000; Ajayi and Fakorede, 2001; Soyelu *et al.*, 2001. The relatively high proportion of total variability in mean values that was accounted for by treatment in each experiment further enhances the reliability of mean values of maize seedling traits as good criteria for comparing treatment differences in seed and seedling Vigour analysis or other physiological investigations. When one of the experimental factors exert an external influence on seed germination and consequently seedling growth, the necessity for replication is very high and the actual differences, inherent in different seed lots and individual seeds from which seedlings result, may be lost and the data may be less useful or misinterpreted if the experiment is not properly replicated.

In summary, there is no economic and statistical advantage in taking linear measurements on more than fifteen maize seedlings per replication when only the mean value will be used for comparative purposes.

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