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Use of millimeter ruler as an alternative tool in the phenotyping of potential descriptors of soybean

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The soybean stands out in the Brazilian agribusiness and part of this success stems from the development of improved cultivars. One of the requirements for the concession of protection to a given cultivar is that it should be distinct from others cultivars. Potential additional descriptors of soybean have been studied. Thus, the aim of this study was to evaluate the performance of the millimeter ruler in the measurement of the lengths of hypocotyl and epicotyl in seedlings of soybean and to compare the methods based on the use of millimeter ruler and the method based on the use of caliper. The hypocotyl and epicotyl of 1.084,0 plants in the stage V2 and 1.252,0 plants in the stage V3 were measured by digital caliper and millimeter ruler in 2014 and 2015. The plants were kept in a greenhouse. The linear regression (Y = βX), bias, concordance index, performance index, sample standard deviation and confidence interval were used to evaluate the performance. The millimeter ruler overestimated the length of epicotyl and hypocotyl by 0.0508 and 0.0147 mm, respectively. The millimeter ruler had an optimum performance in the measurement of the lengths of hypocotyl and epicotyl in seedlings of soybean in V2 and V3. A millimeter ruler is an alternative tool in the measurement of seedlings of soybean in most of the developmental stages.

Key words: Epicotyl, *Glycine max,* hypocotyl, additional descriptors.

INTRODUCTION

Soybean (Glycine max (L.) Merr.) stands out as one of the most important crops in the Brazilian agribusiness. The production of this crop in the 2015/2016 harvest was estimated at 101.18 million tons, which corresponded to an increase of 5.1% as compared to the previous harvest (CONAB, 2016). Part of this success is due to the

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programs of genetic breeding kept by different institutions of research and universities in Brazil (Oda et al., 2015).

Three basic requirements must be met for granting protection to a cultivar: the cultivar must be distinct, homogeneous and stable (Viana, 2013). According to Nogueira et al. (2008), the 38 descriptors, including the additionals descriptors used to differentiate cultivars of soybean are insufficient, which points out to the need of broadening the list of these descriptors.

According to Nogueira et al. (2008), there is genetic variability for the lengths of hypocotyl and epicotyl in the germplasm of soybean. Studies subsequent to this were carried out in order to estimate the genetic variability of the lengths of hypocotyl and epicotyl and for a better understanding of these characteristics (Matsuo et al., 2012). These authors used a digital caliper to measure the lengths of hypocotyl and epicotyl in seedlings of soybean. This device has mobile parts and the reading is presented digitally. With this, the use of this device demands a training and precaution in its handling, besides to require a source of electric power such as batteries.

In this context, it is important to study the possibility of using instruments alternative to calipers in the measurement of soybean seedlings, however, with efficiency similar to that offered by digital calipers. The millimeter ruler is a tool of easy handling that does not require a source of energy and has a cost much inferior as compared to calipers. Thus, the aim of this study was to evaluate the performance of millimeter ruler and caliper in the measurement of the lengths of hypocotyl and epicotyl in plants of soybean and to compare the two methods.

MATERIALS AND METHODS

The study was conducted in a greenhouse at the Federal University of Viçosa, Campus of Rio Paranaíba, in 2014 and 2015. The seeds used had random sizes and came from different cultivars of soybean. The seeds were sown at a standardized depth of 3.0 cm in a soil previously prepared and were kept in pots of 3 dm^3. The cultural practices were carried out according to the recommendations.

The plants were evaluated as to the lengths of hypocotyl and epicotyl, by digital caliper and millimeter ruler at the developmental stages V2 and V3 (Fehr and Caviness, 1977). A total of 1.084,0 plants were evaluated at the V2 stage and 1.252,0 at V3 stage, distributed over five planting seasons (June/2014, November/2014, January/2015, March/2015 and April/2015).

The two methods of evaluation were compared in order to assess their performance and agreement. This comparison was performed using the software GENES (Cruz, 2013). For this, the following reliability measures were adopted:

(i) Angular coefficient (β) and coefficient of determination (r^2) of the simple linear regression (model: Y = βX).

where Y is the value obtained with a caliper, β is the angular coefficient and X corresponds to the value obtained with a millimeter ruler. The value of β was tested using the t-test at α≤0.05, considering the hypotheses $H_0: \beta = 1$ and $H_a: \beta \neq 1$.

(ii) Bias (bias =$\beta - 1$), when β was significant by the t-test (α≤0.05).

(iii) Concordance index(d) proposed by Willmott et al. (1985).

where $d = 1 - \frac{\sum_{i=1}^{n}(X_i - Y_i)^2}{\sum_{i=1}^{n}(|Y_i - \overline{X}| + |X_i - \overline{X}|)^2}$ and X_i is the value obtained with a millimeter ruler, Y_i is the value obtained with a caliper and $\overline{X}$ corresponds to the average of values obtained by measuring with a ruler.

(iv) Performance index(c) proposed by Camargo and Sentelhas (1997), where $c = \sqrt{r^2} * d$; and

(v) Average, sample standard deviation and confidence interval (with 95% of confidence), separately for digital caliper and millimeter ruler, for the lengths of hypocotyl and epicotyl.

RESULTS AND DISCUSSION

Table 1 shows the coefficient of angular regression and the coefficient of determination, the bias, the index of concordance and the index of performance, obtained from the comparison between the results from caliper and ruler.

The estimates of the angular coefficient of regression for the length of hypocotyl at stages V2 (January/2014) and V3 (January/2014) and for the length of epicotyl at stages V2 (June/2014 and November/2014) and V3 (November/2014 and January/2015) had a β non-significant by the t-test (α>0.05). This indicates that the trend line of the ruler follows the values obtained from caliper in a ratio 1:1. β was significant (α≤0.05) for the other planting seasons indicating that the value of the estimate is different from 1. The estimates of determination coefficient were higher than 0.97, which indicates that the models estimated explained well the variables.

The bias for the length of hypocotyl was positive in the evaluations performed at V2 and V3 stages in four planting seasons (June/2014, November/2014, March/2015, and April/2015). This indicates that the millimeter ruler overestimated the values as compared to the measures obtained from the caliper. The maximum overestimation (0.0508) was observed in March/2015 in stage V2. A positive value of bias was observed for the length of epicotyl in stages V2 (January/2015 and March/2015) and V3 (June/2014 and March/2015). The millimeter also overestimated the values as compared to the digital caliper. However, the highest overestimation found had a low magnitude (0.0147 mm), corresponding to the planting season in March/2015 and to the V3 stage. The negative bias (-0.0074) observed in the stage V2 (April/2015) indicates that the millimeter ruler is underestimating the measure as compared to the digital

Table 1. Values of the regression angular coefficient[1] (β), determination coefficient (r^2), concordance index (d) and performance index[2] (c), for the comparisons between measures of hypocotyl and epicotyl from seedlings of soybean obtained with caliper and millimeter ruler in different stages of development and seasons in a greenhouse.

Season	*β*	r^2	*Bies*	*d*	*c*
			Hypocotyl length		
Developmental stage V2					
Jun./2014	1.0250**	0.99	0.0250	0.9997	0.95 - Optmal
Nov./2014	1.0170*	0.98	0.0170	0.9994	0.93 - Optmal
Jan./2015	0.9893^{ns}	0.98	-	0.9993	0.90 - Optmal
Mar./2015	1.0508**	0.99	0.0508	0.9991	0.96 - Optmal
Apr./2015	1.0151**	0.99	0.0151	0.9998	0.95 - Optmal
Developmental stage V3					
Jun./2014	1.0283**	0.98	0.0283	0.9994	0.91 - Optmal
Nov./2014	1.0326**	0.99	0.0326	0.9996	0.95 - Optmal
Jan./2015	1.0017^{ns}	0.99	-	0.9991	0.94 - Optmal
Mar./2015	1.0363**	0.99	0.0363	0.9996	0.97 - Optmal
Apr./2015	1.0113**	0.99	0.0113	0.9999	0.98 - Optmal
			Epicotyl length		
Developmental stage V2					
Jun./2014	1.0063^{ns}	0.99	-	0.9999	0.98 - Optmal
Nov./2014	1.0043^{ns}	0.99	-	0.9999	0.99 - Optmal
Jan./2015	1.0063**	0.99	0.0063	0.9999	0.99 - Optmal
Mar./2015	1.0091**	0.99	0.0091	0.9998	0.99 - Optmal
Apr./2015	0.9926*	0.99	-0.0074	0.9999	0.98 - Optmal
Developmental stage V3					
Jun./2014	1.0111*	0.99	0.0111	0.9999	0.98 - Optmal
Nov./2014	1.0011^{ns}	0.99	-	0.9999	0.99 - Optmal
Jan./2015	0.9921^{ns}	0.99	-	0.9990	0.95 - Optmal
Mar./2015	1.0147**	0.99	0.0147	0.9999	0.99 - Optmal
Apr./2015	1.0028^{ns}	0.99	-	0.9999	0.98 - Optmal

[1] $H_0: \beta = 1$ and $H_a: \beta \neq 1$; [1] **, *Significant at 1 and 5% probability and [ns]non-significant by the t-test, respectively; [2]Performance classification according to Camargo and Sentelhas (1997).

caliper.

The estimates of the concordance index were higher than 0.9999. According to Willmott et al. (1985), this index has a variation from 0, when there is no concordance to 1, when there is complete concordance. The estimates for this index indicate a high concordance for the length of the two characteristics in the two stages and the five growing seasons.

The values of the index of performance were higher than or equal to 0.90 and according to Camargo and Sentelhas (1997), it could be classified as optimal. Thus, the values obtained with a millimeter ruler agreed with those obtained from the digital caliper demonstrating that the millimeter ruler is recommended as an alternative to the digital caliper.

The estimates of the sample standard deviation for the lengths of hypocotyl and epicotyl are shown in Table 2. The greater difference between the estimate obtained for the millimeter ruler and the digital caliper was 0.26 mm and corresponded to the length of epicotyl at stages V2 (January/2015) and V3 (March/2015). According to Gomes (2009), the larger the value of a sample standard deviation, the greater is its variation. With this, it can be observed that the magnitudes of the sample standard deviation were practically similar to the peer-to-peer analysis for the values obtained from caliper and ruler in the different developmental stages and planting seasons.

As shown in Table 2, the confidence interval for the caliper comprises the averages obtained from the ruler and vice versa. This was also observed for other intervals estimated and suggests a similar precision between the two methods. According to Montgomery and Runger

Table 2. Estimates of average, standard deviation (SD) and confidence interval (CI) for the lengths of hypocotyl and epicotyl of seedlings of soybean obtained with a caliper and a millimeter ruler in different stages of development and seasons in a greenhouse.

Season	Average	ED	CI (95%)		Average	ED	CI (95%)	
			IL	SL			IL	SL
					Hypocotyl length			
Developmental stage V2								
Jun./2014	16.95	5.16	16.08	17.78	16.48	4.98	15.64	17.28
Nov./2014	22.08	6.48	21.12	22.99	21.51	6.57	20.54	22.43
Jan./2015	22.14	6.03	21.26	22.99	22.16	6.28	21.24	23.04
Mar./2015	21.94	6.32	21.24	22.60	20.75	6.23	20.06	21.40
Abr./2015	22.76	4.95	21.93	23.54	22.37	4.88	21.56	23.14
Developmental stage V3								
Jun./2014	16.93	4.98	16.09	17.73	16.35	4.82	15.53	17.12
Nov./2014	22.87	6.46	21.92	23.78	22.00	6.48	21.05	22.91
Jan./2015	22.46	6.36	21.77	23.12	22.33	6.31	21.64	22.98
Mar./2015	21.57	5.39	20.97	22.14	20.75	5.29	20.17	21.31
Abr./2015	22.28	4.64	21.51	23.02	22.02	4.58	21.26	22.74
					Epicotyl length			
Developmental stage V2								
Jun./2014	22.56	6.16	21.51	23.54	22.42	6.01	21.40	23.38
Nov./2014	33.06	9.07	31.72	34.33	32.87	9.10	31.54	34.15
Jan./2015	48.79	14.44	46.66	50.81	48.50	14.18	46.42	50.49
Mar./2015	38.01	11.53	36.73	39.22	37.59	11.50	36.32	38.80
Abr./2015	32.44	5.91	31.45	33.37	32.67	5.85	31.69	33.59
Developmental stage V3								
Jun./2014	22.86	6.11	21.82	23.83	22.53	6.16	21.49	23.52
Nov./2014	32.81	9.02	31.48	34.07	32.72	9.07	31.39	33.99
Jan./2015	42.70	14.04	41.17	44.15	42.93	13.80	41.43	44.36
Mar./2015	37.56	11.24	36.31	38.74	36.86	11.50	35.59	38.08
Abr./2015	31.44	5.28	30.56	32.27	31.29	5.45	30.39	32.16

(2012), the confidence interval involves the true value of μ with a confidence of $100(1-\alpha)$ being a precise measure of the estimation of average. According to Gomes (2009), it can affirmed that 100 (1-α) is the fiducial probability that the true value is within a given interval of confidence.

A series of studies report the utilization of the statistics used in this work for comparing the different estimates of reference evapotranspiration (Tagliaferre et al., 2012; Oliveira et al., 2008). In soybean, these statistics were used to validate models of simulation for this crop in Santiago, RG, Brazil (Gomes et al., 2014) and to compare the estimate of leaf area of old and modern varieties by a non-destructive method (Richter et al., 2014).

The statistical indicators showed excellent relation and concordance between the values obtained with a millimeter ruler and those obtained with a digital caliper. The decision of what tool might be used in the evaluation of experiments must consider factors such as the experience, ability and visual acuity of the evaluator, brightness in the place of evaluations as well as the availability of financial resources to acquire the types of equipment.

The results obtained in this work indicate that the lengths of hypocotyl and epicotyl might be used in the tests of distinctiveness, homogeneity and stability of cultivars of soybean. Besides these alternative descriptors, the implementation of computer algorithms based on non-linear approaches represents a modern alternative in the area of genetics and breeding and has provided results with higher precision as compared to

traditional statistical approaches (Arqub and Abo-Hammour, 2014; Arqub et al., 2017).

Conclusion

The millimeter ruler had optimum performance in the measurement of the lengths of hypocotyl and epicotyl in plants of soybean in stages V2 and V3. The millimeter ruler consisted of an alternative tool to caliper in most of the planting seasons.

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