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Emergence and early growth of *Gongronema latifolia* in relation to sowing depth and date

Agbo C.U.
Department of Crop Science, University of Nigeria, Nsukka 410001, Nigeria,
Correspondence email: c_agbogenetics@yahoo.com ; christian.agbo@unn.edu.ng
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ABSTRACT

Objective: This research was designed to determine the optimum sowing depth for *G. latifolia* at two planting seasons in 2007.

Methodology and results: Treatments comprised of 9 sowing depths including 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 cm. The experiment was arranged in a completely randomized design with 10 replications under 65 % shading. The first and second sowing dates were on 5 May and 5 September 2009, respectively. The results showed that sowing depths of 0-1.5 cm gave significantly ($p<0.05$) higher level of emergence, ranging from 93 to 83 % respectively, than in deeper sowing, for the first planting period. In the same way, the coefficient of velocity of emergence (CVE %) for 0 and 0.5 cm sowing depth were the same and significantly ($p=0.05$) higher than the CVE of seedlings emerging from depths beyond 1.5 cm during the former sowing period. The second sowing period led to a low emergence rate of 53 % to 5 % at the sowing depths of 0 to 2.0 cm, respectively. Seeds sown at 2.0 cm depth gave significantly ($p=0.05$) higher hypocotyl lengths in both sowing dates. The hypocotyl diameter was increasing with increasing depth up to 1.5 cm and it decreased thereafter.

Conclusion and application of finding: The findings revealed that *G. latifolia* must be sowed at a depth of 0 to 1.5 cm beyond this depth, emerging rate and CVE will be poorer. The significant influence of sowing date and depth gives room for choice of a better option. In this vein, sowing at the first date and at shallower depths of 0-1.5 cm will be preferred as it led, in addition to the highest emergence rate, to faster growing seedlings with longer internodes reaching transplantable height and size earlier than those sown deeper and latter. This leads to rapid and adequate field establishment

Keywords: Sowing depth; *G. latifolia*; hypocotyls growth; seedling vigor; time of sowing

INTRODUCTION

The establishment of an adequate crop stand is essential to reach an optimal yield. To insure such a good crop stand, quick and high level of emergence are important parameters allowing a good competition against weeds and a rapid growth (Sharma and Anderson, 2003). This explains that rapidity and uniformity of seedling emergence have been suggested as criteria for measuring seedling vigor during emergence (AOSA, 1976; Sharma and Anderson, 2003). Seeds require adequate moisture, oxygen and temperature to germinate. Temperature is among the most influential environmental factors for germination and establishment of crop species. Larsen and Bibby (2005) showed that low soil temperature resulted in relatively slower germination of Kentucky grasses and subsequently poor establishment and poorer competitive ability of this species. Germination of Sunflower was reported to be significantly lower when it occurred at low temperature (21 ^{0}C) than at the temperature of 25 ^{0}C (13). Furthermore, Hatfield and Egli (1974) reported that rate of hypocotyl elongation in soybeans increased with increasing soil temperature until a maximum of 30 ^{0}C. In addition, some soybean cultivars have an inhibition of hypocotyl elongation at 25 ^{0}C (Fehr *et al.*1973; Burris and Knittle, 1975; Samimy and LaMottle, 1976). Rapidity and emergence percentage have also been shown to be influenced by sowing depth in Wheat (*Triticum aestivum* Hordeum) (Sharma and Anderson, 2003). Sowing seeds at an optimum depth is an important aspect to obtain higher emergence and good quality seedlings. Problems encountered if sowing is deeper than necessary includes: delayed and reduced emergence, poor and non-uniform stands as well as reduced competitiveness of the crop (Jun *et al*. 2002). Lower and slower germination have been reported in many crop species sowed at a depth deeper than optimum (Sharma and Anderson, 2003; Abul-Faith and Bazzaz, 2006). It has also been shown that sowing seeds at an optimum depth with particular reference to the size of the seeds gives higher germination and better quality seedlings due to an adequate hypocotyl length (Sharma and Anderson, 2003; Abul-Faith and Bazzaz, 2006). The hypocotyl of crops has been shown to be a critical organ affecting emergence in crop species because it elevates the cotyledons and epicotyl from the planting depth to the soil surface (Knittle and Burris, 1979). Hypocotyl growth of crop species has been shown to experience reduced elongation from the soil surface to the cotyledons with increasing sowing depth (Wanjura and Buxton, 1972). Hypocotyl growth has been well studied in some crops, such as soybean (*Glycine max* L.) (Knittle and Burris, 1979) and not in others, such as *Gongronema latifolia* Benth.

G. latifolia is native from West Africa. It is an Asclepediaceae (Nielson, 1965). It is widely used in Nigeria and other Sub Saharan Africa as a forest Vegetable and for treatment of diverse tropical diseases including, among others, typhoid, worm infections, diabetes mellitus, and malaria (Agbo *et al*. 2005). It has been reported to contain high level of protein 62.5 % and 27.7 % (Okafor, 1997; Eleyinmi, 2007, respectively). The wide variability in the protein levels was attributed to genomic differences (Agbo *et al*. 2009). Production of the plant in regular culture is recently gaining attention and the plant can be propagated by stem cuttings as well as by seeds (Agbo and Obi, 2006; 2007). Seeds of this species are relatively small ranging from 7 mm to 13 mm in length and 5 mm to 8 mm in diameter (thickest part).This research was designed to test emergence rates and early seedling growth of *G. latifolia* sowed at different soil depths. The objective of this research was to determine the optimum sowing depth for *G. latifolia* seeds at two sowing seasons.

MATERIAL AND METHODS

The follicles containing the seeds used for the study were picked on March 20, 2007 at the yellow stage from a field of *G. latifolia* clone (ENS-08-MBU) established in the research farm of the Department of Crop Science, University of Nigeria, Nsukka, Nigeria. The Yellow stage has been identified as the optimum time to pick up follicles for this crop species as seeds are preserved from loss by dispersal and this stage also gives higher germination rates (Agbo and Obi, 2008). The follicles containing the seeds were stored in a laboratory bench of the Department of Crop Science. The average temperature of the laboratory was 29.8 ^{o}C. Nursery beds, each measuring 120 cm x 30 cm, were prepared from a previously plowed and harrowed field. Well decomposed poultry manure was applied on the beds at the rate of 20 tons/ha. The beds were spaced 50 cm x 50 cm and the treatments were performed in completely randomized design (CRD) with ten replications. The experimental site was under 65 % shading to create a homogeneous environment for the treatment. A strong and straight stick of 30 mm thick was calibrated with depth levels of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 cm for sowing the seeds. Two dates of sowing seeds were used, 5 May 2007 and 5 September 2007. The two dates were chosen to test their effect on emergence of the crop under field conditions as seeds of *G. latifolia* sown at varying dates in the laboratory have been shown to exhibit different levels of germination (Agbo and Obi, 2008). Fifty seeds were sown on each bed according to the assigned depth. The seeds were sown on 10 rows of 5 seeds each spaced at 11 cm x 6 cm. The beds were watered regularly to field capacity with 5 litres of water throughout the research period. Emergence of seedlings, recorded daily, was noted as the first appearance of any portion of the seedling above the soil surface. The measured plants were the fifteen plants from the fifth, sixth and seventh rows on each bed. Hypocotyl diameter and length were measured at full cotyledon extension stage. Hypocotyl length of sampled seedlings was measured with a 30 cm metric ruler. The length is the distance from the seed to the cotyledonary leaf. The hypocotyl diameter of the sampled seedlings was measured with Micrometer screw gauge at 1 cm above the soil surface. The internodes lengths of the seedlings were measured at the different leaf stages. Also, days to the attainment of the different leaf stages as well as seedling height at such different leaf stages (1-6) was recorded from the fifteen plants sampled for measurements. Furthermore, the following traits were calculated from the emergence records: percentage of emergence and rapidity of emergence which was estimated by a Coefficient of velocity of emergence (C.V.E.) after Kotowski (1978)

$$\text{C.V.E} = 100 \times \frac{A_1 + A_2 + \ldots + A_x}{A_1T_1 + A_2T_2 + \ldots + A_xT_x}$$

Where:

A = number of seedlings emerging and T =number of days after sowing.

The seed moisture content was determined according to the procedure set by International seed testing Association (ISTA, 2008). The soil temperature and daily rainfall was measured using Fisher (USA) thermometer and rain gauge, respectively. The soil temperature was measured at the different sowing depth. The emergence and other traits data collected were analyzed in a completely randomized design using computer software, Genstat Discovery edition 2.0 (2003). Combined analysis of variance for the two sowing dates was performed on the emergence and early growth parameters of the seedlings. A mixed model of random sowing dates and fixed treatment was used as described by McIntosh (1983). Least significant difference (LSD), as outlined by Obi (2002), was used to compare the means.

RESULTS

The moisture contents of the seeds, soil temperature at the different depths and daily rainfall recorded at the two sowing dates are shown in Table 1. Moisture contents of the seeds as well as rainfall (12.95 versus 10.95 mm/day) were higher during the first period. On the other hand, temperature varied with sowing depths and sowing date. The temperature on the soil surface was 23.3 ^{o}C and 29.2 ^{o}C at morning and evening periods during the first period. The temperature was significantly ($p<=0.05$) reduced with a sowing depth greater than 1.50 cm. The same trend was observed during the second sowing date. But, during the second date, the temperature was 1 ^{o}C lower at each measurement depth.

Table 1: Sowing depth, temperature, moisture and rainfall data during the two periods, in 2007

Sowing depth (cm)	**Temperature ^{O}C**			
	5 May 2007		**5 September 2007**	
	9.00hrs	16.00hrs	9.00hrs	16.00hrs
0.00	23.3	29.2	22.0	27.9
0.50	23.2	29.2	22.0	27.8
1.00	23.1	29.0	21.8	27.8
1.50	23.0	29.0	21.7	27.6
2.00	22.9	28.6	21.6	27.3
2.50	22.8	28.3	21.6	27.2
3.00	22.8	28.3	21.4	27.2
3.50	22.6	28.0	21.3	27.0
4.00	22.5	27.6	21.0	26.5
LSD $_{(P=0.05)}$	0.22	0.59	0.11	0.13
Moisture content of the seeds (%)	13.0		11.5	
Rainfall (mm/per day)	12.95		10.95	

The mean squares of percentage emergence, velocity of emergence, hypocotyl length and diameter and leaf stages of the test crop are presented in Table 2. It showed that the sowing date had a significantly ($p<0.01$) higher emergence rate and growth of the seedlings. The sowing depths also showed significantly ($p<0.01$) higher emergence capacities and early growth dynamic of the seedlings.

Table 2: (a) Results (Mean square and p values) of the two ways analysis of variance performed on the emergence capacity parameters, linear growth curve of *G. latifolia* evaluated for two sowing dates

Source of variation	d.f.	(a) Emergence capacities				(b) Comparison of Linear Growth Portion			
		PE	CVE	HPL	HPD	Cotyl l.	LVS 1	LVS 2	LVS 3
Sowing date (SD)	1	23240**	65.36**	27.07**	0.20**	25.02**	35.21**	49.15**	71.46**
Depth of sowing (DP)	4	850.8**	6.12**	0.94**	0.08**	1.09**	1.80**	2.60**	2.79**
DP XSD	4	149.2^{ns}	0.57*	0.04^{ns}	0.00^{ns}	0.03 ^{ns}	0.08^{ns}	0.23 ^{ns}	0.29 ^{ns}
Error	20	114.2	0.19	0.04	0.02	0.09	0.13	0.32	0.69

Where PE= percentage emergence, CVE= coefficient of velocity of emergence, HPL= hypocotyl length, HPD= hypocotyl diameter, Cotyl l= cotyledonary leaf stage, LVS 1-3= first to third leaf stages, respectively.
** = highly significant level at $p< 0.01$, * = significant level at $p< 0.05$, ns= non-significant effect.

Table 3: Percentage emergence and coefficient of velocity of emergence (C.V.E%) of *G. latifolia* seeds sowed at different depths and dates in 2007

Depth (cm)	sowed on 5 May, 2007		sowed on 5 September, 2007	
	Emergence %	C.V.E. %	Emergence %	C.V.E %
0.00	93.30	9.69	53.30	6.31
0.50	92.00	8.77	40.00	5.80
1.00	90.00	7.02	30.70	5.30
1.50	83.30	7.00	20.00	4.10
2.00	66.75	6.50	5.00	3.20
2.50	43.30	6.32	0.00*	0.00
3.00	30.00	5.82	0.00*	0.00
3.50	20.00	5.33	0.00*	0.0
4.00	3.33	1.67	0.00*	0.00
$LSD_{(P=0.05)}$	19.99	1.75	13.45	0.54

*=Seeds germinated but did not emerge.

The result in Table 3 showed that the rate of emergence and the velocity of emergence were reduced while increasing sowing depth. Seeds emerged at all the depths during the first period while they only emerged at the second sowing date if they were sown within 2 cm depth. The first sowing gave higher emergence and velocity of emergence rates when compared with the latter sowing date at all the depths. Sowing depths of 0-1.5 cm in the first date, with emergence ranged between 93 and 83 %, gave significantly ($p<=0.05$) higher emergence rate than deeper sowing depths. The coefficient of velocity of emergence (CVE %), for sowing depths of 0 and 0.5 cm were not significantly different but were significantly ($p<0.05$) higher than the CVE of other sowing depths at the first sowing date. The second sowing date showed a poor emergence rate evolving from 53 % to 5 % with sowing depths evolving from 0 to 2.0 cm. The CVE values were correspondingly quite low when compared with the values obtained during the first sowing date.

Table 4: Hypocotyl attributes of *G. latifolia* at emergence following the two sowing dates

Sowing Depth (cm)	Sowed on 5 May, 2007		Sowed on 5 September, 2007	
	Hypocotyl length (cm)	Hypocotyl diameter (mm)	Hypocotyl length (cm)	Hypocotyl diameter (mm)
0.00	3.50	1.03	1.64	0.90
0.50	3.73	1.07	1.49	0.90
1.00	3.76	1.18	1.93	1.00
1.50	4.07	1.32	2.27	1.20
2.00	4.43	1.22	2.77	1.00
2.50	4.13	1.20	0.00	0.00
3.00	3.97	1.00	0.00	0.00
3.50	4.40	0.97	0.00	0.00
4.00	4.27	0.27	0.00	0.00
LSD(p=0.05)	0.08	0.33	0.02	0.17

Hypocotyl growth (length and diameter) of emerging seedlings are presented in Table 4. The Table shows that seeds emerging from 2.0 cm depths had significantly longer hypocotyl in both sowing dates. On the other hand, seeds sown at the soil surface and 0.5 cm depth gave significantly shorter hypocotyl at the first and second sowing dates respectively. There was reduction in apparent hypocotyl length (hypocotyl length above soil surface) with increasing sowing depths as well as with latter sowing of seeds. For example, seeds sown at 0-0.5 cm depths in the first sowing date had an apparent hypocotyl length of 3.50 cm and 3.23 cm, respectively while seeds sown at 4 cm depths had a significantly lower apparent hypocotyl length of 0.27 cm. By the second sowing date, seeds placed on the soil surface and 2.0 cm depth had apparent hypocotyl lengths of 1.64 cm and 0.70 cm, respectively. The hypocotyl diameter increased with increasing sowing depth up to 1.5 cm before it decreased at deeper sowing depths. So, hypocotyl diameter of seeds sown at 1.5 cm depth in both sowing dates was significantly higher than those sown at the depths of 3.0 cm and below. In most cases, internodes lengths of the seedlings that emerged from 0-1.5 cm depth were significantly ($p< 0.05$) higher than those that emerged from lower depths in the different leaf stages in both sowing dates (Table 5).

Table 5: Internodes lengths (cm) of the *G. latifolia* seedlings that emerged from different depths at the same sowing periods

	Sowed on 5th May 2007						Sowed on 5th September, 2007					
Sowing Depth (cm)	Leaf Stages						Leaf Stages					
	LS_1	LS_2	LS_3	LS_4	LS_5	LS_6	LS_1	LS_2	LS_3	LS_4	LS_5	LS_6
0.00	0.76	1.14	1.96	5.54	6.19	5.74	0.46	0.80	1.20	2.70	2.42	2.58
0.50	0.90	1.03	1.75	5.76	5.95	5.94	0.50	0.10	1.30	2.20	2.23	3.22
1.00	0.83	1.13	1.80	5.70	5.80	5.44	0.30	0.87	1.87	2.76	1.83	2.80
1.50	0.43	1.10	1.54	5.26	5.50	5.05	0.99	0.60	1.24	2.36	2.20	2.47
2.00	0.60	1.13	1.73	5.10	5.40	3.82	0.20	0.43	1.40	1.37	2.46	2.14
2.50	0.46	1.80	1.20	4.37	3.93	2.40	0.00	0.00	0.00	0.00	0.00	0.00
3.00	0.50	1.10	1.30	3.27	2.89	2.87	0.00	0.00	0.00	0.00	0.00	0.00
3.50	0.30	0.87	0.87	2.76	2.24	1.32	0.00	0.00	0.00	0.00	0.00	0.00
4.00	0.17	0.07	0.20	2.06	0.50	1.53	0.00	0.00	0.00	0.00	0.00	0.00
LSD ($P<0.05$)	0.17	0.13	0.16	0.14	0.14	0.15	0.16	0.16	1.17	0.18	0.18	1.18

Where: LS_{1-6}= Leaf stages 1-6

Seedlings that emerged from 4 cm depth had significantly shorter internodes and lower plant heights in the first sowing date. The internodes lengths in each sowing depth in both sowing dates increased with increasing leaf stage. There was almost three-fold increase in internodes lengths at the fourth leaf stage when compared to the lengths at the third leaf stage.

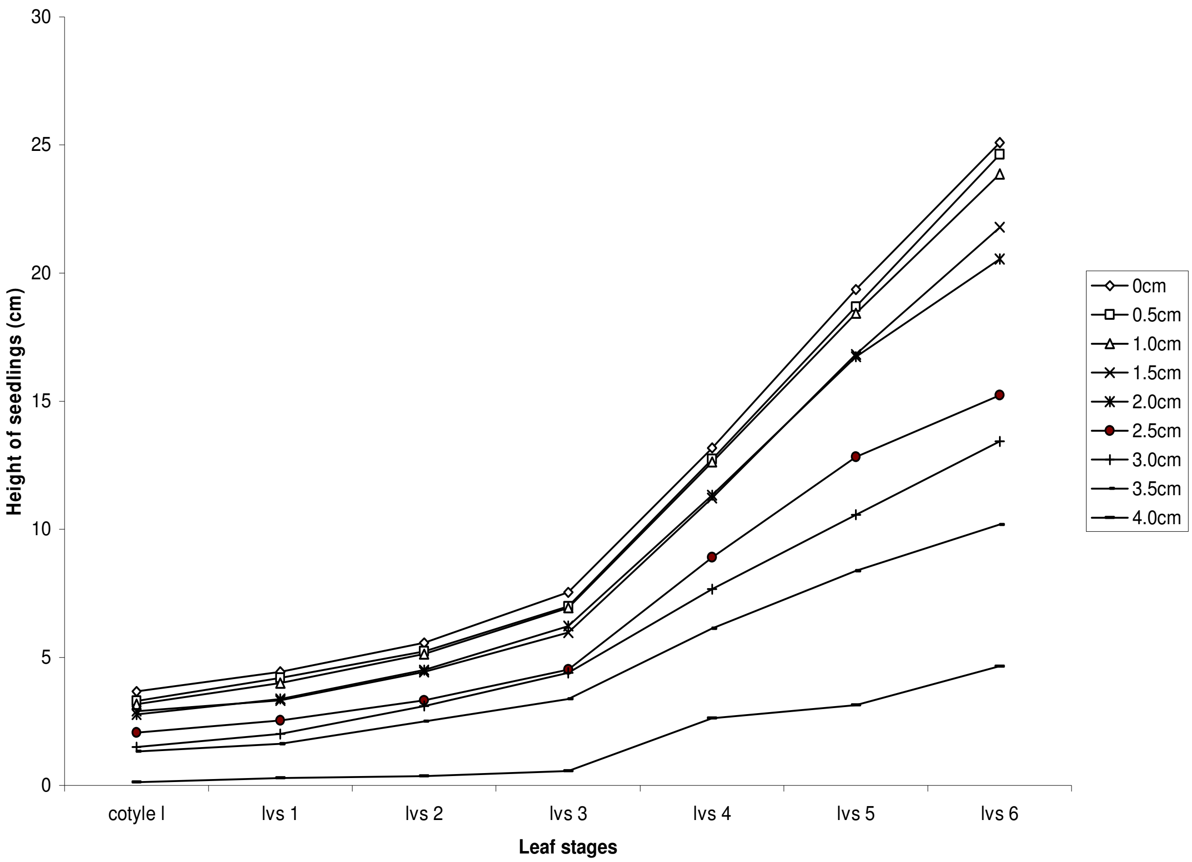

Fig.1: Height of seedlings at different leaf stages of seeds sown at different soil depths (first planting)
Where: cotyl l=cotyledon leaf; lvs 1-6= first to sixth leaf stages, respectively

Evolution of seedling height during the first period (Fig. 1) showed that the effect of sowing depth on the emerged seedlings formed a basal difference in the plant heights of the seedlings. There was a sharp increase in internodes lengths of the seedlings from the third leaf stage resulting in faster plant height growth and longer internodes at all the depths. Seedlings that emerged between 0- 2.0 cm exhibited significantly higher plant height growth from the fourth leaf stage. Conversely, seedlings that emerged from 3.0 – 4.0 cm depths had significantly smaller plant heights and could not reach 15 cm at the sixth leaf stage whereas seedlings sown at shallower depths reached 25 cm and above in height. There were reduced plant heights of the seedlings in the different leaf stages in the second sowing date when compared to the corresponding development stages at the first sowing date (Fig. 2).

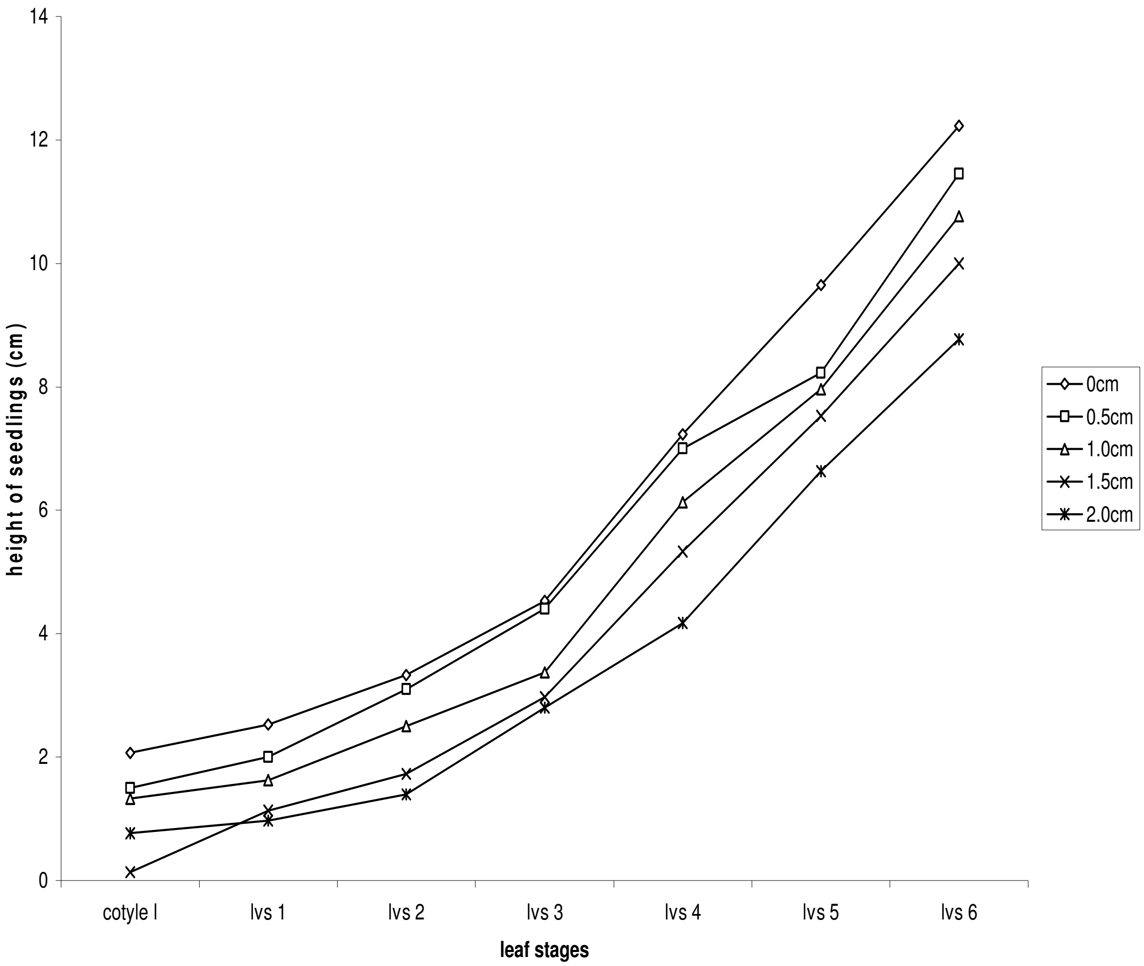

Fig.2: Height of seedlings at different leaf stages of seeds sown at different soil depths (Second planting)
Where: cotyl l=cotyledon leaf; lvs 1-6= first to sixth leaf stages, respectively

DISCUSSION

The significant reduction in the rate as well as in the velocity of emergence with increasing soil depths indicated that this parameter has to be taken into account during the establishment phase of this crop species. The sowing depth of 1.5cm with emergence rate of 83 % at the first sowing date could be regarded as the maximum tolerable sowing depth in order to insure an effective initial establishment of the crop. Hence, the significantly higher CVE observed following the 0- 0.5 cm sowing depth illustrates the higher efficiency of emergence percentage obtained at the shallower depths. Rapidity and emergence percentage have been shown to be influenced by seeding depth in other crop species (Wanjura and Buxton, 1972; Jun *et al*. 2002; Sharma and Anderson 2003). The data also showed that depth of sowing is a significant factor affecting hypocotyl growth dynamic of *G. latifolia* seedlings in the field. The difference in hypocotyl lengths between the different sowing depths with an increase while sowing depth increased in most cases could be attributed to developmental characteristics that are likely to enhance emergence from soil depths to a point. The significantly longer hypocotyl of seeds sown at 2.0 cm is suggestive of the limiting point beyond which the hypocotyl elongation began to reduce. The result of the hypocotyl elongation agrees with reports of other researchers in soybean (Knittle and Burris, 1979) and cotton (Wanjura and Buxton, 1972). Furthermore, the result of smaller hypocotyl diameter for seeds placed on top of the soil (0 cm) than those sown within the depths of 0.5 to 2.0 cm depth at both sowing dates was suggestive of the effects of soil depths in increasing the hypocotyl diameter up to certain soil depths. Such soil depth effects have been reported by other researchers (Jun *et al*. 2002; Eleyinmi, 2007; ISTA, 2008). The elongation and increase in diameter of the hypocotyl of the species from the forgoing seems to be dependent on the resistance or otherwise that the seedling encounters as it emerges from soil surface. The influence of depth of sowing seeds on hypocotyl growth and subsequent emergence rate has a lot of implications in a seedbed situation in different soil textural classes. Direct establishment of the crop species in the field in a predominantly clayey soil with crusting capability has to be done with relatively shallow sowing of not more than 2.0 cm. The different sowing depths resulted in significant differences in soil temperature. Such soil constituents as temperature and moisture have been shown to significantly influence seeds germination (Abul-Faith and Bazzaz, 2006). This implies that the deviation from rapid and high rate of germination observed during the latter sowing period, with seeds from the same seed lot , would be attributable not only to seeds conservation delay but also to differences in soil temperature and moisture during this second period. Indeed, soil temperature was lower even at shallower depths during the second sowing. The second date of sowing was a period when rainfall was at its peak which led to reduced temperature of the soil. The effect of such environmental factor such as temperature that is highly needed for emergence of seedlings but found reduced in this study could be further investigated. This is necessary as non germination of seeds deeply sowed, in correlation, with; reduced soil temperature has been reported by other researchers (Abul-Faith and Bazzaz, 2006).

The significantly higher internodes lengths in seedlings that emerged from 0-1.5 cm depths over seedlings that emerged from higher depths showed the significant influence of soil environments (sowing depths) on the early growth of the seedlings. The shorter internodes of seedlings that emerged from 4.0 cm depths hindered the growth of such seedlings. Longer internodes for nursery seedlings could result in plants that quickly reach transplantable size. The use of height in deciding time for transplanting of seedlings has been reported in tobacco (Onwueme and Sinha, 1999). The results of this study showed that seedlings that emerged from shallower depths (0-1.5 cm) would be selected for transplanting earlier than seedlings from lower depths.

CONCLUSION

The significant influence of sowing date and depth gives room for choice of a better option. In this vein, sowing at the first date and at shallower depths of 0-1.5 cm will be preferred as it led, in addition to the highest emergence rate, to faster growing seedlings with longer internodes reaching transplantable height and size earlier than those sown deeper and latter. This leads to rapid and adequate field establishment.

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