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Research Article

Effects of Nut Cracking and Gibberellic Acid on Emergence and Early Growth of Shea Seedlings

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Abstract

Poor germination, delayed plumule emergence and slow growth associated with the shea tree are major obstacles hindering complete domestication and commercial cultivation of the tree. Studies to develop techniques that improve germination, reduce time to emergence and promote faster seedling growth remain an important research activity on the tree crop (CRIG). As part of these studies, a nursery experiment was conducted to investigate the effects of nut cracking and gibberellic acid concentration (GA3) on plumule emergence and growth of shea seedlings. The lay out was a split plot arranged in randomized complete block design with physical nut handling (cracked and uncracked) and GA3 concentration (1000, 2000, 3000, 4000 and 5000 ppm) as main and subplot factors, respectively. Data on final emergence was taken 4 Months After Sowing (MAS) while those of seedling height, stem diameter and leaf number per plant were taken at 8, 16 and 24 MAS. Data on effects of treatments on dry matter accumulation by seedlings were taken at 24 MAS. The results showed a positive relationship between seedling height and pre-sowing emersion in GA3 up to 16 MAS, irrespective of nut handling. In the long term (24 MAS) seedlings from cracked and uncracked nuts soaked in the 4,000 and 3,000 ppm GA3 solutions, respectively, led to significant stem diameter gain. The implications of these observations on shea seedlings development are discussed.

Key words: Shea tree, dormancy, oil seed, gibberellic acid, growth

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Shea (*Vitellaria paradoxa* C.F. Gaertn) is an indigenous oil seed tree found in the interior savanna of Ghana. Products from the tree play vital roles in the socio-cultural economy of residents of this zone (Abbiw, 1990). Agronomic recommendations for establishment and management of the shea tree however lag behind crops of like status as a result of limited research especially in the area of improved germination, emergence and seedling growth (Awoleye, 1995). Reports of several nursery studies involving shea seeds have shown variable percent germination; 90% (Booth and Wickens, 1988), 10-90% (Yidana, 1994), 60-80% (Ugese *et al*., 2010), 30-65% (Asante *et al*., 2012) and 20-80% (Iroko *et al*., 2013). In all of these studies, final emergence was lower than the values obtained for germination. Added to the poor seed germination and lower percent plumule emergence is a slow growing plumule that takes about 51-79 days to emerge (Ugese *et al*., 2010). The poor germination and slow plumule growth in shea has been attributed, respectively to seed dormancy and rapid fall in viability (Salle *et al*., 1991; Imoro *et al*., 2012; Asante *et al*., 2012) and slow substrate mobilization (Ugese *et al*., 2010). Any pre-sowing treatment that can reduce loss in viability and facilitate substrate mobilization should potentially increase the germination and emergence percentages as well as reduce the time to plumule emergence and enhance faster seedling development.

Seed dormancy is regarded as the failure of an intact viable seed to complete germination under favourable conditions (Amooaghaie, 2009). The inhibition of germination may originate from the hardness and impermeability of the seed coat, dormancy of the embryo or from both. Germination studies of several genera of the Leguminosae family showed that the presence of an impermeable seed coat makes germination difficult (Orozco-Almanza *et al*., 2003). In nature, this may be overcome by drought, temperature changes, passage through animal digestive tracts and bacterial or fungal activity. Artificially, dormant seeds can be stimulated to germinate using treatments that emulate natural conditions or satisfy certain physiological requirements (Rahman *et al*., 2006) through its ability to induce the synthesis of α-amylase (Palmiano and Juliano, 1972). As a result, gibberellic acid is used as a substitute for stratification in horticultural cherry species (Fogle and McCrory, 1960; Nekrasova, 1960) and forest trees (Iroko *et al*., 2013). Several germination stimulation studies with gibberellic acid in other tree crops that resulted in improved seed germination have been reported (Rahman *et al*., 2006; Soyler and Khawar, 2007; Fagge and Manga, 2011; Dhoran and Gudadhe, 2012; Shohani *et al*., 2014). These effects have been attributed to rapid mobilization of stored reserves (Soyler and Khawar, 2007) crucial during seed germination and acceleration of the disappearance of abscisic acid (ABA)-regulated polypeptides abundant in dormant seeds (Nicolas *et al*., 1997).

Apart from promoting germination, gibberellic acid is known to increase shoot and root length in both drought and non-drought situations (Thakare *et al*., 2011; Bahrani and Pourreza, 2012; Afrigan *et al*., 2013). It is also associated with leaf expansion, floral initiation and uniform flowering (Swain and Singh, 2005). In a study under nursery conditions, 50-100 ppm GA3 concentration was found to be effective in most field crops and less recalcitrant tree seeds (Afrigan *et al*., 2013; Thakare *et al*., 2011). However, preliminary work with gibberellic acid solution (100-1,000 ppm) on shea seeds by the Cocoa Research Institute of Ghana (unpublished data) has so far yielded inconclusive results. Since information on effective methods of breaking dormancy in shea seeds and enhancing early growth remain critical to raising economic quantities of seedlings in the nursery. This study therefore sought to investigate the effects of mechanical cracking of shea seeds and treatment with higher GA3 concentrations on final emergence and growth of seedlings in the nursery.

MATERIALS AND METHODS

Experimental design and treatments: The study was laid out in a split-plot arranged in randomized complete block design with physical nut handling as main plot factor and gibberellic acid concentration as subplot factor. Treatments comprise two levels of physical nut handling (C_r-cracked and C_0-uncracked) and six levels of gibberellic acid concentration (0, 1000, 2000, 3000, 4000 and 5000 ppm).

Nut sourcing and sowing: Fifteen shea trees at the Bole substation of Cocoa Research Institute of Ghana (Lat: 09°01'N, Long: 02°29'W and Alt: 305 m ASL) were selected, identified with tags and all fruits found under them collected and disposed to ensure that only freshly dropped nuts were used for the study. Subsequent collections were carried out every morning, depulped and stored in black polythene bags at room temperature until the third day, when sufficient nuts had been accumulated. The nuts were bulked, thoroughly mixed and divided into two groups. Cracks were then gently introduced to the shells of one group using hand pliers, taking care not to damage the seeds. Both cracked and non-cracked nuts were further divided into six equal parts for hormone treatments. Solutions of different GA3 concentrations were then prepared in a laboratory using de-ionized water. Those seeds receiving GA3 treatments were immersed in the respective concentrations for 12 h at 25°C. Seeds not receiving GA3 treatments were immersed in water for the same period at 25°C to eliminate differences attributable to the effect of soaking. Two controls, cracked (C_r) and non-cracked (C_0) nuts without hormone (H_0) treatments were included.

A seed was sown per polybag (30×40 cm^2) for the 50 polybags filled with topsoil per treatment and arranged under 50% shade provided by a shade net. The polybags were thoroughly watered 24 h before and immediately after sowing. Subsequent watering was done every other day.

Data collection and analysis: Data on plumule emergence (final emergence) were taken by simple counts. After final emergence, 20 seedlings were randomly selected and tagged for data collection. Height measurement of all tagged seedlings were taken from the base to the apical region at 8, 16 and 24 Months After Sowing (MAS) with a standard meter rule. Stem diameter of each tagged seedling was measured 5 cm from the soil level at the same interval with digital calipers. The number of leaves present at 8, 16 and 24 MAS on each tagged seedling was also counted. The average height, stem diameter and leaf number per plant was then determined as the sum of the measurements for each treatment divided by the number of seedlings measured. At 24 MAS, 20 plants were harvested per treatment for dry matter analysis. The harvested plants were first separated into leaves, roots and stem and weighed separately. These were placed in well labeled envelopes and oven dried at 80°C until a consistent weight was recorded. The final weights were assumed to the dry weights of the samples. The weights of the 20 plants per treatment were summed and their mean determined as representative weight for each treatment. Pre-analysis of data involved angular transformation of percent final emergence and square root transformation of leaf count. Data was analysed using ANOVA and means separated by the least significant difference at 5%.

RESULTS AND DISCUSSION

Final emergence: There were significant interactions ($p<0.05$) between physical nut handling and GA3 concentration on the final emergence of shea seedlings at 4 MAS (Table 1). Final emergence of shea seedlings was significantly improved ($p<0.05$) by pre-sowing immersion of uncracked nuts in concentrations of GA3 (1,000-2,000 ppm) over the higher concentrations (3,000-5,000 ppm). Emersion of shea seeds in GA3 concentration of 4,000 ppm significantly reduced ($p<0.05$) final emergence compared to the control (transformed data). Final emergence of seedlings after emersion of cracked nuts showed no significant effects of GA3. Emersion of uncracked nuts in GA3 concentrations of 1,000 and 2,000 ppm resulted in the highest emergence of 58.0 and 58.3%, respectively which were similar to the control. These were however, not statistically different from the control in spite of the higher concentrations used and does not compare favourably to the significant positive response observed with lower GA3 concentrations in field crops (Thakare *et al.*, 2011; Afrigan *et al.*, 2013) and other tree crops (Iroko *et al.*, 2013).

Table 1: Effects of physical nut handling and gibberellic acid (GA3) concentration on percent final emergence of shea seedlings (4 MAS)

	Nut handling		
GA3 conc. (ppm)	C_0	C_r	Mean
Control	69.7 (56.6)	69.0 (56.3)	69.3 (56.4)
1,000	72.0 (58.0)	65.7 (54.3)	68.8 (56.2)
2,000	72.3 (58.3)	71.0 (57.5)	71.7 (57.9)
3,000	61.7 (51.7)	67.7 (55.4)	64.7 (53.6)
4,000	62.0 (52.0)	65.7 (54.1)	63.8 (53.0)
5,000	63.3 (52.8)	68.0 (55.6)	65.7 (54.2)
Mean	66.8 (54.9)	67.8 (55.5)	67.3 (55.2)
$LSD_{0.05}$			
GA3		3.70	
Nut handling		ns	
GA3×nut handling		5.23	
CV (%)		5.6	

Values in parenthesis are angular transformations, C_0: Uncracked nuts, C_r: Cracked nuts, MAS: Months after sowing, ppm: Parts per million, Conc.: Concentration, GA3: Gibberellic acid concentration

Table 2: Residual effects of nut handling and GA3 concentration on number of leaves per plant at 8, 16 and 24 months after sowing

	Leaf number per plant		
Treatments	8 MAS	16 MAS	24 MAS
Nut handling			
Cracked	4 (2)	11 (3.32)	9 (3)
Uncracked	4 (2)	11 (3.32)	9 (3)
LSD 0.05	ns	ns	ns
GA3 conc. (ppm)			
Control	3 (1.73)	12 (3.46)	9 (3)
1,000	4 (2)	11 (3.32)	8 (2.83)
2,000	4 (2)	12 (3.46)	9 (3)
3,000	4 (2)	12 (3.46)	10 (3.16)
4,000	4 (2)	11 (3.32)	9 (3)
5,000	4 (2)	11 (3.32)	10 (3.16)
$LSD_{0.05}$	ns	ns	ns
CV (%)	12.8	13.8	23.7

Values in parenthesis are square root transformations, ns: No significant difference between treatment means, MAS: Months after sowing, Conc.: Concentration, ppm: Parts per million, GA3: Gibberellic acid concentration

Table 3: Residual effects of nut handling and GA3 concentration on height and stem diameter of shea seedlings at 8, 16 and 24 months after sowing

	Seedling height (cm)		Stem diameter (mm)			
Treatments	8 MAS	16 MAS	24 MAS	8 MAS	16 MAS	24 MAS
Nut handling						
Cracked (C_r)	5.0	12.5	17.7	1.14	7.75	10.58
Uncracked (C_0)	4.9	13.1	17.5	1.12	8.08	10.72
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns
GA3 conc. (ppm)						
Control (H_0)	3.5	11.2	16.5	0.98	7.61	10.16
1,000 (H_1)	4.1	12.1	16.7	1.11	8.41	10.58
2,000 (H_2)	4.9	12.3	18.4	1.19	7.97	10.73
3,000 (H_3)	5.1	13.1	18.7	1.09	8.56	11.21
4,000 (H_4)	6.0	13.3	17.6	1.31	7.65	10.12
5,000 (H_5)	6.0	13.7	17.7	1.10	7.79	11.11
$LSD_{0.05}$	0.8	1.0	ns	ns	ns	ns
GA3 × Nut handling						
C_0H_0	3.8	11.9	18.0	1.02	7.76	10.18
C_0H_1	4.2	11.6	15.8	1.11	7.77	10.82
C_0H_2	5.6	13.2	18.2	1.18	7.62	10.50
C_0H_3	5.2	12.8	19.8	1.11	8.80	11.50
C_0H_4	6.3	12.3	16.9	1.33	6.64	9.16
C_0H_5	6.8	13.3	17.3	1.08	7.94	11.33
C_rH_0	3.2	10.5	14.9	0.93	7.45	10.13
C_rH_1	4.0	12.6	17.6	1.11	9.05	10.34
C_rH_2	4.2	13.3	18.6	1.19	8.31	10.96
C_rH_3	5.0	13.5	17.7	1.07	8.31	10.92
C_rH_4	5.6	14.3	18.2	1.29	7.68	11.08
C_rH_5	7.3	14.2	18.1	1.12	7.65	10.90
$LSD_{0.05}$	1.1	2.5	4.3	0.2	2.0	2.3
CV (%)	13.7	11.3	14.5	10.6	14.8	12.7

ns: No significant difference between treatment means, MAS: Months after sowing, Conc.: Concentration, ppm: Parts per million, GA3: Gibberellic acid concentration

Despite the high GA3 concentrations used, final emergence (51.7-58.3%) was below the values reported by Booth and Wickins (1988) and within the range reported by Yidana (1994), Ugese *et al.* (2010) and Asante *et al.* (2012) who did not use any hormone to aid germination and Iroko *et al.* (2013) who used H_2SO_4 in pre-sowing treatment of shea seeds. The failure of cracked nuts to respond to GA3 treatment even though the cracks should have potentially facilitated penetration of the hormone was probably due to high hormone imbibition or negative effects of excess water within the seed because of long hours of soaking (Iroko *et al.*, 2013). A seed viability study by Ruyssen (1957) suggested that shea seeds can be stored up to 5 days without significant losses in viability. Since the seeds were sown within 3 days of collection, the poor emergence observed could therefore not be attributed to viability loss due to storage.

Leaf number per plant: Interactive effects of physical nut handling and GA3 concentration did not lead to significant differences (p>0.05) between treatments with regard to leaf number per plant (Table 2). Neither physical nut handling nor GA3 concentration significantly affected (p>0.05) leaf number per plant at 8, 16 and 24 MAS Table 2 although leaf numbers declined by 9.6% (physical nut handling) and 4.8-14.8% (GA3) during the period. The similar leaf numbers obtained suggest that pre-sowing nut cracking and treatment of both cracked and uncracked nuts with GA3 had no residual effects on leaf formation. This agrees with the report of Swain and Singh (2005) suggesting that residual effects of GA3 when used to germinate seeds had no impact on leaf initiation. The subsequent decline in leaf number with seedling age may therefore be attributed to changes in the plant environment such as depletion of nutrient in the medium contained in polybags (Konlan *et al.*, 2014).

Seedling height: Interaction effect between physical nut handling and GA3 concentration with regard to seedling height was significant (p<0.05) at 8, 16 and 24 MAS (Table 3). The heights attained by shea seedlings at 8, 16 and 24 MAS were not significantly affected (p>0.05) by physical nut handling. Seedling height was however, significantly improved (p<0.05) at 8 and 16 MAS by pre-sowing immersion of both cracked and uncracked nuts in GA3 solutions of 4000 and 5000 ppm, respectively. At this early growth stages, increase in seedling height followed GA3 concentration in the order of 0<1,000<2,000<3,000<4,000<5,000 ppm (8 MAS). With the wearing away of the effects of GA3, seedlings recorded similar heights at 24 MAS. Although residual effects of GA3 on stem elongation, seen in both cracked and uncracked nuts were not significant, lower GA3 concentrations were required to achieve such effects in the cracked nuts.

This was probably due to ease of imbibition of the hormone by the cracked nuts since any increase in GA3 concentration which potentially increased uptake led to a corresponding increase in seedling height. Several studies (Afrigan *et al.*, 2013; Bahrani and Pourreza, 2012; Thakare *et al.*, 2011) have associated stem elongation with the use of GA3 to germinate seeds. The response of shea seedlings, which is known to have slow growth rates to such residual effects, therefore opens an important window into shea nursery research and improvement.

Stem diameter: The interaction between physical nut handling and GA3 concentration significantly impacted ($p<0.05$) stem diameter development at 8, 16 and 24 MAS (Table 3). The individual effects of physical nut handling and gibberellic acid concentration on stem diameter of shea seedlings at 8, 16 and 24 MAS were however, not significant ($p>0.05$). Unlike seedling height, data on stem diameter development did not follow a definite pattern at any of the sampling stages. The prolonged interactive effect of uncracked nuts and GA3 on stem diameter increase compared to the observation with regard to height gain suggests that different levels of GA3 were probably required to influence height and stem gain in shea seedlings depending on the way nuts were physically handled. It is therefore worth noting that residual effects of GA3 on seedlings from cracked and uncracked nuts promoted stem elongation and diameter increase, respectively. The different concentrations at which GA3 residual effects on stem diameter became significant for cracked and uncracked nuts probably relates to the ease of imbibition of the hormone and explains why relatively lower concentrations elicited significant residual response in the cracked nuts.

Table 4: Residual effects of physical nut handling and gibberellic acid (GA3) concentration on dry matter accumulated by shea seedlings at 24 months after sowing

	Dry matter per plant (g)			
Treatments	Root	Stem	Leaves	Total
Nut handling				
Cracked (C_r)	48.5	6.9	12.2	67.2
Uncracked (C_0)	43.2	5.1	9.3	57.6
$LSD_{0.05}$	ns	ns	ns	ns
GA3 conc. (ppm)				
Control	57.8	7.1	14.3	79.0
1,000	54.9	6.9	12.6	74.6
2,000	47.7	6.4	10.1	64.3
3,000	49.2	6.2	11.7	67.2
4,000	37.6	4.6	8.8	51.0
5,000	27.7	3.8	6.9	38.5
$LSD_{0.05}$	21.8	4.6	ns	35.2
CV (%)	45.0	66.0	72.1	60.5

Ns: No significant difference between the means, Conc.: Concentration, ppm: Parts per million, LSD: Least signicificant difference, CV: Coefficient of variation

Dry matter accumulation: There were no significant interactive effect ($p>0.05$) of physical nut handing and GA3 concentration on dry matter accumulation (DM) at 24 MAS. Dry matter accumulated by shea seedlings were not significantly affected by physical nut handling either (Table 4). Seed pre-sowing treatment with GA3 however reduced leaf, stem and root dry matter at 24 MAS compared to the control although the reduction was only significant ($p<0.05$) in the case of the root and stem dry matter. The reductions in dry matter were directly related to hormone concentrations. Though GA3 did not show adverse effects on leaf number per plant, declining leaf numbers with increasing GA3 concentration probably led to lower leaf DM at 24 MAS, contrary to what was reported by Afrigan *et al.* (2013), Bahrani and Pourreza (2012) and Thakare *et al.* (2011) in other tree crops. These reductions in leaf numbers probably affected photosynthetic area of those seedlings, resulting in lower seedling DM, which further reduced with increased GA3 concentration. The nature of dry matter partitioning by the shea seedlings under this study favoured the root at the expense of the shoot, allocating between 72.2 and 78.7% of assimilates to the root.

This lopsided allocation of photosynthates confirms earlier report by Frimpong and Adomako (1986) who reported over 60% dry matter allocation to the root system and may explain why the growth of the plumule is very slow. This pattern of dry matter allocation in shea tree is a known survival mechanism designed to survive long dry seasons coupled with wild fires (Frimpong and Adomako, 1986). The inability of higher GA3 concentrations to influence a change in dry matter allocation towards the shoot probably indicates that this behaviour is strongly an intrinsic plant attribute that should be viewed and tackled at the molecular level.

CONCLUSION

The study reveals that GA3 can improve the emergence of shea seedling by a paltry 2.5-3% at relatively higher concentrations of 1,000-2,000 pmm. This comes with economic implications giving the high cost of the hormone. The residual ability of GA3 to influence stem elongation and diameter gain of shea seedlings should however, be further investigated since rapid seedling growth will offer an opportunity for early grafting and reduced time to transplanting of shea seedlings.

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