



## **Effect of Gibberellic Acid (GA<sub>3</sub>) and Kinetin on Seed Germination of *Sesbania sesban* L. and *Sesbania rostrata* L. (Fabaceae)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author SIM designed the study and supervised the research work. Authors CE and NKI performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/AJAHR/2020/v5i230050

#### Editor(s):

(1) Dr. Paola A. Deligios, University of Sassari, Italy.

#### Reviewers:

(1) Grace O. Tona, Ladoko Akintola University of Technology, Nigeria.

(2) Rosendo Balois Morales, Universidad Autonoma de Nayarit, Mexico.

(3) Aparecida Leonir da Silva, Universidade de São Paulo, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55122>

**Original Research Article**

**Received 02 January 2020**

**Accepted 08 March 2020**

**Published 16 March 2020**

### **ABSTRACT**

We investigated the effects of gibberellic acid (GA<sub>3</sub>) and kinetin on seed germination of *S. sesban* L. and *S. rostrata* L. The matured seeds used for this study were obtained from International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria. The seeds were harvested in 2014 and stored dry in a glass container and kept (15°C) in the refrigerator. The viability of the seeds were determined by floating the intact seeds in water and water uptake (imbibition) was carried out. Four replicates of 20 seeds per replicate were germinated and the seeds observed daily and final count was recorded after 14 days of incubation at 30°C. Intact seeds were soaked in gibberellic acid (GA<sub>3</sub>) and kinetin for 24h and germination percentage taken. The results from the water absorption demonstrated that the seeds of *S. rostrata* is more permeable compared to *S. sesban* as indicated by higher water absorption of seeds of *S. rostrata* (70%) to *S. sesban* (25.4%) after 24hrs of incubation. The anatomy of the seed coats indicated the presence of water and gas impermeable tissues namely cuticle, macrosclereids, osteosclereids and disintegrated parenchyma

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layer. Generally, 0.1 mM kinetin and GA<sub>3</sub> enhanced significant germinations compared to the control with 0% germination for the 14 days period of germination. The percentage germination of seeds of *S. sesban* and *S. rostrata* subjected to different treatments and germinated in 0.1 mM GA<sub>3</sub> and water showed a progressive decrease in germination. From our study, 0.1 mM GA<sub>3</sub> and Kinetin significantly enhanced seed germination of *S. sesban* and *S. rostrata*.

**Keywords:** Germination; imbibitions; hormones; water uptake; seed coat.

## 1. INTRODUCTION

In plants, it is important that seed germination occurs in the right place and at the right time, for this reason, most species have mechanisms that delay germination, such as seed dormancy [1]. The definitions of dormancy in seeds have been a source of controversy [1,2]. Different classes of seed dormancy are recognized namely exogenous dormancy (physical dormancy, mechanical dormancy and chemical dormancy), endogenous dormancy (physiological dormancy, morphological dormancy and combined dormancy), combinational dormancy and secondary dormancy [3]. Among these physical dormancy is caused by the seed coat that prevents imbibition of water [4-6].

*Sesbania* L. belongs to the sub-family Papilionoideae and family Fabaceae [7-9]. The genus is widely distributed and cultivated throughout tropical Africa and Asia. It has also been introduced in tropical America [10] and is an exotic plant to Ethiopia [11,12]. The greatest species diversity (33 species) occurs in Africa [9, 13-16]. *S. sesban* and *S. rostrata* have enormous economic importance, among these are nitrogen-fixing [17]; fodder and to alleviation of feed shortages; maintenance of soil fertility and prevention of land degradation [18,19] and serve as protein supplement to poor quality roughages or as substitute for commercial protein supplements [11,14,20].

Ethno-botanical investigations in the central region of Burkina Faso have shown that *S. rostrata* is used frequently and widely in traditional medicine to treat gastrointestinal infections, cardiovascular diseases and have antibacterial and anti-viral activities and natural antioxidants properties [21].

Seed dormancy is regarded as the failure of an intact viable seed to germinate under favorable conditions. Despite the fact that many researchers have studied seed dormancy, there is no unambiguous definition of the phenomenon, perhaps because it is manifested and broken in

different ways in different species [22]. The seeds of some species are prevented from completing germination because the embryo is constrained by its surrounding structures. This phenomenon is known as coat enhanced dormancy; embryos isolated from these seeds are not dormant. In other species, a second category of dormancy is found in which the embryos themselves are dormant (embryo dormancy). Seed germination is affected and regulated by various environmental conditions/factors such as light and temperature [23,24].

Physical dormancy refers to seeds that are water impermeable and is known to occur in 17 families of angiosperms, including the Fabaceae [5,25]. Germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expansion [26-28].

Hormones such as gibberellic acid (GA<sub>3</sub>), kinetin and cytokinin have been known to improve the germination of plant seeds that have germination problem and the mechanism or process by which the hormones act has been well documented [29-38]. Also to note, climate change is reducing viability and that is serious concerns on availability of seeds with its consequence on plants growth and food production. This work attempts to investigate effect hormones (gibberellic acid (GA<sub>3</sub>) and kinetin) on the seed germination of *S. sesban* and *S. rostrata*.

## 2. MATERIALS AND METHODS

### 2.1 Source of Materials

The matured seeds of *S. sesban* and *S. rostrata* used for this study were obtained from International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria. The seeds were harvested in 2014 and stored dry in a glass container and kept at 15°C in the refrigerator. These seeds were subsequently collected for investigations when necessary.

## 2.2 Viability Test by Floating Method

The viability of 100 seeds of *S. sesban* and *S. rostrata* each were determined by floating the intact seeds in water. The viable seeds settled at the bottom of the container, while the other seeds floated to the top and were considered non-viable. The seeds that settled at the bottom of the container were used for this experiment.

## 2.3 Determination of Water Uptake in Intact Seeds

The seeds of *S. sesban* and *S. rostrata* were properly cleaned with tissue paper to remove any dirt. The cleaned, dried seeds were weighed and thereafter placed in petri dish and immersed in distilled water, incubated individually for 1, 4, 8 and 24 hrs in the dark. After the desired time, the dishes were removed and water adhering to the surface of the seeds was blotted with tissue paper and the weight subsequently determined. The water uptake after a specific period of imbibition was determined as noted by weight difference according to Mensah [39].

## 2.4 Germination Procedures

For each treatment carried out, four replicates of 20 seeds per replicate were put in petri dish lined with Whatman filter paper, moistened with 5ml of distilled water and wrapped with aluminium foil. The seeds were observed daily and watered as deemed appropriate. Germination counts were recorded daily, and final count was recorded after 14 days of incubation at 30°C. Germination was scored when radicle protrudes from the seed coat. For all the different treatments, control experiments were set-up alongside.

## 2.5 Treatment with 0.1 mM GA<sub>3</sub> and 0.1 mM Kinetin

Intact seeds of *S. sesban* and *S. rostrata* were soaked in 0.1 mM GA<sub>3</sub> or kinetin for 24h. At the end of this pre-treatment time the seeds were washed severally in distilled water and germinated in water as described in germination procedure.

## 2.6 Germination of Intact Seeds (Control)

The intact seeds were germinated without any treatment and the percentage germination ascertained to know potentials of intact viable seeds to germinate.

## 2.7 Anatomy of Seed Coat

Dry seeds were collected from the specimens collected from IITA, fixed in FAA (formalin, acetic acid and alcohol) for 12hrs. Thereafter, the specimens were dehydrated in series of different percentages of ethanol (30% and 50%) and stored in 70% ethanol and sectioned [40]. The sections were stained in 1% Safranin red for two minutes, counter-stained with Alcian blue, mounted on a slide, viewed and photographed with Optika B-1000 FL LED microscope.

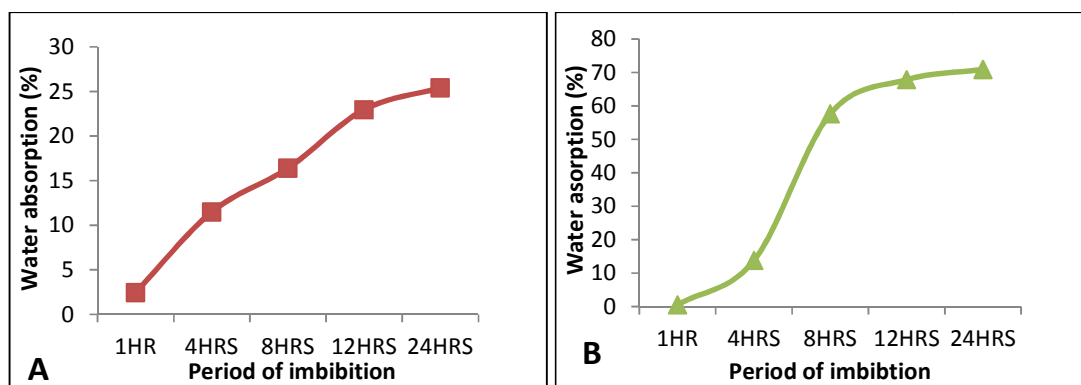
## 3. RESULTS AND DISCUSSION

### 3.1 Germination of Intact Seeds

The results of the intact seeds of *S. sesban* germinated at 25°C in the dark conditions gave 0% germination after 14days of incubation under laboratory conditions. Also, intact seeds of *S. rostrata* germinated at 25°C in the dark condition gave 0% germination after 14days of incubation under laboratory conditions.

### 3.2 Water Absorption

The percentage water absorption of *S. sesban* seeds indicated a progressive increase in water uptake from 1hr imbibition (2.45%) to 24hr imbibition (25.4%), indicating that the seeds of *S. sesban* may not permit substantial water uptake (Fig. 1A). The biphasic pattern of water uptake even though not very obvious may have been attained after 24hrs absorption as indicated by the near presence of plateau. On the other hand, the percentage water absorption of *S. rostrata* seeds indicated a progressive increase in water uptake from 1hr imbibition (0.51%) to 24hr imbibition (70.91%) (Fig. 1B). The biphasic pattern of water uptake is attained after 8hrs imbibition (60% water absorption), indicating substantial water uptake after 8hrs imbibition. For *S. rostrata*, the result indicated that the seed coat may not inhibit water uptake in view of the substantial uptake (70%) at 24hrs imbibition. Though the initial water uptake by *S. sesban* was higher than *S. rostrata*, the final water uptake by *S. rostrata* was much higher than *S. sesban*. Water uptake of 60-78% has been reported by Berrie and Drennan [41] in tomato and oat seeds; Mensah and Agbagwa [42] noted 70% of water uptake for scarified seeds of *Gmelina arborea* and 13.5% has been reported for *Senna obtusifolia* seeds [43].



**Fig. 1. Percentage water absorption of seeds (A) *S. sesban* and (B) *S. rostrata***

The results from the water absorption demonstration that the seeds of *S. rostrata* may be more permeable compared to *S. sesban* as indicated by higher water absorption of seeds of *S. rostrata* (70%) to *S. sesban* (25.4%) after 24hrs of incubation.

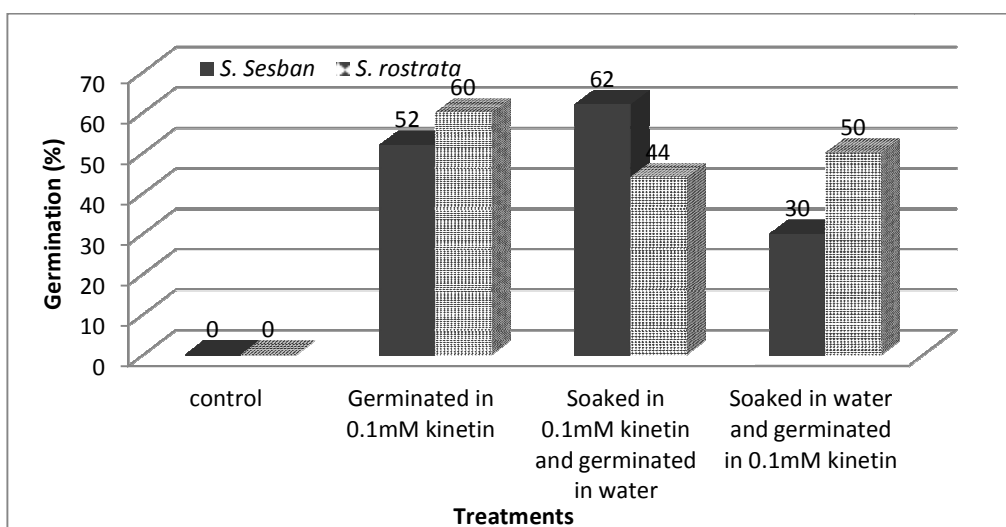
### 3.3 Kinetin Treatment

Seeds germinated in kinetin or soaked in kinetin for 24hrs and later incubated in water gave higher germination percentage for *S. rostrata* and *S. sesban* compared to soaking seeds in water and germinated in kinetin (Fig. 2).

The seeds of *S. sesban* germinating in water after soaking in 0.1 mM kinetin for 24hrs showed enhanced germination (62%) than germinating in kinetin (52%) and soaking in water before germinating in kinetin (30%). However, for *S. rostrata* germinating in 0.1 mM kinetin gave

higher germination percentage (60%) compared to soaking in water and germinating in 0.1 mM kinetin (50%) and soaking in kinetin before germinating in water (44%). For both seeds of *S. sesban* and *S. rostrata* there is enhanced germination for seeds germinated in kinetin and those soaked in 0.1 mM kinetin before germinating in water compared to the ones soaked in water before germinating in kinetin (Fig. 2, Table 1). Generally, with in 0.1 mM kinetin enhanced significant germinations compared to the control with 0% germination for the 14days period of germination (Table 1).

Seeds germinated directly in 0.1 mM kinetin or soaked in 0.1 mM kinetin performed better than seed soaked in water before germinating in 0.1 mM kinetin. The results clearly indicated enhancement of germinations with 0.1 mM kinetin treatment.



**Fig. 2. Comparative percentage germination of *S. sesban* and *S. rostrata* seeds subjected to different treatments (0.1 mM kinetin or water) and germinated for 14days at 25°C**

**Table 1. Effect of 0.1 mM kinetin on the germination of *S. sesban* and *S. rostrata***

	<i>S. sesban</i>	<i>S. rostrata</i>
Control	0 <sup>a</sup>	0 <sup>a</sup>
Germinated in 0.1 mM kinetin	52.0 ± 0.7767 <sup>b</sup>	60.0 ± 1.5275 <sup>b</sup>
Soaked in 0.1 mM kinetin and germinated in water	62.0 ± 1.7009 <sup>c</sup>	44.0 ± 0.5774 <sup>c</sup>
Soaked in water and germinated in 0.1 mM kinetin	30.0 ± 2.2913 <sup>d</sup>	50.0 ± 1.2583 <sup>d</sup>
LSD at P<0.05	0.6971	0.4564

### 3.4 Gibberellic Acid (GA<sub>3</sub>) Treatment

The percentage germination of seeds of *S. sesban* and *S. rostrata* subjected to different treatments and germinated in 0.1 mM Gibberellic acid (GA<sub>3</sub>) or water showed a progressive decrease in germination (Fig. 3, Table 2).

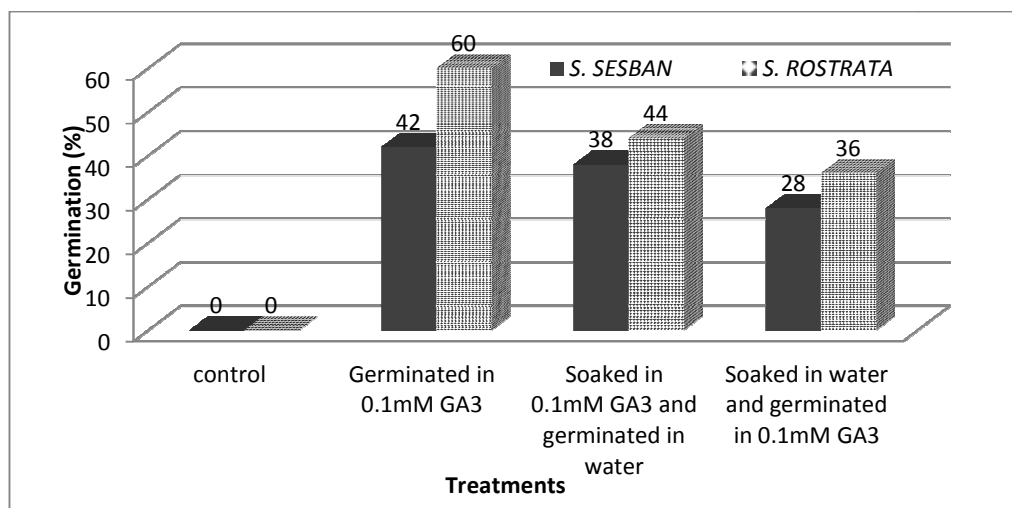
Percentage germinations of *S. sesban* (42%) and *S. rostrata* (60%) were highest in seeds directly in 0.1 mM GA<sub>3</sub>; followed by seeds soaked in 0.1mM GA<sub>3</sub> before germinating in water (*S. sesban* 38%; *S. rostrata* 44%) and the lowest germinations were recorded for soaked water before germinating in 0.1 mM GA<sub>3</sub> (*S. sesban* 28%; *S. rostrata* 36%). The control (germinated in water) gave 0% germination (Fig. 3). The results clearly demonstrated that 0.1 mM GA<sub>3</sub> significantly enhanced germination *S. sesban* and *S. rostrata* seeds (Table 2).

### 3.5 Seed Coat Anatomy

The anatomy of the seed coat indicated the presence of water and gas impermeable tissues like: cuticle; macrosclereids; osteosclereids and

desintegrated parenchyma layers (Plate 1). Dormancy may be broken by treatments which weaken the seed covering or coat, treatments like: sulphuric acid, hydrogen peroxide, KNO<sub>3</sub>, hot water and hormones, etc. The effect of sulphuric acid pre-treatment in breaking dormancy and promoting germination in other species have been reported in previous study [42].

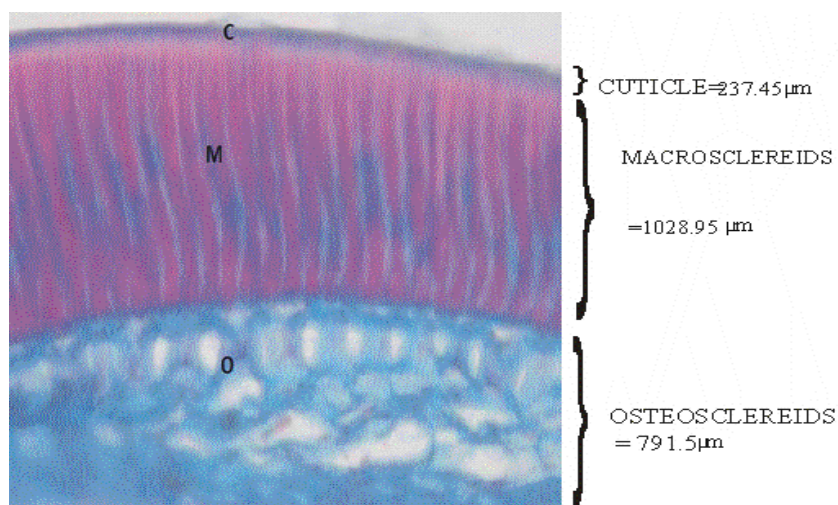
The seed coat of *S. sesban* consists of three distinct layers namely: macrosclereids, cuticle, and osteosclereids layer [44]. In this study, the outermost tissue the cuticle is 237.45 µm thick, macrosclereids 1028.95 µm thick and osteosclereids 791.5µm thick in *S. sesban*. The cuticle is the outer layer which has a waxy and water-repellent character, the second is the macrosclereids cells is referred to as malpighian or palisade cells layer and it belongs to a plant cells type sclereids, the third layer is composed of osteosclereids 791.5 µm thick (Plate 1). Physical dormant seeds are responsible for preventing water. These layers are known to be the major components of seeds [44].



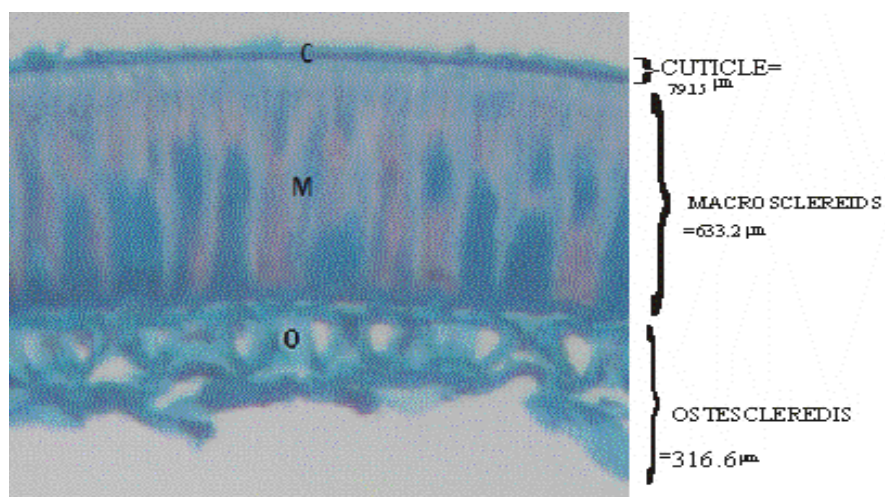
**Fig. 3. Comparative percentage germination of *S. sesban* and *S. rostrata* seeds subjected to different treatments and germinated in 0.1 mM Gibberellin acid (GA<sub>3</sub>) or water and germinated for 14days at 25°C**

**Table 2. Effect of 0.1 mM gibberellin acid on the germination of *S. sesban* and *S. rostrata***

	<i>S. sesban</i>	<i>S. rostrata</i>
Control	0 <sup>d</sup>	0 <sup>d</sup>
Germinated in 0.1 mM GA <sub>3</sub>	42.0 ± 3.0551 <sup>a</sup>	60.0 ± 2.7839 <sup>a</sup>
Soaked in 0.1 mM GA <sub>3</sub> and germinated in water	38.0 ± 2.5059 <sup>b</sup>	44.0 ± 1.9697 <sup>b</sup>
Soaked in water and germinated in 0.1 mM GA <sub>3</sub>	28.0 ± 2.6102 <sup>c</sup>	36.0 ± 2.1008 <sup>c</sup>
LSD at P<0.05	1.1162	0.9441



**Plate 1. Seed coat anatomy of *S. sesban***



**Plate 2. Seed coat anatomy of *S. rostrata***

Dormancy may also be broken by treatments which weaken the seed covering or coat, treatments like water and hormones, etc. Cytokinins have been shown to have stimulatory effects on the germination of seeds of a wide range of plant species [45-47]; Hydrogen peroxide has been reported to

increase germination in pea seeds [48]; it can also be said that ethanol might accelerate germination by promoting the uptake of oxygen [49]. Chris et al. [50] low concentration of ethanol increases seed germination but higher concentration can inhibit germination.

The seed coat of *S. rostrata* (legume) consists of three distinct layers namely; the cuticle, macrosclereids and osteosclereids layers [43]. From our study, the outermost tissue the cuticle of *S. rostrata* is (79.15  $\mu\text{m}$ ) thick and has a waxy and water-repellent character. The second layer is composed of macrosclereid (633.2  $\mu\text{m}$ ) thick, which is also referred to as palisade cell layer, the third layer is composed of osterosclereids (316.6  $\mu\text{m}$ ) Plate 2. Seed coat can be said to impose dormancy on seeds of *S. rostrata* preventing water uptake.

The results of the water uptake by the seeds of *S. sesban* indicated 25.4% water absorption compared to *S. rostrata* which gave 70.91% after 24hrs. Seeds of *S. rostrata* are more permeable to water uptake than *S. sesban*. This relatively rapid uptake of water by *S. rostrata* can be explained by the thin layer of macrosclereid (633.2  $\mu\text{m}$ ), the main layer inhibiting water uptake as compared to *S. sesban* with higher macrosclereid layer of 1028.95  $\mu\text{m}$  thick which slow water uptake.

The influence of water absorption and seed coat anatomy is reflected in the germination percentages, especially *S. rostrata* which showed the higher percentage germinations than *S. sesban* for GA<sub>3</sub> treatments. The GA<sub>3</sub> enters the seed of *S. rostrata* rapidly and in greater amount, thus promoting germination in the seeds of this species compared to *S. sesban*. Dubois and de Vries [51] have demonstrated that GA<sub>3</sub> enhanced the germination of seed having difficulty with germination. Also, Rouskas et al., [30] and Mehanna et al. [31], stated that apple and peach seeds germinations were enhanced by the application of GA<sub>3</sub>.

For kinetin treatments, percentage germinations did not follow a specified trend. *S. rostrata* seeds germinated in kinetin gave slight higher germinations than *S. sesban* seeds; for treatment in which seeds were soaked in the kinetin and germinated in water, *S. sesban* gave higher germination than *S. rostrata*; while the treatment in which the seeds were soaked in water and germinated in kinetin, *S. rostrata* gave higher germination percentage than *S. sesban*.

Generally, kinetin and GA<sub>3</sub> promote seed germinations of *S. rostrata* and *S. sesban* significantly compared to the control. The mechanism or process by which the hormones act has been well documented [29-37]. The hormones might act through *de novo* synthesis

or the release of pre-formed enzymes ( $\alpha$ -amylase). These enzymes are responsible for the conversion of starch to sugar and subsequent absorption by the enzyme, thus the embryo develops and burst through the seed coat resulting in germination. The effect kinetin and GA<sub>3</sub> on the enhanced germinations of *S. rostrata* and *S. sesban* might be suggested through the enhancement of these hydrolytic enzymes, development of the embryo and eventual bursting of the radicle through the seed coat and consequently overcoming limitation imposed by the seed coat. Previous report showed that before a seed can germinate a set of stages must be completed, including the availability of food stores in the seed. Such food stores include starch, protein, lipid and nutrients, which become available to the seed embryo through the activity of specific enzymes and pathways [29]. Thus the findings of our study showed that the dormancy types of these seeds (*S. rostrata* and *S. sesban*) are both physical dormancy (imposed by the seed coat) and physiological dormancy (overcome by the embryo activity).

#### 4. CONCLUSION

The percentage germination of seeds of *S. sesban* and *S. rostrata* subjected to different treatments and germinated in 0.1 mM gibberellic acid (GA<sub>3</sub>) and kinetin were enhanced and the dormancy types of these seeds are both physical dormancy (imposed by the seed coat) and physiological dormancy (overcome by the embryo activity).

#### ACKNOWLEDGEMENTS

The authors wish to thank the of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt for providing enabling environment for this research. More especially we are grateful to Mr. Ogazie, Chinedum Alozie for his expertise advice.

#### COMPETING INTERESTS

Authors have declared that no competing interests.

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