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# Effects of $GA_3$, BAP and $KNO_3$ on the Germination and DNA Content of Cucumber (*Cucumis sativus* L.)

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**ABSTRACT**

**Effects of gibberellic acid ($GA_3$), 6-benzylaminopurine (BAP) and potassium nitrate ($KNO_3$) on the seed germination and DNA concentration of cucumber (*Cucumis sativus*) radicle were assessed. The concentrations of these growth stimulants were 0 mM, 1 mM, 5 mM and 10 mM. The cucumber seeds were surface sterilized in ethanol for 5 minutes and rinsed with distilled water before pretreatment with these growth stimulants. The germination study was allowed to stand for 14 days and DNA concentration of cucumber radicle with the highest germination count was determined for each growth stimulant. The study showed that cucumber seeds had higher germination count under the light condition than in the dark condition. However, it is not statistically different. The study also showed that percentage germination of cucumber seeds was enhanced by $GA_3$ (57 – 72%) and BAP (62 – 70%) when compared to the Control (50%) except $KNO_3$ (41 – 44%). Across the treatments, $GA_3$ gave the highest germination percentage followed by BAP with 5 mM concentration producing the highest germination count while 10 mM recorded the highest in $KNO_3$. The DNA concentration of the cucumber radicle that produced these highest germination percentage are: $GA_3$ (47.40 ng/µl), BAP (98.87 ng/µl), $KNO_3$ (103.23 ng/µl) and Control (79.73 ng/µl). The analysis of variance (ANOVA) showed that treatments are significant at p-value (0.0001) < 5% significant level for cucumber seed. The study recommends the use of 5 mM $GA_3$ in germinating cucumber seeds.**

## INTRODUCTION:

The germination of seed is said to follow a sequential manner starting with seed imbibition which triggers resumption of the metabolic activities therefore enforcing expression of the embryo and emergence of the radicle (Miransari and Smith, 2009; Nonogaki *et al.,* 2010). Imbibition is a passive process and pressures caused by swelling are not sufficient to cause a rupture of the surrounding tissue. This is supported by the work of Mensah and Agbagwa (2004), who reported that embryo expansion is repressed by ABA in some physiologically dormant seeds, so that imbibition alone does not lead to complete seed germination. For complete germination, process of imbibition is followed by activation of hydrolytic enzymes, initiation of growth in the embryo, seed coat rupture and radicle emergence (Miransari and Smith, 2009). The different phases of germination are required for offshoot of seedlings and hence to achieve seedlings with quality yield and reduced disease attack; it is therefore necessary to hasten germination rate for early radicle emergence (Singh *et al*., 2001; Subedi and Ma, 2005).

Seeds have their different moisture requirements to achieve germination known as critical seed moisture content and once that critical level is achieved, the seed is then ready to initiate germination (Bewley *et al*., 2000). Finkelstein (2004) also added that this action will cause an increase in the volume of the seed, resulting to cracking of the testa which may differ from emergence of seedling as seen in Brassicaceae and Solanaceae. Major events after imbibition include DNA repair, initiation of respiration, mitochondrial repair, restoration of cellular integrity, synthesis of germination-related mRNA's and protein. (Nonogaki *et al.,* 2010). The DNA content in the radicle tip cells of wild-type tomato seeds was reported to have increased prior to germination (Bino *et al.*, 1992). Bewley *et al.* (2013) reported that initiation of DNA synthesis during germination is linked with DNA repair following imbibition of dry seeds and also comes before cell division that follows germination. Yanyan *et al.* (2018) further stated that the vigor of the seed can be marked by the time of initiation of DNA replication, moreover it takes a longer time for low quality seeds to achieve DNA repair prior to successful replication.

This research focuses on the germination and DNA studies of Cucumber (*Cucumis sativus*) seed treated with growth promoters.

## MATERIALS AND METHODS:

The matured cucumber (*Cucucmis sativus* L.) seeds (Plate 1) were obtained from fruit garden Port Harcourt, Nigeria. The seeds were properly identified by the Curator at the Herbarium Unit of Department of Plant Science and Biotechnology, University of Port Harcourt. Viability test was carried out on the seeds to ascertain its viability; hence, non-viable seeds were discarded. The viable seeds were surface-sterilized with ethanol for five (5) minutes and rinse with distilled water. Germination studies of cucumber seeds were first carried out both under light and dark conditions.

The growth stimulants used in the study were gibberellic acid ($GA_3$ – 350 g/mol), BAP (6-benzylaminopurine – 225.3 g/mol) and potassium nitrate ($KNO_3$ – 101.1 g/mol). The concentrations (1 mM, 5 mM and 10 mM) of these growth promoters were prepared, respectively. Water was used as the Control treatment. These concentrations were used to pretreat cucumber seeds with 20-seeds per batch. Each treatment was replicated five times. The seeds were germinated under room temperature of 25$^{o}$C, monitored daily and the process lasted for 14 days. Germination percentage of seeds taken for each treatment. Also, the DNA concentration of the radicle with the highest germination count across treatments were assessed using a Quick DNA miniprep kit for isolation of the total DNA from the radicle sample of cucumber ensuring that there was no contamination with RNA.

The data obtained from the study were subjected to statistical analysis using SAS 9.1.3 version.

**Plate 1: Cucumber seeds**

**RESULTS:**

The percentage germination of cucumber seeds germinated under light and dark conditions are presented in Figure 1. The light condition promotes the germination of cucumber seed than under dark condition. However, this is not statistically different at p-value (0.0001) > 5% significant level.

The percentage germination of cucumber seeds treated with different concentrations of $GA_3$, BAP and $KNO_3$ are presented in Figure 2. The study showed that percentage germination of cucumber was enhanced by $GA_3$ and BAP concentrations when compared to the Control except $KNO_3$ concentrations. Across the treatments, $GA_3$ gave the highest germination percentage followed by BAP with 5 mM concentration producing the highest germination count followed by 10 mM for $GA_3$ and BAP treatments.

The cucumber radicles of Treatments with the highest percentage germination were analyzed for DNA contents as shown in Figure 3. There is no positive trend between highest percentage germination and DNA contents in cucumber radicles. Seeds treated with 5 mM $GA_3$ gave 72% germination and DNA content of 47.40 ng/µl; 5 mM BAP gave 70% germination with DNA content of 98.87 ng/µl; 10 mM $KNO_3$ gave 44% germination with DNA content of 103.20 ng/µl while Control gave 50% germination with 79.73 ng/µl DNA content. The results indicated generally that treatments with low percentage germinations ($KNO_3$ and Control) have higher DNA contents while treatments ($GA_3$ and BAP) with high percentage germinations gave lower germinations. The analysis of variance (ANOVA) showed that treatments are significant at p-value (0.0001) < 5% significant level for cucumber seed. Also, the multiple comparisons using least significant difference (LSD) showed that the Control is significantly different at 5% significant level from $GA_3$.

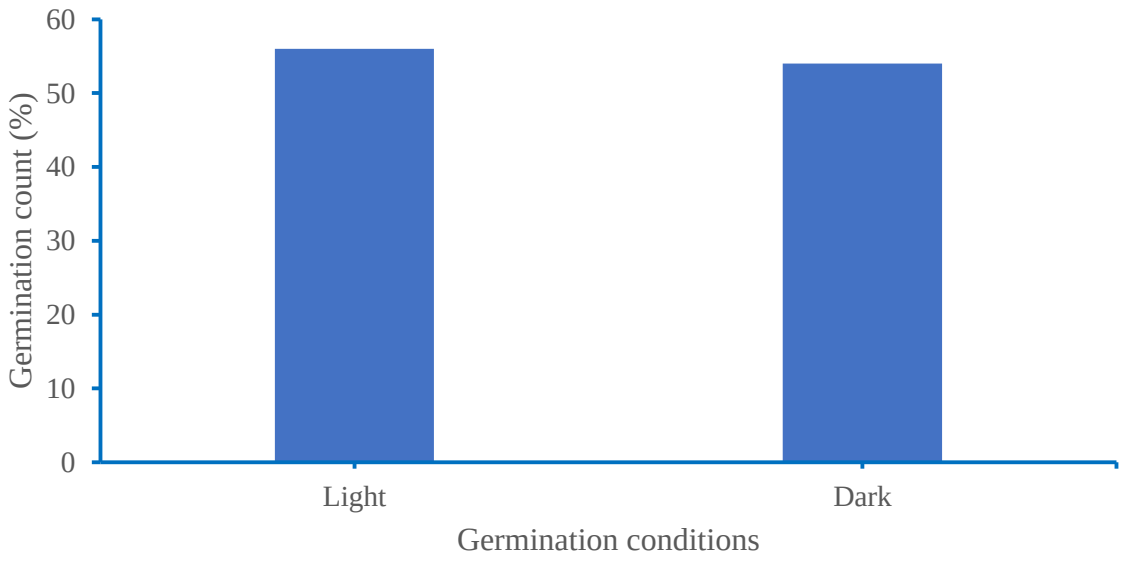


Figure 1: Percentage germination of cucumber seed under light and dark conditions

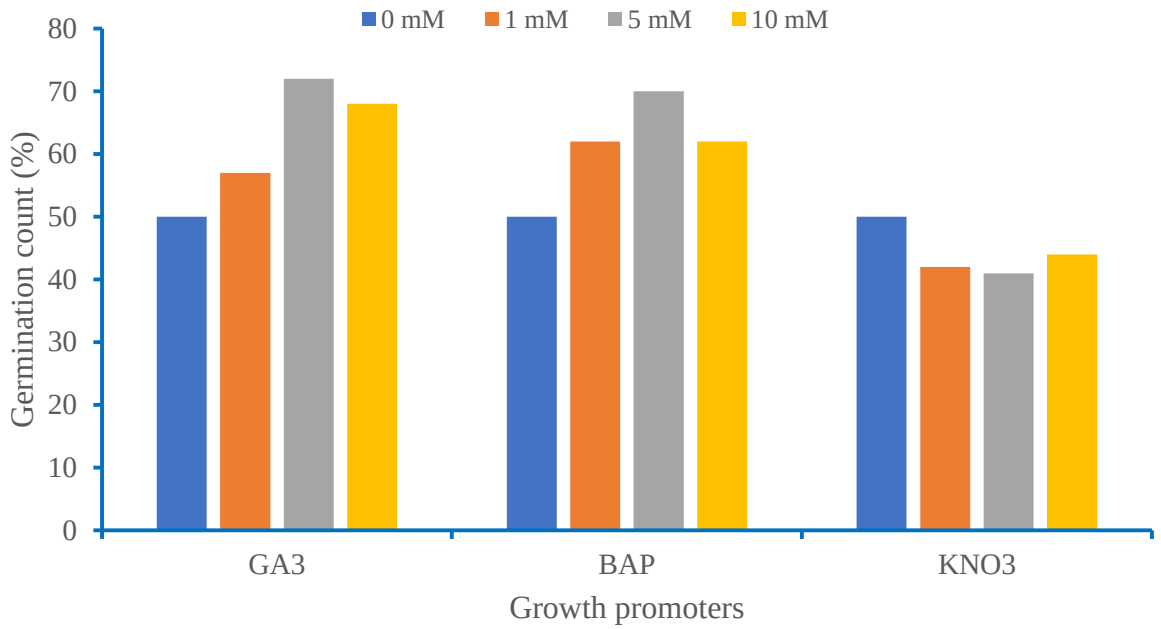


Figure 2: Effects of growth promoters conentrations on the germination of cucumber seed

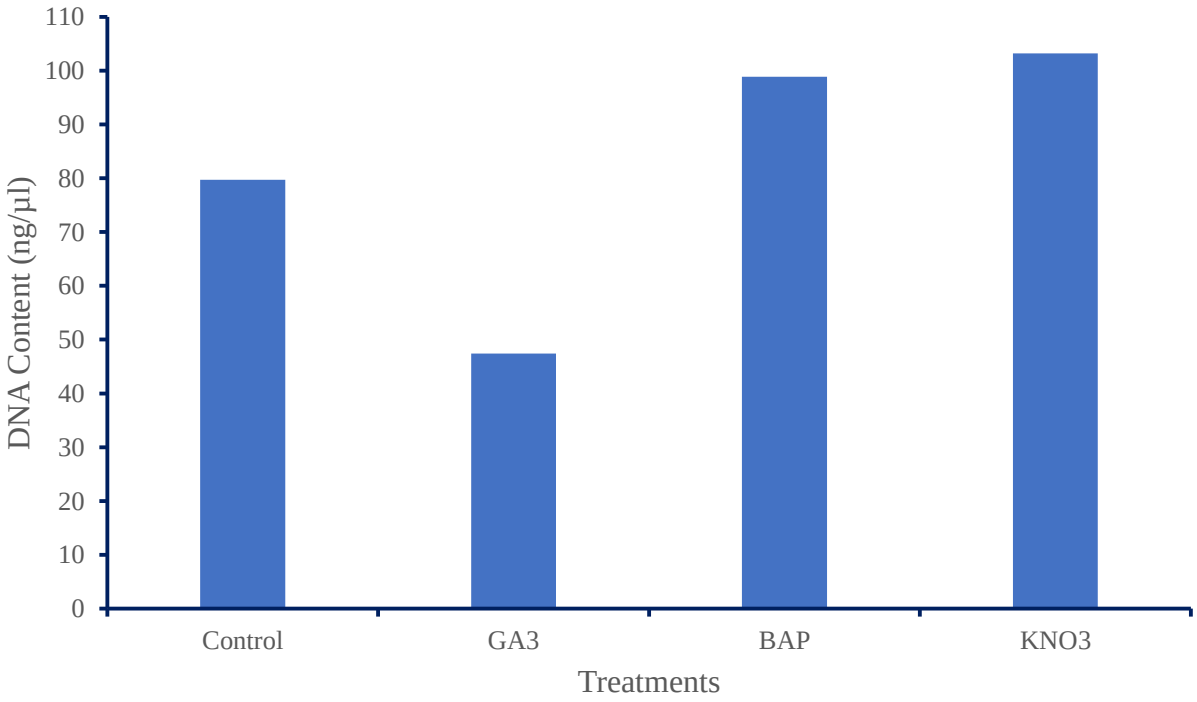


Figure 3: DNA Contents of Cucumber radicle that expressed highest germination

## DISCUSSION:

The findings (Figure 2) indicated that $GA_3$ and BAP enhanced percentage germination compared to the Control. $KNO_3$ treatments did not enhance germination, as the germinations are comparable to Control treatment. The effects of $GA_3$ and kinetin in enhancing germination of dormant and non-dormant seeds are well documented (Miyoshi and Sato, 1997; Mensah and Agbagwa, 2001; Zeb *et al.*, 2018) and supported the findings noted in this study.

This study did not observe positive trend or relationship between highest percentage germinations and DNA contents, indeed the reverse appeared to be the case, that is, treatments with low germination gave high DNA contents and those with high germinations, except 5 mM BAP that gave 70% germination and recorded DNA content of 98.87 ng/µl. Gibberellin and kinetin have been extensively reported to play a role in RNA and protein synthesis, hydrolytic enzymes, substrate mobilization and elongation of embryo axis in dormant and non-dormant seeds (Chrispeels and Varner, 1967; Pinfield and Stobart, 1969; Jones and Armstrong, 1971; Varner and Ho, 1976; Jones and Jacobsen, 1982; Vishal and Kumar, 2018). The study of the action of GA and kinetin has focused on those molecular events that lead to *de novo* protein synthesis (Jones, 1973; Jacobsen *et al.*, 1979; Jones and Jacobsen, 1982). The same suggestions or arguments cannot be inferred in this study because treatments (Control and $KNO_3$) gave high DNA contents. However, it could be suggested that GA and kinetin rather than act on the molecular level through *de novo* synthesis, may act through the release of pre-formed enzymes (hydrolases) in enhancing germination. It is suggested that further work need to be undertaken to clearly establish the relationship between the hormone enhancement of germinations and its molecular action.

The fact that $GA_3$ and BAP effectively enhanced germination is important for early emergence to avoid attack or damage the growing seeds may encounter during unfavourable conditions (Singh *et al.,* 2001; Subedi and Ma, 2005). According to Bewley *et al.* (2013), treated seeds initiate imbibition rapidly and therefore hasten the phase II duration, making the interval between hydration and radicle emergence short. Yanyan *et al.* (2018) reported that the vigour of the seed can be marked by the time of initiation of DNA replication, moreover it takes a longer time for low quality seeds to achieve DNA repair prior to successful replication. Gibberellic acid regulates the production of numerous enzymes by activating the aleurone cells, notably alpha-amylase in growing cereals (Miransari and Smith, 2014).

## CONCLUSION:

The study reveals that $GA_3$, BAP and $KNO_3$ enhanced germination of cucumber seeds with 5 mM concentration of $GA_3$ and BAP been the concentration with highest germination percentage while 10 mM for $KNO_3$ treatment. Control treatment had higher germination count than $KNO_3$ treatment. The DNA concentration of the cucumber radicle depends on the chemical used in treating the seeds of cucumber and there is no clear relationship or trend between treatments and DNA contents.

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