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Research Article



# Effects of $GA_3$ and $KNO_3$ on the Germination and DNA Content of Flax (*Linum usitatissimum* L.)

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## Abstract

The effects of $GA_3$ and $KNO_3$ on the seed germination and DNA concentration of flax (*Linum usitatissimum* L.) radicle were investigated. These growth stimulants were gibberellic acid ($GA_3$), and potassium nitrate ($KNO_3$) with concentrations of 0 mM (Control), 1 mM, 5 mM and 10 mM. The flax 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 flax radicle with the highest germination count was determined for each growth stimulant. The study showed that flax seeds had higher germination count in the dark condition than under the light condition. Concentrations of $GA_3$ and $KNO_3$ enhanced the germination of flax seed. $GA_3$ gave the highest germination percentage with 1 mM concentration been the highest (97%). Also, as the concentration of $GA_3$ and $KNO_3$ increases (1-10 mM), the germination percentage reduces with 1 mM concentration producing the highest germination count (97%,52%), respectively. The DNA concentration of the flax radicle that produced these highest germination percentage in the different treatments are: $GA_3$ (291.50ng/µl), $KNO_3$ (47.77ng/µl) and Control (47.33ng/µl). Also, the multiple comparisons using least significant difference (LSD) showed that the Control is significantly different at 5% significant level from $GA_3$. The study recommends the use of 1 mM $GA_3$ and $KNO_3$ in germinating flax seeds.

**Keywords:** Growth stimulant; Concentration; Germination; Flax seed

## Introduction

Germination rate of seeds has been enhanced either by exogenous application of growth hormone or by seed priming. Batista et al. [1] recorded that seeds of pepper primed with $GA_3$ at 200 mg/L concentration before germination facilitated the rate of germination unlike the unprimed seeds. Also, seeds of *Amaranthus cruentus* pretreated with BAP have shown to have increased germination rate [2]. Exogenous application of hormones and salts have been recorded as promising in removing the factors that led to dormancy in seeds [3,4]. Although there may be some exceptions where application of some growth hormones may not enhance germination; Wall et al. [5] stated that exogenous application of $GA_3$ did not enhance germination in *Pyxidanthera brevifolia*.

Seeds act as sensors to changes to their immediate environment and hence are adjustable to range of environmental factors. Therefore, most seeds have evolved different means of sensing when the environment is convenient to complete germination [6-8], the adjustments create differential seasons for germination to take place. BAP (6-Benzylaminopurine) is an aromatic cytokinin and among the growth stimulants has been involved in seedling enhancement under stress such as; low temperature, salinity and water stress. There is a link between stress and hormonal balance; the pressure stimulates the release of inhibitors and causes a decline in the amount of endogenous growth stimulants, therefore when there is an external application of this growth hormone, it induces the event responsible for uptake of water [9]. Gibberellic acid ($GA_3$) is actively involved in enhancing germination of quite a number of plant species [10-13]. Therefore, application of GA3 is a regular method for releasing seed dormancy [14]. Gibberellin can release dormancy in some seeds that normally require cold (stratification) or light to complete germination [15-17]. Potassium nitrate ($KNO_3$) is a known chemical treatment used to enhance germination; the common application is the use of 0.1-0.2% $KNO_3$ solutions, which are recommended by some International analysts for regular seed germination test of various species [18,19]. It was observed to be effective in improving the seeds of watermelon

germination when subjected to the treatment under unfavourable condition [3]. This research focuses on the germination and DNA studies of Flax (*Linum usitatissimum* L.) seed treated with growth stimulants.

## Materials and Methods

The matured flax (*Linum usitatissimum* 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 were first carried out both under light and dark condition.

The growth stimulants used in the study were gibberellic acid ($GA_3$-350 g/mol) and potassium nitrate ($KNO_3$ -101.1 g/mol). The concentrations (1 mM, 5 mM and 10 mM) of these growth stimulants were prepared for each growth stimulants, respectively. Water was used as the Control treatment. These concentrations were used to pretreated flax seeds with 20-seeds per batch. Each treatment was replicated five times. The seeds were germinated under room temperature of 25 °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 flax 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).

## Result

The percentage germination of flax seed germinated under light and dark conditions are presented in Figure 1. The dark condition promotes the germination of flax seed than under light condition. However, the results of the germinations indicated low germinations.

**Plate 1:** Flax seeds.

The percentage germination of flax seeds treated with different concentrations of $GA_3$ and $KNO_3$ are presented in Figure 2. The study showed that $GA_3$ and $KNO_3$ treatments enhanced the germination of flax seed when compared to Control treatment. Across the treatments, $GA_3$ gave the highest germination percentage with 1 mM concentration been the highest. Also, as the concentration of $GA_3$ and $KNO_3$ increases (1-10 mM), the germination percentage reduces with 1 mM concentration producing the highest germination count. The DNA concentration of the flax radicle that produced these highest germination percentage in the different treatments are: $GA_3$ (291.50 ng/µl), $KNO_3$ (47.77 ng/µl) and Control (47.33 ng/µl). The analysis of variance (ANOVA) showed that treatments are significant at p-value (0.0001) < 5% significant level for flax seed. Also, the multiple comparisons using least significant difference (LSD) showed that the Control is significantly different at 5% significant level from $GA_3$.

## Discussion

The stimulatory effect of $GA_3$ on germination of flax seed is in line with the work of other researchers [10-14]. The study showed that $GA_3$ enhanced germination compared to $KNO_3$ and Control. Again, the $KNO_3$ treatments did not enhanced percentage germinations over Control. Similar reasons were adduced by Bewley et al. [20] that when the damage of the nucleic acids, organelles, enzymes and membranes occurred during storage and maturation by the dry seeds, exceed the ability of the imbibed seeds to repair the systems; then the functional systems start to deteriorate and eventually lead to death which terminate germination. On the other hand, $KNO_3$ treatment shortens the time spent for germination when control of physical environment only is a factor. This was observed by Demir, et al. [3] to be effective in improving germination in the seeds of watermelon subjected to unfavourable growth condition.

According to Alonso Ramirez et al. [11], the exogenous application of $GA_3$ on Arabidopsis seeds in an unfavourable condition greatly enhanced its germination. Potassium nitrate ($KNO_3$) is a known chemical treatment that enhance germination [3].

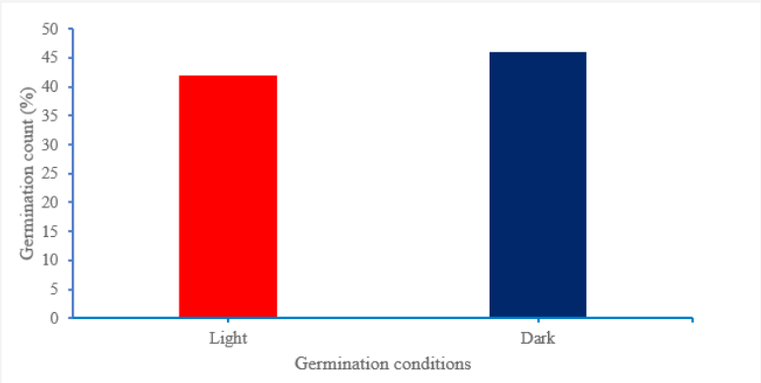


**Figure 1:** Percentage germination of Flax seed under light and dark conditions.

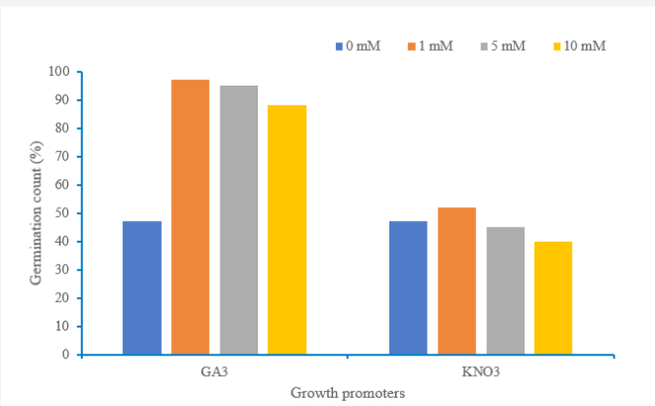


**Figure 2:** Effects of growth promoters' concentrations on the germination of Flax seed.

Seeds of flax treated with 1 mM GA3 probably had made for fast DNA repair when compared to the Control treatment. In comparing the DNA contents with germination percentage, the difference in the trend may be attributed to the type of seeds and the responses of the seeds to the conditions of the treatments. This was also observed by Liu et al. [21], who documented the effect of osmo-priming on DNA synthesis in tomato seeds.

## Conclusion

The study has shown that 1 mM concentration of $GA_3$ and $KNO_3$ enhances the germination. It also reveals that as the concentration of the growth stimulants increases the germination percentage decreases. Again, the DNA concentration of the flax radicle depends on the chemical used in treating the seeds of flax.

## Acknowledgement

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## Conflict of Interest

No conflict of interest.

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