ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 01, pp.9611-9615, January, 2020

DOI: https://doi.org/10.24941/ijcr.37851.01.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

BIO-EFFICACY EVALUATION OF GIBBERELLIC ACID IN ENHANCING GERMINATION OF EXPIRED AND UNEXPIRED MUSTARD GREEN (*BRASICCA JUNCEA*) SEEDS

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ARTICLE INFO

Article History:
Received 12th October, 2019
Received in revised form
28th November, 2019
Accepted 09th December, 2019
Published online 30th January, 2020

Key Words:

Seed Germination, Mustard Greens, Gibberellic Acid, Seed Viability, Seed Repair.

ABSTRACT

The high cost of hybrid seeds and seedlings is one of the key problems faced by famers. Problems in percent germination and germination time make it uneasy for farmers to attain uniform crop establishment and timely harvest. Seed viability decreases over time, and the natural ability in plants for inducing germination weakens with prolonged storage and dormancy. However, the use of plant growth regulators such as the synthetic gibberellic acid (GA) could be an alternative for enhancing germination of expired and long stored seeds. Thus, this experiment aims to test effect of synthetic gibberellic acid (GA) in enhancing germination of expired and unexpired mustard green (Brasicca juncea) seeds. The experiment was laid out in a complete randomized design with four treatments and four replications. Result of the study revealed that applying 900ppm synthetic gibberellic acid (GA) significantly repair expired seeds and improve germination percentage from 64% to 98% (34% enhancement rate) within 80 hours. For unexpired seeds, GA could be used to achieve maximum germination of 100% within 60 hours.

Citation: ***Mcwinner Yawman, Mencho Loayan and Junnie Rex Ambos.*** **2020.** "Bio-Efficacy Evaluation of Gibberellic Acid in Enhancing Germination of Expired and Unexpired Mustard Green (*Brasicca juncea*) Seeds", *International Journal of Current Research*, 12, (01), 9611-9615.

INTRODUCTION

The high cost of hybrid seeds and seedlings is one of the most limiting aspects during crop establishment. Problems in germination rate and sprouting time makes it uneasy for farmers to attain crop growth uniformity and timely harvest. Hybrid seed companies such as East-West Seeds and Kaneko Seeds considers a successful germination rate to be at least 85%. Thus, this percent indicator is the commercially accepted viability of seeds (East-West Seeds, 2019). Seed viability decreases over time. The natural mechanism of seeds to induce germination becomes limited with prolonged storage and dormancy, of which, for farmers, it would be better off buying news seeds than to spend material and labor resources on poor performing seeds. Commercially unsold expired seeds are discarded because of poor germination. However, the use of plant growth regulators such as the synthetic gibberellic acid (GA) might be an alternative for enhancing germination of expired and long stored seeds. Lopez et al., (2009) successfully improved germination of hybrid tomato seeds (*Solanum lycopersicum L.*) by using 900 mg L-1 GA (900ppm). Aside from germination percentage, root length, dry matter, stem and root fresh matter and leaf area were enhanced. The researchers also observed fastest average sprouting rate and tallest height were due to 300 mg L-1 GA3 treatment.

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In field stage, the plant height, stem and total dry matter, leaf and root fresh matter and net assimilation showed the best response at 900 mg L-1 GA3 treatment. The researchers concluded that seed soaking with 900 mg L-1 GA3 yields more vigorous tomato seedlings in less time and with a better development under field conditions. Similarly, Melo (2009) conducted a research on the tittle "Gibberellic Acid Promotes Seed Germination in *Penstemon digitalis"*. From the researchers observation, all concentrations of GA3, except the control, were effective for increasing the speed of germination when seeds were either not covered or were covered with mix. When seeds were not covered with mix, seeds soaked in either the 500 or 1000 mgL–1 GA3 germinated in half the time compared with the control. When seeds were covered with mix, seeds soaked in 1000 mgL–1 GA3 germinated more rapidly. The question arises as whether GA could be effective in enhancing germination of expired commercial mustard seeds. Applying GA to other horticultural crops such as the mustard green (*Brasicca juncea*) seeds might also improve its germination performance. Gibberellins commonly known as gibberellic acid (GA), is a tetracyclic de-terpenoid compound bio-actively synthesized in many vascular plants (Gupta and Chakrabarty, 2013). GA was first discovered in the year 1930s by a Japanese scientist, Kurosawa in the course of his studies on fungal diseases of rice (Karov et al, 2000). A certain fungus *Gibberella fujikuroi* secretes a compound that makes the rice seedlings grew foolishly tall, thus it was named gibberellin (Takahashi et al., 1991 in Pavlista et al., 2012).

Later in the year 1950s, GA compound was successfully extracted from immature seeds of vuner bean plant (Brueckner et al, 1989). It was found out that seed germination is promoted by GA in many plant species (Riley, 1997). Several GA signalling factors are known to induce the expression of genes encoding enzymes that mobilise food reserves, including starches, proteins and lipids, stored in the endosperm during seed germination (Peng and Harberd, 2002). GA is an important growth hormone that exercise different effects on crops. The results vary depending on the plant species (Oliveira et al, 2017). Hence, this experiment tested the effect of synthetic gibberellic acid in enhancing the germination of expired and unexpired commercial mustard green (*Brasicca juncea*) seeds.

Objectives of the Study

- To test the efficacy of Gibberellic Acid in enhancing the germination of expired commercial mustard green seeds; and
- To test the efficacy of Gibberellic Acid in enhancing the germination of unexpired commercial mustard green seeds.

METHODOLOGY

Time and Place of the Study: The experiment was conducted at Kennemer sensory laboratory located in Buhangin, Davao City last September 23, 2019 to September 28, 2019.

Test Crop: The test crop used in the experiment is mustard green (*Brasicca juncea*) seeds. There were two different mustard green seeds used: 4 years commercially expired; and commercially unexpired seeds. All the seeds in the experiment were considered as treatment samples for data gathering and monitoring.

Materials Used

- 4 years expired mustard green seeds. Old and long stored seeds are expected to have very low germination under normal physiological condition.
- Unexpired mustard seeds - used as test crop for point of comparison
- 90% Gibberellic Acid
- Purified water. This prevented potential contaminants from interfering the result of experiment.
- Petri dishes
- Tissue papers
- Forceps
- Digital weighing scale
- 100ml beaker
- Stirring rod
- Digital camera
- Ball pen

Record book

Experimental Design and Treatments: The experiment was laid out in a Completely Randomized Design (CRD) with four treatment replications. Each replication had twenty-five (25) seeds. A total of four hundred (400) seeds were monitored for five (05) days. Following treatments were considered:

Preparation of 0ppm GA Solution (Control): Two separate containers with 20ml pure water were used to soak the Control expired seeds (Treatment 1) and unexpired seeds (Treatment 3).

Preparation of 900ppm GA Solution: About 100mg of 90% Gibberellic Acid (GA) was diluted in 99.9ml water in order to attain 900ppm GA solution. Two separate containers with 20ml 900ppm GA solution were used to soak the expired seeds (Treatment 2) and unexpired seeds (Treatment 4).

Treatment Application

1. About one hundred (100) mustard green seeds per treatment were soaked according to treatment solution for 36 hours:
   - Control or Pure Water. Seeds under Treatment 1 and Treatment 3.
   - 900ppm GA Solution. Seeds under Treatment 2 and Treatment 4.
2. After 36 hours imbibition (soaking), the seeds were laid evenly on a petri dish with wet tissue paper for the germination to take place.
3. First germination reading was done 36 hours after imbibition. Visual appearance of the radicle (rootlet) was considered as the indicator of germination.
4. Subsequent reading of germination was done every 24 hours for 4 days.

Data Gathered

1. Germination Count. All seeds that germinated were counted and recorded.
   - Total germination count at 36 hours.
   - Total germination count at 60 hours.
   - Total germination count at 84 hours.
   - Total germination count at 108 hours.
2. % Germination Rate. This was computed following the formula:

$$\% \text{ Germination} = \frac{\text{Number of Seeds Germinated X}}{\text{Total Number of Seeds}} \quad 100$$

Data Analysis: All descriptive data gathered were analyzed using percentages and means. Analysis of Variance (ANOVA) and Least Significant Difference (LSD) were used to compare means for differences between treatments at 5% level of significance. The statistical software used was SPSS V25 (SAS Institute, 2006)

RESULTS AND DISCUSSION

Germination rate at 36 Hours Soaking: Table 1 below shows the results of the expired and unexpired seeds treated with 900ppm gibberellic acid and seeds treated with purified water for 36 hours. Treatment 4 (900ppm GA unexpired treated seeds) had the highest mean of 24.25 or 97% germination rate. Meanwhile, Treatment 3 (Control – unexpired seeds) had a mean of 14.25 or 57% germination rate. On the other hand, germination of expired seeds treated with 900ppm GA (Treatment 2) and untreated Control (Treatment 1) were only recorded at 3% and 2%, respectively (Table 1 and Figure 1).

Table 1. Germination at 36 hours of expired and expired mustard green seeds treated and untreated with 900ppm gibberellic acid

Treatment	36 hours Imbibition	
	Germination Count	% Germination Rate
T1 -Control Expired	0.50 ^{c}	2% ^{c}
T2- 900ppm GA Expired	0.75 ^{c}	3% ^{c}
T3-Control Unexpired	14.25 ^{b}	57% ^{b}
T4-900 ppm GA Unexpired	24.25 ^{a}	97% ^{a}
%C.V	100.00	100.00

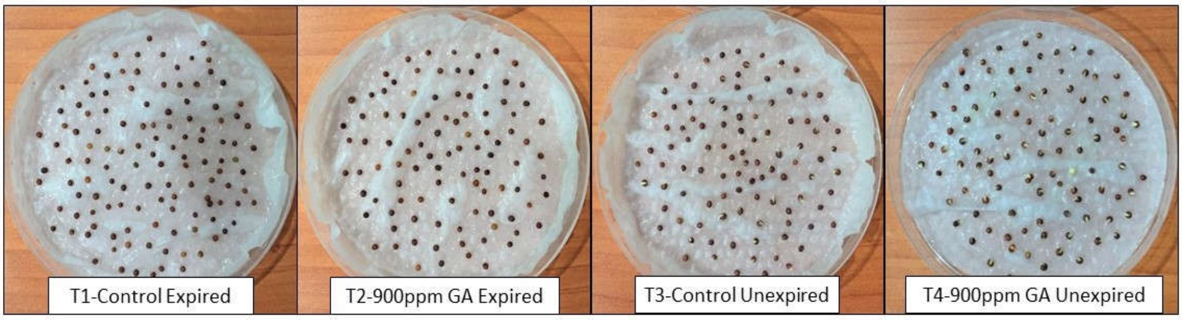

Figure 1. Germination after 36 hours of expired and unexpired mustard green seeds treated and untreated with GA.

Table 2. Germination at 60 hours of expired and expired mustard green seeds treated and untreated with 900ppm gibberellic acid

Treatment	60 hours Germination	
	Germination Count	% Germination Rate
T1 -Control Expired	12.75 ^{d}	51% ^{d}
T2- 900ppm GA Expired	17.50 ^{c}	70% ^{c}
T3-Control Unexpired	20.75 ^{b}	83% ^{b}
T4-900 ppm GA Unexpired	25.00 ^{a}	100% ^{a}
%C.V	73.95	73.95

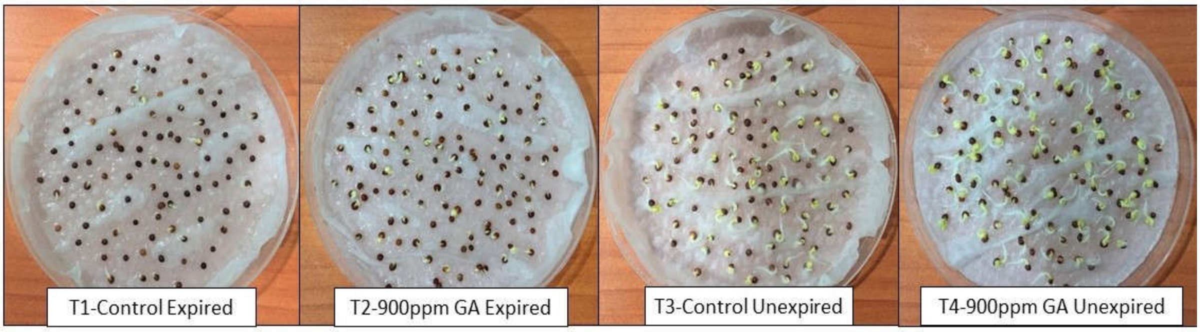

Figure 2. Germination after 60 hours of expired and unexpired mustard green seeds treated and untreated with GA.

Table 3. Germination at 84 hours of expired and expired mustard green seeds treated and untreated with 900ppm gibberellic acid

Treatment	84 hours Germination	
	Germination Count	% Germination Rate
T1 -Control Expired	16.00 ^{b}	64% ^{b}
T2- 900ppm GA Expired	24.50 ^{a}	98% ^{a}
T3-Control Unexpired	24.50 ^{a}	98% ^{a}
T4-900 ppm GA Unexpired	25.00 ^{a}	100% ^{a}
%C.V	25.20	25.20

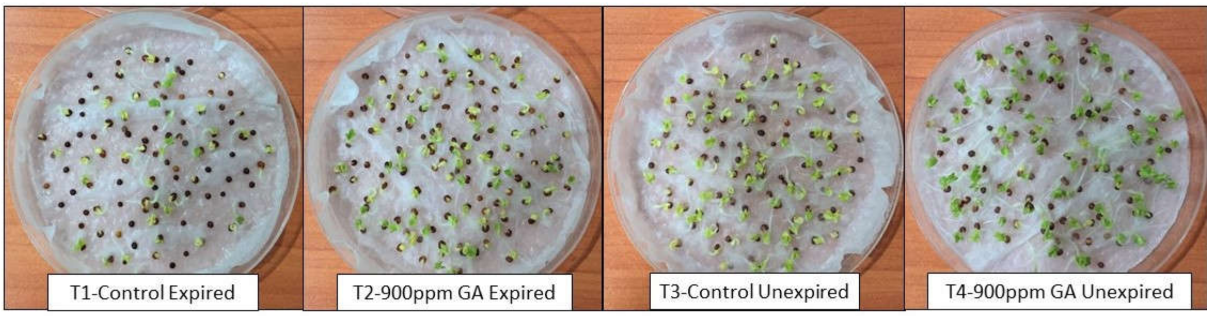

Figure 3. Germination after 84 hours of expired and unexpired mustard green seeds treated and untreated with GA.

Table 4. Germination at 108 hours of expired and expired mustard green seeds treated and untreated with 900ppm gibberellic acid

Treatment	108 hours Germination	
	Germination Count	% Germination Rate
T1 -Control Expired	16.00 [b]	64% [b]
T2- 900ppm GA Expired	24.50 [a]	98% [a]
T3-Control Unexpired	24.50 [a]	98% [a]
T4-900 ppm GA Unexpired	25.00 [a]	100% [a]
%C.V	17.75	17.75

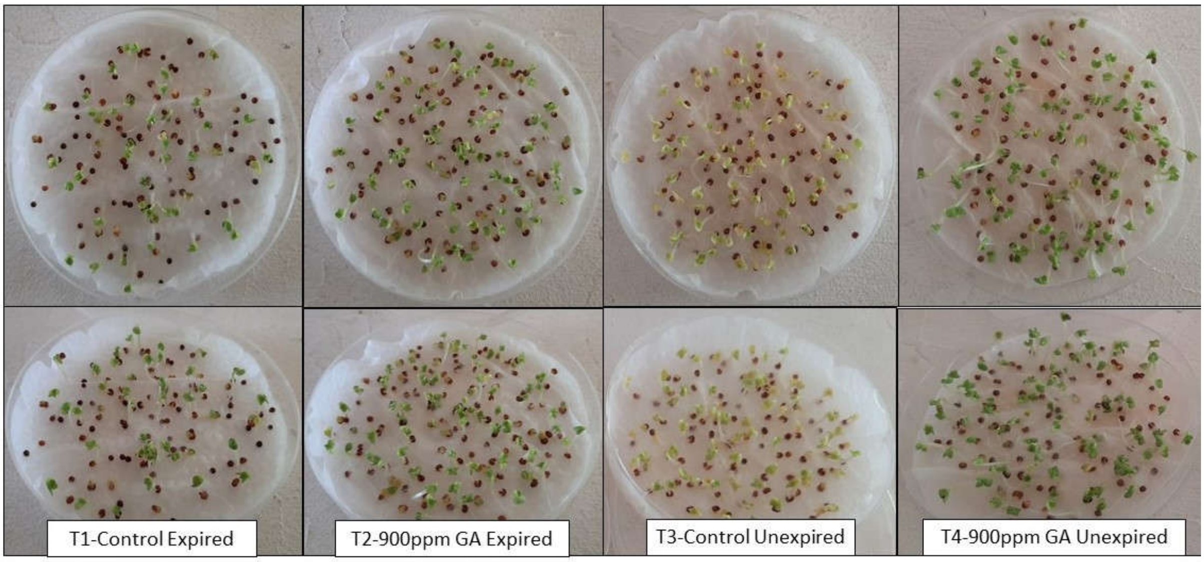

Figure 4. Germination after 108 hours of expired and unexpired mustard green seeds treated and untreated with GA.

From the results, it could be inferred that significant difference exist the germination in T4 compared to T3, T2 and T1. However no significant difference was observed in the germination rate in T2 and T1. This means that the effect of GA in enhancing germination of expired mustard seeds is not significant at 36 hours, but very significant in enhancing germination of unexpired seeds within the same duration.

Germination rate at 60 Hours: The detail of the findings after 60 hours is displayed in Table 2 below. Unexpired seeds treated with 900ppm GA (Treatment 3) reached 100% germination rate with 60 hours. The plumules of a greater percent of the germinated seeds began to emerge at this point. The Control (unexpired seeds -Treatment 3) had an average germination of 20.75 seed count or 83% germination rate within this period. On the other hand, the expired seeds treated with 900ppm GA (Treatment 4) obtained a higher germination mean of 17.5 (70%) compared to Control (Treatment 3) with mean average seed counts of 12.75 (51%). It obvious to mention that significant statistical differences existed among all the treatments at this stage of the germination process. The GA3 seem to have a great effect in invigorating the expired seeds. It seems prudent explain that this could be due repair mechanisms that be going on within the seeds (Yawman, 2012)

Germination Rate at 84 Hours: Seed germination after 84 hours revealed impressive results as shown in Treatments 2, 3, and 4. Unexpired seeds treated with 900ppm GA (Treatment 4) achieved 100% germination earlier at 60 hours germination period. First leaves of Treatment 3 were growing faster compared to other treatments. However, both Control unexpired seeds (Treatment 3) and GA treated expired seeds (Treatment 2) both attained 98% germination. Both treatments were showing-off plumules. On the other hand, germination of Control expired seeds (Treatment 1) did not significantly improved having 64% germination rate (Table 3 and Figure 3). Seemingly, the control expired seeds to have least germination rate because the repair mechanism within the seed was not enhanced (Yawman, 2012)

Germination Rate at 108 Hours: Germination after 108 hours period revealed no changes in reading. The same germination rate was noted during the previous 84 hours observation. This means that the germination period of mustard green seeds only takes 84 hours (3.5 days) (Table 4). Obviously, advanced vegetative growth was observed in 900ppm GA treated unexpired seeds (Treatment 3) and 900ppm GA treated expired seeds (Treatment 2) with visible appearances of greener leaves and epicotyl growth (Figure 4). These findings correspond with the findings of Balaguera-López, Cárdenas-Hernández, and Álvarez-Herrera, (2009) that soaking seeds in 900 mg L-1 GA3 results in the high germination percentage, and average sprouting rate in tomato.
Similarly, Rojas-Arechiga, Aguilar, Golubov, and Mandujano (2011) studied on the tittle effect of gibberellic acid on germination of seeds of five species of cacti from the chihuahuan desert in northern mexico.

The researchers reported that for *Cylindropuntia imbricata,* addition of high concentrations (1,500 ppm) of gibberellic acid gave a 30% germination similar to the control; for *Opuntia rastrera*, medium concentrations (1,000 ppm) gave <40% germination; and for *O. microdasys*, low concentrations (500 ppm) gave 35% germination. Contrary the researchers discussed that high concentrations restricted germination. *Opuntia macrocentra* and *Cylindropuntia leptocaulis* did not differ significantly from the control. In addition, the study of Javanmard, Zamani, Keshavarz Afshar, Hashemi and Struik (2014) also highlights that the highest germination percentage (61.2%) was obtained following seed washing for 24h, followed by 500 mg l–1 GA3 treatment, then 6 weeks of cold stratification.

The counterpart control had germination percentage of 0%. The highest rate of seed germination was observed following 24 h of seed washing, then 1,000 mg l–1 GA3 treatment and 8 weeks of cold stratification (3.8 seed d–1), but this combined treatment did not differ significantly ($P \leq 0.05$) from seed washing, 1,000 mg l–1 GA3, and 6 weeks of cold stratification (3.6 seeds d–1).

Summary and Conclusion

Application of Gibberellic Acid significantly enhances the germination of both expired and unexpired commercial mustard (*Brasicca juncea*) seeds. 900ppm GA is able to improve germination rate of unexpired mustard seeds by 40% within 36 days, and 1% within the same duration for expired commercial mustard seeds. Same concentration of GA is able to increase germination of unexpired mustard seeds by 17% and 19% for unexpired counterpart within 60 hours. Within 84 hours, GA is able to improve germination of expired mustard seeds by additional 34%. The Control Unexpired Seeds (Treatment 3) had the same germination percentage of 98% within 80 hours period. Result of the experiment implied that applying 900ppm GA significantly enhanced germination performance of both expired and unexpired mustard green seeds, and maximum percent germination could be attained within a 84 hours.

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