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Effects of gibberellic acid on seed dormancy of black gram (*Vigna mungo* L.)

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ABSTRACT

Black gram or Urdbean (*Vigna mungo*) is a widely cultivated pulse crop in India. The seeds of this crop show dormancy and are not able to germinate as and when required. Investigation of the effect of gibberellic acid (GA_3) in breaking the dormancy of black gram seeds is the objective of this experiment. To break the dormancy, seeds were soaked with different concentrations of GA_3 along with hydro primed seeds and control seeds for 3 h and shade dried. Evaluation of germination and seedling growth of these primed seeds under controlled environment conditions revealed that the seed treated with 350 ppm of GA_3 observed in maximum physiological parameters. Hence, the treatment of seeds with 350 ppm of GA_3 is an effective dormancy-breaking treatment in black gram.

1. INTRODUCTION

Black gram belonging to Fabaceae family is on among the major pulse crops in area and productivity. Black grams have a high nutritive value containing 20–25% proteins, 40–47% starch, and other essential vitamins and carbohydrates. Major constraints during seed testing and sowing of *Vigna* species are the presence of hard seeds [1].

Leguminous seeds have high dormancy due to the seed structure. Black gram has seed dormancy of about 3–4 months. The classification of dormancy [2] five classes of dormancy based on the embryo or other components of the seed. Dormancy occurring in pulses is physical dormancy, the frequency of physical dormancy increases with the environment [3]. Black gram has only physical dormancy due to water-impermeable layers within the seed coat [4]. When the seeds are exposed to water the seeds that cannot absorb the water due to the impermeable layer, absorption of water is possible only after the removal of the impermeable layer by seed scarification. In black gram, due to the seed dormancy, the germination percentage is less and there will be irregular seed germination and establishment, leading to the subsequent yield loss.

Increased plant establishment and increase in yield productivity are highly related to seed quality. Various technologies are involved in increasing germination, seedling growth, and productivity. Seed priming is one of the techniques, which is highly effective, inexpensive and of low risk [5]. Pre-sowing seed treatment priming includes soaking of seeds in water, chemicals, micronutrients, biocontrol agents, or bio-fertilizers, allowing the metabolic actions before germination in the seeds; hence, there is reduction in the germination time and increase in the efficiency of seedling establishment increasing the germination percentage of seeds [6].

In the chemical treatments to improve seed germination and establishment, seeds are treated with growth regulators like gibberellic acid (GA_3) [7]. GA_3 is a natural regulator highly influencing the physiological parameters of plants and, hence, has multiple uses in the agriculture and horticulture industry. The important impact of GA_3 is the enhancement of seed germination, which has been demonstrated in ornamental plants [8]. During germination, GA_3 is released from the embryo hence stimulating mRNA and production of α-amylase [9].

Improving the seed germination and seedling growth by breaking the seed dormancy can be done by seed priming technique, using

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different solutions when imbibed by the seed improves the seed germination. Several seed priming agents such as potassium nitrate and phytohormone like GA_3 can be used for effective priming of seeds [10]. The results of GA_3 treatments on dormant black gram seeds and their effects on overcoming the seed dormancy are discussed in the paper.

2. MATERIALS AND METHODS

2.1. Collection of Materials

The research was conducted to evaluate the effect of GA_3 on dormancy breaking and seedling growth on the black gram at "SRM College of Agricultural Sciences, SRM Institute of Science and Technology." The source material for the study genetically pure black gram seeds variety ADT 6 was collected from farm office at Tamil Nadu Agricultural University, Coimbatore.

2.2. Treatments

Freshly harvested black gram seeds were used for the study. Initially, a 1000 ppm GA_3 solution was prepared to make different concentrations for treatments. Presoaking of seeds in different concentrations of GA3 solution, that is, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ppm and without any treatment as control. Seeds soaked in the GA_3 for 3 h, then shade dried under room temperature. The physiological growth parameters were analyzed in the treated and control plants which were grown under controlled conditions.

2.3. Germination %

Four hundred seeds used for conducting the seed germination test by roll towel method [11], the temperature and RH maintained at 25 ± 2°C and 95 ± 2%, respectively. At end of the test on 7th day, the total number of normal seedlings was counted and expressed in percentage.

$$\text{Germination } (\%) = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

2.4. Root Length (cm)

At the final count, ten normal seedlings were selected from each treatment, for measuring root length and removing the plant intact with the entire root system. Between the collar and the tip of the root, the root's length was measured, and its mean was given in centimeters.

2.5. Shoot Length (cm)

Measure the shoot length with same seedlings used for root length measurement. The shoot length was recorded length between the collar and tip of the primary leaf and their mean was reported in centimeter.

2.6. Vigor Index

The following formula was used to determine the vigor index, and the mean values were expressed as whole numbers [12].

"Vigour index = Germination percentage × Total seedling length (cm)"

"[Total seedling length = Root length (cm) + Shoot length (cm)]"

2.7. Statistical Analysis

Completely randomized block design was used for the experiment, and there were four replications.

3. RESULTS AND DISCUSSION

Black gram seeds were subjected to GA_3 treatments with different concentrations and recorded seed quality parameters. All physiological measures, including seed germination per cent, seedling lengths, and vigor index, were significantly affected by the treatments. The seeds primed with 350 ppm of GA_3 recorded higher germination per cent (88%) which was on par with 400 ppm and 450 ppm of GA_3, and was significantly greater than the control which showed the lowest germination percentage with 26% [Figure 1]. From the outcomes of the experiments, the germination and growth of dormant black gram seeds are significantly influenced by the GA_3. In the study, GA_3 had significant effect of two functions on the seed germination. Initially, GA_3 increases the growing potential of embryo hence promoting the germination. Later, GA_3 plays significant role in overcoming the mechanical constrains due to hard seed coats by weakening the tissues around the radicle [13].

During the exogenous application of gibberellins, their main function is to compensate for the inhibition caused by abscisic acid and while endogenous application, there will be an increase in GA_3 production playing a major role in seed germination. GA_3 has a major role in overcoming dormancy and controlling the hydrolysis of reserves. The amount of GA_3 present stimulates the synthesis, activation, and secretion of hydrolytic enzymes, primarily-amylase, which releases reducing sugars and amino acids necessary for embryo growth [14].

Black gram ADT 6 variety treated with 300 ppm GA_3 increased the germination and was found effective in breaking the dormancy compared to other seeds. According to the results of [15], the same treatment improved germination percentage and vigor index in lentil seeds. With the increased germination percentage, while soaking the seeds in GA_3, there is the stimulation of cytological enzymes that produce the enzyme α–amylase; hence, there is the fast conversion of insoluble starch into soluble sugars initiating root and shoot development, removing the barriers during metabolism [16]. GA_3 can leach out the inhibitors in germination, in turn, breaking the dormancy of seeds. The role of GA_3 in leaching out inhibitors is considered an important role in germination [17,18].

GA_3 at different concentrations shows increased germination percentage as it induces the production of mRNA molecules that codes for hydrolytic enzymes in the germinating seeds [19]. Degradation of food reserves accumulated in the endosperm is degraded by the hydrolytic enzymes mainly amylase and protease. The energy and nourishment for proper seed germination are attained from the degradation of carbohydrates and proteins in the endosperm [20]. The results prove the findings of [21], the required conditions for absorption of water and cell growth, like an increase in the cell size is done by GA_3, by the release and transition of calcium to the cytoplasm from the cell wall. After the cell growth, calcium returns to the cell wall and stiffens the cell structure. By the absorption of water by seeds, GA_3 is produced by the embryo inducing the synthesis of α- and β-amylase the hydrolytic enzyme in the aleurone layer involved in the conversion of starch and glucose which are absorbed by the embryo. In the endosperm, GA_3 increases DNA replication and dietary material analysis by acting on the proteins that make mRNA [22].

The root's length was measured in centimeters from the collar to the tip, with the mean length being recorded and used as the root length. From the observations, the best result was observed in the concentration of 300 ppm and 350 ppm which expressed a root length of 15.2 cm of GA_3 considering the control which expressed the lowest

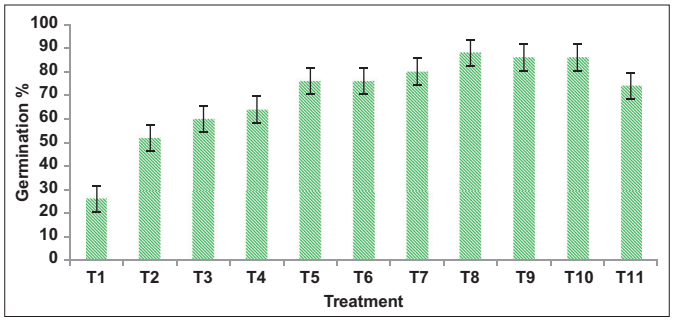

Figure 1: Effect on gibberellic acid (GA_3) treatments on germination percentage of black gram. Treatment details: T_1 – Control, T_2 – 50 ppm, T_3 – 100 ppm, T_4 – 150 ppm, T_5 – 200 ppm, T_6 – 250 ppm, T_7 – 300 ppm, T_8 – 350 ppm, T_9 – 400 ppm, T_{10} – 450 ppm, and T_{11} – 500 ppm.

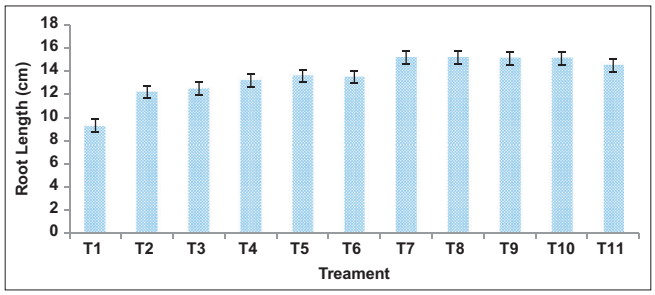

Figure 2: Effect on GA_3 treatments on root length (cm) of black gram. Treatment details: T_1 – Control, T_2 – 50 ppm, T_3 – 100 ppm, T_4 – 150 ppm, T_5 – 200 ppm, T_6 – 250 ppm, T_7 – 300 ppm, T_8 – 350 ppm, T_9 – 400 ppm, T_{10} – 450 ppm, and T_{11} – 500 ppm.

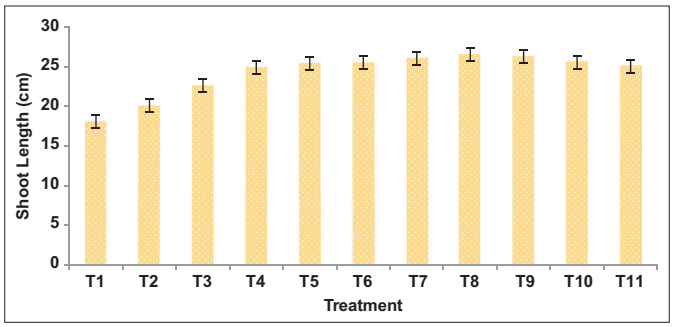

Figure 3: Effect on GA_3 treatments on shoot length (cm) of black gram. Treatment details: T_1 – Control, T_2 – 50 ppm, T_3 – 100 ppm, T_4 – 150 ppm, T_5 – 200 ppm, T_6 – 250 ppm, T_7 – 300 ppm, T_8 – 350 ppm, T_9 – 400 ppm, T_{10} – 450 ppm, and T_{11} – 500 ppm.

root length of 9.3 cm [Figure 2]. The length from base of the seedlings to the top most leaf in each seedling was measured in centimeter and the mean length recorded taken as the shoot length. The longest shoot length was 26.6 cm observed in seeds primed with 350 ppm of GA_3 as compared to control which expressed the 18.1 cm shortest shoot length [Figure 3]. When seeds are treated with GA_3, they showed a higher root length of 15.2 cm in 300 ppm and 350 ppm and shoot length of 26.6 cm in 350 ppm. Hence proving the role of GA3 in inducing root and shoot growth by inducing mitotic division in the respective regions of plants [23]. An increase in root and shoot growth by the increased cell division and cell elongation in the cambium region of the internodes is due to the stimulation of GA_3 [24]. GA_3 treatment

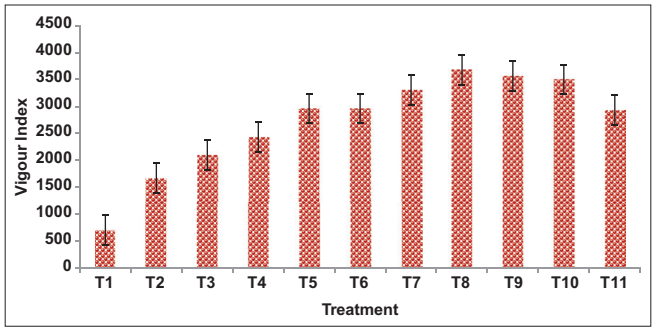

Figure 4: Effect on GA_3 treatments on vigor index of black gram. Treatment details: T_1 – Control, T_2 – 50 ppm, T_3 .100 ppm, T_4 - 150 ppm, T_5 – 200 ppm, T_6 – 250 ppm, T_7 – 300 pp m, T_8 – 350 ppm, T_9 – 400 ppm, T_{10} – 450 ppm, and T_{11} – 500 ppm.

activated the dormant embryo in the seed and improved the shoot growth by increasing the cell division, elongation, and multiplication, which is proved by the increased seedling length. The result coincides with the results of [25,26].

While comparing the calculated values of the vigor index with that of the control, the seeds treated with 350 ppm of GA_3 exhibited the highest value of vigor index of 3678, compared to the control which expressed the lowest vigor index of 712 [Figure 4]. GA_3-treated seed shows maximum vigor index of about of 2941 at 400 ppm. This result agrees with the findings of [16]. Priming has a great impact on seed germination, and seedling length. GA_3 is present in low concentrations in plants by its high impact in the growth and development of plants. The application of GA_3 induces positive impacts when applied at adequate quantities, when the concentration increases that there is an adverse effect on the plant. The germination percentage and seedling length have a direct relation to the vigor index of the seed. Hence, higher vigor index of GA_3-treated seeds is due to the influence of GA_3 on the germination and seedling length in chickpeas in the laboratory [27]. Therefore, it can be concluded that seeds treated with 350 ppm GA_3 were found to be most effective and could be recommended for breaking the dormancy of black gram seeds.

4. CONCLUSION

The present study revealed that seeds treated with 350 ppm of GA_3 showed maximum physiological growth parameters, namely, seed germination per cent, seedling length, and higher vigor index. Hence, the priming with GA_3 at a rate of 350 ppm can be effectively used as a pre-germinative treatment to break the dormancy in black gram.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

Ethics approval was not required for this study.

9. DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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