www.sospublication.co.in Journal of Advanced Laboratory Research in Biology

We- together to save yourself society e-ISSN 0976-7614

Volume 3, Issue 3, July 2012 Research Article

Comparative Study for the Effect of Gibberellic acid, Kinetin and Indole-3-acetic acid on Seed Germination performance of *Dianthus caryophyllus*

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Abstract: Seed germination is the major limiting factor for large-scale production and cultivation of crop species. Such attribute also positively as well as negatively affected by some potent plant growth regulators and other chemical compounds. For this, present experiment was undertaken with an objective to investigate the comparison of the effect of various concentrations of plant growth regulators like Gibberellic Acid (GA_3), Kinetin and Indole-3-acetic acid (IAA) on seed germination of *Dianthus caryophyllus* or Carnation. *Dianthus* seeds were soaked in different concentrations (0 ppm or control, 10, 20, 30 and 40 ppm) of each of GA_3, Kinetin and IAA for 24 h at room temperature (25±2°C). Three replicates of each treatment with ten seeds per replicate were arranged for precise physiological analysis. Significant variation was found in all aspects after analysis of variance (ANOVA) of each mean value. After two weeks of seed soaking, it was noted that germination percentages were significantly accelerated by lower concentrations (10 and 20 ppm) of used hormones. Amongst the three potential growth regulators, 20 ppm was found most effective because it showed highest germination percentage for GA_3 (87.46%), Kinetin (78.92%) and IAA (75.35%). A great deal of information relating to seed germination practices shows that these plant growth regulators were efficient to overcoming dormancy leading to rapid seed germination. GA_3 was selected as best hormone, in this study, which showed highest seed germination. These results could useful to large-scale cultivation of *Dianthus caryophyllus* plants for improving its floricultural impact worldwide.

Keywords: Carnation, *Dianthus caryophyllus*, germination percent, plant.

1. Introduction

Poor seed germination is the major limiting factor of *Dianthus caryophyllus* for large-scale production and cultivation. Seed germination can be controlled by many factors like natural germination (growth) inhibitors [1]. These are the derivatives of benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic and Abscisic Acid (ABA). They interrupt gene expression or evoke enzyme inhibition [2, 3]; thus block the responses induced by any of several growth promoters. This inhibition of such physiological responses was removed by the use of certain growth regulators such as indole-3-acetic acid (IAA), gibberellic acid (GA_3) and in some cases cytokinins [4, 5]. Some plant extracts will inhibit the growth induced by GA_3 in pea and maize seedlings [6]. It has been postulated that seed coat (testa) of many plant species contains a considerable amount of germination inhibitor which prevents their seed germination [7]. The applications of gibberellins increase the seed germination percentage by attributing the fact that they increase the amino acid content in embryo and cause release of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination. GA acts synergistically with auxins, cytokinins and probably

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with the other plant hormones, is what might be called a systematic approach, or synergism. The overall development of the plant is regulated by the growth hormones, nutrient and environmental factors. They also vary in their germination requirement [8]. It is not known that in which concentrations, these hormones will cause a response in the cell. This investigation with growth hormones will help in determining that which of the hormone concentration is suitable for seed germination and proper seedling growth. This analysis has been considered necessary for the beneficial effect of presoaking treatment of seeds with a plant growth regulator and other substances have been reported in the present literature repeatedly.

As for growth regulators, auxins and cytokinins are most frequently used in nutrient media, to increase the percentage of seed germination. As reported by Mitra [9], cytokinins as such or in combination with Indole-3-acetic Acid (IAA) stimulate germination. On the other hand, Hadley [10] found that 1-10 ppm kinetin with or without the auxin: IAA can inhibit seed germination in *Dactylorhiza purpurella*. Gibberellins are used as media additives only marginally. These growth regulators were also linked with several types of abiotic stress responses, especially salinity and water stress in plant species [11]. Gibberellic Acid (GA_3) is known to be concerned with the regulation of plant responses to the external environment [12], also the application of another plant growth bioregulator has increased the saline tolerance of many crop plants [13, 14]. GA_3 has also been shown to alleviate the effects of salt stress on water use efficiency [15]. Das Gupta *et al*., [16] recorded that foliar application of plant growth regulators like IAA and GA helped the plant to restore retardation in water content in Mungbean plants subjected to water stress. Chakrabarti and Mukherji [17] noticed that GA_3 used to overcome the adverse effects on Mungbean plants. The role of plant growth regulators in overcoming the harmful effects of salinity on growth may be due to the change in the endogenous growth regulators which affects plant water balance. In view of the above background, the present investigation was undertaken to study the influence of growth substances like Gibberellic Acid (GA_3), Kinetin and Indole-3-acetic Acid (IAA) by their different concentration on seed germination, radicle and plumule elongation to draw the information of timing and control of seed germination and seedling growth of a species in their natural habitat.

2. Materials and Methods

The present investigation was conducted at the Botany Department (UGC Center for Advance Studies),The University of Burdwan, with an objective to determine the effect of various concentrations of growth regulators, such as Gibberellic Acid (GA_3), Kinetin and Indole-3-acetic Acid (IAA), on the rate of seed germination and growth. Pure line healthy seeds of experimental plant material i.e., *Dianthus caryophyllus* L. or Carnation (annual in nature), were obtained from Globe Nursery, Kolkata. It was suitable to grow in Burdwan agro-climatic conditions under proper sown condition. The moisture content of the seeds was determined by using a hot air oven at 103°C for 12 h. The moisture percent was found within the recommended value of 9.2. Healthy seeds were at the first surface sterilized by 0.01% (w/v) mercuric chloride ($HgCl_2$) for 5 minutes and thoroughly washed thrice with single distilled water for 10 min. in each and then presoaked with double distilled water for 10 h to initiate metabolic activities. After pre-soaking the seeds were blotted to dry [18-20]. To determine the germination response of Carnation seeds in different concentrations of used three potent plant growth regulators, seeds were placed on Whatman No. 1 filter paper in 8.5cm diameter plastic Petri dishes and moistened with 5mL of freshly prepared test solution of Gibberellic Acid (GA_3), Kinetin and Indole-3-Acetic Acid (IAA). Lids of the Petri dishes were closed as an added precaution against the water loss due to evaporation. Three replicates of 10 seeds were used for each treatment and maintained at a 25±2°C temperature in a B.O.D. Incubator cum germinator. Seeds were considered to be germinated at the emergence of the radical. The concentrations of the test solutions used for the treatment were 10 ppm, 20, 30 and 40 ppm for Gibberellic acid, Kinetin and Indole-3-acetic acid with a separate control check using the distilled water treatment (Table 1). These were soaked for 24 h in the above concentrations and only double distilled water for the control set. Seed germination percentage was determined by the method given by Kumari and Dahiya [21], after 14 days of the treatment. Mean values were subjected to analysis of variance (ANOVA) to test the significance for germination percent as per the methodology advocated by Panse and Sukhatme [22].

3. Results and Discussion

The Analysis of Variance (ANOVA) of germination data from growth regulators treated seeds indicated that they were all statistically significant ($P<0.05$). It was also revealed that the value of Coefficient of Variation (CV%) ranges from 6.48 for Kinetin to 9.27 for IAA. Here, Gibberellic acid showed higher Critical Difference (CD) value i.e., 0.758, Control check showed moderate (0.647) and Kinetin represented lower CD value (0.593). The significant Critical Difference (CD) values indicate that *Dianthus* cultivar was suitable for the treatments. The higher CD value indicates higher stability in that experimental environment [23].

Table 1. The treatments of Gibberellic Acid (GA_3) Kinetin and Indole-3-Acetic Acid (IAA) with their different concentrations (parts per million).

Treatments	Growth hormone	Concentration (ppm)	Seed soaked time (h)
T_1 (Control)	----	----	24 h
T_2	GA_3	10	24 h
T_3		20	24 h
T_4		30	24 h
T_5		40	24 h
T_6	Kinetin	10	24 h
T_7		20	24 h
T_8		30	24 h
T_9		40	24 h
T_{10}	IAA	10	24 h
T_{11}		20	24 h
T_{12}		30	24 h
T_{13}		40	24 h

Table 2. Seed germination response (%) of *Dianthus caryophyllus* to three potent plant growth regulators (GA_3, Kinetin and IAA) under different concentrations (10, 20, 30 and 40 ppm) after 14 days of treatment at 25±2°C temperature.

Growth Regulators	Concentrations	Germination percent (%)	S. E. (±)	C. D. (P<0.05)	C.V. (%)
CONTROL	-----	60.23	0.203	0.647	7.19
GA_3	10 ppm	72.08	0.147	0.758	8.62
	20 ppm	87.46	0.142		
	30 ppm	63.34	0.156		
	40 ppm	54.27	0.107		
KINETIN	10 ppm	69.54	0.172	0.593	6.48
	20 ppm	78.92	0.194		
	30 ppm	61.28	0.153		
	40 ppm	47.71	0.182		
IAA	10 ppm	66.64	0.156	0.601	9.27
	20 ppm	75.35	0.157		
	30 ppm	62.75	0.136		
	40 ppm	56.19	0.142		

The moderate seed germination percentage was observed in Control set, i.e. 60.23% (Table 2). Amongst the used plant growth regulators, 20 ppm concentration was found most suitable because it showed the highest germination percentage for Gibberellic acid (87.46%), Kinetin (78.92%) and Indole-3-acetic acid (75.35%). The seeds treated with GA_3 showed significant difference to control. Germination percentage under the GA_3 treatment at 20 ppm concentration (87.46%) was recorded as maximum. Both 30 and 40 ppm concentration of GA_3 did not show any major difference in respect of germination which meant the higher concentration was not as well as the lowest concentration rather it decreased the germination percent. Kinetin in the 40 ppm concentration showed the least germination (47.71%).

Observation showed that germination percent was increased from 10 ppm to 20 ppm treatments, while this parameter decreased when the concentrations were further increased up to 40 ppm. This present study showed that growth regulator in higher concentrations inhibits the seed germination.

In the Kinetin and IAA treatments, plumule elongation was found in decreasing trend with the increase of hormonal concentration (data were not shown). It was observed that for germination enhancement of *Dianthus*, GA_3 with lower concentration was best suited, but in the case of radical and plumule elongation, these three hormones did not show any significant effect. When the germination percentage of three growth regulators were compared (Fig. 1), GA_3 was observed more effective than Kinetin and IAA, which was in accordance with Chakrabarti and Mukherji [12]. The application of another plant growth regulator could increase the seed germination and other physiological activity by the reason of tolerance to the toxic effects/particles which was found inconsistent with the finding of Harous *et al*., [13]; Hoque and Hoque [14]. With the more effectiveness of low concentration of GA_3 (that is ratio of growth hormone and water) could restore retardation in water content, this may able to tolerance to water stress. This result was considered in parallel to the findings of Das Gupta *et al*., [16]. The growth regulating substances may be acting to enhance the synthesis of enzyme proteins and thereby stimulate the germination process. Kinetin helps to overcome decreased protein synthesis of tobacco plants [24]. Similar reports are available for GA_3 and IAA [25].

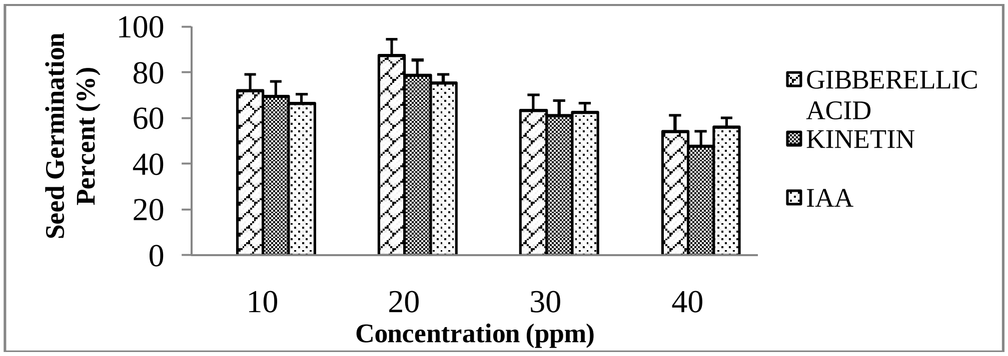

Fig. 1. Graphical representation showing the effect of Gibberellic acid (GA_3), Kinetin and Indole-3-acetic acid (IAA) under four different concentrations (10, 20, 30 and 40 ppm) on seed germination of *Dianthus caryophyllus*.

Although varied in seed germination and root-shoot elongation by different treatments, the pre-soaking with different treatments evident that soaked seed could improve in germination and seedling establishment and this observation was found equivalent the observation of Harris *et al*., [26]. The soaking period of 24 h increased the total uptake of water which helps the maximum imbibition rate. This, in turn, was aid to the quick biochemical changes and the time period was found suitable for seed germination. The same experiment was conducted in Black gram and Horse gram [27].

From the data presented in Table 2, it has been shown that GA_3 could overcome the adverse effects in *Dianthus caryophyllus* than Kinetin and IAA in the seed physiological activity, which supports the finding of Chakrabarti and Mukherji [17]; Mikulik and Vinter [28]. The role of plant growth regulators in overcoming the harmful effects on growth may be due to the change in the endogenous growth regulators [29]. It has been confirmed that exogenous application of Gibberellic acid was founded to promote seed germination of many plants [1, 30].

4. Conclusion

From the above discussion, it was concluded that Gibberellic Acid (GA_3) showed the highest germination percentage in *Dianthus caryophyllus* as compared to Kinetin and Indole-3-Acetic Acid (IAA). Germination percentage was decreased according to the increasing concentrations of the used plant growth regulators. But in the case of radical and plumule elongation, these hormones did not show any significant effect on the said floricultural crop. This indicates that the lower concentration of growth regulators favour the increased enzymatic activity which leads to the favorable environment for the germination as well as the growth of the radical and plumule.

Acknowledgment

We are thankful to Prof. Alok Bhattacharya, Head, Department of Botany (UGC-CAS), The University of Burdwan to give the facilities and funds for doing this research work.

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