Effect of Gibberellic Acid, Kinetin and Indole 3-Acetic Acid on Seed Germination Performance of *Dianthus caryophyllus* (Carnation)

Rajib ROYCHOWDHURY [1] (✉)
Anuj MAMGAIN [2]
Sunanda RAY [3]
Jagatpati TAH [3]

Summary

The experiment was undertaken with an objective to investigate the effect of various concentrations of plant growth regulators, i.e., Gibberellic acid (GA_3), Kinetin and Indole 3-acetic acid (IAA) on seed germination of *Dianthus caryophyllus*. *Dianthus* seeds were soaked in different concentrations (0 ppm or control, 10 ppm, 20 ppm, 30 ppm and 40 ppm) of each of GA_3, Kinetin and IAA for 24 h at room temperature (25±2^{o}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 in overcoming dormancy leading to rapid seed germination. GA_3 was selected as best hormone in this study, which showed highest seed germination (87.46%). These results could be useful in large scale cultivation of *Dianthus caryophyllus* plants to improve its floricultural impact worldwide.

Key words

Dianthus caryophyllus, Gibberellic acid, Indole 3-acetic acid, Kinetin, Seed germination

[1] Visva-Bharati, Department of Biotechnology, School of Life Sciences
Santiniketan - 731235, West Bengal, India
✉ e-mail: rajibroychowdhurybu@gmail.com
[2] Visva-Bharati, Department of Plant Protection, Institute of Agriculture
Sriniketan - 731236, West Bengal, India
[3] The University of Burdwan, Botany Department (UGC-CAS)
Burdwan - 713104, West Bengal, India

Received: March 2, 2012 | Accepted: April 18, 2012

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 (Taiz and Zeiger, 1991). These are the derivatives of benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic and abscisic acid (ABA). They interrupt gene expression or evoke enzyme inhibition (Karssen et al., 1987; Prochazka, 1998); 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 (Phillips, 1962; Wright, 1968). Some plant extracts will inhibit the growth induced by GA_3 in pea and maize seedlings (Corcoran and West, 1968). It has been postulated that seed coat (testa) of many plant species contain considerable amount of germination inhibitor, which prevent their seed germination (EL-Barghathi and EL-Bakkosh, 2005). The application of gibberellins increases the seed germination percentage by increase of the amino acid content in embryo and they 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 with the other plant hormones; what might be called a system approach, or synergism. The overall development of plant is regulated by the growth hormones, nutrient and environmental factors. They also vary in their germination requirement (Chauhan et al., 2009). It is not known in which concentrations these hormones will cause a response in the cell. This investigation with growth hormones will help in determining which of the hormonal concentration is suitable for seed germination and proper seedling growth. This analysis is considered necessary since the beneficial effect of presoaking treatment of seeds with 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 for nutrient media, to increase the percentage of seed germination. As reported by Mitra (1989), cytokinins as such or in combination with indole acetic acid (IAA) stimulate germination. On the other hand, Hadley (1970) found that 1–10 ppm of 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 (Mohammed, 2007). Gibberellic acid (GA_3) is known to be concerned in the regulation of plant responses to the external environment (Chakrabarti and Mukherji, 2003), also, application of another plant growth bio-regulator has increased the saline tolerance of many crop plants (Haroun et al., 1991; Hoque and Haque, 2002). GA_3 has also been shown to alleviate the effects of salt stress on water use efficiency (Aldesuquy and Ibrahim, 2001). Das Gupta et al. (1994) 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 (2002) noticed that GA_3 was used to overcome the adverse effects in 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 that affect plant water balance. In view of the above back ground, 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.

Material and methods

The present investigation was conducted at 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. 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 first surface sterilized by 0.01% (w/v) mercuric chloride ($HgCl_2$) for five minutes and thoroughly washed thrice with single distilled water for 10 minutes and then presoaked with double distilled water for 10 hours to initiate metabolic activities. After presoaking the seeds were blotted dry (Roychowdhury and Tah, 2011; Roychowdhury and Tah, 2011a; Roychowdhury and Tah, 2011c; Roychowdhury et al., 2012). To determine the germination response of Carnation seeds in different concentrations of used three potent plant growth regulators, seeds were placed on Whatman's No. 1 filter paper in 8.5 cm diameter plastic Petri-dishes and moistened with 5 ml 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 25±2°C temperature in a B.O.D. incubator cum germinator. Seeds were considered to be germinated at the emergence of the radicle (Roychowdhury et al., 2012). The concentrations of the

Table 1. The treatments of GA_3, Kinetin and IAA with their different concentrations

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

test solutions used for the treatment were 10 ppm, 20 ppm, 30 ppm 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 Dahiya and Kumari (2007), after 14 days of 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 (1967).

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 also revealed that the value of coefficient of variation (CV %) ranges from 6.48 for Kinetin to 9.27 for IAA. 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 (Roychowdhury and Tah, 2011; Roychowdhury and Tah, 2011b; Roychowdhury et al., 2011d). Here, Gibberellic acid showed higher CD value (0.758), Control check showed moderate (0.647) and Kinetin represented lower CD value, i.e. 0.593.

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 the 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 that meant the higher concentration was not as good as the lower concentration; rather it decreased the germination percent. Kinetin in 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 not shown). It was observed that for germination enhancement of *Dianthus*, GA_3 with lower concentration was best suited, but in case of radicle and plumule elongation, these three hormones did not show any significant effect. When the germination percentage of three growth regulators were compared (Figure 1), GA_3 was observed more effective than Kinetin and IAA, which was in accordance with Chakrabarti and Mukherji (2003). 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 in consistent with the finding of Harous et al. (1991) and Hoque and Hoque (2002). 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 be able in tolerance to water stress. This result was considered in parallel to the findings of Das Gupta et al. (1994). The growth

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	Concentra–tions	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		

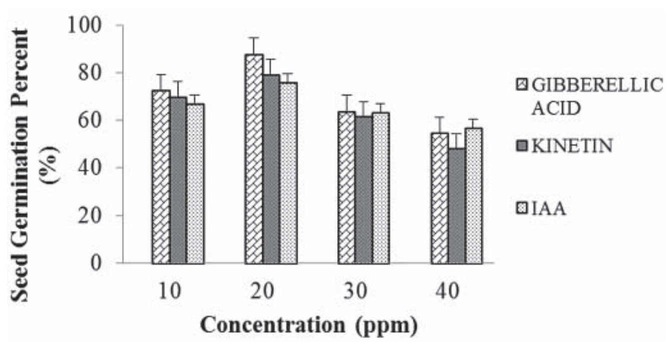

Figure 1. Graphical representation showing the effect of Gibberellic acid (GA_3), Kinetin and Indole 3-acetic acid (IAA) under four different concentrations (10, 20, 30 and 40 ppm) on seed germination of *Dianthus caryophyllus*

regulating substances may be acting to enhance the synthesis of enzyme proteins and thereby stimulate germination process. Kinetin helps to overcome decreased protein synthesis of tobacco plants (Ben-Zioni et al., 1967). Similar reports are available for GA_3 and IAA (Levitt, 1980). Although varied in seed germination and root-shoot elongation by different treatments, the pre-soaking with different treatments was evident that soaked seed could improve in germination and seedling establishment and this observation was found equivalent the observation of Harris et al. (1999). 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 time period was found suitable for seed germination. Same experiment was conducted in Black gram and Horse gram by Mohanty and Sahoo (2006).

From the data presented in Table 2, it has been shown that GA_3 could overcome the adverse effects in *Dianthus caryophyllus* better than Kinetin and IAA in the seed physiological activity, which supports the finding of Chakrabarti and Mukherji

(2002) and Mikulik and Vinter (2002). The role of plant growth regulators in overcoming the harmful effects on growth may be due to the change in the endogenous growth regulators (Izumi and Eiji, 1996). It has been confirmed that exogenous application of Gibberellic acid promotes seed germination of many plants (Carcoren, 1970; Taiz and Zeiger, 2010).

Conclusion

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

References

Aldesuquy H. S., Ibrahim A. H. (20010. Interactive effect of seawater and growth bio-regulators on water relations, absicisic acid concentration and yield of wheat plants. J Agron & Crop Sci 187: 185-193

Ben-Zioni A., Itai C., Vaadia Y. (1967). Water and salt stresses, kinetin and protein synthesis in tobacco leaves. Plant Physiol 42: 361-365

Chakrabarti N., Mukherji S. (2002). Effect of phytohormone pretreatment on metabolic changes in *Vigna radiate* under salt stress. J Environ Biol 23: 295-300

Chakrabarti N., Mukherji S. (2003). Effect of Phytohormone pretreatment on nitrogen metabolism in *Vigna radiate* under salt stress. Biol Planta 46: 63-66

Chauhan J. S., Tomar Y. K., Singh I. N., Ali S., Debarati A. (2009). Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. J American Sci 5(5):79-84

Corcoran M. R. (1970). Inhibitors from carob (*Ceratonia siliqua* L.) II. Effect on growth induced by Indole acetic acid or Gibberellins A_1, A_4, A_5 and A_7. Plant Physiol 46: 531-534

Corcoran M. R., West C. A. (1968). Inhibitors from carob (*Ceratonia siliqua* L.) I. Nature of the interaction with Gibberellic acid on shoot growth. Plant Physiol 43: 859-864

Dahiya, O.S. and Kumari, P. (2007). Seed germination: optimum temperature, moisture and light. In: Plant Physiology research methods (S S Narwal, eds), Scientific Publishers, Jodhpur, India

Das Gupta P., Das D., Mukherji S. (1994). Role of phytohormones in the reversal of stress-induced alteration in growth turgidity and proline accumulation in mungbean (*Vigna radiate* L. Wilczek) plants. Indian Biol 26: 343-348

EL-Barghatgi M. F., EL-Bakkosh A. (2005). Effect of some mechanical and chemical pre-treatments on seed germination and seedling growth of *Quercus coccifera* (Kemes Oaks). J Jerash Private University

Hadley G. (1970). The interaction of kinetin, auxin and other factors in the development of north temperate orchids. New Phytologist 69: 549–555

Haroun S. A., Badawy A. H., Shukry W. M. (1991). Auxin induced modification of *Zea mays* and *Lupinus termis* seedlings exposed to water stress imposed by polyethylene glycol (PEG 6000). Sci J 18: 335

Harris D., Joshi A., Khan P. A., Gothkar P., Sodhi P.S. (1999). On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. Exp Agric 35:15-29

Hoque M., Haque S. (2002). Effects of GA_3 and its mode of application on morphology and yield parameters of mungbean (*Vigna radiate* L.). Pak J Biol Sci 5: 281-283

Izumi Y., Hirasawa E. (1996). Gibberellin induces endopeptidase activity in detached cotyledons of *Pisum sativum*. J Plant Growth Regul 19 (1): 55-60

Karssen C. M., Groot S. P. C., Koornneef M. (1987). Hormone mutants and seed dormancy in *Arabidopsis* and tomato. In: Developmental Mutants in Higher Plants (H Thomas, D Grierson, eds), Cambridge University Press, London, UK, 119-134

Levitt J. (1980). Responses of plants to environmental stresses. Vol. II. Academic Press, New York

Mikulik J., Vinter V. (2002). Evaluation of factors affecting germination of *Dianthus superbus L.* subsp. *Superbus.* Biologica 40: 13-18

Mitra G. C. (1989). Biology, conservation, and culture of orchids. In: *in vitro* culture of orchid seeds for obtaining seedlings, East-West Press Pvt Ltd., India, 401–409

Mohammed A. H. M. A. (2007). Physiological Aspects of Mungbean Plant (*Vigna radiata* L. wilczek) in Response to Salt Stress and Gibberellic Acid Treatment. Res J Agric Biol Sci 3(4): 200-213

Mohanty S. K., Sahoo N. C. (2000). Effect of soaking period, seed size and growth regulators on imbibition and germination of seeds of some field crops. Orissa J Agric Res

Panse V. G., Sukhatme P.V. (1967). Statistical Methods for Agricultural Workers. 2nd Ed., ICAR publication, New Delhi, India

Phillps I. D. J. (1962). Some interactions of gibberellic acid with naringenin (5, 7, 4- trihydroxy flavanone) in the control of dormancy and grcwth in plants. J Exp Bot 13: 213-226

Prochazka J. (1998) Fyziologie rostlin. Academia, Praha

Roychowdhury R., Tah J. (2011). Mutation breeding in *Dianthus caryophyllus* L. for economic traits. Electronic Journal of Plant Breeding 2(2): 282-286

Roychowdhury R., Tah J. (2011a). Chemical mutagenic action on seed germination and related agro-metrical traits in M1 *Dianthus* generation. Current Botany 2(8): 19-23

Roychowdhury R., Tah J. (2011b). Genetic variability study for yield and associated quantitative characters in mutant genotypes of *Dianthus caryophyllus* L. International Journal of Biosciences 1(5): 38-44

Roychowdhury R., Tah J. (2011c). Assessment of chemical mutagenic effects in mutation breeding programme for M1 generation of Carnation (*Dianthus caryophyllus*). Research in Plant Biology 1(4): 23-32

Roychowdhury R., Tah J., Dalal T., Bandyopadhyay A. (2011d). Selection response and correlation studies for metrical traits in mutant carnation (*Dianthus caryophyllus* L.) genotypes. Continental Journal of Agricultural Science 5: 06-14.

Roychowdhury R., Alam M. J. F., Bishnu S., Dalal T., Tah J. (2012). Comparative study for chemical mutagenesis on seed germination, survivability and pollen sterility in M1 and M2 generations of *Dianthus*. Plant Breeding and Seed Science 65(1): 29-38.

Taiz L., Zeiger E. (2010) Plant Physiology. Sinauer Associates Inc., USA.

Wright S. T. C. (1968). Multiple and sequential roles of plant growth regulators. In: Biochemistry and Physiology of Plant Growth Substances (F Wightman, G Setterfield, eds), Runge Press, Ottawa, 521-542

acs77_30