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Screening of finest concentratrion of brassinolide by germination tests with pea (*Pisum sativum* L.)

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Abstract
Brassinosteroids have strong and unique biological activities when applied to plant tissue at nano or micro molar levels. As result of extensive investigation these were found to show characteristic physiological actions on the growth of plant in micro quantities. The experiment is a selection test consisting of 4 doses of brassinolide (BL) along with control i.e. distilled water. Here we have taken seeds of pea *(Pisum sativum* L.) for germination test and also some seedling growth parameters viz. germination rate index, speed of germination, vigour index. Best results were seen with 0.01mMBL and 0.05mMBL. This study is enlightening a way for the detailed observation in biochemical and molecular parameters, which will be the answers to all the "whys'" behind this.

Keywords: Screening, finest concentratrion, brassinolide, germination tests, (*Pisum sativum* L.)

Introduction
Brassinosteroids (BRs) are growth promoting natural products similar to animal steroidal hormone, found at low level in pollen, seeds and young vegetative tissues throughout the plant kingdom. The BRs are a class of plant originated steroidal lactones that exert pronounced growth promoting activities. Therefore, BRs can be regarded as a new class of plant hormone, in addition to auxin, gibberellin, cytokinin, abscisic acid and ethylene (Fujioka and Sakurai, 1997; Clouse and Sasse, 1998) [5, 3]. The first BR was isolated in 1979 from rapeseed (*Brassica napus* L.) pollen. At that time, 40 mg of the active substance was extracted from 40 kg of pollen. This discovery promoted an intensive research on the plant hormones. Nowadays, around 70 different BRs have been successfully isolated from plants not only in free form, but also in conjugation with sugars and fatty acids. BRs are able to influence plant growth and cell division even at very low concentrations, thus affecting growth performances. Moreover, BRs can influence plants' architecture, flowering and shedding of leaves. The positive effects of BRs on seed germination provided an important incentive to conduct this study. Many experiments also show that the application of brassinosteroids to seed improves both its germination and vigour.

Pea is the third most important pulse crop at global level, after dry bean and chickpea and third most popular *Rabi* pulse of India after chickpea and lentil. It provides a variety of vegetarian diet hence liked throughout the world. The mature seeds are used as whole or split into dal and put to use in various ways for human consumption. Beside vegetable purposes, it is also grown as a forage crop for cattle and cover crop to prevent soil erosion but mainly for matured seed for human consumption. Highly nutritive not only having protein 22-25%, Calcium 64 mg/100g, fat 0.8- 1%, iron 4.8 mg/100g, dietary fiber 13.4%, moisture 11%, carbohydrate 62.1% but also provides agronomic significance that being leguminous crop it leaves 25-30kg N/ha to the succeeding crops. Canada rank first in area (21%) and production (35%) at Global level. China stands second position in area (13.70%) followed by Russian Fed. (12.94 %). India occupy forth position in area (10.53 %) and 5th position in production (5.36 %). Highest productivity is recorded in Ireland (5000 kg/ha) followed by Netherland (4766 kg/ha), and Denmark (4048 kg/ha), while India's productivity is only 822 kg/ha. Due to the drought situation especially in Bundelkhand region of UP, farmers are discouraged to cultivate field peas. Therefore, the overall acreage for field peas has shortfall by 4%. Due to unsupportive weather the yield rate of the crop has fall down, as a result there is a major shortfall of 13.6% in Field pea / matar production in UP. Seed is the most basic need of a farmer for his livelihood. Seed germination is the first action performed by the seed, which gives us an idea about the whole crop life, although other factors are responsible for the status of crop.

Hence if we are improving seed germination and controlling seedling mortality, than we can control 30-35% crop loss.
In the current study our objective is to check the variation in seed germination and associated parameters of pea in different concentrations of BR.

Material and methods
Different concentrations of BL were taken i.e. 0.01, 0.025, 0.05 and 0.1mM along with control. The seeds of pea were washed and sterilized within 0.1% HgCl2 for 5 minutes. This process was followed by rinsing of seeds twice with distilled water and now the seeds were placed on filter paper kept in Petri-dishes of 15 cm diameter and 2 cm depth. For each replication, 8 Petri-dishes, each containing 10 seeds were allowed to germinate separately, at various concentrations against control. This pattern was replicated thrice. The parameters like percentage of germination, seedling length, vigour index etc. were measured at regular time interval. Germination parameters were determined according to following formulae at final days of observation:
A. Germinability (%G) = Total No. of Seeds Germinated X 100/Total No. of Seeds Sown
B. Vigour index (VI) = Germination (%) x seedling length (Abdul-Baki & Anderson, 1973).

Result and discussion
The observed data from the present investigation is tabulated below. In case of germination percentage the 0.05mM has performed best with 91.67%. This is followed by 2 concentrations of BL i.e. 0.01mM and 0.025mM with 73.33%. Minimum percentage of germination was found in the highest concentration i.e. 0.1mM, but it was higher than control. In case of root length of the seedling the highest length was found in 0.01mM (3.7cm), followed by control (2.93). Lowest was found in case of 0.1mM i.e. 0.9cm. Seedling shoot length measure was found non- significantly different from each other. Apart from that highest was found in 0.05mM and lowest length was measured in case of 0.1mM.
The root dry weight was found significantly high in case of 0.01mM (0.028g), which was followed by the distilled water i.e. the control (0.020g). The latter is closely related to 0.025mM i.e. 0.018g. The lowest was found in 0.1mM (0.008g), which was even lower than the control. The shoot dry weight difference among the various concentrations was non-significant. Still the highest weight was observed in case of 0.05mM followed by control and then 0.01Mm BL. Significant difference was found in case of vigour index and the highest VI was with 0.01mM followed by 0.05mM. Lowest VI was with the concentration of 0.1mM, which was lower to control also.
It is well known fact that Brassinosteroids promote seed germination and other germination attributing parameters, similar to other groups of hormones. The treatment of *Lepidium sativus* seeds (Jones Held *et al*., 1996) [7] with brassinolide improved germination percentage. Likewise, Brassinosteroids promoted seed germination in case of *Brassica napus* (Chang and Cai, 1988) [2], rice (Dong *et al*., 1989) [4], wheat (Hayat *et al*., 2003) [6] and tobacco (Leubner-Metzger, 2001) [8]. Young vegetative tissue is mostly responsive to BL and if endogenous BL is straight involved in the control of cell expansion, it must be present in such tissue. Approaches to establishing this include the analysis of levels in a Brassinolide-sensitive zone of pea stem (Sasse *et al*., 1992) and localization of an exogenously supplied 125I-BR, which accumulated in the elongating zone of mung bean epicotyls and the apex of cucumber seedlings (Xu *et al*., 1994) [9]. This experiment on the interaction of Brassinosteroids with morpho-physiology of pea seeds provides a credible body of evidence that these plant steroids in micro quantities are critical regulators of plant growth and development.
Effect of various concentrations of Brassinolide, KNO_3 and Thiourea on different seed germination parameters in pea. All the data is an average of triplicates.

Concentration	%G	Root Length (Cm)	Shoot Length (Cm)	Root Dry Weight(G)	Shoot Dry Weight(G)	VI
CONTROL	55	2.93	1.33	0.020	0.014	232.5
0.01mM	73.33	3.7*	1.57	0.028*	0.012	384.5*
0.025mM	73.33	2.5	0.53	0.018	0.002	223.0
0.05mM	91.67*	2.7	2.07	0.017	0.015	292.5
0.1mM	61.67	0.9	0.37	0.008	0.003	119.17
CD @5%	13.25	1.4	NS	0.009	NS	166.36

Conclusion
Above investigation can lead us towards a number of concluding points, which is showing a new ray of hope to a number of problems in the field of agriculture. From the above study we can finalize that the best concentration for the seed germination was 0.01 mM, which is more or less similar to 0.025mM and 0.05mM. The least performing concentration was 0.1mMBL, which was poorer than control. This kind of result is arising a number of queries in our mind, which can only be answered after the interdisciplinary study and activity on this topic.

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