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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Seed Treatment and Foliar Application of Brassinolide on Morpho-Physiological Parameter of Pea (*Pisum sativum* L.) Under Drought Stress

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ABSTRACT
To endow with nutrition to the growing population is a great challenge. Apart from that climate change is making barrier to increase production. Drought stress is one of the major abiotic stresses affecting plant growth and productivity globally. Keeping this in mind, the present investigation was undertaken in poly house of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during rabi season of this year to study consequence of seed treatment and foliar application of two concentrations i.e. 0.05mM and 0.01mM brassinolide on morpho-physiological parameters of pea (Pisum sativum L.) under drought. This study will enlighten the way to manage crop with new agricultural weapon like brassinolide to fight against drought stress.
Keywords*: drought, brassinolide, seed treatment, foliar spray, pea*

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INTRODUCTION

Water is the synonym of life. Water in excess or in deficit both causes devastation. World population is increasing in a geometric progression and quality water deficiency due to water pollution and ground water depletion is decreasing in a same rate. In such case to maintain or increase the productivity to feed such a huge population is a great challenge.

Drought causes a number of significant changes in the morpho-physiological characters of plants. The reduced amount of water during drought causes an increase in the osmotic pressure of plant cell. This increase in osmotic pressure permits the plant to utilize better soil moisture. Decreased leaf area is an early adaptive response to water deficit. In general, stomata lose their function and may die, because wilting after certain limit denatures the starch in guard cells and also in the mesophyll cells. Drought stress caused a large decline in the chlorophyll 'a' and 'b' contents and the total chlorophyll contents in all sunflower varieties investigated [16]. On the other hand CO_2 diffusion into the leaf is prevented due to decrease in stomatal opening and there by reduces photosynthetic activity in green cells [21]. Water shortage alters the chemical composition. For example starch is converted to sugar. Soluble sugars may also function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor [8]. Besides this, there is a considerable increase in nitrate nitrogen and protein synthesis is adversely affected. Apart from this the permeability to water and urea increases during drought. Drought is also a significant yield limiting factor in crop production. Decreasing water availability under drought generally results in limited total nutrient uptake and their diminished tissue concentrations in crop plants. An important effect of water deficit is on the acquisition of nutrients by the root and their transport to shoots. Proline accumulation is believed to play adaptive roles in plant stress tolerance. Proline metabolism in plants, however, has mainly been studied in response to osmotic stress [20].

Pea, being a legume crop of cool season of Northern India, is naturally, slightly resistant towards drought. Abiotic stresses are main factors negatively affecting growth and yield worldwide. Plants are

continuously confronted with the harsh environmental conditions such as salinity, drought, heat, cold, flooding and heavy metal contamination. Drought stress has been reported to severely reduce the germination and seedling stage [12]. In a study on pea, drought stress impaired the germination and early seedling growth of five cultivars tested [17].
The question arises as to how to mitigate drought stress and or making the plants tolerant for maintaining growth and development of plants under stress. Certain organic chemicals and/or phytohormones/plant growth regulators have been reported in the recent years to have their potentials in alleviating harmful effects of some abiotic stresses by controlling plant growth regulations. Amongst known phytohormones and/or plant growth regulators, brassinolide (BL) has been identified as one of the brassinosteroids (BRs), which is a natural plant growth regulating substance with structural similarities to animal steroidal hormones [9]. They have a wide distribution in the plant kingdom and have been demonstrated to play role in stem elongation, pollen tube growth inhibition, induction of ethylene biosynthesis, proton pump activation, xylem differentiation and regulation of gene expression [14]. Exogenous application of BR to plant can have a growth promotion effect that increase carbonic anhydrase and nitrate reductase activities [10]. BRs were discovered after about 10 years of research, where only 40 mg of pure BL were finally isolated from 40 kg of rapeseed pollen [15] in order to determine its structure. Initial interest in BR was based on the growth promoting properties of pollen extract [1]. So far about 50 naturally occurring BRs have been identified with BL being the most active compound. BRs are involved in numerous plant processes such as: cell expansion and division, seed germination, xylem differentiation, reproductive development, pollen elongation and pollen tube formation. Furthermore, exogenous applications of BR have led to a broad spectrum of disease resistance [4]. Apparently BRs are also involved in responses to insects and might affect the development of anti-hervibory structures in tomato [5].
With regard to growth and development, Hunter [11] reported that treatment of soybean seedlings with 24-epibrassinolide at a concentration range between 0.1 and 10 nM contributed to inhibition of root and shoot length, dry weight and lateral root numbers. Bajguz [3] demonstrated by means of synchronously dividing cultures of the alga *Chlorella vulgaris* that accelerated increase in cell number and marked increase in nucleic acid and protein levels followed BR treatment. Fatkhutdinova *et al*. [6] reported that the mitotic rate increased in roots of wheat after treatment with 24-epibrassinolide while volumes of nucleoli were also increased, similar to the plant's response to treatment with cytokinin. Leubner–Metzger [13] compared exogenously applied BL and gibberellins to tobacco seed and observed different responses depending on the state of dormancy, or on whether imbibitions occurred in the dark or light. It was concluded that the two hormones acted in distinct pathways and it was assumed that gibberellin and light act in a common pathway, whereas BR directly enhances the growth of the emerging embryo independent of gibberellin.
Additionally, inhibitory effects were observed at the highest concentration confirming the typical hormonal action of BRs namely that a concentration below or above the optimum can contribute to opposite results. The current study is having target to access the effect of BL on morpho-physiological attributes in pea under drought stress. Apart from that it will focus on comparative study of seed treatment and foliar application of BL on pea.

MATERIALS AND METHODS

Location of the Experimental Site
The experimental site is located in the south eastern part of Varanasi city at 25°18' N latitude, 83°3' E longitude and at an altitude of 80.71 meter above mean sea level.
Weather and Climate of the Location
Varanasi falls in a sub tropical climate and is subjected to extremes of weather condition i.e., extremely hot summer and cold winter. The temperature begins to rise from middle of February and reaches its maximum by May-June (mean maximum temperature 43.6°C) but has a tendency to decrease from July onwards touching the minimum in December- January (mean minimum temperature 8.2°C). The normal annual rainfall is about 1100 mm of which 88% are received from June to September as monsoon season rain, 5 to 7% in October to December as post monsoon and about 3.3% from January to February as winter season or pre monsoon season rains. The mean relative humidity of the area is about 66%, which rises up to 92% during July to September and falls down to 39% during the end of April to early June.
Soil
Varanasi exists almost in the middle of Indo-Gangetic plain. In general, the soil is alluvial with sandy loam texture, deep, well drained and moderately fertile, being low in available phosphorus and potassium. The pH range is neutral to slightly alkaline.
Experimental Details:

Seed source Seeds of field pea (*Pisum sativum* L.), variety HUP-2, were provided from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Chemical Source:

Brassinolide and other chemicals required for the biochemical analysis was obtained from the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Pot Filling and Seed Sowing:

Soil and FYM was collected from agricultural farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. It was cleaned by removing the stones, weeds, etc. and the soil was used in the pots was dried, powdered and mixed with FYM in 1:3 (1FYM: 3 soil) ratio thoroughly. The pots were washed with tap water and then kept for drying. After then the pots were filled with soil.

Seed treatment

Pea seeds (HUP-2) were subsequently washed in sterilized with 0.1% $HgCl_2$ for 5 minutes and then washed with distilled water 3-4 times and dried. Seeds were treated in three categories in petridis containing water (for control) and BL (concentration 0.05mM and 0.01mM) respectively.

Pot Experiment

30 pots were kept under net house condition and consistent care and precaution was taken. Seeds were sown in plastic pots filled with 3 Kg pulverized soil on. Seedlings were maintained at normal supply of moisture. Plants were watered in alternate day. Water stress treatment was started at 30 DAS. Normal plants were given alternate irrigation with tap water from the date of sowing to maturity and over there no stress is created. Drought stress or pre-anthesis drought stressed plants received irrigations with 5 days gap.

The experiment was having following treatments.

T0	CONTROL
T1	BL @ 0.01Mm
T2	BL @ 0.05mM
T3	BL @ 0.01mM + DROUGHT
T4	BL@ 0.05mM + DROUGHT
T5	BL @ 0.01mM (seed treated)
T6	BL @ 0.05mM (seed treated)
T7	BL @ 0.01mM (FOLIAR SPRAY + DROUGHT)
T8	BL @ 0.05mM (FOLIAR SPRAY + DROUGHT)
T9	BL UNTREATED + DROUGHT

MORPHO-PHYSIOLOGICAL PARAMETERS

PLANT HEIGHT (cm)

Plant height of one plant from each treatment and under each replication was measured from the base of the plant to the top of the main stem with meter scale and expressed in centimetre. The average of the height of four plants was considered.

ROOT LENGTH (cm)

Root length of a plant was measured in cm from the root tip to the base of the root. Root length of four plants was averaged.

LEAF NUMBER

Leaves number were counted as grown every after two day

RELATIVE WATER CONTENT (RWC)

Leaf relative water content (RWC) was determined from the fully expanded young leaves according to [7]. Leaf relative water content was calculated according to the equation: RWC (%) = Fresh weight - Dry weight X 100 Saturated weight -Dry weight Fresh weight is the samples fresh weight, dry weight is the dry weight after oven-drying the leaves at 70°C for 48 h, and saturated weight is the turgid weight after rehydrating the leaves at 4°C.

MEMBRANE STABILITY

Membrane stability was estimated according to Sairam *et al*. [18]. Leaf material (0.1 g) was taken in 10 cm^3 of double distilled water in two sets. One set was subjected to 40 °C temperature for 30 min and conductivity of medium was recorded using a conductivity bridge (C1). Second set was kept in a boiling water bath (100 °C) for 10 min and its conductivity was also recorded (C2). Membrane stability index (MSI) was calculated as below:

$$MSI = [1- (C1/C2)] \times 100$$

ESTIMATION OF PROLINE CONTENT:

Free Proline content in the leaves was determined following the method of Bates *et al.* [2].

Reagents used: (a) Acid Ninhydrin Reagent : For the preparation of acid ninhydrin reagent, 1.25 g of ninhydrin was dissolved in a mixture of warm 30mL of glacial acetic acid and 20 mL of 6 M Orthophosphoric acid with agitation.(b) Sulphosalicylic Acid (3 per cent) :Sulphosalicylic acid was prepared by dissolving 3.0 g of sulphosalicylic acid in 80 mL of distilled water then make up to 100 mL. (c) Glacial Acetic Acid (99.7 %), (d) Toluene (99.5 %).

Procedure for Proline Estimation:

Leaf sample (0.5 g) was homogenized in 5 mL of sulphosalicylic acid (3 %) using mortar and pestle. It was centrifuged at 6000 g for 10 min and supernatant was saved. Residue was again extracted twice with 5 mL of 3 per cent aqueous sulphosalicylic acid. All the supernatant fractions were pooled and final volume was made to 15 mL. Two mL of the extract was taken in the test tube and 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were added. The reaction mixture was boiled in water bath at 1000 for 30 min till brick red colour developed. After cooling the reaction mixture, 5 mL of toluene was added and then transferred to separating funnel and the absorbance read at 520 nm in spectrophotometer against toluene blank. Concentration of proline in the plant samples was estimated by referring to a standard curve of proline.

RESULTS AND DISCUSSION

PLANT HEIGHT (cm)

The data on shoot length with different treatments were recorded under four growth periods (21 DAS, 27 DAS, 33 DAS, 39 DAS) are presented in Table 1 There was a significant decrease in shoot length under drought stress. The plants under drought condition treated with BL @ 0.01mM and 0.05mM showed continuous reduction in shoot length and the plants under normal condition treated with BL @ 0.01 mM exhibited better responses towards plants height, as compared to plants treated with BL @ 0.05mM as well as control plants. The plants under normal condition treated with BL @ 0.05mM showed slightly increased the plant height after foliar application as compared to plants treated with BL @ 0.01mM under normal condition.

Table: 1 Effect of BL on root length (cm) of pea (*Pisum sativum* L.) at different days after sowing (DAS)

	21 DAS	27DAS	33 DAS	39 DAS
T0	24.83	29.33	31.5	33.5
T1	23.16	28	30.1	31.5
T2	20.6	25.5	27.6	29.3
T3	24.16	26.83	28.4	30.3
T4	23.5	27	29.5	32.1
T5	19.5	22.5	23.5	24.3
T6	21.66	21.1	22.6	23.8
T7	17.33	19.6	22.6	23.4
T8	16.6	21.6	22	22.4
T9	17	20	21.8	24
SEm±	0.36	0.49	0.53	0.51
CD5%	2.3	1.9	2.18	2.4

As observed, drought stress reduced plant height whereas foliar application of brassinolide increased plant height as well as leaf number. Increased root growth may result in reduced growth of shoot and resulted in change of the root: shoot ratio. This might be due to a greater decrease in growth of tops with a view to minimize water loss and a rapid increase in root growth in search of moisture under moisture stress condition. Seeds treated with different concentration of brassinolide showed high frequency of germination.

ROOT LENGTH (cm)

The data on root length at different treatment was recorded at four growth stages, i.e., 21 DAS, 28 DAS, 34 DAS and 39 DAS which are presented in Table 2. The morphological observation exhibited increased length of roots under drought condition as compare to control plant (T0). The plants under induced drought when treated with foliar spray of BL @ 0.01 and BL @ 0.05 showed increased root length while root length reduced in BL untreated plants under induced drought stress. Plants grown under normal condition treated with foliar spray of BL @ 0.01 and BL @ 0.05 exhibited slight increase in the length of roots.

Table 2. Effect of BL on root length (cm) of pea (*Pisum sativum* L.) at different days after sowing (DAS)

TREATM	21 DAS	27 DAS	33 DAS	39 DAS
T0	9.5	9	8	7
T1	7.5	10.5	9	8
T2	10.5	9.5	7	9.5
T3	7.5	11	11.5	9
T4	10.5	6	6	10
T5	8	9	8	7
T6	8.5	7.5	9	10
T7	9.5	8.5	9	7
T8	7	11	7	13
T9	9	11	8	8
SEm±	0.54	0.39	0.45	0.51
CD5%	2.16	1.56	1.8	2.3

NUMBER OF LEAVES PLANT^{-1}

The data of leaves number plant^{-1} was recorded at seven days interval under four growth periods (21 DAS, 27DAS, 33 DAS and 39 DAS) are presented in Table 3. The plants under normal condition treated with brassinolide (BL) @ 0.05mM showed high number of leaves as compared to the plants treated with BL @ 0.01mM whereas plants under drought condition treated with BL @ 0.05mM exhibited increased number of leaves as compared to other plants, which grown under drought condition. The foliar spray of BL showed increased leaf number under normal condition. Under drought condition plants treated with foliar spray of BL @ 0.05mM showed barely good response for increased number of leaves.

Table 3. Effect of BL on number of leaves plant^{-1} on pea (*Pisum sativum* L.) at different days after showing (DAS)

TREATMENT	21 DAS	27 DAS	33 DAS	39 DAS
T0	24.83	26.5	29.1	29.6
T1	23.16	18.3	21	24.3
T2	20.6	24.6	28.6	30.66
T3	20.8	22	24.6	26.3
T4	23.5	20.8	25.5	21.6
T5	19.5	14.8	15.1	14
T6	21	19	20.5	20.6
T7	17.3	14.16	18	20
T8	16.3	17.1	16.1	16.3
T9	17	15	14.6	15.6
T10	18.66	13.3	13.6	15
SEm±	0.25	0.8	0.12	0.33
CD5%	1.73	2.45	0.91	2.5

RELATIVE WATER CONTENT (%)

In the present investigation it was found that per-cent of RWC level decreased under drought condition as compared to control plant. RWC increased after 28 DAS under normal condition. Foliar treatment of BL @ 0.05mM showed increment in RWC level as compared to the plats treated with BL @ 0.01mM. In this experiment it is observed that reduction of the RWC level with the decrease in the amount of water available in the pots.

Table 4. Effect of brassinolide on leaf relative water content (%) in field pea (*Pisum sativum* L.) at different days after showing (DAS)

TREATMENT	21 DAS	27 DAS	33 DAS
T0	48	60	81
T1	83	88	90
T2	84	59	75
T3	78	60	73
T4	77	59	78
T5	83	68	59
T6	59	66	57
T7	70	72	85
T8	78	63	65
T9	79	67	58
SEm±	0.55	0.63	0.78
CD5%	1.22	2.06	1.99

Further, relative water content, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. Relative water content of wheat leaves was higher initially during leaf development and decreased as the dry matter accumulated and leaf matured [19]. In present investigation, RWC decreased under drought condition. A decrease in the relative water content in response to drought stress has been noted in wide variety of plants..

MEMBRANE STABILITY INDEX (MSI)

The data of membrane stability index was recorded under three growth periods (21 DAS, 23 DAS, 33 DAS) which are presented in Table 5 In present investigation the data exhibited level of membrane stability index decrease under drought condition. The plants under normal condition treated with BL @ 0.05mM and 0.01mM showed high level of MSI as compared to the control plants.

Table 5. Effect of brassinolide on membrane stability index (MSI) in field pea (*Pisum sativum* L.) at different days after showing (DAS)

TREATMENT	21 DAS	27 DAS	33 DAS
T0	23.95	20.5	22
T1	28.7	22.5	24.6
T2	31.8	25.49	23.84
T3	24.1	21.6	19.1
T4	28.3	24.38	22.2
T5	26.4	17.4	24.1
T6	19.98	22.75	20.03
T7	26.6	24.17	11.17
T8	24.19	22.2	16.9
T9	19.18	25.94	18.9
SEm±	0.32	0.16	0.9
CD5%	1.05	1.23	1.77

PROLINE CONTENTS (μg g^{-1} fresh weight)

In this present investigation the amount of proline contents was estimated under drought and normal condition at 33 DAS which are presented in Table 6. It was found that amount of proline contents increased under drought condition. The plants under drought condition treated with BL @ 0.01 showed high increasement of proline contents. Under normal condition plants exhibited decreased the amount of proline level.

Table 6. Effect of brassinolide on proline content (μg g^{-1} fresh weight) in field pea(*Pisum sativum* L.) at 33 days after showing (DAS)

TREATMENT	33 DAS
T0	428.09
T1	255.093
T2	257.11
T3	244.83
T4	383.84
T5	368.78
T6	284.45
T7	515.41
T8	577.28
T9	489.72
SEm±	0.86
CD5%	2.33

CONCLUSION

On the basis of present investigation, it was concluded that drought stress caused significant reduction in plant height and leaves number. Drought also results in alteration in plant metabolism. The present study indicated that foliar sprayed brassinolide caused a number of physiological and biochemical changes in the seedlings, including increased content of antioxidants and free proline content. The response of the antioxidant enzymes to exogenously applied BR was different. It depended on the level of stress and the BR concentration. The most effective dose of brassinolide under stress conditions was found to be 0.05mM.

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