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Evaluation on effect of brassinolide and salicylic acid on morpho-physiological parameters in mustard (*Brassica juncea* L.) under rainfed conditions

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Abstract
The Indian mustard variety *viz.* varuna were grown in field under randomized block design with three replications and 6 treatments. The plots were maintained under rain-fed condition. The spray of brassinolide and salicylic acid was taken during flower initiation and after 50 per cent flowering stage. Different morpho-physiological parameters were recorded at flower initiation and 50 per cent flowering stage. The results showed that different morpho-physiological parameters like plant height, number of primary branches, secondary branches, leaf area per plant, dry matter accumulation, relative water content, membrane stability index and showed maximum at hormonal spray of brassinolide at 20 ppm, which is followed by hormonal spray of salicylic acid at 200 ppm. The results of the study finally concluded that all the growth and morphological parameters increased with respect to brassinolide foliar spray even under rainfed conditions and suggested that the variety varuna is suitable for cultivation under rainfed conditions with the foliar spray of both plant growth regulators.

Keywords: Mustard, morpho-physiological parameters, brassinolide, salicylic acid

Introduction
Rapeseed mustard group of crops are grown under diverse agro-climatic conditions in India ranging from north-eastern or north western hills to down south under-irrigated or rain-fed area either timely or late sown in saline soils and mixed cropping systems. They are mainly cultivated in the northern and eastern parts of the country. Generally, Brassica species have been developed in the areas with high rainfall so perform poorly in the areas with low rainfall (Resketo and Szabo, 1992; Richards, 1978) [13, 14]. Growth and seed yield production of Brassica species have greatly decreased owing to drought conditions. Like other crop, mustard suffers from stringent droughts with fluctuating and un-predicting intensities, so there is an urgent need for drought proofing of mustard crop. The knowledge about the magnitude of morpho-physiological parameters of mustard's component traits for different conditions is essential for an effective breeding programme. The most important state of rapeseed mustard growing in India is Rajasthan. It ranks first with 30.87 lakh ha area, 38.28 lakh tones production and 1263 kg/ ha of average productivity during the year 2014- 15 and Uttar Pradesh is the second largest state of growing mustard in the country. The area, production and productivity of mustard in Uttar Pradesh were 6.93 lakh ha, 7.7 lakh tones and 1161 kg per ha, respectively during the year 2014-2015, 7.97 million tonnes of rapeseed and mustard production in 2016- 2017. Plant growth regulators are known to affect growth, assimilate translocation, flowering and ion transport (Arteca, 2000) [1]. Brassinolide are a new group of plant hormones with a growth promoting activity (Mandava, 1988) [8]. The ability of certain pollen extracts to promote growth led to the discovery of this group of substance in plants. These hormones have been isolated from the active factor of pollen grains of rape plant, which was named as brassinolide (Grove *et al.* 2009) [4]. Brassinolide improve the resistance of the plants against environmental stresses such as water stress, salinity stress, low temperature stress and high temperature stress (Rao *et al.* 2002) [11]. These hormones increased the content of chlorophyll, soluble protein, nitrate reductase activity, uptake of nitrogen, grain yield, grain protein and total sugar content. Brassinolide are shown to improve germination and seedling growth of groundnut (Vidyavardini and Seetharamarao, 2016) [15], grain yield of mustard (Ram raj *et al.*, 1997) [10] and increased the rate of carbon dioxide assimilation in mustard and wheat (Brawn and Wild, 1984) [2]. The blending of two traits *i.e* growth promotion and stress tolerance as imparted by brassinolide will have great economic bearing in future agriculture for enhanced crop production. It regulates root responses to gravity because root development is highly responsive to fluctuated environmental conditions (Vieten, *et al.* 2016) [16], it also regulate lateral root initiation (Lynch, 2013) [7].

Salicylic acid is an endogenous growth regulator of phenolic in nature and provides protection against a number of abiotic stresses (Raskin 1992) [2]. Salicylic acid is known as endogenous signalling molecule, which is involved in various physiological processes in plants, such as growth regulation, plant water relations and mechanisms of plant resistance and tolerance to biotic and abiotic stresses (Hayat, *et al*. 2017) [5]. The salicylic acid sprayed at lower concentration to mustard plants produced larger amount of dry matter and had higher photosynthetic rate when compared with controlled plants. Exogenous application of salicylic acid influences a range of physiological process *i.e* transpiration rate, stomatal conductance, photosynthesis (Wang and Li, 2016) [17]. It also serves as a regulator to stimulate the synthesis of protein, retard or enhance the accumulation of proline and induce chlorophyll content. The stimulated nitrate reductase activity was also observed in salicylic acid treated plants. This might be due to enhancement of nitrogen uptake by plants (Fariduddin 2003) [3]. It also influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea.* The mean data were subjected to analysis of variance and analysis was done through OPSTAT software. The main objective of this study was to determine the morpho-physiological parameters under rainfed condition and showed maximum with foliar spray of brassinolide @ 20 ppm which was followed by foliar spray of salicylic acid @ 200 ppm.

Materials and Methods

A field experiment was carried out at Chandra Shekhar Azad university of Agriculture and Technology, Kanpur in Department of Crop Physiology, during rabi season 2017-2018 to investigate the response of brassinolide and salicylic acid on plant growth, development and yield of mustard [*Brassica juncea* L.] under rainfed condition. Homogeneous composite soil sampling from 0-30 cm depth were subjected to mechanical, physical and chemical analysis and reported data for soil sample where EC (Electrical conductivity) (dSm^{-1}) is 1.10, pH is 7.61, field capacity (%) is 20.3 and permanent wilting point is 4.5.

The experiment was conducted in randomized block design with three replications. The genotype of Indian mustard namely Varuna was used for investigation under control and water stress conditions. Under control condition the plants were irrigated at flowering and pod formation stages while in water stress the plants were maintained under rain-fed condition by withdrawing irrigation. Following morphological and physiological parameters were measured and recorded after 1st spray, 2nd spray and at complete flowering. The plants were observed daily to see the appearance of first flowering. When 50 percent flowers appeared in the plot the date were recorded. The different morphological and physiological parameters such as plant height, number of leaves per plant, number of primary, secondary branches, dry matter accumulation, leaf area, specific leaf weight, crop growth rate and absolute growth rate was recorded from five randomly selected plants was counted and their mean was computed.

Plant height (cm): Plant height was measured for three plants from base of the plant to the tip of main shoot and mean of plant height was worked out and expressed in centimeters.

Number of leaves per plant: The number of leaves of five randomly selected plants were counted after 1st and 2nd spray and mean of those plants were recorded.

Number of primary and secondary branches per plant: The number of primary and secondary branches of five randomly selected plants were noted and mean of those plants were recorded.

Total dry matter (g $plant^{-1}$): Three plants were uprooted at random from each plot and were air dried. The samples were placed in a hot air oven at approximately 70 ±1 ℃ for 48 hrs until a constant weight was obtained and their dry weight was expressed in g $plant^{-1.}$

Leaf area (cm^2 $plant^{-1}$): Three plants were uprooted at random from each plot 10 random leaves from each three plants were used to measure leaf area per plant and the average of 10 leaves from each three plants was computed by using an instrument Leaf area meter (Biovis Bio science) and was expressed as cm^2 $plant^{-1}$

Specific leaf weight (mg cm^{-2}): It was calculated by the following formula.

$$SLW = \frac{\text{Leaf dry weight}}{\text{Leaf area}} \text{ mg cm}^{-2}$$

Crop growth rate (g m^{-2} day^{-1}): It was calculated by using the below mentioned formula

$$CGR = \frac{W_2 - W_1}{T_2 - T_1}$$

Absolute growth rate (cm day^{-1}): It was calculated for two growth variables by using following formula

$$AGR = \frac{H_2 - H_1}{T_2 - T_1}$$

Where

H_1 and H_2 and W_1 and W_2 refer to the plant height (cm) and dry matter weight (g) at the time T_2 and T_2, respectively.

Statistical Analysis

Mean values were taken from each treatment of three independent replications; and Statistical Package for Social Science (SPSS Version 16.0) was used for the analysis of random block design (RBD). Significant differences among various treatments were determined using Duncan's test.

Result and Discussion

The results of plant height, number of leaves, number of primary, secondary branches per plant, dry matter accumulation, leaf area, specific leaf weight, crop growth rate and absolute growth rate in variety varuna are followed.

Plant height (cm): Plant height were observed at 1st, 2nd spray and at complete flowering under different treatments. The highest plant height was observed with the foliar spray of brassinolide @ 20 ppm (149.81), which is 13.1% higher when compared with the control which was followed by foliar spray of salicylic acid @ 200 ppm. At complete flowering stage, the foliar spray of brassinolide @ 20 ppm again showed maximum plant height (178.0 cm) closely followed by the

foliar spray of salicylic acid @ 200 ppm (174.43 cm). Plant height improved with application of both hormones but it was recorded significantly higher in plants treated with brassinolide @ 20 ppm under rain-fed condition because the main physiological effect of brassinolide is bit similar to cytokinin i.e. cell division and due to random cell division and cell enlargement in plant the increased height was observed. The results are in finding with (Ramraj, 1997) [10].

Number of leaves per plant: The number of leaves were observed at 1st, 2nd spray and at complete flowering under different treatments. the maximum leaves were recorded in brassinolide @ 20 ppm (35.0) which is 26.3% increase over water spray and minimum was recorded in brassinolide @ 10 ppm (30.0) closely followed by salicylic acid @ 200 ppm which is 17.3% increase over water spray. At complete flowering stage plant sprayed with brassinolide @ 20 ppm produced maximum leaves (41) whereas, 34.4% increase over water spray which is closely followed by the foliar spray of salicylic acid @ 200 ppm (39.2) where as 28.5% increase over water spray. It might be attributed that all plant organs influenced by the application of these hormone which improves the transport mechanism for formation of leaves under rain-fed condition and presented in table 1. The results are in finding with (Ramraj, 1997) [10].

Number of primary and secondary branches per plant: The number of primary and secondary branches were observed at 1st, 2nd spray and at complete flowering under different treatments. The primary and secondary branches were recorded maximum in plants sprayed with brassinolide @ 20 ppm (5.51 and 11.9) where it was 20.6 and 29.3% increase over water spray and minimum was recorded at lower concentration brassinolide spray @ 10 ppm (4.61 and 10.20). At complete flowering, again the foliar spray of brassinolide @ 20 ppm showed maximum (5.72 and 12.40), where it was 24 and 26.5% increase over water spray which is followed by foliar spray of salicylic acid @ 200 ppm (4.99 and 11.92). This may be due to enhance transport mechanism of photosynthates used in the formation of branches in plants. These results are in continuity with (Pipattanawong, *et al.* 2015) [9].

Table 1: Effect of brassinolide and salicylic acid on plant height (cm) and number of leaves (plant^{-1}) in mustard at flower initiation stage, at 50% flowering stage, at complete flowering stage. Presented data in table are the mean of three replication and ± represent standard deviation between the replications

Treatments	Plant height (cm)			Number of leaves (plant^{-1})		
	After 1st spray	After 2nd spray	Complete flowering	After 1st spray	After 2nd spray	Complete flowering
Control	97.81	132.23	156.00	19.2	24.8	29.7
Water spray	98.77	132.47	161.16	20.5	27.7	305
SA @ 100 ppm	104.75	143.51	173.33	21.3	31.2	37.5
SA @ 200 ppm	112.81	146.62	174.43	22.2	32.5	39.2
BR @ 10 ppm	99.63	139.75	169.33	21.2	30.0	35.5
BR @ 20 ppm	109.85	149.81	178.00	21.7	35.0	41.0
Mean	103.91	140.73	168.70	21.01	35.0	35.56
S.Em ±	0.63	0.88	1.22	0.53	0.52	0.48
CD @5%	1.26	1.76	2.44	1.09	1.08	0.99

SA*= Salicylic acid BR*= Brassinolide

Total dry matter accumulation (g plant^{-1}): Total dry matter accumulation was observed at 1st, 2nd spray and at complete flowering under different treatments. The total dry matter accumulation was recorded maximum in plants sprayed with the foliar spray of brassinolide @ 20 ppm and gave a maximum value of (24.7, 36.9 and 75.9 g plant^{-1}) with the increase of 36.2% and 30.8% at respective stages over water spray which was followed by foliar spray of salicylic acid @ 200 ppm (23.5, 36.4 and 67.4 g plant^{-1}). Vegetative growth of the plant in terms of dry matter accumulation per plant did not show any remarkable effect at early stage but with the advancement of plant age, it influenced by the application of these substances. It might be attributed due to efficient utilization of soil moisture and its proper utilization by crop plants in enhancing photosynthetic efficiency of leaves and increase the rate of transport of photosynthate to grow better under rain-fed condition. These results are in continuity with (Rao, 2002) [11].

Leaf area (cm^2 plant^{-1}): Leaf area was observed at 1st, 2nd spray and at complete flowering under different treatments. The leaf area was recorded maximum in plants sprayed with the foliar spray of brassinolide @ 20 ppm and gave maximum value of leaf area (1541.3 and 1198.6 cm^2 plant^{-1}) with the increase of 10.7% and 8% at respective stages over water spray which was followed by foliar spray of salicylic acid @ 200 ppm (1508.8 and 1184.5 cm^2 plant^{-1}). The foliar application of hormonal solution increased leaf area meaning thereby it improves the transport mechanism and utilization process of available soil moisture and nutrient for stimulation of leaf growth and presented in table 2. These results are in continuity with (Grove, *et al.* 2009) [4].

Table 2: Effect of brassinolide and salicylic acid on leaf area (cm^2 plant^{-1}) and total dry matter accumulation (g plant^{-1}) in mustard at flower initiation stage, at 50% flowering stage, at complete flowering stage. Presented data in table are the mean of three replication and ± represent standard deviation between the replications

Treatments	Leaf area (cm^2 plant^{-1})			Total dry matter accumulation (g plant^{-1})		
	After 1st spray	After 2nd spray	Complete flowering	After 1st spray	After 2nd spray	Complete flowering
Control	638.5	1324.9	1023.9	11.8	18.7	33.5
Water spray	692.8	1391.8	1108.9	12.3	20.9	35.0
SA @ 100 ppm	776.5	1466.5	1158.5	13.8	22.4	35.7
SA @ 200 ppm	814.8	1508.8	1184.5	14.7	23.5	36.4
BR @ 10 ppm	766.2	1356.3	1142.8	13.6	22.2	35.2

BR @ 20 ppm	791.5	1541.3	1198.6	14.5	24.7	36.9
Mean	746.7	1431.6	1136.2	13.45	22.06	35.45
S.Em ±	4.03	13.90	4.37	0.44	0.86	0.94
CD @5%	8.06	27.81	8.94	0.83	1.75	1.88

SA*= Salicylic acid BR*= Brassinolide

Specific leaf weight (mg cm^{-2}): Specific leaf area was observed at 1st, 2nd spray and at complete flowering under different treatments. Specific leaf weight represents the thickness of leaf increased with the age of the plant and value of it was recorded significantly higher in plants sprayed with brassinolide and salicylic acid against the water spray. Under rain-fed condition, the foliar spray of brassinolide @ 20 ppm (15.2), whereas 19.3% increase over water spray showed maximum specific leaf weight, which is closely followed by salicylic acid spray @ 200 ppm. At complete flowering, again foliar spray of brassinolide @ 20 ppm showed maximum specific leaf weight (16.3) which is followed by spray of salicylic acid @ 200 ppm (15.1), which is 6.3% increase over water spray. It might be attributed due to physiological effects of hormones to enhanced transport mechanism of assimilate and nutrients available soil moisture in crop plants to grow better under rain-fed condition. Similar findings have also been reported by (Rao, *et al.* 2002) [11].

Crop growth rate (g m^{-2} day^{-1}): Crop growth rate was observed at 1st, 2nd spray and at complete flowering under different treatments. Brassinolide and salicylic acid sprayed at each concentration improves crop growth rate of mustard variety varuna but under rain-fed condition the foliar spray of brassinolide @ 20 ppm (10.54 g m^{-2} day^{-1}) which is 54.3% increase over water spray showed maximum crop growth rate which is closely followed by salicylic acid sprayed @ 200 ppm (9.90 g m^{-2} day^{-1}) which is 45% increase over water spray. It might be attributed due to efficient utilization of available moisture to many physiological effects of hormones such as shoot elongation, cell division and intermodal elongation in crop plants to grow better under rain-fed condition in fig. 5.7. Similar findings have also been reported by (Wang and Li, 2016) [17].

Absolute growth rate (cm day^{-1}): Absolute growth rate was observed at 1st, 2nd spray and at complete flowering under different treatments. It might be attributed due to many physiological effects of hormones through better utilization of available moisture in mechanism of transport in shoot elongation, internodal elongation in crop plants to grow better under rain-fed condition. The foliar spray of brassinolide @ 20 ppm (38.23 cm day^{-1}) showed maximum absolute growth rate which is 68.8% increase over water spray which is closely followed by salicylic acid spray @ 200 ppm (35.71 cm day^{-1}) which is 54.3% increase over water spray and presented in table 3. Similar findings have also been reported by (Khan, 2003) [6].

Table 3: Effect of brassinolide and salicylic acid on SLW (mg cm^{-2}), CGR (g m^{-2} day^{-1}) and AGR (cm day^{-1}) in mustard at flower initiation stage, at 50% flowering stage, at complete flowering stage. Presented data in table are the mean of three replication and ± represent standard deviation between the replications

Treatment	CGR (g m^{-2} day^{-1})		AGR (cm day^{-1})		SLW (mg cm^{-2})	
	B/w 1st and 2nd spray	B/w 2nd and complete flowering	B/w 1st and 2nd spray	B/w 2nd and complete flowering	B/w 1st and 2nd spray	B/w 2nd and complete flowering
Control	1.81	3.88	18.91	19.21	13.11	14.00
Water spray	2.38	5.03	19.61	20.25	13.92	14.23
SA @100 ppm	3.42	9.09	23.06	32.45	14.70	14.97
SA @200 ppm	3.74	9.90	24.89	35.71	14.90	15.18
BR @10 ppm	2.50	8.69	21.08	27.62	14.33	14.82
BR @20 ppm	3.56	10.54	24.28	38.23	15.21	16.35
Mean	2.90	7.80	21.9	28.9	14.35	14.88
S.Em ±	0.49	1.24	2.25	7.32	0.08	0.17
CD @5%	2.94	4.66	4.37	14.63	0.16	0.37

SA*= Salicylic acid BR*= Brassinolide

Conclusion

Growth regulators considerably increased morpho-physiological characters at all observational stages as against water spray as well as control (no spray). Observations such as plant height, number of leaves, primary and secondary branches per plant, leaf area, total dry matter accumulation absolute growth rate, crop growth rate, relative growth rate were taken at after 1st, 2nd foliar spray and at the time of complete flowering. Under rain-fed condition, the foliar spray of brassinolide at their higher concentration i.e @ 20 ppm showed maximum among all the treatments tried. All the treatments showed higher than brassinolide spray at their lower concentration *i.e* @ 10 ppm.

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