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Influence of Sowing Time, Varieties and Salicylic Acid Application on Different Physiological Parameters of Indian Mustard (*Brassica juncea* L)

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A field experiment was conducted at Agronomy Farm, S.K.N. College of agriculture, Jobner, Jaipur, Rajasthan to evaluate the effect of sowing time, varieties and salicylic acid (SA) application on different physiological parameters (i.e. relative water content, photosynthetic rate, transpiration rate, stomatal conductance, leaf temperature, Membrane stability index, chlorophyll stability index, heat susceptibility index) of Indian mustard. The experiment was laid out in split plot deign and replicated thrice. The experiment consisted of three sowing dates [20th October (timely sowing), 15th November (late sowing) and 30th November (very late sowing)], two varieties [RGN-236, RGN-229] and four levels of Salicylic acid (Control, SA 50 ppm, SA 100 ppm and SA 150 ppm). Physiological traits like relative water content, photosynthetic rate transpiration rate, stomatal conductance, leaf temperature, chlorophyll stability index Content, heat susceptibility index and membrane stability index are directly correlate with heat stress tolerance in crop plant. Results were revealed that effect of different sowing time, varieties and concentration of SA has shown significant effect on all tested physiological parameters of Indian mustard and those are associated with high temperature stress tolerance.

Keywords: Mustard, sowing time, varieties, salicylic acid, physiological parameters.

Indian mustard is the most important winter oilseed crop. It is generally grown under rain fed conditions and moderately tolerant to soil acidity, it requires well drained soil having pH near to neutral. Oil content in seeds varies from 37 to 49 per cent. The seed and oil of mustard have a peculiar pungency due to presence of glucosinolate and its hydrolysis products such as allyl-isothiocynate making it suitable to use as condiment in the preparation of pickles and for flavoring curries and vegetables. Its oil mainly used in cosmetics, medicines and various other industries.

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India is one of the largest producers of rapeseed and mustard in the world. The contribution of India in global rapeseed mustard production is 72.62 lac tonnes occupying 67.17 lac hactares area with average yield of 1080 kg/ha (Anonymos, 2012-13). The major mustard growing states are Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Gujarat, West Bengal, Assam, Bihar, Punjab and Jammu & Kashmir. Rajasthan ranks first both in area and production of rapeseed and mustard in the country.

Out of the various abiotic stresses, high temperature is the most important stress, which can strike a crop at any time and imposed many limitations on growth and development. High temperature stress negatively affects plant growth,

development and crop yield (Boyer, 1982). Under field conditions, high temperature stress is frequently associated with reduced water availability (Simoes-Araujo *et al*., 2003). Shekhawat *et al.,* (2012) also found same result in Indian mustard. It has negative impact on physiology of plant and ultimately reduces the yield in all agricultural crops. Mustard crop is very sensitive to temperature stress during reproductive stage. In general, the negative impacts of abiotic stresses on agricultural productivity can be minimized by a combination of genetic improvement and cultural practices. Among crop production factors, sowing time and variety contributes a lot towards the yield potential. A good variety often fails to express its potential even under good management conditions, unless it is grown at optimum time. Mustard is an important oil seed crop and its early sowing implies many important advantages such as escape from aphids infestation, while late sown crop encounters high temperature stress at seed development stage, which causes a great yield reduction (Abolfazl *et al*., 2009). The application of plant growth regulators is known to play an important role in plant response to stress (Chakrabarti and Mukherjee, 2003). Salicylic acid (SA) has recently been recognized as a plant growth hormone and plays diverse physiological roles in plants including thermogenesis generate a wide range of metabolic and physiological responses thereby affecting their growth and development (Hayat *et al*., 2010). It also plays a major role in osmotic adjustment and there by restricts to water loss from cell.

MATERIALS AND METHODS

The present investigation was conducted at Agronomy Farm, S.K.N. College of agriculture, Jobner (260 05' N latitude, 750 28' E longitude and at an altitude of 427 m above mean sea level), Jaipur, Rajasthan, India during *rabi* season 2013-14. The soil of the experimental site was classified as loamy sand in texture and alkaline in reaction. The climate of this region is typically semi-arid which is characterized by the aridity and extremity of temperature fluctuations in summer and winter. During summer, maximum temperature ranges between 35-46 ^{o}C while in winter, it may falls down to as low as -1^{o}C. The average rainfall of this locality is approximately 400 mm, most of which is received during rainy season from July to September.

The experiment was conducted in split plot deign with three replications. The experiment consisted of three sowing dates [20th October (timely sowing), 15th November (late sowing) and 30th November (very late sowing)], two varieties [RGN-236, RGN-229] and four levels of Salicylic acid (Control, SA 50 ppm, SA 100 ppm and SA 150 ppm). The crop was fertilized with 60 kg N and 40 kg P_2O_5 ha^{-1}. One half of nitrogen and full dose of phosphorus were given as basal application. The remaining dose of nitrogen was top dressed at first irrigation 30 days after sowing (DAS). The foliar spray of SA was applied at flowering and siliqua formation.

Procedure and techniques

Relative water content

Fresh weight of the leaves was taken then the leaves were kept in distilled water for 4 hours (Barrs and Weatherly, 1962) to obtain turgid weight. The turgid weight was recorded after blotting the excess water on the surface of the sample. Dry weight was obtained after drying the samples in oven at 60 ^{0}C till constant weight was achieved. The relative water content (RWC) was calculated by the formula given by Slavik (1974).

$$RWC\ (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})

The rate of photosynthesis was measured with the help of Infra Red Gas Analyzer (CID 301, USA). The net exchange of CO_2 between a leaf and the atmosphere was measured directly by enclosing leaf in the assimilation chamber and the rate was monitored at which the CO_2 concentration changed over a definite time interval. The system automatically calculates the rate of photosynthesis on the basis of preloaded flow and leaf area as per the chamber used. Measurements were taken between 10 to 12.00 AM in triplicate for all the treatments.

Transpiration rate (µmol H_2O m^{-2} s^{-1}), Stomatal conductance (mmol m^{-2}S^{-1}) and Leaf temperature (^{o}C)

These observations were measured directly by using Infra Red Gas Analyzer (CID 301, USA).

Chlorophyll stability index: Total chlorophyll content (mg g^{-1} fresh weight) (The sum of chlorophyll 'a' and chlorophyll 'b') were estimated according to the method of Arnon (1949).

The total chlorophyll content was calculated by the formula

$$\text{Total chlorophyll (mg g-1)} = \frac{\text{Total chlorophyll (mg g}^{-1}\text{)}}{1000} \times \frac{\text{Total volume of the extract}}{\text{Sample weight (g)}}$$

Total chlorophyll (mg/l extract) was calculated as:

Total chlorophyll (mg/l) = 20.02 A645 + 8.02 A663

Heat susceptibility index (HSI)

Heat susceptibility index (HSI) was the mean values of these traits under optimum and heat stress conditions were used to estimate the Heat susceptibility index and calculated by the method suggested by Fischer and Maurer (1978) with the following formula.

Heat susceptibility Index (HSI) = (1-YD/YP)/D

Where,

YD=Mean seed yield in stress.

YP= Mean seed yield in non stress.

D= 1- (Mean YD of all genotypes / Mean YP of all genotypes.)

Membrane stability index (%)

Membrane stability index (MSI) was calculated by taking the electrical conductivity (EC) of leaf leachates in double distilled water at 40 (C_1) and 100°C (C_2) by following the method of Sairam (1994).

Membrane stability index (MSI) = 1- C_1/C_2 x 100

RESULTS AND DISCUSSION

Data presented in table 1 and 2 showed that different times of sowing, varieties and levels of SA bring significant effect on different physiological parameters (i.e. relative water content, photosynthetic rate, transpiration rate, stomatal conductance, leaf temperature, Membrane stability index, chlorophyll stability index, heat susceptibility index) of Indian mustard.

Relative water content

Planting of mustard at 20th October recorded significantly higher relative water content at flowering and siliqua initiation stage as compared to late planting. The variety RGN-229 recorded significantly higher relative water content at flowering (86.00%) and siliqua initiation (81.52%) stage as compared to RGN-236. In case of SA, the maximum increase in relative water content with spray of 150 ppm was at flowering stage (18.08%) and at siliqua initiation stage(14.67%).

Photosynthetic rate

The photosynthetic rate was decreased with delayed sowing of mustard. The significantly highest photosynthetic rate was recorded with 20th October and lowest at 30th November planted crop at flowering and siliqua initiation stage. Between varieties, the variety RGN-229 showed significantly higher Photosynthetic rate. The maximum increase in photosynthesis rate with the spray of 150 ppm SA at flowering stage (21.5%) and at siliqua initiation stage (19.08%).

Transpiration rate

Data pertaining to transpiration rate showed that sowing time had significant effect on transpiration rate. The transpiration rate was significantly highest under 30th November sown crop as compared to 20th October and 15th November sown crop. The significantly lowest transpiration rate at flowering (3.60 mmol $H_2O/m^2/s$) and siliqua initiation stage (3.30 mmol $H_2O/m^2/s$) was recorded under cultivar RGN-229 as compared to RGN-236. With increasing levels of SA increases transpiration rate at all the growth stages in three different date of sowing. The maximum increase in Transpiration rate with spray treatment of 150 ppm SA was 21.41% at flowering stage and 15.38% at siliqua initiation stage.

Stomatal conductance

A perusal of data revealed that delayed sowing of mustard significantly reduces the stomatal conductance. Sowing of mustard on 20th October being at par with 15th November sowing, but the significantly lower stomatal conductance was recorded under 30th November sowing. Results further revealed that the different cultivars were also having different stomatal conductance in a given environment. Mustard cultivar RGN-236 recorded significantly highest stomatal conductance at flowering stage and at siliqua initiation stage (42.59 and 41.05 mmol/m^2/s, respectively) followed by RGN-229. The maximum decrease in stomatal conductance with spray treatment of 150 ppm SA was 22.80% at flowering stage and 23.23% at siliqua initiation stage.

Leaf temperature

The effect of sowing times was found to

Table 1. Effect of sowing time, genotypes and SA application on relative water content (%), photosynthetic rate (µ mol CO_2 m^{-2} s^{-1}), transpiration rate (µ mol H_2O m^{-2} s^{-1}) and stomatal conductance (mmol/m^2/s)

Treatments	Relative water content (%)		Photosynthetic rate (µ mol CO_2 m^{-2} s^{-1})		Transpiration rate (µ mol H_2O m^{-2} s^{-1})		Stomatal conductance (mmol/m^2/S)	
	Flowering stage	Siliqua initiation stage	Flowering stage	Siliqua initiation stage	Flowering stage	Siliqua initiation stage	Flowering stage	Siliqua initiation stage
Varieties								
RGN-236	81.56	77.16	26.6	24.13	4	3.68	42.59	41.05
RGN-229	86	81.52	30	27.93	3.6	3.3	39.41	35.87
S.Em.+	0.956	0.62	0.36	0.33	0.048	0.042	0.56	0.48
C.D. (P=0.05)	2.709	1.76	1.01	0.93	0.136	0.118	1.58	1.37
Date of sowing								
Timely sowing	87.71	82.55	30.52	28.13	3.4	3.26	45.28	42.01
Late sowing	83.92	80.14	29.08	26.93	3.7	3.45	42.07	39.01
Very late sowing	79.72	75.33	25.3	23.02	4.3	3.76	35.65	34.36
S.Em.+	1.171	0.76	0.44	0.4	0.059	0.051	0.68	0.59
C.D. (P=0.05)	3.318	2.16	1.24	1.14	0.166	0.145	1.93	1.68
Salicylic acid								
Control	76.9	74	25.5	23.79	4.25	3.77	46	42.99
SA 50 ppm	81	77	27.2	25.2	3.97	3.6	43	40.96
SA 100 ppm	86.41	81.5	29.5	26.8	3.64	3.4	37.5	36.89
SA 150 ppm	90.81	84.86	31	28.33	3.34	3.19	37.5	33
S.Em.+	1.352	0.88	0.51	0.47	0.068	0.059	0.79	0.69
C.D. (P=0.05)	3.832	2.49	1.43	1.32	0.192	0.167	2.23	1.94

Table 2. Effect of sowing time, genotypes and SA application on Leaf temperature (^{0}C), Chlorophyll stability index (%),Cell membrane stability (%) and Heat susceptibility index

Treatments	Leaf temperature (^{0}C)		Chlorophyll stability index (%)		Cell membrane stability (%)		Heat susceptibility index
	Flowering stage	Siliqua initiation stage	Flowering stage	Siliqua initiation stage	Flowering stage	Siliqua initiation stage	
Varieties							
RGN-236	31.97	32.91	41	38.08	60.8	56.38	0.76
RGN-229	30.46	30.84	45.54	41.34	66.88	63.88	0.88
S.Em.+	0.466	0.462	0.538	0.459	0.73	0.89	0.029
C.D. (P=0.05)	1.322	1.31	1.526	1.302	2.07	2.52	0.083
Date of sowing							
Timely sowing	29.22	29.85	46.47	42	68.84	65.16	0.94
Late sowing	30.8	31.66	44.28	40.13	65.31	62.02	0.83
Very late sowing	33.61	34.11	39.06	37	57.37	53.21	0.69
S.Em.+	0.571	0.566	0.659	0.563	0.97	1.09	0.036
C.D. (P=0.05)	1.619	1.605	1.869	1.595	2.53	3.08	0.101
Salicylic acid							
Control	32.1	32.5	37.58	36	58.06	53.6	0.62
SA 50 ppm	31.5	32.1	40.5	37.94	61	57.66	0.74
SA 100 ppm	30.8	31.7	45.3	41.07	65.85	62.45	0.89
SA 150 ppm	30.45	31.2	49.7	43.83	70.45	66.81	1.03
S.Em.+	0.66	0.654	0.761	0.65	1.13	1.26	0.041
C.D. (P=0.05)	1.87	1.853	2.158	1.842	2.92	3.56	0.117

be significant on leaf temperature. The leaf temperature of Indian mustard was progressively increased with delayed sowing but 20th October and 15th November sown crop was recorded statistically similar leaf temperature at both stages. The significantly lower leaf temperature was recorded under variety RGN-229 then by RGN-236 which was 1.51 and 2.07^{0}C more at flowering and siliqua initiation stage, respectively. The maximum decrease in leaf temperature with spray treatment of 150 ppm SA was 30.45 ^{0}C at flowering stage and 31.20 ^{0}C at siliqua initiation stage.

Chlorophyll stability index

It is evident from the data (Table 2) that different times of showing brought about significant effect on chlorophyll stability index. Delayed sowing of mustard crop significantly decreased the chlorophyll stability index. Chlorophyll stability index was also influenced significantly under various cultivars of Indian mustard. Mustard cultivar RGN-236 recorded significantly lower chlorophyll stability index (38.08 per cent). However, significantly highest chlorophyll stability index was recorded in RGN-229 (41.34 per cent). The maximum increase in chlorophyll stability index with spray treatment of 150 ppm SA was 32.25% at flowering stage and 21.75% at siliqua initiation stage.

Membrane stability index

The membrane stability index was significantly reduced with late sowing of Indian mustard than early sowing. Crop sown on 30th November and 15th November were significantly reduced the membrane stability index by 16.66 and 5.14% , at flowering stage and 18.33 and 4.81%t at siliqua initiation stage respectively, over 20th October sowing. RGN-229 cultivar recorded significantly highest membrane stability index (66.80%) followed by RGN-236 (60.80%). The maximum increase in membrane stability index with spray treatment of 150 ppm SA was 21.33% at flowering stage and 24.64 % at siliqua initiation stage.

Heat susceptibility index

A critical examination of data revealed that the heat susceptibility index of different mustard cultivars were influenced significantly by time of sowing. The significantly lowest heat susceptibility index for 30th November sown crop was recorded in RGN-236 followed by RGN-229. The mustard cultivar RGN-229 recorded significantly highest heat susceptibility index for 15th November (0.83) as well as for 30th November (0.69) sown crop. The maximum increase in heat susceptibility index with spray of 150 ppm SA with an increase 66.12% over control.

Mustard crop is very sensitive to temperature stress during reproductive stage.Heat stress due to high ambient temperatures is a serious threat to crop production all over worldwide (Hall, 2001). In general, the negative impacts of abiotic stresses on agricultural productivity can be minimized by a combination of genetic improvement and cultural practices (*viz.* planting time, planting density, varieties, irrigation managements and fertilizer management etc.). Optimum time of sowing and a good cultivar use maximum resources and coincides with optimum environmental condition such as GDDs, temperature, humidity, moisture etc. that is why the positive effect of date of sowing and varieties on physiological parameters were recorded. Plant growth and development is determined by several endogenous and exogenous factors.

Among the internal factors, hormones play a vital role in regulation of growth and development. SA is important growth hormone and expressed positively in biotic and abiotic stress (Horvath *et al*., 2007. It has been reported that exogenous application of SA enhanced photosynthesis efficiency, metabolism and growth of mustard plant under elevated temperatures (He *et al*., 2005, Kaur *et al*., 2009, Chhabra *et al*., 2013). However, exogenous application of SA reverses the effect of heat stress on *Brassica juncea*. A similar growth promoting effect of salicylic acid in *Brassica juncea* plants exposed to temperature stress was reported by Cong *et al*. (2008). Canopy temperature is the index of crop water status by measurement of difference between ambient temperature and leaf temperature (Jackson *et al*., 1981). Salicylic acid activates a novel gene BjDREB1B encoding a DRE (dehydration responsive element) binding protein, leading to elevated level of proline thereby provided tolerance to the plants against harmful effects of temperature stress (Cong *et al*., 2008). The induction of chalcone synthase and phenylalanine ammonia-lyase by salicylic acid application which results in the synthesis of certein phenolic compounds that

play an important role in conferring resistance against various abiotic stresses including temperature stress (Campos *et al*., 2003) further supports our results. At the sub-cellular level major modifications occur in the chloroplast, leading to significant changes in photosynthesis. For example, heat stress reduced photosynthesis by changing the structural organization of thylakoids (Karim et al., 1997). Such changes result in the formation of antenna-depleted photosystem-II (PS-II) and hence reduced photosynthetic and respiratory activities (Zhang *et al*., 2005). Stomatal conductance and net photosynthesis are inhibited by moderate heat stress in many plant species due to decrease in activation state of rubisco (Monson et al., 1982). In the present study, however, application of SA enhanced the SPAD chlorophyll value and photosynthetic rate and also partially overcame the deleterious effect of the heat stress, if administered as a follow-up treatment. This is deduced from the present observation that application of SA was beneficial in enhancing total chlorophyll and PN value under normal conditions and also under temperature stress. Enhanced PN and chlorophyll by SA application was also reported earlier (Fariduddin *et al*., 2003). The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) and the level of proline exhibited increases in response to SA or heat stress application (Kavi Kishore *et al*., 2005 and Chakraborty and Tongden, 2005).). Heat stress induces oxidative stress. For example, generation and reaction of activated oxygen species (AOS), which causes the autocatalytic peroxidation of membrane lipids and pigments subsequently leads to membrane permeability and modification of its functions (Xu, *et al*., 2006). Recent studies show that some signaling molecules may increase the antioxidant capacity of cells (Gong *et al*., 1997; Dat *et al*., 1998). Extreme temperatures led to accumulation of certain organic compounds termed osmolyte, including sugars, polyols, and proline.

CONCLUSION

It can be concluded from the present study that timely sowing (20th October) of Brassica juncea variety RGN-229 with foliar spray of 150 ppm SA at flowering and siliqua formation stage proved supremacy over other treatments for all the tested physiological parameters.

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