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Role of antitranspirants on transpiration rate and physio-biochemical aspects in ber

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Abstract
This two-year experiment was carried out in earthen pots of 40kg soil holding capacity on *Ziziphus rotundifolia* root stock of ber during 2018-19 under cage house, Department of Plant Physiology, S.K.N. College of Agriculture, Jobner. The *Ziziphus rotundifolia* root stock of ber were planted in pots. The recommended doses of FYM, fertilizers and other inputs provided at proper time. These Plants were irrigated by tape water as and when required in pots. The plants were foliar sprayed by Kaoline 2%, Kaoline 4%, Kaoline 6%, PMA 2ppm, PMA 4 ppm, PMA 6 ppm silicon oil 1% and caster oil 1%. A significant decrease was observed in the transpiration rate, proline content, cell membrane injury, reducing sugar content with increase in relative water content in the plant leaves by the use of Kaoline 6% followed by Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 2ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 6 ppm, silicon oil 1%, and caster oil 1%.

Keywords: Ber, transpiration rate, antitranspirants, Kaoline, silicon oil, caster oil, phenyl mercuric acetate

Introduction
Ber (*Ziziphus mauritiana* L.) is a member of family Rhamnaceae. The genus Zizyphus contains 135 to 170 species (Islam and Simmons, 2006) [6]. *Ziziphus mauritiana* is a spiny, evergreen shrub or small tree up to 15 m height. It is a fast-growing tree, with an average bearing life of 25 years. It is well adapted to arid and semi-arid conditions with adequate rain during the vegetative period. The fruit's skin is smooth, glossy, and thin but tight. The flesh is white and crispy. When semi-ripened, it is bit juicy and possesses a pleasant aroma (Pareek, 2013) [10]. The fruit is a good source of vitamin C, carotene, phosphorus and calcium. Leaves contain 6% digestible crude protein which is an excellent source of ascorbic acid and carotenoids (Abbass *et al*., 2012; Ismail, 2013) [1, 6]. Average fruit yield per tree ranges from 50-250 kg tree^{-1} and is relatively easy and cheap to cultivate. The worldwide annual production of ber fruit is 0.90 million tons and cultivated on an area of 88, 000 hectares. Ber trade has expanded over the last decade and is expected to continue in future. India, Thailand and Pakistan export ber to the Middle East, Malaysia and Far East but only Thailand exports on a year-round-basis (Szabolcs, 1994) [12]. As compared to other agricultural and horticultural crops, Indian Jujube is known to grow successfully under a low erratic rainfall. Temperature extremes and saline soils with low fertility (Meena *et al*., 2006) [8].

Plant through various mechanisms such as stomatal closure, reduced transpiration, root weight and length, keeping up photosynthesis, respiration and osmoregulation can increase the resistance to drought (J. Levitt, 1980) [7]. Antitranspirants were grouped into three categories, namely film-forming types (which coat leaf surface with films that are impervious to water vapor), reflecting materials (which reflect back a portion of the incident radiation falling on the upper surface of the leaves) and stomatal closing types (which affect the metabolic processes in leaf tissues [Rossum, Olivier, Sarah Le Pajolec, Olivier Raspé, 2020] [13]. Kaolin (surround WP) is a non-abrasive, non-toxic aluminosilicate (Al4Si4O10 (OH) 8) clay mineral that has been formulated (Engelhard Corporation, Iselin, NJ) as a wettable powder for application with conventional spray equipment (Velikova V, Yordanov I, Edreva A.,2000) [14]. A reflective Kaolin spray was found to decrease leaf temperature by increasing leaf reflectance and to reduce transpiration rate more than photosynthesis in many plant species grown at high solar radiation levels (Wolucka BA, Goossens A, Inze D.,2005) [15]. Reflecing antitranspitants were found that to be non-toxic and have effectiveness in longer period than metabolic types (J. Levitt, 1980) [7]. This experiment was carried out to minimizing the transpiration rate, proline content, cell membrane injury and reducing sugar content with increase in moisture content in leaves. Cantore, *et al*, 2009 [4], investigated the underlying mechanism asserted by kaolin on tomato physiology by evaluating its effect on leaf, canopy and inner fruit temperatures, gas exchange at the leaf and canopy scales, above ground biomass, yield and fruit quality. In treated plants, stomatal conductance decreased by 53%, resulting in reductions of 34 and 15% in transpiration and internal CO_2 concentration, respectively.

Materials and Methods

Plant materials and experimental details

The study was conducted in the cage house under natural conditions. Six-month-old plants of *Ziziphus rotundifolia* grown in ceramic pots of 40 x 40 cm diameter were taken for study. The pots were filled with 40 kg of loamy sandy soil having a bulk density of 1.5 g cm^{-3}, electric conductivity (EC) 0.6 dSm^{-1}, P^H 8.2, sodium absorption ratio 12.5 and $CaCo_3$ 0.14%. The field capacity and permanent wilting point of the soil were 11.8 and 2.8%, respectively. About 21 pots were used for this study. The recommended doses of manures, fertilizers and other inputs were provided at the appropriate time. The planting will be done in R_1, R_2 and R_3 replications. The treatments applied after establishment of these plants in pots. The experimental design is CBD. The water loss controlled by using following chemicals i.e. water spray (without antitranspirants), Kaoline 2%, Kaoline 4%, Kaoline 6%, PMA 2ppm, PMA 4 ppm, PMA 6 ppm silicon oil 1% and caster oil 1%. (stomatal closing), Castor bean oil 1% and silicon oil 1%. The solutions were sprayed on the leaves by a hand-held sprayer. Before spraying antitranspirants, irrigation will be applied at every 7days after foliar spray of antitranspirants the irrigation in these pots were interrupted in all treatments and irrigation intervals increased from 7 days to 10, 13 and 15.

The Physio-biochemical observations recorded after treatment application. Two liter of the water was provided to each pot as and when required. The control plants were irrigated with tape water. The top most mature leaves were sampled after treatment application to plants for observation.

Transpiration rate

Transpiration rate was measured by Infra-Red Gas Analyzer (CI- 301, gas analyzer, USA). Three topmost fully expanded leaves from each treatment were selected randomly for the measurements.

Relative water content

Properly washed 0.2g fresh leaves discs or parts were weighed to record fresh weight (FW), followed by dipping half of their portion in Petri dish distilled water for 12 h. The leaves were blotted to wipe off excess moisture, weighed to record fully turgid weight (TW), subject to oven drying at 70 °C till constant weight to record dry weight (DW). Relative water content was calculated as per formula and expressed as RWC= [FW-DW] X100/ [TW-DW] of standard method of (Barrs and Weatherley, 1962) [2].

Reducing sugar content

Reducing sugar was estimated in the ethanol soluble fraction as described by Nelson (1994) [9]. 0.5 ml aliquot was taken in test tube and volume was made to 1 ml with double distilled water. After adding 1 ml copper reagent, the tubes were bath for 20 minutes and then to cooled at room temperature. Added 1 ml aresnomolybdate reagent then to each test tube. Final volume was made to 10 ml with double distilled water and the absorbance was measured at 570 nm.

Cell membrane injury (CMI)

Leaves of control (C) and salt stressed plants(T) were collected and thoroughly washed with distilled water.1g of a leaf sample was placed in 25 ml of double distilled water at 40 °C for 30 min and thereafter an electric conductivity(C1) was measured with conductivity meter. Subsequently, the same samples were placed on boiling water bath (100 °C) for 10 min and their electric conductivity was recorded. CMI% was calculated using the method given by Sullivan, C.Y. 1971 [11]

$$CMS\% = 1 - \left[\frac{1-(T1-T2)}{1-(C1-C2)}\right] x100 \text{ or \% injury=100-CMS}$$

Where T and C refer to treatment and control, respectively and subscripts 1 and 2 refer to initial and final conductance, respectively.

Determination of proline

Leaves of plants were homogenized with 3% sulphosalicylic acid and the homogenate was centrifuged at 3,000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 hr. and then absorbance at 520nm was determined. Contents of proline were expressed as µg/g fr. wt. of leaf (Bates, *et al*., 1973) [3].

Statistical analysis

There were three replication for each treatment. Statistical analysis of data was processed using completely randomized block design (Gomez and Gomez, 1984) [5].

Results and Discussion

Effect of Antitranspirants on transpiration rate

A study of the data in the table 1 indicated that a significant decrease was observed in the transpiration rate in the plant leaves by the use of Kaoline 6% followed by Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2ppm, silicon oil 1%, and caster oil 1%.

The decrease in mean transpiration rate in plant leaves by the foliar treatment of Kaoline 6%, Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1% is 30.70, 31.23, 18.23, 24.93, 23.62, 15.48, 16.01 and 14.96 over control. The lowest transpiration rate was recorded due to treatment with 6% kaoline as foliar treatment.

Effect of Antitranspirants on relative water content

A study of the data in the table 1 indicated that a significant decrease was observed in the relative water content content in the plant leaves by the use of Kaoline 6% followed by Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1%.

The decrease in relative water content in leaves by the foliar treatment of Kaoline 6%, Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1% is 14.28, 12.72, 8.28, 9.43, 7.09, 4.63. 7.69 and 5.88 over control. The highest relative water content was recorded due to treatment with 6%kaoline as foliar treatment.

Table 1: Effect of antitranspirants on Physiological traits of *ziziphus rotundifolia* under salinity.

Treatments	Physiological traits of ber leaf					
	Transpiration rate of leaf (mMol/m²s¹)			Relative leaf water content (%)		
	2018	2019	Pooled mean	2018	2019	Pooled mean
Control	3.76	3.86	3.81	73	71	72.0
Kaoline 2%	3.12	3.08	3.10	79	78	78.5
Kaoline 4%	2.67	2.57	2.62	85	80	82.5
Kaoline 6%	2.66	2.61	2.64	86	82	84.0
PMA 2 ppm	3.26	3.18	3.22	77	74	75.5
PMA 4 ppm	2.92	2.89	2.91	79	76	77.5
PMA 6 ppm	2.88	2.85	2.86	81	78	79.5
Silicon oil 1%	2.78	3.62	3.20	80	76	78.0
Caster oil 1%	2.82	3.66	3.24	79	74	76.5
CD(P=0.05)	0.12	0.16	0.14	1.0	1.3	1.15

Effect of Antitranspirants on proline content

A study of the data in the table 1 indicated that a significant decrease was observed in the proline content in the plant leaves by the use of Kaoline 6% followed by Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1%.

Table 2: Effect of antitranspirants on Physiological traits of *ziziphus rotundifolia* under Salinity.

Treatments	Biochemical traits of ber leaf								
	Proline content (µg/g fr wt of leaf)			Cell membrane injury (%)			Reducing sugar content (mg/g fr wt of leaf)		
	2018	2019	Pooled mean	2018	2019	Pooled mean	2018	2019	Pooled mean
Control	42.6	40.7	41.65	24.2	24.66	24.43	15.22	14.89	15.05
Kaoline 2%	38.66	37.55	38.11	22.56	21.15	21.85	14.62	14.11	14.36
Kaoline 4%	32.14	30.86	31.5	20.88	18.14	19.51	14.1	14.52	14.31
Kaoline 6%	30.22	30.1	30.16	19.56	18.36	18.96	12.33	13.22	12.77
PMA 2 ppm	38.92	37.88	38.4	22.98	21.66	22.32	14.89	14.44	14.66
PMA 4 ppm	32.64	31.12	31.88	21.28	18.56	19.92	14.53	14.82	14.67
PMA 6 ppm	30.72	30.5	30.61	19.96	18.76	19.36	12.72	13.62	13.17
Silicon oil 1%	36.82	37.12	36.97	22.14	21.66	21.9	14.18	13.56	13.87
Caster oil 1%	34.15	34.44	34.29	21.42	21.12	21.27	13.61	13.96	13.78
CD (P=0.05)	5.44	4.87	5.15	3.62	4.12	3.87	2.14	1.42	1.78

The decrease in proline content in leaves by the foliar treatment of Kaoline 6%, Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1% is 27.58, 24.36, 8.49, 26.5, 23.45, 7.81, 11.23 and 17.67 over control. The lowest proline content was recorded due to treatment with 6% kaoline as foliar treatment.

Effect of Antitranspirants on cell membrane injury

A study of the data in the table 1 indicated that a significant decrease was observed in the cell membrane injury in the plant leaves by the use of Kaoline 6% followed by Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6 ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2ppm, silicon oil 1%, and caster oil 1%.

The decrease in cell membrane injury in leaves by the foliar treatment of Kaoline 6%, Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1% is 22.39, 20.13, 10.56, 20.75, 18.46, 8.64, 10.35 and 12.93 over control. The lowest cell membrane injury was recorded due to treatment with 6%kaoline as foliar treatment.

Effect of Antitranspirants on reducing sugar content

A study of the data in the table 1 indicated that a significant decrease was observed in the reducing sugar content in the plant leaves by the use of Kaoline 6% followed by Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6 ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1%.

The decrease in reducing sugar content in leaves by the foliar treatment of Kaoline 6%, Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1% is 15.14, 4.91, 4.58, 12.49, 2.52, 7.84 and 8.43 over control. The lowest reducing sugar content was recorded due to treatment with 6% kaoline as foliar treatment.

Conclusion

The transpiration rate of plants may be reduced by the use of these antitranspirants. The maximum decrease in transpiration rate is recorded by foliar treatment of plant from kaoline at 6% concentration of it as compared to Kaoline 4%, Kaoline 2%, phenyl mercuric acetate 6ppm, phenyl mercuric acetate 4ppm phenyl mercuric acetate 2 ppm, silicon oil 1%, and caster oil 1%.

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Conflict of interest

None

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