Original Research Article

Priming Induction in Neighbouring Plants of *Gossypium hirsutum* under Salt Stress

ABSTRACT

Plants are subjected to various types of environmental stresses throughout their lifecycle. It has been found that plants are able to communicate with the neighbouring plants under stress conditions through volatile organic compounds. These volatiles act as signals for the neighbouring plants thus preparing them for the upcoming stress, a phenomenon known as priming. So, the present study explores the effects of salt stress on cotton plants and the resultant induction of priming in the nearby plants. For this purpose, salt tolerant cotton (*Gossypium hirsutum*) variety was used. Two concentration levels, 100 mM, and 150 mM were used to study the impacts of the stress. The experiment was divided into two steps for each treatment. In the first step, a set of plants (emitters) was given salt stress. A second set of plants (receivers) was placed adjacent to the stressed plants (emitters), while the third set of plants was placed separately as a control for both the treatments. Various physiological and morphological parameters were measured at the beginning and the end of the first step. In the second step, the receiver plants now termed as "primed" were given same levels of stress while a new set of non-primed plants was placed near the primed plants. These non-primed plants were now treated with 100mM and 150mM of NaCl respectively and the results were compared. The result demonstrates that cotton plants were able to perceive stress signals emitted from stressed neighbours and responded by changing physiology and morphology, possibly preparing themselves for a future stress. The effects were more prominent at 150mM concentration. The result showed that the plants might have communicated with each other through airborne signals and priming was induced on reception of those signals. It is assumed that the tolerance of cotton plants against abiotic stresses may be increased by artificially applying these volatile organic compounds.

1. INTRODUCTION

Communication among plants has been an area of interest for quite a long time now. This communication is done by releasing and sensing volatile organic compounds. These compounds are released by the plants under normal circumstances [1] and are found in almost all plant tissues including roots, stem, leaves and flower tissues.

However, under conditions of stress, the level and composition of these volatile organic compounds has been reported to fluctuate [2]. Several studies suggest that plants are also able to receive signals from neighbouring plants in the form of these VOCs. Plants can thus detect any change in the concentration of such signals, from a neighbouring plant, caused by biotic or abiotic stress [3, 5]. Interaction among plants through roots via common mycorrhizal networks (CMNs) has already been reported in several studies

The present study focuses on plant to plant communication as a result of VOC emission through the leaves. VOCs released by leaf tissues are called "Green Leaf Volatiles" or GLVs [6, 7]. Alterations in the levels of GLVs can induce defensive responses in the form of physiological and morphological changes in the unstressed neighbours [8]. Green leaf volatiles mainly consist of alcohols, aldehydes, ketones, monoterpenes and esters [9]. Several studies show the effects of biotic stresses on GLVs concentration such as viral infection [10, 12].

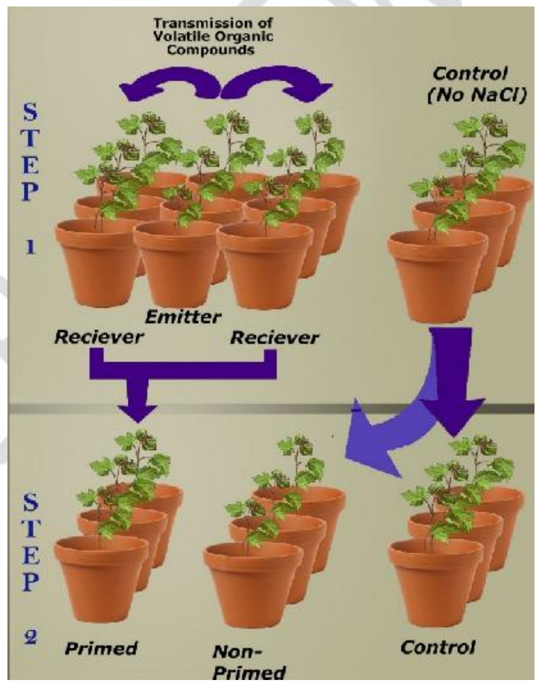
However, there is not much work done over the effects of abiotic stress on the levels of VOCs and the resultant induction of resistance (priming) in the receiver plants. Salinity is an abiotic stress and is associated with several changes in the treated plants at both morphological as well as physiological level. Changes in the levels of isoprene and other volatile organic compounds have been associated with salinity stress. So the following experiment was conducted in order to study effects of salinity stress on both treated and untreated cotton plants as well as the induction of priming in the untreated neighbouring plants.

2. MATERIAL AND METHODS

The experiment was carried out at Cotton Research Station, Ayub Agricultural Research Institute Faisalabad. Fuzzy seeds of cotton (*Gossypium hirsutum*) variety FH-142 were soaked under water overnight. These seeds were then sown in polythene bags containing a mixture of compost and alluvial soil (mixture of sand silt and clay) for good germination. 210 bags were filled and 4-5 seeds were sown in each bag on 02 March, 2018 and placed it in green house.

Transplantation was done after three weeks from polythene bags to plastic pots having one feet height. These pots were filled with soil, alluvial soil and FYM mixture in 4:4:1 proportion. Selected 150 pots and place them under the tunnel and watered it frequently.

The experiment was arranged into three plots for treatment 1, 2 and control respectively and these plots were partitioned by polythene paper. Total 36 plants were placed in 1st and 2nd plot each, while third plot contained 72 plants. In 1st and 2nd plot, arrange the plants in three rows and each row contain 12 plants. The mid row called emitter while other two rows called receiver, we named it because according to hypothesis, emitter release volatile organic compounds when we gave any stress while receiver which is placed at the periphery of emitter receive those compounds (Fig. 1). After two weeks of transplantation a salt stress was given to emitters only. Two levels of NaCl concentration were used, 100mM and 150mM respectively. The emitters placed in the 1st plot was given 100 mM solution while other emitters placed in 2nd plot was given 150 mM solution while rest of the plants were on tap water. 100 ml salt solution to emitters and 100 ml tap water to rest of the plants were given.



Since the whole experiment was carried out in two steps. In step one a group of plants called receivers placed in the proximity of salt stressed plants called emitter for twenty days. Another group of plant called control placed apart from emitter and receiver. In second step, receiver plants were taken from step one (Fig. 1). Now it is called primed plants, gave a salt stress to primed and non-primed plants (taken from the control of step 1) and compare it with control. This whole experiment was repeated under different dose of salt concentration.

The parameters that studied are Leaf area, Total dry weight, Total Fresh weight, Relative growth rate, Proline content, No. of leaves per plant, Shoot length, Root length, Relative growth rate, H_2O_2 species, Dry shoot weight, Dry root weight, Moisture content, Root to shoot ratio, Leaf Area.

The plant material with sample size of six were taken at the beginning of experiment (day 0), at the end of step 1 just after 20 days of experiment started and at the end of step 2 , after 20 days of step 1 finished. Plants were uprooted carefully, gently washing away the soil and take fresh weight of each sample by using weighing balance. Different morphological trait that are mentioned above was then analyse. Leaf area was measure using leaf area meter. Plant material was then oven dried at 70 °C for 48 hours. Relative Growth rate was measured by using total dry weight according to Pérez [13] method using the formula RGR = $(\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W is a total dry weight and t is a time between two reference period which is 20 days.

Proline content was determined by Bates [14] method with little modification. Take 250 g sample of ground leaf and add 10ml of 3% solution of salphosalicyclic acid, then centrifuged it under 3000rpm for 10 min, transfer 2ml of supernatant to new test tube after centrifugation. Add 2ml of 6M ortho phosphoric acid, 2ml acid ninhydrin and 2ml glacial acetic acid. Keep it in water bath for one hour at 100°C. After it add 4ml of toluene, shake it well and wait for few minutes. Separate the upper layer of solution and observe it in spectrophotometer under 520nm. The reading was compared with standards. H_2O_2 analysis were determined by Orozco-Cardenas and Ryan [15] method.

3. STATISTICAL ANALYSIS

LSD test was used to compare the variation within and between the two treatments for each parameter, while paired t-test was used to compare the step 1 with step 2. These statistical analysis was conducted using the software Statistix 8.1. One-way ANOVA, Mean comparison test and Student's t-test, depending on the dataset, were used to identify significant differences between and within the treatments ($P \leq 0.05$).

Table no. 1

Step 1		Control	Treatment 1		Treatment 2	
			Emitter	Receiver	Emitter	Receiver
Physiological Data	Proline Content (520nm) µmol g^{-1}	0.078^{C}	0.088^{B}	0.091^{B}	0.079^{C}	0.102^{A}
	H_2O_2 Species (390nm)	0.392^{D}	0.499^{B}	0.363^{D}	0.574^{A}	0.471^{C}
	Moisture Content	15.042^{A}	9.091^{B}	9.629^{B}	6.835^{C}	6.566^{C}
Morphological Data	Relative Growth Rate (g)	0.170^{A}	0.131^{B}	0.137^{B}	0.107^{C}	0.107^{C}
	Total Fresh Weight (g)	19.218^{A}	12.498^{B}	12.418^{B}	9.756^{C}	9.498^{C}
	Total Dry Weight (g)	4.177^{A}	3.407^{B}	3.517^{B}	2.922^{C}	2.932^{C}
	Dry Shoot Weight (g)	3.263^{A}	2.818^{AB}	2.79^{AB}	2.414^{BC}	2.245^{C}
	Dry Root Weight (g)	0.913^{A}	0.588^{C}	0.720^{B}	0.507^{D}	0.687^{B}
	Root Length (cm)	14.200^{A}	12.420^{C}	12.440^{C}	12.867^{BC}	13.250^{B}
	Shoot Length (cm)	23.125^{A}	21.867^{B}	21.333^{BC}	20.967^{C}	20.683^{C}
	Root / Shoot in Weight (g)	0.280^{A}	0.209^{B}	0.257^{AB}	0.210^{B}	0.306^{A}
	Root / Shoot in Length (cm)	0.614^{AB}	0.568^{C}	0.583^{BC}	0.614^{AB}	0.641^{A}
	No. of Flowers / 6 plants	13	7	5	6	4
	No. of Leaves	9^{A}	8^{A}	8^{AB}	8^{AB}	7^{B}
	No. of Nodes	7^{A}	6^{AB}	6^{AB}	6^{B}	6^{B}

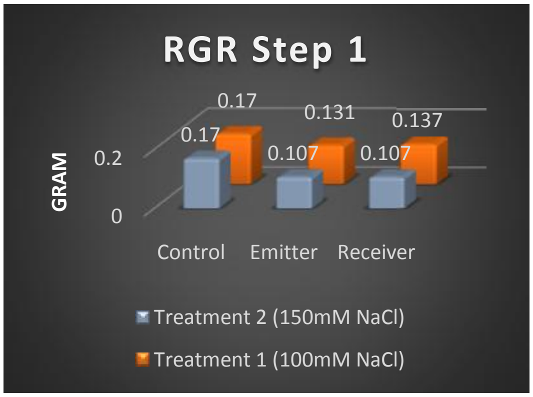
Table No. 2

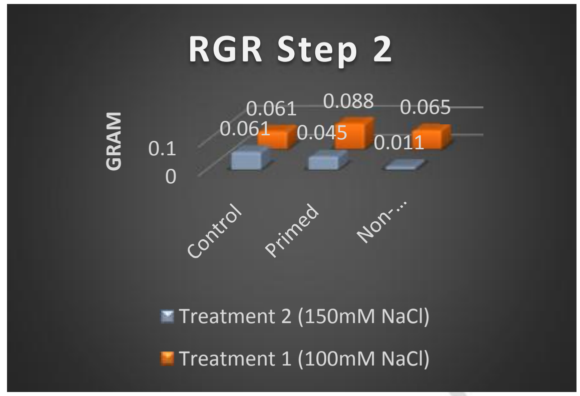
Step 2		Control	Treatment 1		Treatment 2	
			Primed	Non-Primed	Primed	Non-Primed
Physiological Data	Proline Content (520nm)	0.205^{B}	0.183^{C}	0.139^{D}	0.209^{B}	0.294^{A}
	H_2O_2 Species (390nm)	1.029^{B}	0.777^{C}	0.569^{D}	1.096^{B}	1.45^{A}

	Moisture Content	13.514^{A}	10.206^{C}	12.421^{B}	6.951^{E}	8.297^{D}
Morphological Data	**Relative Growth Rate (g)**	0.061^{BC}	0.088^{A}	0.065^{B}	0.045^{C}	0.011^{D}
	Total Fresh Weight (g)	18.916^{A}	14.754^{C}	17.907^{B}	10.782^{E}	12.701^{D}
	Total Dry Weight (g)	5.403^{A}	4.548^{B}	5.485^{A}	3.832^{C}	4.404^{B}
	Dry Shoot Weight (g)	4.154^{B}	3.385^{B}	4.235^{A}	2.816^{C}	3.266^{BC}
	Dry Root Weight (g)	1.249^{A}	1.163^{AB}	1.250^{A}	1.015^{B}	1.138^{AB}
	Root Length (cm)	23.400^{A}	21.650^{B}	14.300^{D}	18.833^{C}	14.260^{D}
	Shoot Length (cm)	23.683^{B}	22.750^{C}	24.800^{A}	20.967^{D}	23.220^{BC}
	Root / Shoot in Weight (g)	0.301^{A}	0.343^{A}	0.295^{A}	0.360^{A}	0.348^{A}
	Root / Shoot in Length (cm)	0.988^{A}	0.952^{A}	0.577^{C}	0.898^{B}	0.614^{C}
	No. of Flowers / 6 plants	13	8	11	5	7
	No. of Balls / 6 Plants	2	6	3	4	1
	No. of Leaves	9^{AB}	8^{AB}	10^{A}	7^{D}	7^{CD}
	No. of Nodes	8^{AB}	7^{AB}	9^{A}	7^{B}	7^{AB}

Table No. 3

Change in reading in % from step 1 to step 2		**Control**	**Treatment 1**		**Treatment 2**	
			Primed	**Non-Primed**	**Primed**	**Non-Primed**
Physiological Data	**Change in Proline Content (520nm)**	162.82*	101*	78*	104*	276*
	Change in H2O2 Species (390nm)	162.5*	114*	45.1*	132.7*	269.9*
	Change in Moisture Content	-10.15 N.S	5.99 N.S	-17.42 N.S	5.86 N.S	-44.84 *
Morphological Data	**Change in Total Fresh Weight (g)**	-1.59 N.S	18.81 N.S	-7.32 N.S	13.51 N.S	-51.31 *
	Change in Total Dry Weight (g)	29.35 *	29.31 *	31.31 N.S	30.69 *	5.43 N.S
	Change in Dry Shoot Weight (g)	27.3 *	21.32 *	29.78 N.S	25.43 N.S	0.09 N.S
	Change in Dry Root Weight (g)	36.8 *	61.52 *	36.91 N.S	47.74 *	24.64 N.S
	Change in Root Length (cm)	64.79 *	74.03 *	0.7 N.S	42.13 *	0.42 N.S
	Change in Shoot Length (cm)	2.41 N.S	6.64*	7.24 *	1.37 N.S	0.41 N.S





4. RESULTS

4.1 PROLINE CONTENT

The experiment was organized in to three treatments with two doses of NaCl concentration and one is control. As we see in Table 1, the emitter and receiver of treatment 1 is non-significant while both are significant with control. It means that the Emitter which is under salt stress produce more proline than control and it induces a receiver plant to produce proline. When we move this receiver to step 2, it become primed and shows a significant result with control as well as Non-primed but it seems that when plant grow the rate of proline content in primed plant reduces and it produce less amount of proline than control while non primed plant also reduces its rate as compared with control and receiver.

In treatment 2 (under 150 mM NaCl), emitter shows non-significant with control. The treatment 2 have no effect on proline while it induces proline in receiver. It is assume that emitter plant produce another compound and reduces the amount of proline and that another compound induces a proline in receiver. When we go to step 2, a receiver gives better result than treatment one, its proline content increase up to 104% and becomes equal to control. Non primed plants went to more stress and it proline content rises up to 276%.

4.2 H_2O_2 SPECIES

At the end of step one, the emitter produce more H_2O_2 than control and have no effect on receiver under treatment 1. When this receiver move to step 2, it shows a negative response compared with control. The non-primed plant coming from control of step 1 also shows a negative response. Overall treatment 1 do not induces a priming effect on neighboring plants in case of H_2O_2 species.

In treatment 2 emitter produce large amount of H_2O_2 and induces in neighboring plant. Receiver show a priming effect in step 2 and increases its production up to 132.7% and tried to near control. Non-primed plants increases H2O2 up to 269.9%.

4.3 MOISTURE CONTENT

At the end of step 1, salt greatly effect on emitter and this emitter induce a priming effect on receiver. Moisture content decreases compared with control. In step 2 receiver and control undergoes no changes while non-primed plant coming from control of step 1 shows a negative response. Non-primed plant reduces its moisture content in treatment 1 and 2 up to -17.42% and -44.84 respectively.

4.4 RELATIVE GROWTH RATE

Relative growth rate means, how much increase in total dry weight per day. The emitter and receiver shows a non-significant results with each other while both shows a significant result with control. This result shows that the emitter and receiver reduces its growth rate in both treatment but if we compare treatments with each other, it shows that the treatment 2 reduces more growth rate with treatment 1.

In step 2 receiver of both treatments show a non-significant result with control. This shows that receiver which is now primed speed up its growth rate and becomes equal to control. Non-primed plant in treatment 1 shows non-significant with control, this is due to stagnant growth of plants in step 2. Non-primed in treatment 2 greatly reduces its growth rate due to high level of NaCl concentration.

4.5 PLANT WEIGHT

Plant weight is a major character to study salt stress in plants. Total fresh and dry weight of emitter and receiver in both treatments of step 1 shows a significant reduction in weight. The fresh weight decrease in emitter as well as receiver might be due to decrease in moisture content and dry root weight. In step 2 total fresh weight of receiver shows a non-significant result due to no change in moisture content.

Under treatment 2, total fresh and dry weight of emitter and receiver both show non-significant result with each other, which shows that the emitter induces a priming effect on receiver. In step 2 the increase in fresh weight was non-significant because receiver after induction start to increase total dry weight of shoot, while moisture content remains stagnant.

4.6 PLANT HEIGHT

At the end of step 1, both root and shoot length increases up to definite length but emitter and receiver of both treatment show significant reduction in length of both character compared with control. Root length of primed in step 2 of treatment 1 and 2 increases up to 74.03% and 42.13% respectively. While non-primed plant shows no increase in root length compared with control. Shoot length in step 2 show non-significant due to stagnant growth.

4.7 NO OF FLOWERS AND BOLLS

Number of flowers per six plants were counted at the end of step 1. Controls showed the maximum number of flowers per six plants. The number of flowers were significantly reduced in the emitters and receivers in both the treatments.

The no. of flowers per six plants remained the same for control plants. However, the no. of flowers in treatment 1 primed plants increased from 5 flowers to 8 flowers. No. of flowers in the non-primed plants of treatment 1 decreased from 13 to 11. In treatment 2, no. of flowers in the primed plants increased from 4 to 5 while their no. reduced from 13 to 7.

At the end of step 2, an average of two bolls appeared on the control plants. 6 bolls appeared on the primed while 3 appeared on the non-primed plants of treatment 1. Similarly, 4 bolls were formed on the primed while 1 boll was appeared on average in the non-primed plants of treatment 2.

5. DISCUSSION

Priming is a phenomenon whereby plants once exposed to a particular stress, become tolerant towards it as a result of physiological and morphological changes. Several studies have been conducted to study plant-to-plant communications caused by biotic and abiotic stresses [16]

This experiment was conducted to study whether the neighboring cotton plants were able to receive air borne signals from the salinity stressed plants. As indicated by the table, although the receivers were not directly exposed to the salt stress, their dry and fresh weights were still reduced compared to the controls. Salinity has been associated with lowering of both fresh and dry weights and it is inversely proportional to the salt concentration [17, 19].

The reduction in relative growth rate of the receivers compared with the control indicates the possibility of some sort of air borne communication between the emitters and the receivers.

As indicated (Table 1), the fresh and dry weights of cotton emitter and receiver plants affected by 150mmol salt concentration were much lower than those affected with 100mmol salt concentration. Similar effects were observed in the case of dry root weight. Salt stress has been found to be associated with reduction in total moisture content of the plant [20, 21].

Same was the case with the emitters in both the treatments. Interestingly, the moisture content of the neighboring plants were also lowered indicating possibility of plant to plant communication. Salinity causes reduction in root and shoot length leading to stunted growth [22, 25].

By the end of step 1, flowering had been induced in the cotton plants. The number of flowers in the emitter and receiver plants of both the treatments were much less than the control plants.

5.1 STEP 2

Step 2 was conducted to check whether the receivers in step 1 had developed resistance against the same stress they were exposed to as receivers.

The data for various morphological and physiological parameters was collected after a 20 days interval.

Therefore, 100mmol treatment had a little effect on the non-primed and primed plants. Whereas, 150mmol treatment caused significant decline in the moisture content of non-primed plants. No significant effect was observed on the primed plants indicating that the plants had developed resistance against the stress.

Similar to the moisture content, no significant change was observed in primed and non-primed plants in treatment 1 while the moisture content of non-primed plants was significantly lower than the primed plants. This can be attributed to the loss in moisture content due to salinity stress.

There was no increase in total dry weight of non-primed plants in both the treatments. While the dry weight increased for control and the primed plants, it remained stagnant in the non-primed plants indicating stagnant growth. Root length is reduced by salt stress. Though the root length increased normally in the control and the primed plants, there was no significant increase in the root length of the non-primed plants in both the treatments. Low levels of salinity have positive effects on shoot length at certain time of development.

When the treatment 1 primed and non-primed plants were treated with 100mmol salinity, there shoot length increased. There was no significant increase in the shoot length of treatment 2 primed and non-primed plants, however, in comparison with the non-primed plants, the shoot length increased more in the primed plants. Flowering is inversely affected by salinity.

As indicated by the graphs, the flowering was greatly reduced in treatment 2 non primed plants in comparison to the controlled. Salinity induces boll formation in the cotton plants. Rapid boll formation in the primed plants in both the treatments indicate acclimatization to salinity.

5.3 CHANGES IN PHYSIOLOGY

In step 1, the proline content of both the emitters and the receivers increased slightly at 100mmol concentration. At 150mmoml concentration, there was no increase in the proline content but the proline content of the receivers was much higher [26].

This is probably due to the formation of some other compound at higher salt concentration [28] Salinity causes increase in the H_20_2 species [27].

As indicated, the H2O2 species increased at both treatment levels. Interestingly, the H2O2 concentration also increased in the receivers indicating communication.

In step 2, the proline content of treatment 2 non primed plants was more than the primed plants indicating preparedness. However, the proline content of control also increased which was due to growth of the plant [28]. Same was true for H_2O_2 species.

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