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RESEARCH PAPER

Physiological and biochemical basis of PGR's induced insect resistance in cotton

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Abstract: The experiment on physiological and biochemical basis of PGRs induced insect resistance in cotton was conducted during *kharif* 2018 at ARS, Dharwad farm, UAS, Dharwad. The experiment consisted of seven sub plot treatments (T_1: Control T_2: CCC 50 ppm, T_3: CCC 100 ppm, T_4: SA 50 ppm, T_5: SA 100 ppm, T_6: MC 50 ppm and T_7: MC 100 ppm) maintained at two main plot treatments (M_1: Sucking pest protected and M_2: Sucking pest unprotected) laid in split plot design with three replications. The sucking pest susceptible non-*Bt* cotton genotype, Suraj was grown with recommended package and PGRs are sprayed at 60 DAS. Pesticides for sucking pests were sprayed only for treatments of protected condition. Periodical, morphological, biochemical and pest load parameters were recorded along with yield and yield attributes. Foliar application of MC at 100 ppm recorded significantly lower pest load (Thrips-23.87, Aphids-7.3, Jassids-24.5) lower reducing sugar (4.51 mg /g fr.wt.), lower protein content (8.04 mg /g fr.wt.), higher phenol (3.96 mg /g fr.wt.) and gossypol content (33.83 µg /g fr.wt.) under protected condition over other treatments. However, foliar spray of SA at 100 ppm recorded significantly more boll numbers (45.38), boll weight (4.79 g), seed cotton yield (19.16 q ha^{-1}) and harvest index (42.44%) compared to other treatments including control.

Key words: Cotton, Host plant, Insect resistance, Physiological

Introduction

Among the four currently cultivated cotton *Gossypium* species, two allotetraploids (*Gossypium hirsutum and Gossypium barbadense*) and two diploids (*Gossypium herbaceum and Gossypium arboreum*), *Gossypium hirsutum* also known as upland cotton, occupies major area about 90 per cent of the world's cotton whereas, the remaining species are cultivated only in small areas (Fryxell, 1979).

Cotton is cultivated over 70 countries, with total coverage area of 331 lakh ha and 1165 lakh bales of production with productivity of 765 kg lint per ha. India is the traditional home for cultivation of cotton and textiles, it covers 117.71 lakh ha area of cotton cultivation by producing 349 lakh bales with productivity of 504 kg lint per ha. In Karnataka, it is cultivated in area of 6.12 lakh ha and 20 lakh bales of production with productivity of 556 kg lint per ha (Anon., 2017).

Even though India has the largest area under cultivation of cotton, but it's productivity is only 504 kg lint per ha. as compared to world's productivity of 765 kg lint per ha. Major reasons for the low productivity are biotic stress and abiotic stress, among which the major biotic factor is insect pests. The average loss due to insect pest is about 8 to 10 per cent, but under favorable conditions, infestation of insect pest can cause heavy qualitative as well as quantitative losses varying from 45 to 48 per cent. (Naqvi, 1976). The major sucking pests that plague cotton are Jassid (*Amrascadevastans*), whitefly (*Bemisiatabaci*), Aphid (*Aphis gossypii*) and Thrips (*Thysanoptera*) which cause most physiological and morphological damage to the cotton crop.

The host plant resistance mechanisms in response to infestation of insect pest consist of a several morphological, biochemical, physiological events, such as increase in leaf thickness, trichome density, variation in sugar, protein, gossypol and phenol content which help in resisting the incidence of insect pests. The primary metabolites like carbohydrates and proteins also function as precursors of secondary metabolites, which act as major resisting elements in cotton plants. These secondary metabolites help to colonize and exploit the herbivores insect pests, thus controlling the host plants.

Recently, resistance to boll feeding and foliage insects has been achieved by transgenic technology in *Bt.* cotton. But, there is no achievement in resistance against sucking pests in both *Bt.* cotton as well as non-*Bt.* cotton. The only available management practice to control the sucking pest is to apply systemic insecticides, which in turn will lead to negative effect on environment. Thus, it is imperative for us to go for ecologically effective options to control the pest in a sustained manner.

Among various options available, plant growth regulators which are similar to natural plant hormones offer a safer approach. These have been used in regulation of plants growth, playing an important role in increasing agricultural production. These growth regulators will be safer to human health, if they are used in recommended dose coupled with good agricultural practice. Most of the growth regulators also help to gain certain degree of resistance against stress by manipulating source-sink relation and also improving the quality of plant produce.

It is hypothesized that PGRs may alter the plant biochemistry leading to induction of acquired resistance to sucking pests. Hence, the present experiment.

Material and methods

The field experiment was carried out, at Agriculture Research Station, Farm (Hebballi), Dharwad on *hirsutum* cotton genotype to study the variations in insect pest resistance due to PGR's spray. The experiment was laid out in split plot design consisted of seven sub plot treatments (T_1: Control T_2: CCC 50 ppm, T_3: CCC 100 ppm, T_4: SA 50 ppm, T_5:SA 100 ppm, T_6: MC 50 ppm and T_7: MC 100 ppm) maintained at two main plot treatments (M_1: Sucking pest protected and M_2: Sucking pest unprotected) and three replications with the row spacing of 60cm and plant to plant spacing of 30cm with plot size 2.4m width and 4.5m length. The crop was raised with recommended package of practice except sucking pest management.

Sucking insect pest load was recorded as number of pests (aphids, thrips and jassids) per three leaves of plants. Number of each pest on top, middle and bottom leaf of every plant were before three days and after 7th and 15th day of PGR spray.

Four biochemical parameters *i.e.* Total sugar content, gossypol content, protein content and phenol content were estimated. Total sugar estimation was done as per the method of Du Bois *et al*. (1956). Gossypol content was determined by the method of Bell (1967). Protein content was determined by Lowry's method suggested by Geiger and Bessman (1972) and phenol estimation was done by FCR method suggested by Singleton *et al.* (1999).

Total number of bolls picked from the five tagged plants was counted and average was worked out. Seed cotton yield per plant was worked out by weighing total number of bolls picked from the five tagged plants and average was expressed in grams. Boll weight was obtained from 20 bolls selected randomly from the net plot was weighed and the mean single boll weight was worked out and expressed in grams. Seed cotton yield per hectare was worked out from plot yield in each treatment and expressed as quintals per hectare. Harvest index was calculated by dividing economic yield by biological yield and expressed as percent.

Results and discussion

Population of pests did not vary significantly before spray of PGR's with respect to both protected and unprotected condition. After spraying of PGR's at 60DAS, there was a

Table 1. Effect of plant growth regulators on thrips load in cotton grown under sucking pest protected and unprotected condition

Treatments	Thrips load (no./3 leaf)								
	Before spray			After 7th of spray			After 15th of spray		
	P	UP	Mean	P	UP	Mean	P	UP	Mean
S_1- Control	41.55	53.00	47.28	29.97	44.26	37.11	26.43	40.17	33.30
S_2 - CCC at 50 ppm	45.53	49.87	47.70	25.70	38.14	31.92	23.48	35.00	29.24
S_3 - CCC at 100 ppm	46.53	53.47	50.00	23.97	35.17	29.57	22.56	34.46	28.51
S_4- SA at 50 ppm	49.00	51.67	50.33	19.50	32.90	26.20	20.94	32.21	26.58
S_5- SA at 100 ppm	43.60	51.60	47.60	17.19	30.42	23.81	19.99	29.74	24.86
S_6 - MC at 50 ppm	47.20	53.00	50.10	16.23	29.71	22.97	22.43	32.62	27.53
S_7 - MC at 100 ppm	45.59	48.40	46.99	13.55	29.48	21.51	21.36	31.13	26.24
Mean	45.57	51.57		20.87	34.30		22.46	33.62	
ANOVA	S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%	
M	2.753	NS		0.144	0.878		0.113	0.685	
S	1.193	NS		0.243	0.708		0.029	0.083	
M × S	1.688	NS		0.343	1.002		0.040	0.118	

CCC - cycocel (Chlormequate Chloride), C - Mepiquat Chloride, SA - Salicylic Acid, P - Protected, UP - Un protected

Table 2. Effect of plant growth regulators on jassid load in cotton grown under sucking pest protected and unprotected condition

Treatments	Jassids load (no./3 leaf)								
	Before spray			After 7th of spray			After 15th of spray		
	P	UP	Mean	P	UP	Mean	P	UP	Mean
S_1- Control	12.59	13.12	12.85	6.88	9.20	8.04	8.77	13.09	10.93
S_2 - CCC at 50 ppm	12.50	12.91	12.70	6.19	7.55	6.87	8.62	12.35	10.48
S_3 - CCC at 100 ppm	12.23	12.55	12.39	6.16	7.36	6.76	8.57	11.41	9.99
S_4- SA at 50 ppm	12.22	12.50	12.36	6.13	7.68	6.91	8.13	11.22	9.67
S_5- SA at 100 ppm	12.19	12.65	12.42	6.22	7.26	6.74	7.53	10.87	9.20
S_6 - MC at 50 ppm	12.00	12.84	12.42	6.19	7.20	6.69	7.10	10.62	8.86
S_7 - MC at 100 ppm	12.34	12.37	12.36	5.64	6.96	6.30	6.37	10.48	8.42
Mean	12.29	12.71		6.20	7.60		7.87	11.43	
ANOVA	S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%	
M	0.006	NS		0.020	0.120		0.043	0.263	
S	0.003	NS		0.008	0.024		0.011	0.033	
M × S	0.005	NS		0.012	0.034		0.016	0.047	

Table 3. Effect of plant growth regulators on aphid load in cotton grown under sucking pest protected and unprotected condition

Treatments	Aphids (no./leaf)								
	Before spray			After 7th of spray			After 15th of spray		
	P	UP	Mean	P	UP	Mean	P	UP	Mean
S_1- Control	40.14	52.47	46.30	23.97	37.90	30.93	27.30	41.50	34.40
S_2 - CCC at 50 ppm	43.07	50.67	46.87	21.13	34.05	27.59	24.26	36.16	30.21
S_3 - CCC at 100 ppm	38.14	48.93	43.54	19.51	33.84	26.68	23.31	35.60	29.45
S_4- SA at 50 ppm	53.00	51.13	52.07	15.86	29.39	22.62	21.64	33.28	27.46
S_5- SA at 100 ppm	47.33	51.73	49.53	15.86	26.55	21.21	20.65	30.72	25.69
S_6 - MC at 50 ppm	43.72	52.60	48.16	15.05	24.12	19.59	23.18	33.70	28.44
S_7 - MC at 100 ppm	44.43	49.53	46.98	17.08	25.54	21.31	22.06	32.16	27.11
Mean	44.26	51.01		18.35	30.20		23.20	34.73	
ANOVA	S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%	
M	3.101	NS		0.092	0.557		0.130	0.790	
S	1.776	NS		0.033	0.098		0.033	0.096	
M × S	2.511	NS		0.047	0.138		0.047	0.136	

P - Protected, UP - Un protected

Table 4. Effect of plant growth regulators on total sugar and protein content in cotton grown under sucking pest protected and unprotected condition

Treatments	Total sugar (mg /g fr.wt)						Protein content (mg /g fr.wt)					
	After 7th of spray			After 15th of spray			After 7th of spray			After 15th of spray		
	P	UP	Mean	P	UP	Mean	P	UP	Mean	P	UP	Mean
S_1- Control	8.04	9.78	8.91	11.92	13.99	12.96	6.74	8.96	7.85	10.96	12.55	11.75
S_2 - CCC at 50 ppm	6.51	8.50	7.51	10.15	12.06	11.10	6.25	7.92	7.08	10.44	11.51	10.97
S_3 - CCC at 100 ppm	5.84	7.79	6.82	9.00	10.51	9.75	5.73	7.06	6.39	9.89	10.65	10.27
S_4- SA at 50 ppm	4.94	7.25	6.10	7.49	10.15	8.82	4.69	7.11	5.90	9.19	10.71	9.95
S_5- SA at 100 ppm	4.41	6.40	5.41	7.02	7.86	7.44	4.15	6.36	5.26	8.23	9.96	9.09
S_6 - MC at 50 ppm	6.93	8.27	7.60	8.19	9.27	8.73	5.14	7.37	6.26	9.27	10.96	10.12
S_7 - MC at 100 ppm	8.07	9.44	8.76	9.22	9.83	9.52	4.12	4.26	4.19	8.34	7.73	8.04
Mean	6.39	8.20		9.00	10.52		5.26	7.01		9.47	10.58	
ANOVA	S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%		S.Em.±	C.D. @ 5%	
M	0.051	0.313		0.017	0.104		0.010	0.060		0.014	0.083	
S	0.121	0.353		0.021	0.060		0.037	0.109		0.013	0.037	
M × S	0.171	0.500		0.029	0.085		0.053	0.154		0.018	0.052	

P - Protected, UP - Un protected

significant difference for pest load both in protected and unprotected condition. Significantly higher pest load was found in unprotected condition compared to protected condition. Infestation of aphids and jassids reduced protein content significantly in cotton, because they suck the phloem sap to fulfill their nitrogen requirements and deplete protein content. This finding reiterates the results of Shao *et al.* (1994) who observed depletion of protein, starch and soluble sugar content in pulse beetle (*Callosobruchus maculatus*) infested cowpea seeds. Zhou and Xu (2013) reported that infestation of woolly apple aphid (*Eriosoma lanigerum*) reduced soluble sugar, protein, and amino acid content in fuji apple twigs. Wei *et al.* (2015) who stated that reduction in soluble sugar content in various host plants due to infestation of the sucking insect, *Lygus lucorum*. Kler *et al.* (1989) observed reductions in protein content and deterioration of starch properties in rose-grain aphid (*Metopolophium dirhodum)* infested plants.

Infestation of these sucking pests altered various biochemical parameters, ultimately reduce cotton yield. In present study the plants treated with MC (100 ppm) recorded higher levels of phenol content (4.16 mg/g), gossypol content (35.17 µg/g) (Table 5) in unprotected condition indicating induction of pest resistance. Incidence of pest was comparatively low compared to control mainly because of their role in altering their components to act as resistance mechanism against pests. Kanher *et al.* (2016), Van Sumere *et al.* (1975), Acharya *et al.* (2008) and Butter and Vir (1992) also reported that total sugar is higher in susceptible genotypes and lower in resistant genotypes. It is also noticed that phenolic and gossypol content may act as repellent to insects to bring resistance in such genotypes. MC and SA application recorded significant reduced pest load possibly because of increased phenol and gossypol content which are reported as principle biochemical constituents for inducing resistance to sucking pests. Similar results are reported by Nishant *et al*. (2016) who evaluated resistance and susceptible cotton genotypes.

In our findings yield components such as number of bolls, single boll weight and yield per hector (Table 6, Table 7) were varied significantly with MC and SA spray, highest was recorded in salicylic acid sprayed at 100 ppm which was followed by

Table 5. Effect of plant growth regulators on phenol and gossypol content in cotton grown under sucking pest protected and unprotected condition

Treatments	Phenols (mg /g fr.wt)						Gossypol (µg/g fr.wt)					
	After 7th of spray			After 15th of spray			After 7th of spray			After 15th of spray		
	P	UP	Mean	P	UP	Mean	P	UP	Mean	P	UP	Mean
S_1- Control	1.44	1.67	1.55	2.38	2.76	2.57	16.94	19.49	18.21	23.12	25.28	24.20
S_2 - CCC at 50 ppm	1.70	1.88	1.79	2.59	3.08	2.83	18.32	21.30	19.81	24.84	27.45	26.15
S_3 - CCC at 100 ppm	1.62	2.01	1.82	2.76	3.23	2.99	20.27	22.16	21.21	25.30	29.59	27.44
S_4- SA at 50 ppm	1.76	2.22	1.99	3.13	3.40	3.26	21.03	23.77	22.40	27.54	31.44	29.49
S_5- SA at 100 ppm	2.05	2.39	2.22	3.21	3.53	3.37	21.31	24.26	22.78	28.39	32.79	30.59
S_6 - MC at 50 ppm	2.11	2.69	2.40	3.55	3.76	3.65	22.22	25.06	23.64	29.86	33.95	31.91
S_7 - MC at 100 ppm	2.28	2.90	2.59	3.76	4.16	3.96	22.85	25.80	24.32	32.49	35.17	33.83
Mean	1.85	2.25		3.05	3.42		20.42	23.12		27.36	30.90	
ANOVA	S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%	
M	0.009	0.052		0.005	0.028		0.045	0.272		0.070	0.425	
S	0.008	0.024		0.010	0.028		0.036	0.105		0.069	0.200	
M × S	0.011	0.033		0.014	0.040		0.051	0.149		0.097	0.283	

P - Protected, UP - Un protected

mepiquat chloride at 100 ppm and least was observed in unprotected control.

Seed cotton yield was influenced sucking pest populations irrespective of protected and unprotected condition. In the present experiment, mean yield in protected condition was significantly higher (19.16 q ha^{-1}) over unprotected condition (13.18 q ha$^{-1)}$. These results are in confirmatory with Bhosle *et al.* (2009) and Bhute (2010) who reported that seed cotton yield indicated significant differences between protected and unprotected treatments with a yield of 15.03 and 12.62 q/ha, respectively and mean loss of 16.29 % in the seed cotton yield was recorded under unprotected conditions as compared to protected due to sucking pests. Shahawy, *et al.* (2000) reported that spraying MC decreased plant height, number of main stem internodes, monopodial, sympodial branches, number of unopened bolls per plants and increased the boll set percentage, number of open bolls which result in increased boll weight and seed cotton yield. Plants treated with SA at 150 ppm recorded enhanced all growth parameters and yield compared with control. This positive effect of SA may be attributed to the increase of CO_2 assimilation (photosynthetic rate) and increased mineral uptake. This effect probably is related to SA inhibition of Cl^- and Na^+ absorption and its assistance for absorption of Mg, Fe, Mn, N and Cu (Agamy *et al.,* 2013).

Harvest index indicates the translocation efficiency of plants and it is measured in terms of percent of dry matter being utilized for the production of economic yield. In this study treatments, salicylic acid at 100 ppm recorded the maximum harvest index and it has significant positive correlation with yield Basu and Bhatt (1987).

Total sugar and protein content differed significantly with the application of salicylic acid, cycocel and mepiquat chloride and the control recorded significantly higher sugar content (12.96 mg/g fr.wt) over other treatments. Application of salicylic acid at 100 ppm resulted in lower sugar content (7.44 mg/g fr.wt) it shows increasing the concentrations of salicylic acid decreased the sugar content. Total phenol and gossypol content showed significant differences between the treatments and more phenol and gossypol content was recorded in mepiquat chloride which was followed by salicylic acid treatments. Insect pests (thrips, aphids, jassids) will be affected by the bio chemical contents. As the sugar and protein content increase the sucking pests also increase, and as the concentration of phenol and gossypol increases, decrease in number of pests is noticed. So, we can say that mepiquat chloride followed by salicylic acid is the best treatment for controlling sucking pests. The results on yield and yield attributes indicated that all the yield contributing characters *viz.*, number of bolls (45.38), single boll weight (5.19 g), seed cotton yield (19.16 q ha^{-1}) and harvest index (42.44 %) increased significantly with the application of salicylic acid over control. Application of mepiquat chloride proved next best treatment for yield improvement.

The application of PGRs resulted in increase in biochemical constituent's *viz.*, reducing sugar (37.06 %), protein (33.26 %), phenol (21.62 %) and gossypol (13.22 %) content measured at 7th and 15th day of spray under insect unprotected condition over protected leading to induced insect resistance in cotton. In present study the plants treated with MC (100 ppm) recorded higher levels of phenol content (3.96 mg/g), gossypol content (33.83 µg/g), but lower reducing sugar content (4.5 mg/g) which was followed by SA (100 ppm). Incidence of pest was comparatively low compared to control mainly because of their role in altering their components to act as resistance mechanism against pests. It can be concluded that phenol and gossypol content are more in resistant genotypes than the susceptible ones. Whereas, reducing sugar is higher in susceptible genotypes and lower in resistant genotypes. It is also noticed that phenolic and gossypol content may act as repellent to insects, to bring resistance in such genotypes. These results were on par with Kanher *et al.* (2016), Acharya *et al.* (2008), Butter and Vir. (1992), Singh and Agarwal (1983). MC and SA application recorded significant reduced pest load possibly because of increased phenol and gossypol content which are reported as principle biochemical constituents for inducing resistance to sucking pests.Significantly higher seed cotton

Table 6. Effect of plant growth regulators on boll number, boll weight, seed cotton yield and harvest index in cotton grown under sucking pest protected and unprotected condition

Treatments	Number of bolls per plant			Single boll weight (g)			Seed cotton yield (q / ha)			Harvest index (%)		
	P	UP	Mean	P	UP	Mean	P	UP	Mean	P	UP	Mean
S_1- Control	16.72	13.66	15.19	3.81	3.29	3.55	18.16	10.21	14.19	36.34	33.64	34.99
S_2 - CCC @ 50 ppm	21.63	18.83	20.23	4.09	3.70	3.90	19.44	12.04	15.74	36.73	34.98	35.86
S_3 - CCC @ 100 ppm	25.35	22.48	23.92	4.31	4.12	4.22	20.44	13.04	16.74	39.22	36.43	37.83
S_4 - SA @ 50 ppm	39.92	30.29	35.11	5.09	4.49	4.79	22.36	13.95	18.15	42.10	39.11	40.61
S_5 - SA @ 100 ppm	49.64	41.11	45.38	5.27	5.11	5.19	23.18	15.14	19.16	43.80	41.09	42.44
S_6 - MC @ 50 ppm	29.98	28.88	29.43	4.67	3.97	4.32	21.26	13.77	17.52	34.62	31.86	33.24
S_7 - MC @ 100 ppm	40.77	36.76	38.77	5.11	4.32	4.72	21.99	14.13	18.06	37.65	34.28	35.96
Mean	32.00	27.43		4.62	4.15		20.97	13.18		38.64	35.91	
ANOVA	S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%		S.Em±	C.D. @ 5%	
M	0.024	0.144		0.008	0.050		0.005	0.032		0.033	0.200	
S	0.042	0.123		0.010	0.029		0.011	0.031		0.039	0.115	
M × S	0.060	0.174		0.014	0.041		0.015	0.043		0.056	0.163	

P - Protected, UP - Un protected

yield (19.16 q/ha) was recorded in salicylic acid treatments, and lower insect pest load was noticed in mepiquat chloride than control (35-40 %). So, we can conclude that by foliar application of salicylic acid and mepiquat chloride can help to get better yield.

Conclusion

Sucking pests (thrips, aphids and jassids) load depends on the biochemical contents in cotton. As the sugar and protein content increases the sucking pests also increases and as the concentration of phenol and gossypol increases, decrease in number of pests is noticed. The application of PGRs resulted in increase in biochemical constituent's *viz.*, reducing sugar (37.06 %), protein (33.26%), phenol (21.62 %) and gossypol (13.22 %) content measured at 7th and 15th day of spray under insect unprotected condition over protected leading to induced insect resistance in cotton. Significantly higher seed cotton yield (19.16 q/ha) was recorded in salicylic acid treatments and lower insect pest load was noticed in mepiquat chloride than control. Hence, foliar spray of mepiquat chloride or salicylic acid @100 ppm found to be effective for controlling sucking pests through induced resistance and to get higher cotton yield.

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