Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 2642 - 2649
Received 15 April 2021; Accepted 05 May 2021.

The Synergistic Effect for *Pseudomonas Fluorescens* and Salicylic Acid in Chlorophyll A, B and Carotene Content of Wheat Plant (*Triticum Aestivum* L.)

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Abstract

The aim to study the effect of salicylic acid at level (50-100 and150 ppm) and *pseudomonas fluorescens* at (10^5-10^6-10^7 c.f.u./ml)on chlorophyll a, b and carotene for two varieties *(Abu-graib ,Abaa 99)*of *Triticum aestivum* L. The results indicates the significant effect of *pseudomonas fluorescens* and salicylic acid at the combination concentration *P. fluorescens* (10^6c.f.u./ml) (FP2) + SA concentration of (100 ppm) (SA2)) for variety (Abu-graib) was more impact than (Abaa 99) on the physiological aspect like chlorophyll a, b , and carotene content (2.832 , 0.963 , 0.969) mg/g fresh weight respectively while the value of control was (2.113, 0.612,0.618)mg/g respectively. on other hand the results showed that the use of salicylic acid and *pseudomonas fluorescens* induced better as compared with the sole application of SA or *pseudomonas fluorescens*.

Key words : *pseudomonas fluorescens* **,** salicylic acid **,** chlorophyll content , carotene content .

Introduction

Wheat (*Triticum aestivum* L.) is a grain crop of significant economic importance among (cool-season) cereals due to its high grain yield potential (Chenu *et al*., 2017)

Pseudomonas fluorescens is a non-pathogenic bacterium that shares the capacity to make pyoverdines with additional *Pseudomonas* (Ringel and Brüser, 2018) . The effecting plant nutrition can use *Pseudomonas* sp. bacterized plants have a higher chlorophyll level. When compared to the control, bacterization increased chlorophyll a, chlorophyll b, and total chlorophyll contents by 34, 48, and 39 percent respectively (Sharma *et al.*, 2003) .

The effect of foliar salicylic acid applications on chlorophyll content in salt-stressed plants. seedlings were given foliar SA treatments at various concentrations (0.0, 0.25, 0.50, and 1.00 mM). The SA was sprayed twice, once before and once after the transplanting. SA foliar applications, resulted in In both saline and non-saline settings, 1.00 mM SA treatment produced the highest chlorophyll content (Yildirim, Turan and Guvenc, 2008) Salicylic acid is

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a plant hormone that regulators a amount of aspects of plant growth and development, and also the creation of defences against biotic and abiotic stress. In 1933, it was suggested that plants could develop acquired immunity after being infected with a pathogen.(Ku *et al.*, 2018) .

The total pigment molecules present in the leaf are chlorophyll a, chlorophyll b, and carotenoids, and they are needed for photosynthesis. Most plants contain the essential photosynthetic pigments (Chl-a,Chl-b). Chlorophyll-rich plants are expected to grow faster than chlorophyll-deficient plants.(Campbell and Reece, 2005) Description of the synergistic influence of salicylic acid and *Pseudomonas fluorescens* on wheat plant for chlorophyll a, chlorophyll b, and carotenoids, , as well as the changes that occur in terms of reaction to salicylic acid and *Pseudomonas fluorescens* treatment, for two wheat varieties. It is easier to detect some concentration.

MATERIAL AND METHODS

In all experiments, wheat seeds were used, variety (Abaa 99) and variety (Abu Ghraib), which were taken from Al-Najmi Research Station of the National Program for Wheat Development in Iraq . Isolation of Rhizobacteria *Pseudomonas fluorescens* were isolated from different soil samples collected from rhizosphere for the purpose of obtaining pure colonies of *P. fluorescens*, a number of single colonies were selected that gave similar morphological characteristics to *P. fluorescens*. The streaking method was used for the purpose of subculturing these bacteria on the surface of a King B medium , Morphological of properties ,cultural characteristics and biochemical tests according to (Holt *et al*. 1994). .The seeds used in the study were surface sterilized by washing the seeds with The tap water to remove the fogging substance, then they were transferred to a Clorox solution, which is a 4% sodium hypochlorite, and kept in it for three minutes, then the seeds were removed and washed with sterile distilled water 7 times (Pikovskaya , 1948) .

Plant Cultivation

This experiment was conducted under natural conditions in a Greenhouse of (Al Muthanna university /College of sciences) by using two variety of plant seeds *(Triticum aestivum L.)*(Abaa 99) and (Abu–graib). Some of them are foliar in salicylic acid but other are inoculation *pseudomonas fluorescens* and are put in pots that weight 5 kg in 3 replicates for each treatment. The plant cultivation is summers as following : Seeds were cultivated in (1/12/2020). Irrigation of the pots : with 75% field capacity ,Put more than (20 seed/pot). When the seedlings began to grow, reduce their number to(8 seedlings).Age for plant around 10 days the plants were foliar in

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SA + inculation PF. at three stage leaves (Zadoks *et al.*1974) for two weeks ,The harvesting for some plants in after 60 days from sowing for physiological parameters and then the practical experimental was conducted. season : during the wheat growing season (2020-2021) .

measurement of Chlorophyll Type and Carotenoid Content .

The contents of chlorophyll and carotene in fresh leaves are measured using the procedure of (Mackinney .,1941) modifying by (Arnon., 1949) ,to estimate the amount of chlorophyll, the following relationships were applied :

$$Chl.a = \left(12.7\,(D663) - 2.69\,(D645)\right) * \frac{V}{(1000 \times W)}$$

$$Chl.b = (22.9\,(D645) - 4.68\,(D663)) * \frac{V}{(1000 \times W)}$$

$$Carotenoids = 4.2 \times D452.5 - (0.0264\,Chl.a + 0.426\,Chl.b) * \frac{V}{(1000 \times W)}$$

(D) = the optical density reads of chlorophyll extracted at the wavelengths 663 and 645 nm, respectively

(V)= The final volume of the diluted acetone is (80%).

(W)= fresh weight per gram of extracted vegetable tissue.

Experimental Design and Treatments

The experiment was factorial based on a completely randomized design (CRD) with three replicates There were nine treatments for each *(Triticum aestivum* L.*)* cultivar plus the control one the treatment as follows (Khandan-mirkohi, Halbi and Salami, 2017) , the treatment as follows :

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Treatment	Concertation	Methods
T1 : (SA)	SA1 ,SA2 ,SA3=(50+100 and150) ppm	foliar shoot method
T2 : (PF)	PF1,PF2,PF3=Concentration *P. fluorescens* (4×10^5), (4×10^6), (4×10^7) c.f.u./ml	inoculation roots
T3 : (SA+PF)	different concentration that use in number T1 , T2	Combination between T1+T2 .

Results :

Effect of *P. fluorescens* and SA on the Chl. a Content :

The Impact of *Pseudomonas fluorescens* and salicylic acid on chlorophyll a content in (*Triticum aestivum*) varieties (Abaa 99) , (Abu-graib). Table (1) shows .For SA concentration of 100 ppm (SA2) +the concentration *P. fluorescens* (10^6 c.f.u./ml) (PF2), which is (2.832) mg / g the highest relevant concentration is below the probability rate of (5%) and represents the share of the variety Abu-graib. In comparison to the regulation, it has (31.6%) ratio. That being said, there are significant differences between them, with a likelihood rate of 5%, and they use different letters. Related to variety (Abaa 99), SA2+PF2 it has a higher value in the concentration *P. fluorescens* (10^6 c.f.u./ml) PF2+SA concentration of 100 ppm (SA2), which is (2.634) mg/g, although the lowest value is (2.113) mg/g. in another side, since they take different letters, there are substantial variations between them at the level of chance of 5%.

Table (1) : Effect of P. fluorescens and SA on the Chl. a Content (mg/g)

mucitirTmuvitsea .L	AS	*Pseudomonas fluorescens*			
		0	10^5 cfu.ml^{-1}	10^6 cfu.ml^{-1}	10^7 cfu.ml^{-1}
Abaa 99	0	2.113 T	2.162 r	2.176 Q	2.145 S
	50 ppm	2.252 O	2.532 d	2.425 Ef	2.329 K
	100 ppm	2.273 N	2.345 j	2.634 C	2.332 K
	150 ppm	2.261 O	2.351 Ji	2.365 H	2.534 D

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*Abu-*Graib	0	2.143 S	2.202 p	2.202 P	2.182 Q
	50 ppm	2.283 N	2.735 b	2.432 E	2.364 Hi
	100 ppm	2.315 L	2.383 g	2.832 A	2.361 Hi
	150 ppm	2.302 M	2.393 g	2.416 F	2.530 D

Effect of *P. fluorescens* and SA on the Chl. b Content :

The impact of *Pseudomonas fluorescens* and SA on the chlorophyll b of the *Triticum aestivum* varieties (Abaa 99) , (Abu-graib) ,is seen in table (2). there seem to be substantial differences of different concentrations of *Pseudomonas fluorescens* and salicylic acid of chlorophyll b content, with the highest value at *P. fluorescens* (10^6) c.f.u./ml PF2+SA concentration of 100 ppm (SA2), which is (0.963) mg/g and reflects the share of the variety Abu-graib, and the lowest value at same variety (0.612) mg/g .

Table (2): Effect of P. fluorescens and SA on the Chl. b Content (mg/g)

mucitirTmuvitsea .L	AS	*Pseudomonas fluorescens*			
		0	10^5	10^6	10^7
Abaa 99	0	0.612 P	0.613 p	0.615 P	0.612 p
	50 ppm	0.607 P	0.870 e	0.836 Fg	0.711 m
	100 ppm	0.630 O	0.743 k	0.926 C	0.727 l
	150 ppm	0.616 Op	0.754 jk	0.758 J	0.843 f
*Abu-*Graib	0	0.612 P	0.617 op	0.617 Op	0.616 op
	50 ppm	0.676 N	0.940 b	0.850 F	0.773 i
	100 ppm	0.704 M	0.803 h	0.963 A	0.783 i
	150 ppm	0.701 M	0.813 h	0.826 G	0.903 d

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Effect of *P. fluorescens* and SA on the Carotene Content :

Table (3) shows the effect of *P. fluorescens* and SA on carotene content in (*Triticum aestivum* L.) varieties (Abaa 99) , (Abu-graib) . The highest relevant concentration is below the probability rate of 5% for (*P. fluorescens* 10^6 c.f.u./ml) (PF2) + SA concentration of (100 ppm) (SA2), which is (0.969 mg/g) and reflects the share of the variety Abu-graib and the concentration (*P. fluorescens* (10^6) c.f.u./ml) (PF2)+SA concentration 100ppm ,Nevertheless, there are significant differences between them, with a likelihood rate of 5%, and they use different letters. The variety Abaa 99 has the lowest value of (0.618) mg/g. In other direction, because they take the disparities letter into account, there are substantial differences between them at the degree of chance of 5%.

Table (3):Effect of P. fluorescens and SA on the Carotene Content(mg/g)

mucitirTmuvitsea .L	AS	*Pseudomonas fluorescens*			
		0	10^5	10^6	10^7
Abaa 99	0	0.618 D	0.681 z	0.692 Z	0.672 z
	50 ppm	0.733 W	0.943 d	0.913 G	0.802 q
	100 ppm	0.752 U	0.823 o	0.964 Ab	0.813 p
	150 ppm	0.742 V	0.842 n	0.851 M	0.934 e
*Abu-*Graib	0	0.663 Z	0.716 x	0.722 X	0.702 y
	50 ppm	0.761 T	0.960 b	0.923 F	0.862 l
	100 ppm	0.791 R	0.885 j	0.969 A	0.872 k
	150 ppm	0.782 S	0.892 i	0.902 H	0.953 c

Discussion

The total pigment molecules present in the leaf (Chl-a, chl-b), as well as carotenoids, are needed for photosynthesis. Most plants contain the essential photosynthetic pigments Chl-a and Chl-b. Chlorophyll-rich plants are expected to grow faster than chlorophyll-deficient

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plants (Campbell and Reece, 2005) ,As a natural pigment, chlorophyll works a critical role in the absorption of light energy and the promotion of growth. Plant chlorophylls and carotenoids are brightly colored conjugated polyenes that are important for photosynthesis (Fahey *et al*., 2005). The presence of the green pigment chlorophylls is responsible for these abilities. SA and *Pseudomonas fluorescens* are used in various proportions on wheat *Triticum aestivum* L. , either in combination or alone, and then SA is used in methods of treatment foliar shoots, followed by *Pseudomonas fluorescens* inoculation roots. In the current research, all forms of Chl-a and Chl-b and carotene were calculated in the same concentration and found to have a greater effect than others, providing high values (2.832, 0.963,0.969) mg/g.in the (PF2+SA2) in the same wheat *Triticum aestivum* L for the chl.(a, b, and carotene) respectively, but the content plant from chlorophyll and carotene reducing whenever increasing of concentrations from 100 ppm and *Pseudomonas fluorescens* concentrations from 10^6 c.f.u./ml .This is what they identified: *Pseudomonas fluorescens* and SA mixture or solely improved chlorophyll (Khan, Banu, and Babar 2019). The use of SA alone increased chlorophyll content and photosynthetic rate while also counteracting the stress's negative effects. SA can increase photosynthetic rate due to increased activity of Rubisco and PEP carboxylase (Popova *et al*. 2003; Singh and Usha 2003).

Conclusion :

Physiological parameters in wheat plants *Triticum aestivum* is enhanced by the treatment of *pseudomonas fluorescens* and salicylic acid. The use of *pseudomonas fluorescens* and SA increased levels of chlorophyll a,b, and carotene.

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