RESEARCH ARTICLE

The Effect of *Pseudomonas fluorescens* and Salicylic Acid, and their Synergistic Effect on Protein, Carbohydrates Content and Leaf Area of Two Varieties of *Triticum aestivum* L. Plant

Hussein M. Hameed, Faiq H. Ali*, Maytham A. Makki

College of Science, University of Al-Muthanna, Samawah, Iraq

Received: 24th February, 2022; Revised: 17th April, 2022; Accepted: 08th May, 2022; Available Online: 25th June, 2022

ABSTRACT

The study aims to show the effect of salicylic acid at concertation (50–100 and 150 ppm) and *Pseudomonas fluorescens* at (10^5,10^6,10^7 cfu/mL) on protein, carbohydrates content and leaf area for two varieties (Abu-Ghraib, Abaa 99) of *Triticum aestivum* L. The results are indicated the significant effect of *P. fluorescens* and salicylic acid (SA) at the combination of concentration of *P. fluorescens* (FP2) and SA (SA2) for variety (Abu-Ghraib) was more impact than (Abaa 99) on the physiological aspect like protein, carbohydrates content and leaf area (7.427, 55.55, 26.42) mg/g dry weight respectively while the value of control was (0.969, 4.158, 4.100) mg/g, respectively. The results showed that salicylic acid and *P. fluorescens* induced better than the sole application of SA or *P. fluorescens*.

Keywords: Carbohydrates content and leaf area, Protein, *Pseudomonas fluorescens*, Salicylic acid, *Triticum aestivum* L.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.2.34

How to cite this article: Authors. The Effect of *Pseudomonas fluorescens* and Salicylic Acid, and their Synergistic Effect on Protein, Carbohydrates Content and Leaf Area of Two Varieties of *Triticum aestivum* L. Plant. International Journal of Drug Delivery Technology. 2022;12(2):662-665.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Triticum aestivum L. is one of the most widely cultivated and consumed human crops (Zahoor *et al*. 2020). Bread wheat (*T. aestivum* L.), which is high in protein, carbohydrate, and minerals, is one of the world's most significant cereal grain crops, feeding 30% of the world's population (Lobell *et al*., 2011)**,** a carbohydrate reserve is a key carbon source for grain output in wheat and barley (Bonnett and Incoll, 1993; Schnyder, 1993).

P. fluorescens is a naturally found bacteria with several properties that make it a good choice for Plant Growth-Promoting Root Bacteria (PGPR). The most important *Pseudomonas* strains have been fluorescent *Pseudomonas* spp. A lot of work has been done worldwide to bring the promise of a bacteria called fluorescent *Pseudomonas* to life (Lata *et al*., 2002).

Salicylic acid regulates various physiological processes in plants by acting as a non-enzymatic antioxidant and a plant growth regulator (PGR). Salicylic acid is linked to the activation of the stress-induced antioxidant system, the induction of flowering in many species, the enhancement of flower life, and the regulation of ion absorption through roots and stomatal conductivity (Muthulakshmi and Lingakumar, 2017).

P. fluorescens alone, as well as in interaction with PGRs (such as SA) and co-treatment, results in a greater rise in leaf protein content in the sensitive variety than in the resistant variety (Khan *et al*., 2019) Bacillus cereus (P2. Khan found that *P. fluorescens* substantially increased leaf protein content in both types compared to uninoculated untreated plants cultivated in sandy soil (Khan *et al*., 2020) P-solubilisation, antibacterial and antifungal activities and catalases and oxidases activities and were also screened for the production of indole-3-acetic acid (IAA. Inoculation with chosen PGPB and SA application, either alone or in combination, relieved the chromium, as evidenced by better glucose metabolism in maize plants subjected to Cr contamination (Islam *et al*., 2016).

MATERIAL AND METHODS

Wheat seeds, variety (Abaa 99), and variety (Abu Ghraib) were sterilized by washing them in tap water to remove the fogging substance, then transferring them to a Clorox solution, which is a 4% sodium hypochlorite, and keeping them in it for three minutes, then removing them and washing them seven times with sterile distilled water (Pikovskaya, 1948). *P. fluorescens* was isolated from various soil samples taken from the rhizosphere. To acquire pure colonies of *P. fluorescens*, several single colonies with comparable morphological features to

**Author for Correspondence: faiq_alradi73@mu.edu.iq*

P. fluorescens were chosen. The streaking method was utilized to subculture these bacteria on the surface of a King B medium and perform morphological, cultural, and biochemical testing (Holt *et al*., 1994).

Two types of plant seeds were used in the experiment in a greenhouse at Al-Muthanna University's College of Sciences in natural circumstances (*T. aestivum* L.). Some are salicylic acid foliar, while others are inoculation *P. fluorescens* inoculated in 5 Kg pots in three replicates for each treatment. Summer is when the plants are cultivated as follows: Seeds were grown in (1/12/2020). Irrigation of the pots: the field capacity is 75%, so put more than 20 seeds each. Reduce the number of seedlings once they have begun to grow (8 seedlings). Plants were foliar in SA and inculation PF. at three stage leaves (Zadoks *et al*.1974) for two weeks, and some plants were harvested 60 days after planting for physiological parameters, and then the practical experiment was done during the growing season (2020-2021).

Estimation of Total Soluble Protein

The protein was calculated using the Follen method. The technique of (Schacterale and Pollak,1973) has been rehabilitated (Lowry *et al*., 1951). Calculate the apparent density at 650 nm using an optical spectrometer.

Estimation of Carbohydrates

0.2 g well-grounded leaves, 10 mL distilled water, 15 minutes at 3000 rpm centrifuge, aspirate the solution using filter paper, then extract 1-mL of leachate into a fresh test tube Add 5 mL of phenol at 5% and 5 ml of 80 % sulfuric acid after stirring and rotating. Using a spectrophotometer, the tubes are cooled and photo-resisted after appropriate dilution across a 488 nm wavelength (Al Wahbi and Bassallah, 2003).

Estimation of Leaf Area

Weigh a fresh leaf from the highest point on the stem, then cut a disk (1-cm^2) off it and weigh it. The following formula is used to determine the leaf area: (total leaf area=1cm^2 × weight of leaf / weight of the disc), (Shaheen M.A,1987).

Statistical Analysis, Experimental Design and Treatments

The study was factorial, with three replicates and a completely randomized design. Each variety (*T. aestivum* L.) received nine treatments, including a control, as follows (Antar and Alwakaa, 2010):

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

RESULTS

Effect of *P. fluorescens* and Salicylic Acid in the Leaf Area

The effect *of P. fluorescens* and *salicylic aci*d on the Leaf Area in *T. aestivum*. L varieties (Abaa 99, Abu-graib) can be seen in Table 1. The highest concentration is below ($p \leq 0.05$) for the concentration *P. fluorescens* (10^6) c.f.u./mL (FP2) for a SA concentration of 100 ppm (SA2)), which was (26.42) cm^2 for variety in Abu-graib, compared with the control.

Table 1: Effect of *P. fluorescens* and salicylic acid in Leaf Area between the two varieties of *T. aestivum*

		P. fluorescens			
T. aestivum	*SA*	*0*	*10^5 c.f.u./ml*	*10^6c.f.u./ml*	*10^7 c.f.u./ml*
Abaa 99	0	4.100 u	8.523 s	8.593 s	5.571 t
	50 ppm	10.71 q	18.21 e	16.42 g	12.18 m
	100 ppm	11.07 o	12.62 l	21.62 c	12.17 m
	150 ppm	10.80 qp	12.87 l	13.36 k	18.31 e
Abu-graib	0	4.215 u	9.51 r	9.427 r	9.314 r
	50 ppm	11.21 o	23.45 b	17.31 f	14.22 j
	100 ppm	11.77 n	15.10 i	26.42 a	14.86 i
	150 ppm	11.52 n	15.62 h	16.62 g	18.65 d

Table 2: Effect of *P. fluorescens* and salicylic acid in carbohydrates (mg/g dry weight) between the two varieties of *T. aestivum*

		P. fluorescens			
Triticum aestivum	*SA*	*0*	*10^5 c.f.u./mL*	*10^6 c.f.u./mL*	*10^7c.f.u./mL*
Abaa 99	0	4.158 u	6.272 t	7.903 s	5.556tu
	50 ppm	15.388 pq	32.630 de	31.42 ef	21.466 l
	100 ppm	16.520po	27.450 jk	35.363 c	26.416 k
	150 ppm	16.466 po	27.540 Jki	27.566jki	31.72ef
Abu- graib	0	4.587 u	12.550 r	14.660 q	11.633 r
	50 ppm	17.406 n	37.490 b	31.540 ef	28.29jhi
	100 ppm	19.526m	29.686 gh	55.553 a	29.20gh
	150 ppm	18.507mn	30.333 gh	30.660 gf	33.613 d

Table 3: Effect of *P. fluorescens* and salicylic acid in (protein)mg/g between the two varieties of *T. aestivum*

		P. fluorescens			
Triticum aestivum	*SA*	*0*	10^5 *c.f.u./ml*	10^6 *c.f.u./ml*	10^7 *c.f.u./ml*
Abaa 99	0	0.969 m	1.750 k	1.770 k	1.770 k
	50 ppm	2.270 j	4.031 e	3.145 g	2.729 hi
	100 ppm	2.208 j	2.645 hi	5.721 d	2.666 Hi
	150 ppm	2.125 j	2.604 hi	2.687 hi	3.541 f
*Abu-*Graib	0	1.384 l	2.145 j	2.125 j	1.854 K
	50 ppm	2.541 j	6.918 b	3.437 f	2.770 H
	100 ppm	2.583 hi	3.000 g	7.427 a	3.041 G
	150 ppm	2.604 hi	3.041 g	3.000 g	4.646 d

Effect of *P. fluorescens* and Salicylic Acid in the Carbohydrates

The *P. fluorescens* and salicylic acid effected the carbohydrates in *T. aestivum*. L varieties (Abaa 99, Abu-graib) can be seen in Table 2. The highest concentration is below ($p \leq 0.05$) for the concentration *P. fluorescens* (10^6) c.f.u./mL (FP2) for a SA concentration of 100 ppm (SA2)), which was (55.553 mg/g dry weight) for a variety Abu-graib, compared with the control.

Effect of *P. fluorescens* and Salicylic Acid in the Protein

The effect *of P. fluorescens and salicylic aci*d on the protein in *T. aestivum*.L varieties (Abaa 99, Abu-graib) can be seen in Table 3. The highest concentration is below ($p \leq 0.05$) for the concentration *P. fluorescens* (10^6) c.f.u./ml (FP2) for a SA concentration of 100 ppm (SA2)), which was (7.427 mg/g), compared with the control.

DISCUSSION

Plants create carbohydrates as product materials during the photosynthesis process, which involves turning raw materials into complex chemicals in the chloroplast, a small organelle. As a result, in terms of the amount of chlorophyll in a cell's chloroplast. In either scenario, growing carbs benefits both the cell and the plant. As a result of the results in table (2), SA and *P. fluorescens* influenced the quantity of carbohydrates in wheat *T. aestivum* L., particularly at concentration SA2 and PF2 in both varieties (Abaa 99 and Abu-graib). They has values of carbohydrates at (55.55,35.36) mg/g. The increase in carbohydrates at this concentration (SA2+PF2) may be due to the increase in the leaf area. Although some research has found that SA and *P. fluorescens* work together to enhance carbohydrate concentrations by activating and maintaining organelles and their functions, such as chloroplasts, Others have discovered that SA and *P. fluorescens* work to increase the concentration of carbohydrates because it has a role in regulating the inner genes of the cell in inducing an ion exchange. Others have discovered that SA and *P. fluorescens* increase the concentration of carbohydrates because it has a role in regulating the inner genes of the cell in inducing an ion exchange (Kaydan *et al*., 2007). *P. fluorescens* and SA combination or alone for carbohydrate improvement (Khan *et al*. 2019).

Proteins are highly essential to the cell since they may be used to create hormones or enzymes, which can be studied to generate amino acids in some form. Proteins are essential in the cell plant life. Therefore, any substance enhancing protein is popular on the plant Ps. According to the findings in table 3, *P. fluorescens* and SA both affected raising protein content, especially at *P. fluorescens* (10^6) c.f.u./mL (FP2) at a SA concentration of 100 ppm (SA2), which was (7.427 mg/g). On the other hand, increasing *P. fluorescens* and SA concentrations in combination or alone increases protein content, implying that there is a positive relationship between a high degree of SA concentration and protein content, implying that salicylic acid may be used as an internal phytohormone again. Plants treated with SA and Placed had somewhat greater foliar protein content in their leaves than *P. fluorescens*. The leaf protein content of the sensitive cultivar was considerably raised by *P. fluorescens* alone, in conjunction with PGRs and co-treatment. PGPR alone, or their consortium, or PGPR consortium, resulted in a higher rise in leaf protein concentration in the responsive variety than in the resistant variety when employed in combination with PGRs (Khan *et al.,* 2019).

Exogenous treatment of *P. fluorescens* and SA, on the other hand, can enhance leaf area, especially when combined, according to results. This is in line with the findings of (Naserzadeh *et al*., 2018), who discovered that the influence of bacteria increases leaf area and number of leaves, and (Larqué-Saavedra and Martin-Mex, 2007), who discovered that the effect of SA increases leaf area and several leaves.

CONCLUSION

Physiological and growth parameters in wheat plants *Triticum aestivum* is enhanced by the treatment of *P. fluorescens* and salicylic acid. Using *P. fluorescens* and SA increased protein, carbohydrates content and leaf area of the wheat plant (*T. aestivum* L.).

REFERENCES

1. Al Wahbi, M. H., and Bassallah, M. O. (2003).Analysis Plant tissue Practica.
2. Antar, S.H., Alwakaa A.H. (2010). Statistical Analysis In Scientific Research and The SAS Program. Dar Ibn Al Atheer Printing And Publishing. University Of Mosul.

3. Bonnett, G. D., & Incoll, L. D. (1993). Effects on the Stem of Winter Barley of Manipulating the Source and Sink during Grain-filling: II. CHANGES IN THE COMPOSITION OF WATER-SOLUBLE CARBOHYDRATES OF INTERNODES. Journal of Experimental Botany, 44(1), 83-91.
4. Holt, John G., Noel R. Krieg, B. H. Seneath, J. T. Staley, and S. T. Williams. (1994). Bergey's manual of determinative bacteriology 9th edition.William & Wilken. Baltimore, 175-248.
5. Islam, F., Yasmeen, T., Arif, M.S., Riaz, M., Shahzad, S.M., Imran, Q. and Ali, I. (2016). Combined ability of chromium (Cr) tolerant plant growth promoting bacteria (PGPB) and salicylic acid (SA) in attenuation of chromium stress in maize plants. Plant Physiology and Biochemistry, 108, 456-467.
6. Kaydan, D. ; Yagmur, M. and Okut, N. (2007). Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). Tarim Bilimleri Dergisi, 13(2),114-119.
7. Khan, N., Bano, A. and Babar, M.A. (2019). The stimulatory effects of plant growth promoting rhizobacteria and plant growth regulators on wheat physiology grown in sandy soil. Archives of microbiology, 201(6), 769-785.
8. Khan, N., Bano, A.M. and Babar, A. (2020). Impacts of plant growth promoters and plant growth regulators on rainfed agriculture. PloS one, 15(4), e0231426.
9. Larqué-Saavedra, A. and Martin-Mex, R. (2007). Effects of salicylic acid on the bioproductivity of plants. In Salicylic acid: a plant hormone (15-23). Springer, Dordrecht.
10. Lata, S.A. and Tilak, K.V.B.R. (2002). Biofertilizers to augment soil fertility and crop production. Soil Fertility and Crop Production Science Publishers, USA. Edited by Krishna KR, 279-312.
11. Lobell, D.B., Schlenker, W. and Costa-Roberts, J.(2011). Climate trends and global crop production since 1980. Science, 333(6042), 616-620.
12. Lobell, D.B., Schlenker, W. and Costa-Roberts, J.(2011). Climate trends and global crop production since 1980. Science, 333(6042), 616-620.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193, 265-275.
14. Muthulakshmi, S, and K Lingakumar. (2017). Role of Salicylic Acid (SA) in Plants—a Review. International Journal of Applied Research 3 (3), 33–37.
15. Naserzadeh, Y., Kartoolinejad, D., Mahmoudi, N., Zargar, M., Pakina, E.L.E.N.A., Heydari, M.O.H.A.M.A.D., Astarkhanova, T. and Kavhiza, N.J. (2018). Nine strains of Pseudomonas fluorescens and P. putida: Effects on growth indices, seed and yield production of Carthamus tinctorius L. Research on Crops, 19(4), 622-632.
16. Pikovskaya RI. (1948).Mobilization of phosphorous in soil in the connection with vital activity of some microbial species. Mikorobiologiya,17,362–370.
17. Schacterle, G. P., & RL, P. (1973). A simplified method for the quantitative assay of small amounts of proteins in biologic material.
18. Schnyder, H. (1993). The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling—a review. New phytologist, 123(2), 233-245.
19. Shaheen M. A., J.(1987). Prediction of leaf area of three cultivars of grape (vitis vinifera L.) from leaf measurement J. Coll. Agric, King Sand University, 9(2), 81-88.
20. Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. Weed research, 14(6), 415-421.
21. Zahoor, I., Hasan, H., Gul, A., Khursheed, A., Ali, M., Amir, R., Afzal, F., Kubra, G., Basharat, A., Aziz, F. and Zarrar, F. (2020). Molecular mechanism of drought tolerance in wheat. In Climate Change and Food Security with Emphasis on Wheat (129-154). Academic Press.