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CHANGES IN BIOMASS GAIN, PROTEIN CONTENT AND HORMONAL LEVELS IN WHEAT KERNELS IN RESPONSE TO SOIL DRENCH WITH SODIUM SALICYLATE

By

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تأثير إشباع التربة بساليسيلات الصوديوم على الكتلة الحية والمحتوى البروتيني والهرموني لحبوب القمح

سامي أبو القاسم أبو حامد وفتحي عواد منصور وحشمت سليمان الدسوقي

لقد وجد أن اشباع التربة بساليسيات الصوديوم بالتركيزات (٨٠٠٠,٤٠٠٠,٨٠٠ ملجم/لتر) أدي إلى زيادة الوزن الطازج والجاف للحبوب المتكونة وتبين أيضاً أن التركيز الأدنى خاصة عند اليوم الثالث والعشرين والستين من تكون الحبوب وكذلك التركيزات العليا منها أثناء تكوين الحبوب قد أدوا إلى زيادة ملحوظة في المحتوى البروتيني للحبوب .

وتبين من هذه الدراسة أن لساليسيلات الصوديوم القادرة على زيادة محتوى الحبوب من مثبط النمو (حمض الأبسيسيك) زيادة معنوية في حين تؤدي إلى نقص ملحوظ في المحتوى الأكسيني والجبريلليني لها عدا التركيز الأدنى منها وقد أدى إلى زيادة محتويتها الجبريلليني . أما عن المحتوى السيتوكينيني فلقد أتضح أنه يزداد في الحبوب التامة النضج مع كل التركيزات المستخدمة من الساليسيلات .

Key Words: Antitranspirant, Biomass, Hormonal levels, Kernels, Sodium salicylate, Soil drench, *Triticum aestivum*

ABSTRACT

Drenching of soil with sodim salycylate (800, 4000 and 8000mg/l) increased grain f. wt. and d. wt. Protein content was increased in grains at days 23 and 60 with 800 mg/l salicylate. With higher doses "4000 and 8000mg/l protein increased during grain filling.

Sodium salicylate increased significantly the ABA content in grains compared to control. A significant decrease in auxins and gibberellins in grains was noticed in sodium salicylate- treated plants, except with 800mg/l which increased gibberellins at day 80. Cytokinin content was also increased in ripened grains with all concentrations used.

INTRODUCTION

In developing seeds, dry matter accumulation has been shown to be affected by the application of several antitranspirants.

The magnitude of the effect was found to depend on the type and/or concentration of the anitranspirant employed (Agarwal, 1979; Sheikh and Mall, 1980 and Gurbaksh and Sharma, 1982). Thus salicylate was reported to induce the synthesis of pathogenically related proteins (White, 1979; Antoniw and White, 1980, and Pennazio *et al,* 1983). Also, salicylate sometimes acts as an antiauxin (Lee and Skoog, 1965), in other reports it provided protection to auxins against oxidation (Schneider and Whitman, 1974).

To our knowledge the role or salicylate on kernel growth in cereals and the concomitant changes which might occur either directly or indirectly are not understood. The objectives of this work are to find out (a) if drenching of soil with sodium salicylate would increase dryweight and fresh weight, (b) if protein content would change, and (c) any appreciable changes in endogenous hormones e.g. ABA, IAA, GA_3 and cytokinins in wheat grains as a result of the soil drenching.

MATERIALS AND METHODS

1. Growth conditions:

Pot experiments were carried out in a greenhouse at the Faculty of Science, University of Mansoura, Egypt. The wheat variety used was Giza 157. Wheat grains (*Triticum aestivum* L.) were sown in earthern-ware pots (30 cm in diameter) filled with a loamy-sand soil. Fifteen grains were sown in each pot and the pots were kept in a greenhouse in which plants were subjected to normal day/night conditions. Irrigation was carried out with water when it was required. After two weeks, thinning was started so that ten uniform seedlings were left in each pot. The pots were either irrigated with sodium salicylate as a soil drench (at a concentration of 800, 4000, or 8000mg/l) or with water alone (control). Sampling was carried out at 23, 46, 53, 60 and 80 days from anthesis.

Methods:

For fresh and dry matter determinations, triplicate samples each of 10 grains from each concentration were randomly taken and dried in an oven at 80°C for 24 hours after their initial fresh weights were recorded. The mean values were estimated as mg per grain.

The protein content of fresh plant material, (triplicate samples from fresh grains) was determined colorimetrically as described by (Lowry, *et al,* 1951).

The method of (Shindy and Smith, 1975) was employed for extraction of endogenous hormones from fresh grains. For estimation of the compound with hormonal activity in the purified extracts, the method of (Wright 1969) was applied for abscisic acid, for auxins the method of (Foda and Radwan, 1962) for gibberellins the method of (Frankland and Wareing, 1960) and for cytokinins the method of (Esashi and Leopold, 1969).

The results were first subjected to an analysis of variance (ANOVA). If the ANOVA showed significant ($P < 0.05$) effects, the least significant difference was performed (Snedecor and Cochran, 1967).

RESULTS

1. Changes in fresh and dry matter of developing wheat grains:

In controls, maximum fresh weight of grains was recorded at 53 days post-anthesis. The applications of sodium salicylate (at all doses used) seemed to cause a significant increase ($P < 0.01$) in grain fresh weight at almost all stages of grain development except with 8000mg/l at day 23 and 800mg/l at day 60 and day 80 where a significant or a non significant decrease in grain fresh weight was obtained (Table 1).

The grain d. wt. reached maximum values in control after 60 days. Soil drench with sodium salicylate led to a significant increase ($P < 0.01$) in grain dry matter at almost all stages of grain maturation. Salicylate seemed generally to shorten the time required for maximum dry matter accumulation, by one week compared to control (Table 1).

2. Changes in protein content of developing wheat grains:

It is obvious in (Table 2) that in control plants there was an increase in the protein content during the growth period (23-53d), then it decreased sharply on day 60 and on day 80 the protein content was increased.

Salicylate application to the soil at dose 800mg/l caused a significant increase in grain protein content on days 23 and 60 ($P < 0.01$) and a significant decrease on days 46, 53 and 80. Higher doses of salicylate (4000 and 8000mg/l) caused a marked and significant increase in protein content of wheat grains during the whole studied periods, except with 8000 mg/l at 23d where the protein content was lower than that of control.

Table 1

Changes in fresh and dry weights of developing wheat grains as affected by different concentrations of sodium salicylate. Values are expressed as mg. f. wt. or dry wt. grain^{-1}.

(a) Fresh weight.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	25.00	36.67	47.00	39.47	30.63
Sodium salicylate					
800mg/l	31.25	37.10	58.00	35.72	30.09
4000mg/l	30.00	61.11	64.10	43.03	37.60
8000mg/l	18.14	51.23	62.80	41.32	37.60
L.S.D. 5%	3.72	2.43	2.31	2.93	3.97
5%	5.18	3.38	3.22	4.09	5.54

(b) Dry weight.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	8.24	18.93	25.97	26.77	24.81
Sodium salicylate					
800mg/l	10.07	17.74	31.06	30.70	27.21
4000mg/l	9.13	36.70	38.11	35.90	33.84
8000mg/l	4.61	30.56	34.56	32.18	30.71
L.S.D. 5%	1.71	1.50	1.22	1.01	3.05
1%	2.39	2.08	1.69	1.41	4.26

Table 2

Changes in protein contents of developing wheat grains as affected by different concentrations of sodium salicylate. Values are expressed as mg protein grain^{-1}.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	1.7	2.3	3.9	1.5	3.5
Sodium salicylate					
800mg/l	2.1	1.4	3.2	1.9	3.0
4000mg/l	2.4	5.2	5.2	1.9	4.2
8000mg/l	1.3	3.9	4.4	1.9	3.9
L.S.D. 5%	0.008	0.005	0.007	0.030	0.027
1%	0.010	0.010	0.010	0.060	0.050

3. Changes in growth inhibitory substances in the developing wheat grains:

In control plants, ABA seemed to increase gradually during the early period (23-53 days) followed by a rapid accumulation of ABA in grains at day 60, after which the ABA declined slightly. On the other hand, the different concentrations of sodium salicylate appeared to increase the levels of ABA significantly compared to control with more ABA at 46 and 60 days. More ABA was detected on day 46 with an increase in salicylate concentration, while at day 60, the maximum ABA level in grains was obtained with 4000mg/l sodium salicylate (Table 3).

Table 3

Changes in growth inhibitors in developing wheat grains as affected by different concentrations of sodium salicylate. Values are expressed as μg ABA. equivalent g^{-1} f. wt.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	9.71	11.86	11.88	27.26	24.19
Sodium salicylate					
800mg/l	10.09	22.55	21.31	36.33	24.47
4000mg/l	22.57	38.39	24.85	42.12	27.21
8000mg/l	37.56	48.51	28.69	26.97	23.59
L.S.D. 5%	7.29	11.25	12.79	11.68	19.87
1%	10.16	15.67	17.82	16.28	27.69

4. Changes in growth promotory substances in the developing wheat grains:

Soil drench with sodium salicylate, in general, led to a significant decrease ($P < 0.01$) in auxin and gibberellin levels throughout, except with 800mg/l which increased ($P < 0.01$) gibberellin content at day 80 (Table 4). The cytokinin levels were markedly increased ($P < 0.01$) during early stages of grain development at all doses of salicylate. This was followed by a sharp decline ($P < 0.01$) up to day 60, whereas at grain ripening, a pronounced increase ($P < 0.01$) in cytokinin levels was obtained.

DISCUSSION

The present investigation further indicated that soil drench with sodium salicylate at all doses was able to increase significantly both fresh weight ($P < 0.05$) and dry matter ($P < 0.01$) of wheat grains at almost all stages of grain development. This may be attributed to the fact that salicylate reduces the rate of transpiration from leaves (Larque, Saavedra, 1978), which could possibly lead to the accumulation of excessive water, thus resulting consequently in an increase in grain f. wt. But the resulting increase in grain d. wt. might have occurred from the accumulation of more assimilate in the grains. These results in fact agree with those of (Agarwal, 1979) working with barley plants. Moreover, (Gurbaksh and Sharma, 1982) found an increase in dry pod yield of ground nuts after application of salicylic acid.

The noticed decline in grain protein content with 800mg/l salicylate-treated plants is probably due to the fact that salicylate (a phenolic compound) possesses chelating properties (Clementson and Anderson, 1966). Moreover, it has been demonstrated that salicylic acid accelerates leaching of soluble nitrogen from maize endosperm (Jain and Srivastava, 1981). Presumably this might explain the accumulation of proteins in grains due to the higher doses of salicylate applied in our study.

The increase in grain ABA during its development and ripening when sodium salicylate at 4000 and 8000mg/l were applied as soil drench may suggest that salicylate acts as an inducer for the production and/or accumulation of ABA in the developing grains.

Table 4

Changes in auxins, gibberellins and cytokinins levels in developing wheat grains as affected by different concentrations of sodium salicylate. Values are expressed at μg g^{-1} f. wt.

(a) Auxins.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	310.59	360.13	760.88	1339.96	342.44
Sodium salicylate					
800mg/l	151.10	338.20	306.08	75.77	290.13
4000mg/l	85.80	149.70	369.27	105.81	325.01
800mg/l	55.96	153.24	400.03	192.12	265.56
L.S.D. 5%	18.76	14.350	45.31	47.32	34.72
1%	26.19	20.00	63.14	65.93	84.41

(b) Gibberellins.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	207.97	258.00	21.92	228.59	70.02
Sodium salicylate					
800mg/l	106.54	61.04	44.16	45.21	109.12
4000mg/l	133.59	43.20	34.93	37.14	34.35
8000mg/l	193.29	14.26	36.81	87.33	29.87
L.S.D. 5%	6.06	4.29	3.41	4.10	6.74
1%	8.45	5.98	4.75	5.71	9.40

(c) Cytokinins.

Treatments	Days after anthesis				
	23	46	53	60	80
Control	262.19	449.02	52.45	35.98	7.75
Sodium salicylate					
800mg/l	294.79	157.64	18.37	8.23	12.30
4000mg/l	331.92	172.19	42.84	13.95	25.28
8000mg/l	301.97	115.64	32.07	16.80	12.98
L.S.D. 5%	5.02	1.38	1.56	10.30	2.08
1%	6.94	1.93	2.17	14.35	2.90

The remarkable decline in auxin levels at all stages of grain development due to sodium salicylate application in the soil might be due to activation of IAA-oxidase resulting in degradation of auxins (Ray, 1960; Lee and Skoog, 1965 and Sharma and Kaushik, 1983).

The marked decrease in gibberellin levels of grains after application of sodium salicylate might suggest that the latter interferes with the metabolism of gibberellins, thus causing deactivation of GAs or inhibiting their biosynthesis in the grains.

Sodium salicylate was obviously able to enhance the biosynthesis of cytokinins in grains during the period of growth, while during maturation salicylate led to a marked reduction in cytokinin levels. The reason for such an effect of salicylate on cytokinins in grains is difficult to interpret but we might say that, in general, the demand for cytokinins is greater during grain growth and lower at grain maturation. Salicylate, an antitranspirant (Larque, Saavedra, 1978) might cause a reduction in the levels of translocated cytokinins originating inside the root through the transpiration stream, or it may enhance the utilization of cytokinins during grain development.

The data of the present work reveal that although sodium salicylate, when applied to the soil, was able to alter the balance of endogenous hormones in the developing grains in a way that induced such grains to attain more dry matter. This might be a result of the presence of more cytokinins as being able to attract the assimilates towards grains.

Based on the aformentioned pattern of results, it emerged out that the application of sodium salicylate (as soil drench at 4000 - 8000mg/l) in wheat cultivation is valuable for improvement of the quality of wheat grains since it enhances protein accumulation in grain during grain filling.

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