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Low concentrations of salicylic acid increase nitrate accumulation in roots of *Pinus patula*

(With 1 table)

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Abstract. Seedlings of *Pinus patula* were sprayed with salicylic acid (SA) at concentrations between 10^{-4} and 10^{-10} M. At the end of the treatment root and shoots were harvested and nitrate and protein content determined. All the salicylic acid treatments increased significantly nitrate concentration in the roots; the 10^{-10} and 10^{-8} M treatments increased the amount of proteins in the roots, but in the shoots no significant effect was detected.

Key words: salicylic acid (SA), protein-nitrates, *Pinus patula*, roots

Salicylic acid (SA) is a phenolic compound recently recognized as a plant growth regulator (19) involved in many physiological events such as flowering induction (2, 10), stomatal closure (12, 13), thermogenesis in *Arum* (17, 4), protection of nitrate reductase from degradation (9), induction of multiple stress tolerance (21). Furthermore SA, is involved in disease resistance, inducing the biosynthesis of pathogenesis-related (PR) protein (3, 22).

Other effects of exogenous application of SA and its close analog acetylsalicylic acid (ASA) to plants have been reported: inhibition of ethylene biosynthesis (8) seed germination, blocking wound responses, affecting nastic leaf movements (18, 20), and the enhancement of somatic embryogenesis in *Coffe arabica* tissue cultures (16).

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One of the early discoveries on salicylates was that phenolic compounds such as SA in combination with indolacetic acid stimulate rooting in mung bean, *Phaseolus aureus*, *Acer saccharinum* and *A. griseum* (14).

Our laboratory reported since 1995 that very low concentrations of SA have a profound effect on physiological responses and that 10^{-8} M concentrations of SA for instance, when sprayed on the shoots, increase significantly root length of soybean plants up to 45 to 100%. Further work has shown that similar responses of root development have been found in other angiosperms (5, 6). Following this discovery, the effect was tested and reproduced in *Pinus patula*, a gymnosperm (unpublished).

Since (a) N is one of the most important plant nutrients, (b) the main pathway of N into plants is absorption of nitrate by roots and (c) SA increases root length, it was considered important to test if pine seedlings sprayed with SA could accumulate larger amounts of nitrate in the shoots and in the roots.

MATERIALS & METHODS

Three-month old seedlings of *P. patula* Schl. et Cham were transplanted in black polyethylene bags (10 cm internal diameter and 60 cm long), containing 6.0 kg of soil (mixture of 70% limesand soil, 30% agrolite), maintained under greenhouse conditions.

SA solutions were 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M, with pH adjusted to 5.5 with KOH (1.0 N); Tween-20 was added as a surfactant. The solutions were sprayed to the shoots of the plants late in the afternoon, once a month, for 9 months. A randomized design with 20 replicates per treatment was used.

Plants were harvested one month after the last application by cutting the plastic bags; the roots were cleaned under a running tap water stream and the plants divided in roots and shoots for nitrate and protein determination. Leaf chlorophyll concentration was determined according to Harborne (7), nitrate content by the Cataldo method (1) and the total soluble protein content, using the Lowry method (15), with bovine serum albumin as protein standard.

RESULTS & DISCUSSION

As seen in table 1, the chlorophyll, protein and nitrate contents show no increase by any of the SA treatments. In the roots however, the 10^{-10} M and 10^{-8} M SA treatments increased by 34 and 30% respectively the protein content. All SA treatments increase

Table 1.– Effect of shoot SA sp
concentration of *Pinus patul*

Treatment SA (M)	Chlorophyll content (mg g^{-1} dry weigth)	Nitr cont (µg dry we
Control (H_2O)	3.6 a*	10
10^{-10}	2.9 a	10
10^{-8}	2.5 a	9
10^{-6}	3.6 a	11
10^{-4}	4.0 a	8

*Means followed by the same letter a

significantly nitrate concentra
results that the effect of SA on
that could be considered a dose-
content might be related to its
which, once affected, remains
Therefore it is important to de
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Higher nitrates and protei
are difficult to explain, althoug
be detected later, since we
bioproductivity of the shoot (5
the leaves was not affected by
data.

Further work must be don
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systems.

Finally it is important to s
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physiological processes.

LITERA

1. Cataldo DA, M Aaron, LE Schrade
Analysis 1 (1975)71
2. Cleland CF, A Ajami, *Plant Physi*

Table 1.– Effect of shoot SA spray on the chlorophyll, nitrate and protein concentration of *Pinus patula*. Mean values of ten replicate samples.

Treatment SA (M)	Chlorophyll content (mg g^{-1} dry weigth)	*Shoot* Nitrate content (µg g^{-1} dry weigth)	*Shoot* Protein content (µg g^{-1} dry weigth)	*Root* Nitrate content (µg g^{-1} dry weigth)	*Root* Protein content (µg g^{-1} dry weigth)
Control (H_2O)	3.6 a*	1023 a	103 a	257 b	148 b
10^{-10}	2.9 a	1006 a	97 a	510 a	200 a
10^{-8}	2.5 a	910 a	99 a	573 a	193 a
10^{-6}	3.6 a	1127 a	119 a	429 a	115 b
10^{-4}	4.0 a	874 a	106 a	556 a	140 b

*Means followed by the same letter are not significantly different at $p \geq 0.05$, Tukey's.

significantly nitrate concentration in the root. It seems from these results that the effect of SA on nitrate content does not follow a pattern that could be considered a dose-response effect. Such increase in nitrate content might be related to its transport system at the membrane level which, once affected, remains open regardless of the amount of SA. Therefore it is important to determine whether the increase of nitrate concentration in the roots caused by SA is due to new nitrate taken up by the roots. The absorbed nitrate is reported to be exported to the xylem, reduced in roots and stored in vacuoles. Moreover, it is known at present that during the induction of nitrate absorption, RNA and proteins are always synthesised as in the present work.

Higher nitrates and proteins levels in the root but not in the shoot are difficult to explain, although it is expected that such increase could be detected later, since we have shown that SA increased the bioproductivity of the shoot (5). The fact that chlorophyll content of the leaves was not affected by the treatments agrees with the shoot data.

Further work must be done since the present finding is interesting in that any treatment that favours the uptake of N from the soil by the roots is of great importance in nature especially for agricultural systems.

Finally it is important to stress that again, as it has been found elsewhere, low concentrations of salicylates affect important physiological processes.

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