Pergamon

PII: S0031-9422(96)00468-2

*Phytochemistry*, Vol. 44, No. 2, pp. 221–223, 1997

0031-9422/97 $17.00 + 0.00

# STIMULATION OF ETHYLENE PRODUCTION IN AGED POTATO TUBER SLICES BY SALICYLIC ACID

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(*Received in revised form* 13 *June* 1996)

**Key Word Index**—*Solanum tuberosum*; Solanaceae; tuber slices; ageing; ethylene production; salicylic acid.

**Abstract**—The influence of salicylic acid (SA) on ethylene formation in aged potato (*Solanum tuberosum*) tuber slices was investigated. SA treatments significantly stimulated ethylene production of the slices during 24 hr of ageing. Up to 90 $\mu$M SA (the highest concentration tested), the stimulation was positively correlated with concentrations. SA showed stimulation effects on ethylene production at pH 5.4, 6.4 and 7.4, with the greatest stimulation at pH 6.4. These results show that SA enhances endogenous ethylene formation in aged potato tuber slices. This stimulation effect of SA is different from the general conception that SA ultimately inhibits ethylene biosynthesis in plants. Copyright © 1996 Elsevier Science Ltd

## INTRODUCTION

Salicylic acid (SA), a naturally occurring compound in a large number of plants, is known to influence a wide variety of plant physiological processes. It has been suggested recently that SA may be a new kind of plant hormone. However, knowledge about its functional mechanism(s) is still limited [1]. Studies of the relationship between SA and other plant hormones will be very useful in helping to understand the possible functional mechanism(s) of SA. In fact, it has been shown that SA can interfere with the biosynthesis or action of ethylene [1], abscisic acid [2] and cytokinin [3] in plants.

Most of the reported work has focused on the effects of SA on ethylene formation. Inhibition of ethylene biosynthesis was demonstrated in pear cell suspension cultures [4], in apple discs and in mung bean hypocotyls [5], and has led to the general conception that SA ultimately inhibits ethylene biosynthesis [1].

However, SA and ethylene can be shown to have a number of similar functions. For example, both can induce certain pathogenesis-related proteins (PRPs) [1], and both can induce the alternative respiration pathway in many kinds of plant tissues [1, 6, 7, 8]. These results seem contradictory to the generally accepted inhibitory effect of SA on ethylene formation. In addition, there are two reports showing that SA increases endogenous ethylene formation in alfalfa tissue cultures [9], and in suspension cultures of carrot [10]. Taken together, these results suggest further studies are needed to fully assess the effect of SA on ethylene biosynthesis in plants. Here we report a stimulation by SA on ethylene production in aged potato tuber slices.

## RESULTS AND DISCUSSION

When potato tuber slices were aged for 24 hr, their ethylene production rates increased from $0.57 \pm 0.14$ nl $g^{-1}$ fresh weight $hr^{-1}$ at 1 hr to $6.48 \pm 1.01$ nl $g^{-1}$ fresh weight $hr^{-1}$ at 24 hr. With 30 $\mu$M SA, ethylene production rates were significantly enhanced during the whole ageing process of 24 hr, although to gradually decreasing extents with time (Fig. 1). These data indicate a large stimulatory effect of SA on endogenous ethylene formation of excised, washed slices. A further study showed that higher concen-

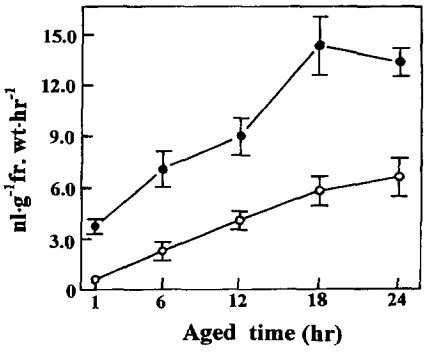


Fig. 1. Time courses of ethylene production rate of the aged potato tuber slices with (●) or without (○) salicylic acid (SA) (30 $\mu$M solutions) treatment. Results are means ±S.D. of three experiments.

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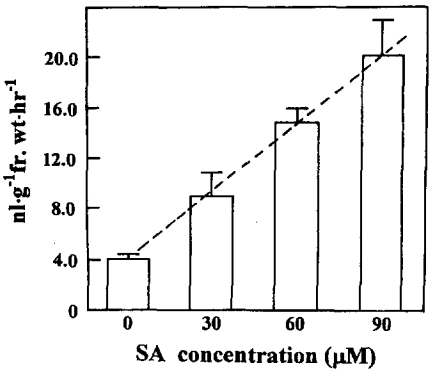


Fig. 2. Effects of salicylic acids (SA) of different concentrations on the ethylene production rate of tuber silces aged for 12 hr. The correlation coefficient ($r$) between SA concentrations and the ethylene production rates is 0.99. Results are means ±S.D. of three experiments.

trations of SA (60 and 90 $\mu$M) could bring greater stimulations than 30 $\mu$M when assayed over 12 hr. Here there was a positive linear correlation between SA concentration and ethylene production (Fig. 2). In a previous study [10], in which a stimulation effect of SA on the endogenous ethylene formation was shown in carrot suspension cultures, the positive correlation between ethylene production and SA concentration also ranged from 10 to 100 $\mu$M.

A previous report [4] showed that the pH values of SA solutions could influence the effects of SA on ethylene formation. Here using SA solutions buffered to three different pH values (5.4, 6.4 and 7.4), it was found that all stimulated ethylene production, the greatest stimulation effect being found at pH 6.4. In slices aged for 12 hr, the treatment in 30 $\mu$M SA at pH 6.4 gave an average stimulation of 121.5%. At pH 7.4 and 5.4, the stimulations were 68.1% and 42.9%, respectively (Table 1). These influences of pH on the effect of SA on ethylene formation are different from those for pear cell suspension cultures, which showed inhibitory effects in acidic conditions, but no effect near neutral pH values [4]. However, they are close to the two examples which also showed a stimulation of SA on endogenous ethylene formation at pH 5.8 [9, 10]. The stimulation by SA of ethylene formation at near neutral pH may have great significance, for normal plant cytoplasmic pH values are *ca* 7.4–7.6, even those in vacuoles are *ca* 5.5–5.8 [11, 12].

Table 1. Influences of pH values on the stimulation effects of salicylic acid (SA) (30 $\mu$M solutions) on the ethylene production rate of tuber slices aged for 12 hr

| | pH values | | |
|---|---|---|---|
| Treatment | 5.4. | 6.4 | 7.4 |
| Without SA | 5.24±0.73 | 4.30±0.55 | 4.33±0.65 |
| With SA | 7.49±0.61* | 9.52±1.48** | 7.28±1.32* |

Data are means ±S.D. of three experiments and express nl ethylene produced $g^{-1}$ fr. wt $hr^{-1}$.

Student's $t$-test: *$P < 0.05$; **$P < 0.01$.

The stimulation of ethylene formation by SA in aged potato tuber slices reported here is at variance from the previously reported inhibitory effect of SA in some other experimental tissues [4, 5]. The reason for this difference is still unknown and warrants further studies [10]. However, the similar biological roles of SA and ethylene in some plant physiological processes may point to possible mechanisms. A recent experiment with tobacco leaves indicated that the accumulation of some SA-induced pathogenesis-related proteins (PRPs) was ethylene-dependent, which suggested a possible downstream role of ethylene in some SA functions in regulating plant disease resistance [13]. Considering that ethylene biosynthesis probably involves free radical step(s) [14], the recent findings that SA can bind catalase and inhibit its activity, thereby increasing $H_2O_2$ concentration *in vivo* [15], perhaps provides a clue, in that $H_2O_2$ can act as a substrate in some free radical generating reactions [16].

## EXPERIMENTAL

*Plant material.* Freshly harvested potato tubers (*Solanum tuberosum* L.) were stored at 5° in darkness. The tubers were in an endogenous rest-period for *ca* 2 months after harvest. Thereafter they were in a dormant state induced by low temperature. Dormant tubers used here were those stored for less than 4 months.

*Preparation and ageing of slices.* Potato tubers were thoroughly washed and cylindrical cores excised with a 6-mm diameter stainless steel borer. Slices of 1 mm thickness were cut from these cores into ice-distilled-water using a microtome blade. Slices were then given a 5-min-infiltration in the different ageing solns under red. pres. (*ca* 350 mm Hg) followed by incubation of the submerged slices under normal pressure for a further 10 min. Slices were then placed onto one layer of tightly stretched gauze to age in air at 30° for 24 hr, as previously reported [6]. The periphery of the gauze was immersed in the same solns as those used for infiltration. Therefore, capillary solns through the gauze, plus spraying the ageing solns onto the slices every 30 min, kept the potato slices fully moistened during the whole process of ageing.

*Ageing solutions.* The treatments were carried out with 30, 60 and 90 $\mu$M SA dissolved first in EtOH (not exceeding 0.1% final concn) and then diluted with distilled water, or with 40 mM HOAc–NaOAc buffer (pH 5.4) and 40 mM K-Pi buffer (pH 6.4 and pH 7.4). Distilled water or buffers alone of the same pH values were used as controls. The pH values of distilled water and unbuffered SA solutions are *ca* pH 6.4.

*Incubation of slices for ethylene determination.* At specified intervals, slices (*ca* 3 g fr. wt) were placed into little glass bottles with known volumes of *ca* 10 ml. Then the bottles were sealed with rubber stoppers. Incubations were carried out under the same conditions as ageing.

*Ethylene determination.* After an incubation of 1.5 hr, 2 ml samples were withdrawn from the head space and analysed for ethylene by FID-GC using an activated Porapak Q column. The injection temp. was 120°. The column oven was at 90°. The carrier gas ($N_2$) flow was 40 ml $min^{-1}$. Ethylene production rates were calculated according to the method of ref. [17].

*Acknowledgement*—This work was supported by the National Natural Science Foundation of China.

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