Pergamon

PII: S0031-9422(96)00869-2

Phytochemistry, Vol. 46, No. 1, pp. 61–64, 1997

0031-9422/97 $17.00+0.00

MODIFICATIONS TO POLYAMINES PROVOKED BY SHORT-CHAIN SATURATED FATTY ACIDS IN *CICER ARIETINUM* SEEDS

MARIA DEL CARMEN GOMEZ-JIMENEZ, FATIHA CHIBI, ISABEL SANCHEZ-CALLE and ANGEL MATILLA*

Departamento de Biologia Vegetal, Laboratorio de Fisiología Vegetal, Facultad de Ciencias, Universidad de Granada, 18001 Granada, Spain

(*Received in revised form* 22 *October* 1996)

Key Word Index—*Cicer arietinum*; Fabaceae; chick-pea; short-chain saturated fatty acids; embryonic axis; germination; polyamines; proline.

Abstract—Short-chain saturated fatty acids (SCSFA), ranging from pentanoic (C_5) to decanoic acid (C_{10}), when added at micromolar concentrations to the germination medium of chick-pea seeds, did not significantly affect radicle emergence, although fresh weight diminished. Only heptanoic acid (C_7) and octanoic acid (C_8) stimulated ethylene production and free spermidine and spermine content, whereas putrescine reached a maximum with nonanoic acid (C_9). All the SCSFA tested increased the polyamines (PAs) bound to substances of low M_r; however, only hexanoic acid (C_6) and C_9 acid induced a strong appearance of PAs bound to substances of high M_r. Proline synthesis was stimulated in the presence of C_7 acid and C_9 acid, but values lower than control were obtained with the other SCSFA studied.

INTRODUCTION

Short-chain saturated fatty acids (SCSFA) ranging in chain length from 6 to 10 are known to be synthesized during senescence in some plants by the acetate pathway [1]. For example, in petunia and carnation flowers, these acids are synthesized in the styles [1, 2]. In the columns and perianths of orchid flowers, SCSFA increased following pollination, the C_8 acid being the main SCSFA found [3]. In addition, such acids have been detected in the endosperm, cotyledons and embryonic axes of some dry [4], or in the hull and pericarp during maturation of some dormant seeds [5, 6]. The mechanism of action of SCSFA is still unknown, but little doubt remains that they contribute to membrane fluidity and permeability [7, 8]; in addition, the sensitivity of various plant tissues of ethylene increases as a result of SCSFA incorporation into the lipid bilayer of cell membranes and subsequent alteration of the lipid order [3, 9, 10].

We have found that the phytohormone, ethylene, is involved in controlling the germination of *Cicer arietinum* seeds by accelerating radicle emergence [11, 12]. Thermoinhibition in this seed is alleviated by exogenous ethylene [13], inhibition of polyamine (PA) biosynthesis [14] and exogenous putrescine (Put) [15]. Since ethylene and polyamines (PAs) arise from the same precursor, *S*-adenosyl methionine (SAM), it has been suggested that ethylene and aliphatic PA formation impose competitive demands on this precursor and that allocation of SAM to either pathway may constitute a regulation point in ethylene and PA biosynthesis [16]. The mechanism by which PAs influence growth and development, however, is not yet known [16, 17].

In the present paper, we describe the changes in the levels of free and bound PAs, osmoregulating proline and ethylene production in embryonic axes of chick-pea seeds induced to grow in the presence of SCSFA (C_5–C_{10}).

RESULTS AND DISCUSSION

With the exception of the C_7 acid (18–20% inhibition), the SCSFAs tested had little effect on the speed of radicle emergence (Table 1). However, all SCSFAs examined, except C_9 (10 μM) and C_6 (100 μM), sharply reduced the fresh weight of the embryonic axis (Table 2). Ethylene production, whilst slightly stimulated by the C_7 and C_8 acids (10 μM), was strongly promoted by the C_7–C_9 acids (100 μM) (Table 1). In some biological systems, certain SCSFAs are synthesized probably to increase sensitivity to ethylene [9, 10, 18]; these saturated fatty acids do not

* Author to whom correspondence should be addressed.

Table 1. Effect of SCSFA on germination of chick-pea seeds (results as %) and ethylene production (results as nmol g fr. wt^{-1} hr^{-1}) by embryonic axis

	10 μM		100 μM	
Fatty acid	%	Ethylene	%	Ethylene
None	100±0	1.3±0.1	100±0	1.3±0.1
C_5	95±1	1.0±0.1	95±3	0.9±0.1
C_6	98±2	1.2±0.2	95±2	0.9±0.1
C_7	80±3	1.7±0.1	82±4	2.4±0.3
C_8	95±2	1.8±0.2	91±3	2.4±0.2
C_9	94±1	0.9±0.3	96±2	1.8±0.1
C_{10}	94±1	0.8±0.2	92±1	0.8±0.2

Mean of 4–5 replicate experiments ± s.d.

contribute to ethylene production [2]. In the seeds of *C. arietinum*, as in *Hordeum distichum* [4], SCSFAs were not detected (data not shown). This legume requires ethylene for radicle emergence [11]. However, the stimulation of ethylene production by C_7–C_9 acids (Table 1) does not favour germination for two possible reasons: (a) the endogenous ethylene level produced in the presence of these SCSFA is not sufficient to provide germination; or (b) SCSFAs in cells of chick-pea seeds act more effectively as inhibitors than does ethylene as a stimulator of germination. The second reason is perhaps the more feasible possibility, given that sensitivity to ethylene in the embryonic axis of *C. arietinum* seeds appears to be quite high, in view of the fact that it has been demonstrated that this seed can germinate with very low levels of ethylene produced in the presence of aminoethoxyvinylglycine (AVG) [13]. Recently, it was suggested SCSFAs decrease the order of specific regions in the membrane lipid bilayer of orchid flowers, thereby altering ethylene activity [3]. In *C. arietinum* seeds, the continued presence of SCSFAs alters the integrity of the embryonic-axis cell membranes [8].

Proline is accumulated in plants as a consequence of various types of environmental stress, such as altitude, drought, salinity or temperature [19–21]. However, no information about proline is available with respect to growth induced by the presence of SCSFA. All concentrations of the C_7 and C_9 acids tested in the present work provoked an accumulation of proline (Table 2). This appears to be due to the fatty acid and not only to the water-stress induced, given that, except for the C_9 acid, in the presence of SCSFAs (1 mM), a decrease in the accumulation of proline was found (Table 2).

The accumulation of PAs is well known under certain stress conditions [22, 23], playing an important part in maintaining membrane integrity [24]. Since SCSFA can be intercalated into membranes [3] modifying the permeability to K^+, glucose or ABA in some cellular compartments [7, 8] and altering conductivity of external medium, we might assume that, *in vivo*, an alteration of the apoplastic space is induced and, thus, hypothesize that SCSFAs might be related to metabolic pathways present in the cell wall, such as those in PAs. The presence of SCSFAs in the germination medium induces an accumulation of free Put in the embryonic axis, which is concomitant with the number of carbons and the concentration of fatty acid, the accumulation with C_9 being the most important (Fig. 1). However, the free spermidine (Spd) and spermine (Spm) maxima were found with C_7 and C_8 acids, and C_7–C_9 acids, respectively. With regard to alterations in PAs bound to substances of low M_r (SH), all SCSFAs induced their accumulation, Spd and Spm being the most affected; in the presence of C_9 and C_{10} acids, the SH levels were between 2- and 4-fold greater than controls (Tables 3 and 4).

The C_5 acid induced the least quantity of PAs bound to substances of high M_r (PH). However, C_6 and C_9 acids strongly stimulated PH synthesis, Spd increasing a striking 7-fold in response to the latter at 100 μM. Although the physiological role of bound PAs is not yet known SCSFAs stimulate the appearance of these substances to levels previously unknown in the embry-

Table 2. Effect of SCSFA on fresh weight (mg per embryonic axis) and free proline content (μg g fr. wt embryonic $axis^{-1}$) in embryonic axis of chick-pea

	10 μM		100 μM		1 mM	
Fatty acid	fr. wt	proline	fr. wt	proline	fr. wt	proline
None	23.5±0.3	128±4	23.5±0.3	128±4	23.5±0.3	128±4
C_5	23.5±0.2	103±3	20.5±0.4	119±3	16.5±0.3	34±5
C_6	20.0±0.3	100±3	25.0±0.5	94±1	20.0±0.4	72±6
C_7	15.5±0.1	135±1	18.0±0.6	152±2	16.5±0.5	130±2
C_8	17.5±0.2	117±1	15.0±0.4	72±4	12.5±0.1	22±2
C_9	25.0±0.4	166±2	15.0±0.3	262±5	11.5±0.1	287±5
C_{10}	20,0±0.5	120±5	17.5±0.1	188±2	12.5±0.4	125±4

Mean of four to five replicate experiments ± s.d.

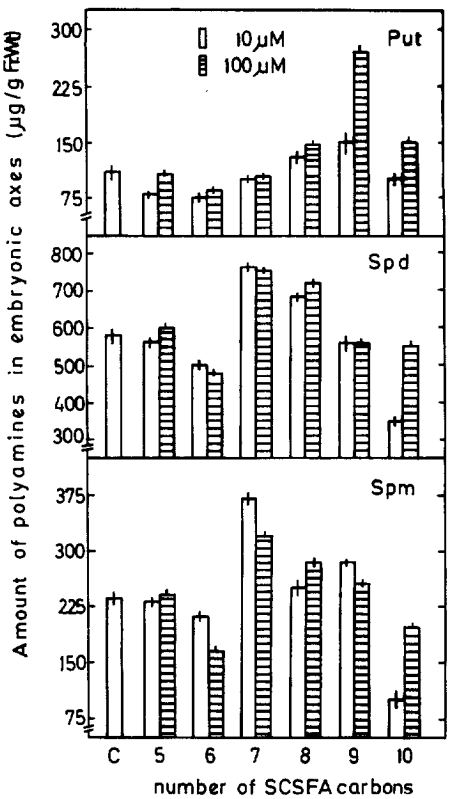

Fig. 1. Content of free polyamines in embryonic axes of chick-pea seeds grown in the presence of SCSFA for 24 hr. Data are means ± s.d. of three replicate experiments.

onic axes of *C. arietinum* seed subjected to stress conditions, such as thermoinhibitory temperatures [11, 14], ABA (unpublished data) or other conditions under which ethylene synthesis was inhibited [14].

EXPERIMENTAL

Chemicals. Cadaverine, hexane diamine, putrescine, spermine, spermidine, proline, short-chain saturated fatty acids and dansyl chloride from Sigma.

Plant material. Cicer arietinum L. cv. Castellana seeds, harvested in 1994, were stored dry at 0–4° until used.

Seed germination. Seeds were allowed to germinate at 25° in darkness on H_2O or (C_5–C_{10}) SCSFA-moistened filter paper in sterile plastic trays. Each SCSFA was used at 0.01, 0.1 and 1 mM and solubilized in the presence of Tween-20 (0.02% w/v). The germination time was 24 hr and the embryonic axes were removed aseptically as described previously [25]. Radicle emergence was taken as evidence of germination.

Determination of free and bound polyamines. PAs were determined as described elsewhere [11, 26]. Samples of embryonic axes were ground with TCA and 1,6-hexane diamine. After centrifugation at 27 000 *g*, the supernatant was used for free-PA determination. A portion of the ppt., after dissolving in 1 M NaOH, was hydrolysed in 12 M HCl for 18–24 hr at 110°. The resulting supernatant and ppt frs were used for determination of PAs bound to molecules of low and high M_r [11].

Determination of proline. Embryonic axes (500 mg) were homogenized in 5 ml of 3% sulphosalicylic acid and centrifuged at 1000 *g*. The supernatant (1 ml was reacted with 1 ml acid ninhydrin and 1 ml HOAc for 60 min at 100°. The reaction was stopped in an ice-bath and the mixt. extracted with 3 ml toluene. The chromophore containing toluene was sepd, warmed to room temp. and the *A* read at 520 nm [19].

Determination of ethylene. Samples (250 mg) of embryonic axes from appropriately treated seeds were transferred to 20 ml flasks containing 0.25 ml of the various SCSFA solns. After a 30-min incubation period, the atmosphere in the vial was sampled for ethylene determination as described in ref. [25].

Acknowledgements—This research has been supported by a grant from 'La Dirección General de Investigación Científica y Técnica' (DGICYT) PB93/1112. M. C. G. J. is the recipient of a fellowship of 'Fundación Ramón Areces' (Spain). We thank David Nesbitt for correction of the English in the text.

Table 3. Effect of SCSFA on polyamines bound to substances of low M_r in embryonic axis of chick-pea seeds germinated for 24 hr (μg g fr. wt embryonic axis^{-1})

	10 μM			100 μM		
Fatty acid	Put	Spd	Spm	Put	Spd	Spm
None	43±3	293±15	87±7	43±3	293±15	87±7
C_5	22±1	275±11	106±8	76±5	393±20	149±9
C_6	47±4	372±10	180±6	29±4	333±14	212±8
C_7	30±2	320±13	180±3	30±1	580±19	215±8
C_8	30±3	390±18	135±4	300±8	120±12	150±4
C_9	85±2	650±20	220±6	85±3	1010±17	420±9
C_{10}	240±9	1320±16	415±9	115±7	625±15	210±5

Mean of three replicate experiments ± s.d.

Table 4. Effect of SCSFA on polyamines bound to substances of high M_r in embryonic axis of chic-pea seeds germinated for 24 hr (μg g fr. wt embryonic axis^{-1})

	10 μM			100 μM		
Fatty acid	Put	Spd	Spm	Put	Spd	Spm
None	44±2	135±10	48±3	44±2	135±10	48±3
C_5	5±1	41±4	14±2	15±1	54±4	22±1
C_6	125±8	101±8	36±4	439±9	592±14	231±9
C_7	44±2	110±7	70±6	30±2	100±6	35±4
C_8	25±1	60±5	25±2	30±1	65±4	30±4
C_9	25±2	125±7	40±3	65±4	950±12	200±11
C_{10}	85±4	200±14	55±6	40±3	100±8	25±5

Mean of three replicate experiments ± s.d.

REFERENCES

1. Whitehead, C. S. and Halevy, A. H., *Plant Growth Regulation*, 1989, **8**, 41.
2. Whitehead, C. S. and Valsiljevic, D., *Physiologia Plantarum*, 1993, **88**, 243.
3. Halevy, A. H., Porat, R., Spiegelstein, H., Borochov, A., Botha, L. and Whitehead, C. S., *Physiologia Plantarum*, 1996, **97**, 469.
4. van Beckun, J. M. M. and Wang, M., *Plant Science*, 1994, **102**, 153.
5. Majumder, M. K., Seshu, D. V. and Shenoy, V. V., *Crop Science*, 1989, **29**, 1298.
6. Seshu, D. V. and Dadlani, M., *Seed Science Research*, 1991, **1**, 187.
7. Babiano, J., Aldasoro, J. J., Hernández-Nistal, J., Rodriguez, D., Matilla, A. J. and Nicolás, G., *Physiologia Plantarum*, 1984, **61**, 391.
8. Babiano, J., Nicolás, G. and Matilla, A. J., *Biologia Plantarum* 1989, **31**, 92.
9. Sutcliffe, M. A. and Whitehead, C. S., *Journal of Plant Physiology*, 1995, **145**, 271.
10. Whitehead, C. S. and Nelson, R. M., *Journal of Plant Physiology*, 1992, **139**, 479.
11. Muñoz De Rueda, P., Gallardo, E., Bueno, M., Gallardo, M., Sánchez-Calle, I. M. and Matilla, A. J., *Journal of Plant Physiology*, 1993, **142**, 347.
12. Sánchez-Calle, I. M., Delgado, M. M., Bueno, M., Díaz-Miguel, M. and Matilla, A. J., *Physiologia Plantarum*, 1989, **76**, 569.
13. Gallardo, M., Muñoz De Rueda, P., Matilla, A. J. and Sánchez-Calle, I. M., *Biologia Plantarum*, 1994, **36**, 201.
14. Muñoz De Rueda, P., Gallardo, M., Sánchez-Calle, I. M. and Matilla, A. J., *Plant Science*, 1994, **97**, 31.
15. Smith, T. A., *Annual Review of Plant Physiology*, 1985, **36**, 117.
16. Evans, P. T. and Malmberg, R. L., *Annual Review of Plant Physiology*, 1989, **40**, 325.
17. Tassoni, A., Antognoni, F. and Bagni, N., *Plant Physiology*, 1996, **110**, 817.
18. Whitehead, C. S. and Bossè, C. A., *Journal of Plant Physiology*, 1991, **137**, 358.
19. Angosto, T. and Matilla, A. J., *Physiologia Plantarum*, 1993, **87**, 329.
20. Delavney, A. J. and Verma, D. P. S., *Plant Journal*, 1993, **4**, 215.
21. Krackhardt, M. and Guerrier, G., *Journal of Plant Physiology*, 1995, **146**, 725.
22. Friedman, R., Altman, A. and Levin, N., *Physiologia Plantarum*, 1989, **76**, 295.
23. Erdei, L., Trivedi, S., Tadeka, K. and Matsumoto, H., *Journal of Plant Physiology*, 1990, **137**, 165.
24. Tiburcio, A. F., Besford, R. T., Capell, T., Borrell, A., Testillano, P. S. and Risueño, M. C., *Journal of Experimental Botany*, 1994, **45**, 1789.
25. Muñoz De Rueda, P., Gallardo, M., Matilla, A. J. and Sánchez-Calle, I. M., *Journal of Experimental Botany*, 1995, **46**, 695.
26. Gallardo, M., Bueno, M., Angosto, T., Gallardo, E. and Matilla, A. J., *Phytochemistry*, 1992, **31**, 2283.