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Abscisic Acid Accelerates Adaptation of Cultured Tobacco Cells to Salt[1]

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P. CHRISTOPHER LAROSA, AVTAR K. HANDA, PAUL M. HASEGAWA, AND RAY A. BRESSAN*
Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Adaptation of tobacco (*Nicotiana tabacum L. var* Wisconsin 38) cells to NaCl was accelerated by (±) abscisic acid (ABA). In medium with 10 grams per liter NaCl, ABA stimulated the growth of cells not grown in medium with NaCl (unadapted, S-0) with an increasing response from 10^{-8} to 10^{-4} molar. ABA (10^{-5} molar) enhanced the growth of unadapted cells in medium with 6 to 22 grams per liter NaCl but did not increase the growth of cells previously adapted to either 10 (S-10) or 25 (S-25) grams per liter NaCl unless the cells were inoculated into medium with a level of NaCl higher than the level to which the cells were adapted. The growth of unadapted cells in medium with Na_2SO_4 (85.5 millimolar), KCl (85.5 or 171 millimolar), K_2SO_4 (85.5 millimolar) was also stimulated by ABA. ABA (10^{-8}–10^{-4} molar) did not accelerate the growth of unadapted cells exposed to water deficits induced by polyethylene glycol (molecular weight 8000) (5–20 grams per 100 milliliters), sorbitol (342 millimolar), mannitol (342 millimolar) or sucrose (342 millimolar). These results suggest that ABA is involved in adaptation of cells to salts, and is not effective in promoting adaptation to water deficits elicited by nonionic osmotic solutes.

Cell lines of several glycophytic species which are tolerant to NaCl stress have been obtained (4, 12, 14, 20, 26, 32). Cell lines of tobacco have been isolated in our laboratory which are capable of growth in liquid medium containing up to 600 mM NaCl (5). Because cultured cells must adapt to saline conditions through cellular processes, investigations of such cells should help us to elucidate the cellular mechanisms involved in NaCl tolerance.

The generally held view that ABA enhances adaptation to stresses is based largely on indirect evidence. ABA accumulates in plant tissues in response to various stresses, suggesting that ABA has a role in adaptation (2, 7, 10, 21, 25). ABA accumulation, which has been shown to be heritable in some plants (22), has been found to be correlated with heritable stress tolerance (27). ABA also has been shown to stimulate physiological responses believed to have stress-adaptive value (1, 13, 27). More direct evidence that ABA is involved in stress adaptation is provided by the findings that ABA can increase the tolerance of plant tissues to stresses caused by water deficit (25), chilling (6, 10), freezing (11), and a herbicide (29). However, heretofore evidence has been lacking to show that ABA increases the tolerance of plant cells or tissues to salinity. In this study, we show that ABA stimulates the adaptation of tobacco cells to salt stress by accelerating their growth in the presence of NaCl and other salts. Furthermore, we present evidence that the effect of ABA is specific to cells exposed to salts, but not to water deficits caused by addition of nonionic solutes to the medium.

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MATERIALS AND METHODS

Nicotiana tabacum L. var Wisconsin 38 cell suspensions were initiated and maintained as described by Hasegawa *et al.* (20). Cells were grown either in 1-L Erlenmyer flasks containing 250 ml medium or in 125-ml Erlenmyer flasks containing 25 ml of medium. Cells were grown to a fresh weight of about 300 mg/ml, a density corresponding to the late exponential phase of growth, for use in experiments. These cells were inoculated into fresh medium at a fresh weight of 10 mg/ml. Stock solutions of 10^{-3} M (±) ABA (Calbiochem-Behring Corp., catalog No. 100111), were prepared in culture medium using ethanol as a co-solvent (0.1%, v/v) and filter sterilized prior to use in experiments. ABA was added to sterile culture medium just prior to inoculation. When used as osmotic solutes, Na_2SO_4, KCl, K_2SO_4, mannitol, sorbitol, sucrose, NaCl, or PEG (mol wt 8000, J. T. Baker Chemical Co., catalog No. 1-U222) were added during preparation of the medium and autoclave sterilized in the medium. The cell wall is impermeable to PEG at the mol wt used and PEG does not degrade appreciably and enter the cells (8, 17, 18).

Fresh and dry weights were measured as described by Hasegawa *et al.* (20). For these experiments the lag period is defined as the time elapsed before fresh weight of the cells exceeds the fresh weight at inoculation. Fresh weight doubling times were calculated from linear regression lines established from the data taken during the exponential phase of growth. The correlation coefficients in all cases were greater than 0.90.

RESULTS

In this report, and from prior experiments (5, 20), we conclude that tobacco cells are capable of adapting to medium with high concentrations of NaCl. After adaptation, these cells grew more rapidly in NaCl than they could prior to initial exposure to the salt. Furthermore, the presence of ABA accelerated the process of adaptation. Cells previously growing in the absence of NaCl (unadapted, S-0) began growing in medium with NaCl and reached the adapted or more tolerant state sooner in the presence of ABA than in its absence (Figs. 1, 2; Table I). ABA enhanced the growth of unadapted cells subjected to 10 g/L NaCl (Fig. 1) but did not increase final yield of unadapted cells in media with either 0 to 10 g/L NaCl, with the possible exception of cells grown in medium containing 10 g/L NaCl with 10^{-4} M ABA. Growth of unadapted cells in the absence of NaCl was unaffected by ABA except at 10^{-4} M where growth was slightly inhibited (Table I). Concentrations of ABA from 10^{-8} to 10^{-4} stimulated

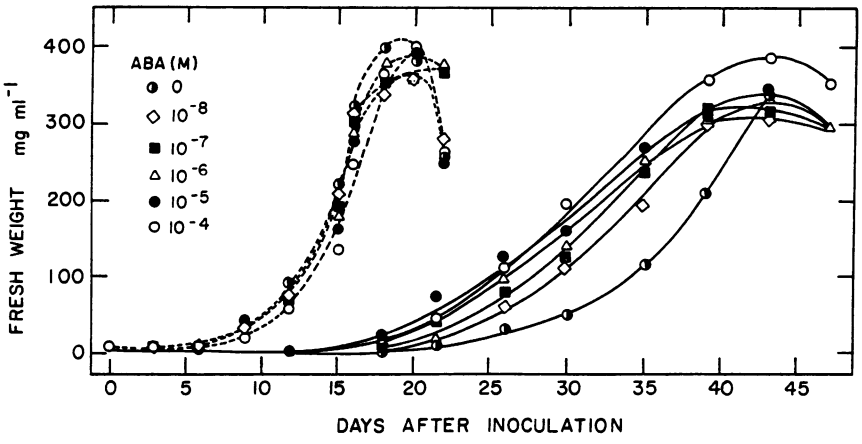

FIG. 1. Kinetics of fresh weight gain of unadapted cells grown in medium with 0 (- - -) or 10 (——) g/L NaCl and containing 0 (◑), 10^{-8}, (◇), 10^{-7} (■), 10^{-6} (△), 10^{-5} (●), or 10^{-4} (○) M ABA. Points represent the average of two 4-ml samples per flask from two 1-L Erlenmyer flasks initially containing 250 ml of cell suspension.

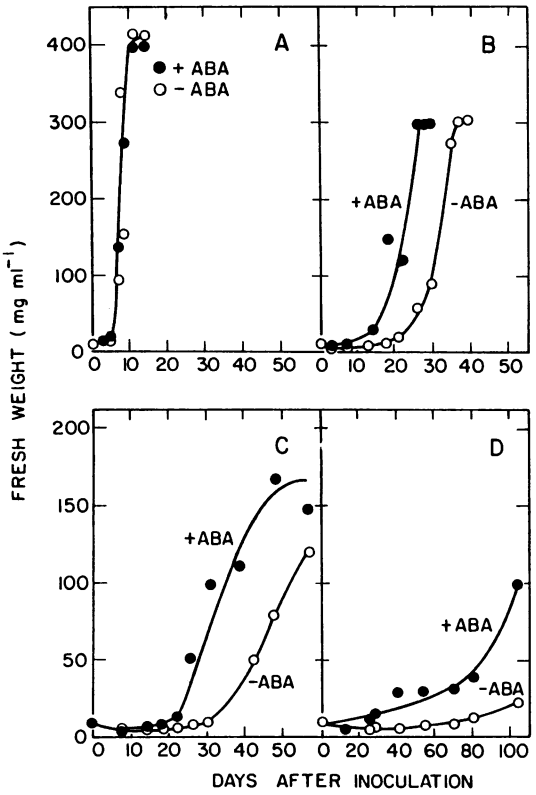

FIG. 2. Kinetics of fresh weight gain of tobacco cells transferred to medium containing 0 (○) or 10^{-5} M ABA (●) and A, 0; B, 10; C, 14; and D, 22 g/L NaCl. Data points represent the average mass harvested from two 125-ml Erlenmyer flasks.

Table I. *Growth Kinetics of Unadapted Tobacco Cells in Medium with 0 and 10 g/L NaCl with Various Concentrations of ABA*

Doubling times and lag periods were calculated from the data in Figure 1.

ABA	Fresh Weight Doubling Time	Fresh Weight Doubling Time	Lag Period	Lag Period
M	*d*	*% of control*	*d*	*% of control*
0 g/L NaCl				
0	2.6	100	3.9	100
10^{-8}	2.5	96	4.2	108
10^{-7}	2.6	100	3.9	100
10^{-6}	2.5	96	4.3	110
10^{-5}	2.5	96	4.3	110
10^{-4}	2.1	81	6.4	164
10 g/L NaCl				
0	4.7	100	19	100
10^{-8}	4.0	85	18	95
10^{-7}	4.3	91	15	79
10^{-6}	4.3	91	15	79
10^{-5}	3.6	77	15	79
10^{-4}	3.5	74	16	84

the growth of unadapted cells in medium with 10 g/L NaCl in a dose-dependent fashion (Fig. 1; Table I). In other experiments, similar dose-dependent responses were observed when unadapted cells were transferred to medium containing 4, 6, 8, 10, and 12 g/L NaCl (data not shown). ABA (10^{-5} M) also enhanced adaptation of these cells to higher levels of NaCl up to 22 g/L NaCl (Fig. 2).

Growth of tobacco cells adapted to and grown in 10 g/L NaCl (S-10) for over 200 generations was not enhanced with the addition of 10^{-5} M ABA to the medium (Fig. 3). ABA also did not stimulate the growth of cells adapted to 25 g/L NaCl (S-25) when grown in medium with 25 g/L NaCl (data not shown). Growth of cells adapted to 10 g/L NaCl was inhibited in medium with 16 g/L NaCl; however, the presence of ABA stimulated their growth and coincident adaptation to the elevated level of salt (Fig. 3).

ABA did not accelerate the process of adaptation to water deficit induced by PEG (Fig. 4; Table II); in fact, in some instances growth in PEG was inhibited by ABA (Table II). ABA stimulated growth of cells subjected to osmotic stress only in the presence of ionic solutes (*i.e.* NaCl, KCl, Na_2SO_4, or K_2SO_4), but not in the presence of nonionic (*i.e.* mannitol, sorbitol, sucrose or PEG) solutes (Table III).

DISCUSSION

The effect of ABA may be viewed in two ways. First, ABA may be considered to enhance the tolerance of the cells since they grow faster when challenged with elevated concentrations of NaCl if exogenous ABA is present. Second, cells which grow for the first time in NaCl become changed (adapted) since they then can grow more rapidly in NaCl when challenged with NaCl again (5, 20, and data not shown). ABA can be said to increase this rate of adaptation since it can cause the cells to grow faster in NaCl during the first exposure. The effect of ABA is clearly related to this adaptation or tolerance and not simply to growth since ABA has no stimulating effect on the growth of unadapted cells in medium without NaCl, nor does it stimulate the growth of adapted cells in medium containing NaCl at the concentration

Table II. *Growth Kinetics of Unadapted Tobacco Cells in Medium with PEG (g/100 ml) with Various Concentrations of ABA*

Fresh weights shown were taken when the cells without ABA (control) reached the stationary phase of growth. This data represents the average of two experiments.

ABA	Fresh Wt Doubling Time	Fresh Wt Doubling Time	Lag Period	Lag Period	Fresh Wt	Fresh Wt
M	*d*	*% of control*	*d*	*% of control*	*mg/ml*	*% of control*
0	4.2	100	13	100	365	100
10^{-8}	4.9	117	18	138	235	64
10^{-7}	5.4	129	20	154	213	58
10^{-6}	6.0	143	16	123	215	59
10^{-5}	5.6	133	19	146	218	60
10^{-4}	10.1	240	29	223	32	9

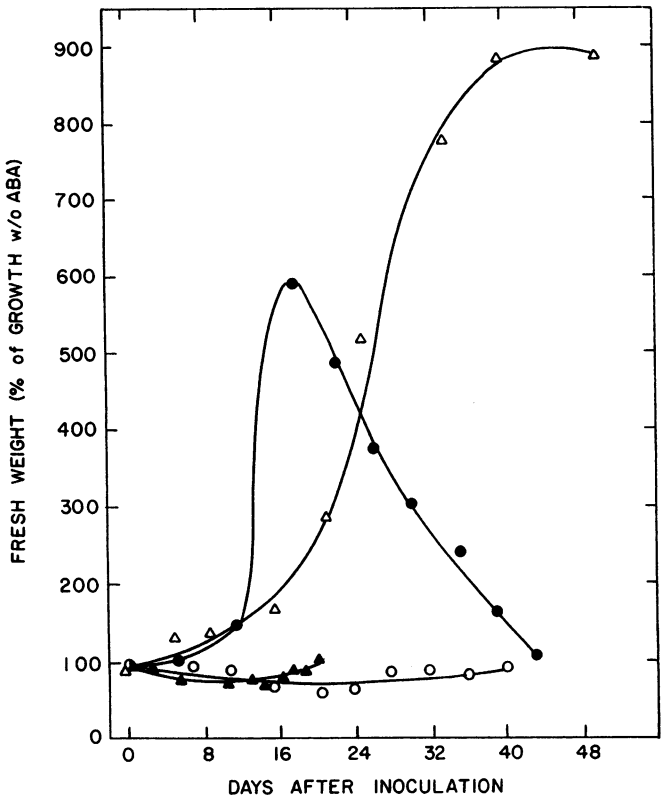

FIG. 3. Effect of 10^{-5} M ABA on relative fresh weight gain of unadapted tobacco cells in medium with 0 (▲) or 10 (●) g/L NaCl and cells adapted to 10 g/L NaCl in medium with 10 (○) or 16 (△) g/L NaCl. Adapted cells were maintained in medium with 10 g/L NaCl for over 200 generations. Points represent the average of two 4-ml samples per flask from two 1-L Erlenmeyer flasks.

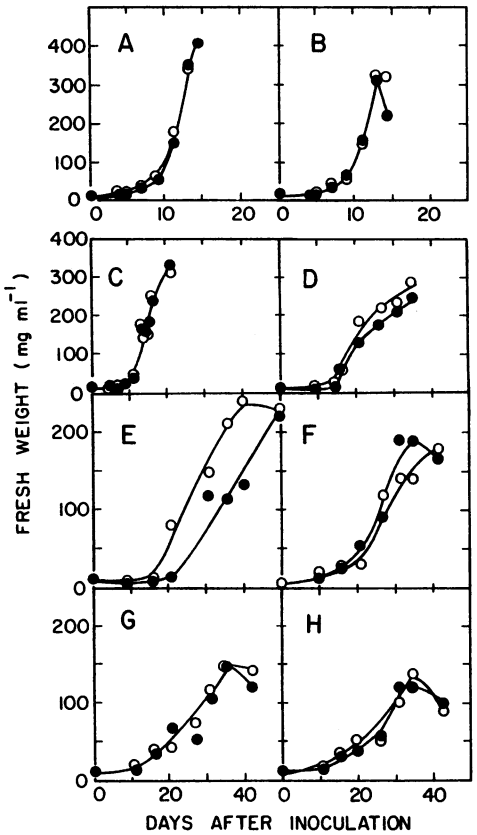

FIG. 4. Kinetics of fresh weight gain of unadapted tobacco cells transferred to medium containing A, 0; B, 5; C, 7.5; D, 10; E, 12.5; F, 15; G, 17.5; and H, 20 g/100 ml PEG with 0 (○) or 10^{-5} (●) M ABA. Points represent the average of the mass harvested from three 125-ml Erlenmyer flasks per treatment.

to which the cells are adapted.

This is the first report in which ABA has been shown to enhance the rate of adaptation of cells to salinity. The mechanism by which ABA stimulates adaptation upon exposure to salinity is unknown although ABA increases osmotic adjustment of pea shoots and roots in the absence of salinity (21), and it is reasonable to suspect that ABA may be acting to stimulate osmotic adjustment in the cultured cells.

From our results, we suggest that exogenous ABA supplements the synthesis of ABA in response to stress, and consequently that ABA can stimulate adaptive processes. Thus the stimulation of growth by ABA may reflect a normal process. Alternatively, we cannot rule out the possibility that the stimulation of growth is pharmacological.

ABA stimulates responses in some plant tissues that may confer tolerance to NaCl. For instance, proline accumulates in cultured tomato cells subjected to water deficit induced by PEG (19) and in tobacco cells in response to NaCl stress (32, data not shown). Accumulation of proline in plants can be stimulated by NaCl, water deficit, or ABA treatment (1, 19, 28). Unique proteins, including a major 26 kD protein, accumulate in cells adapted to NaCl stress (15, 30), and the synthesis of this major 26 kD protein appears to be stimulated by ABA. Net accumulation of Na^+, K^+, Cl^-, and unspecified solutes in root tissues of various species is stimulated by ABA (3, 9, 31). ABA may also influence ion fluxes and compartmentation in plant cells. Compartmental analysis of beet tissues treated with ABA grown under low salt conditions showed that ABA reduces the cytoplasmic

Table III. *Growth of Unadapted Tobacco Cells in Medium with No Osmotic Solutes, or with NaCl, KCl, Na_2SO_4, K_2SO_4, Mannitol, Sorbitol, Sucrose or PEG in the Presence or Absence of 10^{-5} M ABA*

Solute concentrations were chosen to have approximately the same osmolarities or the same cation concentrations as 171 mM NaCl except for PEG and 85.5 mM KCl.

Solute			Fresh Wt ± SE (*n*)	Dry Wt ± SE	Time to Harvest	Fresh Wt/Time to Harvest	
	mM		*mg/ml*		*d*	*(mg/ml)/d*	*% ABA*
Control		−ABA	363 ± 22 (5)	12.0 ± 0.60	12	30.3	
		+ABA	370 ± 28 (4)	12.4 ± 0.56	12	30.8	102%
NaCl	171	−ABA	104 ± 18 (4)	8.7 ± 0.62	28	3.7	
		+ABA	151 ± 7 (5)	10.3 ± 0.43	28	5.4	146[a]
Na_2SO_4	85.8	−ABA	114 ± 6 (5)	8.4 ± 0.46	28	4.1	
		+ABA	198 ± 7 (5)	12.3 ± 0.34	28	7.1	173[a]
KCl	85.5	−ABA	202 ± 27 (5)	10.9 ± 0.76	14	14.4	
		+ABA	349 ± 16 (5)	14.0 ± 0.12	14	24.9	174[a]
KCl	171	−ABA	8 ± 1.3 (5)	2.1 ± 0.52	28	0.3	
		+ABA	37 ± 12 (5)	8.5 ± 0.71	28	1.3	433[a]
K_2SO_4	85.5	−ABA	24 ± 6 (5)	2.1 ± 0.51	28	0.9	
		+ABA	104 ± 17 (5)	8.5 ± 0.68	28	3.7	411[a]
Sorbitol	342	−ABA	284 ± 10 (5)	19.2 ± 1.46	21	13.5	
		+ABA	296 ± 17 (5)	17.8 ± 0.48	21	14.0	104
Mannitol	342	−ABA	246 ± 4 (5)	17.8 ± 0.48	21	11.7	
		+ABA	246 ± 4 (5)	14.7 ± 0.67	21	11.7	100
Sucrose	342	−ABA	145 ± 7 (4)	16.1 ± 0.85	28	5.2	
		+ABA	135 ± 14 (5)	15.2 ± 1.95	28	4.8	92
g/100 ml							
PEG	10	−ABA	7 ± 0.8 (5)	0.88 ± 0.04	28	0.3	
		+ABA	8 ± 0.8 (5)	0.73 ± 0.16	28	0.3	100

[a] $P < 0.0005$ by two-tailed *t* test.

sodium content and increases the vacuolar sodium content (16). Similar effects on the cytoplasmic and vacuolar sodium contents by ABA is barley roots have been observed (24). It appears that ABA can stimulate physiological responses thought to be involved in adaptation to NaCl.

Since there appeared to be an actual decrease in tolerance to PEG caused by addition of ABA, it seems unlikely that the failure of ABA to enhance tolerance of cells subjected to water deficits induced by PEG, mannitol, sorbitol, or sucrose was due to inactivity of ABA. ABA stimulated the growth of cells in medium with NaCl and in media with other salts including Na_2SO_4, K_2SO_4, and KCl. The basis of the specificity of the ABA effect is unelucidated but appears to be correlated with the ionic nature of the solutes used to alter the external water potential of the medium. Perhaps the combination of ABA and ions may stimulate osmotic adjustment by increasing the uptake and compartmentation of Na^+ and Cl^- as well as other ions. This is a reasonable supposition because Na^+ and Cl^- make up a significant portion of the solutes used for osmotic adjustment to NaCl (23, 32, data not shown).

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