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BRIEF COMMUNICATION

The effect of abscisic acid and methyl jasmonate on carbonic anhydrase activity in pea

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Abstract

Short-term (2 h) treatment with 10 μM abscisic acid decreased stomatal conductance and net photosynthetic rate, and increased carbonic anhydrase activity in pea seedlings. The treatment with 10 μM methyl jasmonate did not significantly affect these parameters.

Additional key words: intercellular CO_2 concentration; net photosynthetic rate; plant hormones; stomatal conductance; *Pisum sativum*.

The intensive research on plant response to various environmental stresses during the last years has revealed a role for both abscisic acid (ABA) and jasmonates (jasmonic acid, JA, and its methyl ester, JA-Me) as signalling molecules or stress-modulating compounds. Increased endogenous levels of ABA and/or jasmonates have been found in plants suffering drought, osmotic stress, wounding, *etc.* Leaf photosynthetic activity is considerably reduced by water stress as well as by long-term treatment of plants with ABA or JA (Popova *et al.* 1987, 1988). Carbon dioxide input to the leaf and its supply to the carboxylation sites within the chloroplast are generally limited in those cases. However, a very recent study on short-term application of both effectors to barley seedlings has revealed marked differences in ABA and JA influence on stomatal conductance for CO_2 and biochemical capacity for photosynthesis (Metodiev *et al.* 1996).

The CO_2 diffusion within leaf mesophyll during C_3 photosynthesis is facilitated by the enzyme carbonic anhydrase (CA, E.C. 4.2.1.1). Although CA activity appears to

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Abbreviations: C_a - ambient CO_2 concentration; C_i - intercellular CO_2 concentration; g_s - stomatal conductance for CO_2; P_N - net photosynthetic rate at ambient CO_2 concentration.
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be non-limiting to the net photosynthetic rate (P_N) at ambient CO_2 concentration (Majeau *et al.* 1994, Price *et al.* 1994), its role may become substantial to maintenance of the photosynthetic carbon assimilation when carbon dioxide input through stomata is reduced. Thus, an increased CA activity has been found in salt- and drought-stressed plants, and after long-term treatment with ABA and JA, when stomatal conductance for CO_2 is low (Popova *et al.* 1991, 1996, Kicheva and Lazova 1997). The aim of present study is to determine if the activity of soluble CA changes in response to short-term treatment of pea seedlings with either ABA or JA-Me.

Pea (*Pisum sativum* L. cv. Ran) seedlings were grown for 10 d in a growth chamber at irradiance of 160 μmol m^{-2} s^{-1} PAR, 12 h-photoperiod, 24 °C, and relative humidity of 50 %. Seedlings were treated with either 10 μM ABA or 10 μM JA-Me through the transpiration stream for 2 h. P_N and stomatal conductance for CO_2 (g_s) were measured by the Portable Photosynthesis System *LI-6000* (*Li-Cor*, USA) at irradiance of 170 μmol m^{-2} s^{-1} PAR. The activity of soluble CA was determined in leaf extract by measuring the pH decrease at 2 °C as described by Popova *et al.* (1996). Enzyme activity was estimated in Wilbur Anderson units [1 unit = $10(t_0-t)/t$, in which t and t_0 represent the time for a pH decrease from 8.3 to 7.8 with and without enzyme, respectively]. Soluble protein was determined according to Bradford (1976).

Short-term treatment of pea seedlings with ABA caused decrease in P_N and g_s similarly as in the long-term experiments (Popova *et al.* 1987, Seemann and Sharkey 1987). In contrast to ABA, JA-Me neither reduced P_N nor g_s when applied to seedlings for 2 h (Table 1). These observations in pea corresponded to the very recent findings of Metodiev *et al.* (1996) in barley seedlings subjected to both exogenous ABA and JA for a period up to 2 h. Under JA-Me treatment, the C_i/C_a ratio did not change while it decreased slightly after ABA treatment (Table 1). Our measurements indicated that short-term treatment with JA-ME has a positive effect on leaf photosynthesis.

Table 1. Leaf gas exchange characteristics of pea seedlings treated with abscisic acid or methyl-jasmonate for 2 h: P_N - net photosynthetic rate at 650 mg m^{-3} ambient CO_2 concentration; g_s - stomatal conductance for CO_2; C_i/C_a - ratio of intercellular to ambient CO_2 concentration. Means ± SE of four independent experiments.

Treatment	P_N [mg(CO_2) m^{-2} s^{-1}]	g_s [cm s^{-1}]	C_i/C_a
Control	0.143 ± 0.015	0.55 ± 0.06	0.98
10 μM JA-Me	0.167 ± 0.025	0.53 ± 0.03	0.98
10 μM ABA	0.068 ± 0.012	0.06 ± 0.01	0.93

The activity of soluble CA increased significantly only in response to ABA treatment, and the increase was 75 % (Table 2). Our results indicated that CA activity was high when g_s was decreased by the treatment applied, *i.e.* exogenous ABA. This observation resembled previously reported effects of both ABA and water stress on CA activity in barley and wheat (Popova *et al.* 1996, Kicheva and Lazova 1997).

Higher CA activity in pea leaves had been also found when plants were transferred to a low C_a (Majeau and Coleman 1996). It seems that CA activity is inversely proportional to C_i. Experiments with transgenic tobacco plants possessing low-CA activity support the speculation that plants possibly compensate for the low chloroplastic CA activity by increasing stomatal conductance, and thereby increasing C_i (Majeau *et al.* 1994). We suggest that increased CA activity detected in response to ABA treatment could compensate for the reduced C_i.

Table 2. Carbonic anhydrase (CA) activity and soluble protein content in leaf extracts from pea seedlings treated with abscisic acid or methyl-jasmonate for 2 h. Means ± SE of three independent experiments.

Treatment	CA activity [10^6(unit) kg^{-1}(protein)]	Protein content [g kg^{-1}(fresh mass)]
Control	1837 ± 139	29.3 ± 0.3
10 μM JA-Me	1916 ± 103	28.4 ± 0.5
10 μM ABA	3229 ± 148	28.6 ± 0.2

In conclusion, short-term effects of ABA and JA-Me on pea CA activity were found to differ markedly. They were connected with the changes in stomatal conductance.

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