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SHORT COMMUNICATION

INTERACTION OF JASMONIC ACID AND HYDROGEN CYANIDE ON EARLY SUGAR CATABOLISM IN APPLE EMBRYO DURING GERMINATION

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Effects of jasmonic acid (JA) and HCN on apple embryo germination, on fructose-2, 6-bisphosphate content (F-2, 6-P_2) as well as on the activities of pyruvate kinase (PK), PPi-dependent phosphofructokinase (PPi-PFK) and ATP-dependent phosphofructokinase (ATP-PFK) were studied in cultured, dormant apple embryos in darkness. JA and HCN stimulated embryo germination. Additive effect of JA with HCN on embryo germination was also observed. JA and HCN increased the F-2, 6 content as well as the activities of PPi-PFK and PK, whereas the activity of ATP-PFK was inhibited both by JA and HCN. Additive interaction between JA and HCN on PK and PPi-ATP activities and non-additive interaction on the activity of ATP-PFK suggests that HCN acts independently of JA in the regulatory complex controlling embryonic dormancy in apple.

***Key words*: Apple embryo, germination, HCN, jasmonic acid, sugar catabolism**

Dormancy in apple embryos is expressed by delayed germination, which results in several morphological abnormalities (Bogatek and Lewak 1991). The most important are the inhibition of hypocotyls and internodes elongation, and asymmetric growth and greening of cotyledons. Dormancy and its removal are under temperature and light control and several plant growth regulators are involved in the regulation of these processes (Ranjan and Lewak 1994). Jasmonic acid (JA) has been identified in apple embryos and quantified in the course of dormancy removal (Ranjan *et al*. 1994). Moreover, stimulating effect of JA on germination of dormant embryos and its interaction with light and other growth regulators together with its involvement in the control of hydrolytic enzymes have been demonstrated (Ranjan and Lewak 1995). Studies have also indicated that JA affects dormancy through regulation of lipids and sugar catabolism in germinating embryos (Ranjan 1998).

The observation as well as the finding that the concentration of free HCN in apple embryos changes during removal of dormancy (Dziewanowska *et al*. 1979) led to postulate that HCN has a specific role in the control of dormancy. It has been demonstrated that a short pre-treatment of dormant apple embryos with gaseous HCN leads to their increased germinability and disappearance of growth abnormalities of young seedlings (Ranjan and Lewak 1994). Data collected so far indicate that HCN acts on dormancy removal of apple embryos through regulation of sugar catabolism (Bogatek and Lewak 1991). Since both endogenous JA and HCN play an important role in the regulatory complex controlling the germination of dormant apple embryos, the interaction of exogenous jasmonic acid and HCN on early sugar catabolism was studied.

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Seeds of apple (*Malus domestica* Borb. Cv. Antonowka) harvested in 2000 and provided by Centre for Apple Research in Ozarow (Poland) were used in the study. The embryos were prepared from imbibed seeds and cultured according to Ranjan and Lewak (1992). Lots of 30 embryos were cultured for 7 days in petri dishes on a filter disc moistened with 5 ml of distilled water or JA (20 µM) solution in dark at 25/20°C, 12/12 h day/night. This concentration of JA was found the most effective in previous experiments (Ranjan and Lewak 1992).Treatment of isolated embryos with gaseous HCN (1.0 mM) was carried out as described earlier (Bogatek and Lewak 1991) and the embryos were cultured under the conditions described above. An embryo was considered as germinated when geotropic curvature of the axis was observed. The activities of pyruvate kinase (PK), PPi-dependent phosphofructokinase (PPi-PFK) and ATP-dependent phosphofructokinase were assayed according to Nakayama *et al.* (1976) and Van Schaftingen *et al.* (1982) respectively. Fructose-2, 6-bisphosphate (F-2, 6-P_2) levels were determined as described by Van Schaftingen *et al.* (1982). All experiments were repeated 3 times with 3 replicates in each. The results were presented as mean values ± SD.

Data in Table 1 show the stimulatory effects of JA and HCN on apple embryo germination cultured for 7 days. The addivity (calculated according to Nitsch and Nitsch, 1961) was observed between the effects of JA and HCN (Table 1, lines 5 and 6). This calculation and its usefulness have been used earlier (Ranjan and Lewak 1992, 1994, 1995). Hence, these two factors act independently on apple embryo germination as suggested in our previous paper (Ranjan and Lewak 1994). When the joint effect of two factors is greater than the sum of effects of separate treatments, the term synergism is used, whereas a lower value is described as non-additive inhibition or negative synergism (Nitsch and Nitsch 1961). Additivity indicates independent modes of action, in contrast to non-additive interaction.

Maximum level of F-2, 6-P_2 was observed in one-day-old embryo culture (Table 1), but decreased after the first day of culture (data not shown). Treatment of embryos with JA and HCN caused a sharp increase in F-2, 6-P_2 concentration in one- day- old embryo culture, but had no effect during the days that followed (data not shown). This is in agreement with earlier data (Ranjan, 2001). Since maximum increase in F-2, 6-P_2 content was observed in one- day- old embryo culture, the interaction study was conducted at that time. Combined treatment of embryos with JA and HCN caused a significant (4-folds) increase in F-2, 6-P_2 levels (Table 1).

Table 1. Effects of jasmonic acid (20 µM) and HCN (1.0 mM) on germination of apple embryos after 7 days of culture and on the changes in F-2, 6-P_2 content (nmol g FW^{-1}) in one- day- old embryo culture in darkness. (Values are means ± SD. In the case of F-2, 6-P_2 SD did not exceed 0.05 nmol FW^{-1}).

	Culture conditions	% Germination after 7 days	F-2, 6 – P_2 content after one day
1	Control (H_2O)	24.4 (±1.9)	1.25
2	JA	42.2 (±1.2)	3.60
3	HCN	36.4 (±0.7)	3.20
4	HCN + JA	56.0 (±1.7)	5.2
5	(4) – (1)	31.6	
6	(3) – (1) + (2) – (1)	29.8	

The effect of JA and HCN on the activities of PK, PPi-PFK and ATP-PFK is shown in Table 2. Maximum PK, PPi-PFK and ATP-PFK activities were observed in one- day- old embryo culture followed by gradual decline. Treatment of embryos with JA and HCN significantly (20-25%) inhibited the ATP-PFK activity. In contrast, treatment of embryos with JA and HCN resulted in maximum increase in the activities of PK and PPi-PFK (Table 2). Maximum stimulation in the activity of PPi-PFK was observed at that time when F-2, 6-P_2 content was maximal (Table 1), indicating glycolytic degradation of sugars. Earlier experimental data support the results (Ranjan, 2001). The effects of JA and HCN on PK and PPi-PFK activities were additive (Table 2, lines 5 and 6) when both were applied together at that period. This indicates the lack of their interaction on these enzyme activities. In contrast, a non- additive

Table 2. Effects of JA (20 μM) and HCN (1.0 mM) on the changes in activities of PK (nmol NADH min^{-1} $embryo^{-1}$), PPi-dependent and ATP-dependent phosphofructokinases (nmol NADH min^{-1} $embryo^{-1}$) in one- day- old embryo culture in darkness. Values are means ± SD. In the case of phosphofructokinases SD did not exceed 0.005 nmol $embryo^{-1}$.

Culture conditions	PK activity	PPi-PFK activity	ATP-PFK activity
	after one day	after one day	after one day
1 Control (H_2O)	14.8 (±1.5)	0.170	1.100
2 JA	23.0 (±1.1)	0.850	0.854
3 HCN	21.8 (±1.0)	0.820	0.900
4 HCN + JA	30.0 (±1.7)	1.510	0.830
5 (4) – (1)	15.2	1.340	(-) 0.270
6 (3) – (1) + (2) – (1)	15.2	1.330	(-) 0.446

inhibitory interaction between JA and HCN on ATP-PFK activity was observed, indicating that there is a common site of JA and HCN action on enzyme activity.

The assumption that the additivity of effects of JA and HCN on apple embryo germination and on PK and PPi-ATP activities is the result of their independent action was the fundamental principle of the present paper. Therefore, the additive effect of JA with HCN on apple embryo germination indicates that HCN is not involved in the control of the chain of events leading to JA-stimulated germination. Previous results showed that JA stimulates the germination of dormant apple embryos through activation of alkaline lipase which initiates the mobilization of reserve lipids (Ranjan and Lewak 1992). Fatty acids released from storage lipids by lipase undergo gluconeogenesis. The activity of isocitrate lyase, the key enzyme of gluconeogenesis, is stimulated by JA (Ranjan 2004). HCN does not affect either the activity of alkaline lipase or isocitrate lyase (Bogatek *et al*. 2002).

Dormant apple seeds contain only a small amount of storage oligosaccharides and the hydrolysis of storage oligosaccharides takes place during dormancy removal, thus supplying the substrates for glycolysis. The hydrolysis of oligosaccharides is controlled by JA and HCN (Bogatek *et al*. 2002). Previous results showed the catabolism of soluble sugars occurs through fructose 6-phosphate 2-kinase (F-6-P, 2K) activation. This leads to accumulation of F-2, 6-P_2, which in turn, activates PPi-PFK and enhances glycolysis with the activation of PK activity (Ranjan 2001). Substantial increment in the activities of PK and PPi-PFK and inhibition of ATP-PFK activity was observed by JA and HCN in one day old embryo culture when they were applied separately. It has been proposed that PPi-PFK is an alternate enzyme to ATP-PFK and acts in the direction of fructose-6-phosphate consumption (Van Schaftingen *et al*. 1982). F-2, 6-P_2 is potent stimulator of PPi-PFK and the rate of glycolytic flux is controlled by its concentration (Black *et al*. 1987). The additive action of JA and HCN on the activities of PPi-PFK and PK suggests that they act independently on this process.

The mobilization of reserves in apple embryo, which is the main feature of the removal of dormancy, is controlled by several environmental and hormonal factors that comprise the regulatory complex. The hydrolysis of reserve proteins is under the control of low temperature, ABA and JA (Ranjan and Lewak 1995). The breakdown of stored lipids is under the control of light, GA, ABA and JA (Zarska-Maciejewska and Lewak 1976 and Ranjan and Lewak, 1995). Oligosaccharide hydrolysis and the catabolism of its products are regulated by HCN (Bogatek *et al*. 1999) and JA (Ranjan 1999) whereas; gluconeogenesis is regulated by light, GA, ABA and JA (Bogatek *et al*. 1989 and Ranjan 2004). The additivity of JA effects on apple embryo germination with those of light and GA has been previously demonstrated (Ranjan and Lewak 1994), indicating that JA does not act on the regulatory pathway that is initiated by light and which induces germination through GA accumulation and storage lipid mobilization. ABA and HCN control this pathway (non-additive relation with light effect). However, while between the JA and ABA effects an antagonistic non-additive interaction was observed, the effects of JA and HCN were additive. Together with earlier data, the results presented here allow us to assume that both JA and HCN act independently on sugar catabolism, through different mechanisms.

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