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SHORT COMMUNICATION

EFFECT OF 1-MCP ON MALIC ENZYME ACTIVITY AND ETHYLENE PRODUCTION IN MANGO DURING RIPENING

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1-methylcyclopropene is a novel, magic gas that has proven itself to be non-toxic and odourless inhibitor of ethylene in wide range of fruits and vegetables. The effect of 1-MCP on malic enzyme, a marker enzyme, during ripening in climacteric fruits like mango, was studied. Malic enzyme activity was found to be completely inhibited for 8 days after 1-MCP treatment, the period for which endogenous ethylene evolution was also checked. Malic enzyme activity appeared after 8 days when 1-MCP lost its control to inhibit endogenous ethylene and autocatalytic evolution started, triggering cascade of biochemical changes leading to ripening in mango cv. Amrapali. 1-MCP was found to be effective for 8 days in delaying fruit ripening in mango, suggesting the role of endogenous ethylene in regulating malic enzyme activity during ripening.

Key words: **Climacteric fruits, ethylene, malic enzyme, *Mangifera indica*, 1-methylcyclopropene, respiration.**

1-Methylcyclopropene (1-MCP) has recently become available as a stable powder (Ethylbloc™) from which the gaseous form can be released by addition of dilute base. The magic gas, 1-MCP has been used as tool to enhance shelf life of perishable flowers, as it blocks ethylene receptors and prevents ethylene effects in plant tissues for extended periods (Sisler and Serek 1997). This chemical, therefore, provides a valuable tool to investigate ethylene metabolism and has tremendous potential to extend keeping quality of climacteric fruits in which ethylene is the key player that promotes ripening.

Ethylene stimulates the production of new mRNAs and proteins required to bring about the changes that are integral part of the fruit ripening process (Jiang and Fu 2000). Malic enzyme is used as another marker of ripening state, which catalyzes the decarboxylation of the malic acid, the major organic acid for respiration during ripening in climacteric fruits like mango (Goodenough *et al*. 1985). Srivastava *et al.* (1996) reported that, silver ions that antagonize ethylene action during ripening, cause inhibition of malic enzyme, suggesting some relation between the two processes. In the present investigation, an attempt has been made to find the relation between malic enzyme and ethylene evolution in ripening mango, when treated with 1-methylcyclopropene to mature unripe fruits. Duration of 1-MCP effectivity in mango was also examined.

Mature, unripe fruits of mango, (*Mangifera indica* cv. Amrapali) were harvested from the orchard of Indian Agricultural Research Institute, New Delhi. Fruits were kept in lab at $27^0C \pm 2^0C$. A ripening stimulator calcium carbide (CaC_2) was used to initiate the ripening process. Thereafter, the fruits were divided into two lots (6 fruits in each lot) for 1-MCP treatment and control. 1-MCP (1 ppm) treatment was given by keeping fruits in an air tight container to expose them to gas evolved by 1-MCP for 24 h. The lid of the container was sealed immediately after placing 5 mg 1-MCP dissolved in 1 ml distilled water

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to release 1 ppm concentration of 1-MCP in container of 5 litre capacity. The lid was opened after 24 h and fruits were transferred to baskets that provided good aeration, till full ripe stage. Control set was given similar treatment but without 1-MCP.

Malic enzyme activity was assayed as described by Dubery *et al*. (1984) by measuring the reduction of NADP at 340 nm in Beckman Spectrophotometer. Mango tissue (15 g) was homogenized with 30 ml of 0.2 M Tris –HCl buffer pH 8.0 at 4^0C in presence of a pinch of polyvinyl pyrrolidone (PVP). The homogenate was adjusted to pH 7.1 and centrifuged at 15,000 g for 10 min at 4^0C. The supernatant was used for determining the malic enzyme activity. The reaction mixture contained 1.6 ml of 10 mM Tris HCl buffer at the optimum pH of 7.1, 0.1 ml of 1 mM $MnSO_4$, 0.6 ml of 5 mM malate, 0.4 ml of 0.5 mM NADP and 0.3 ml of enzyme. The level of ethylene was measured using Gas Chromatograph (Perkin-Elmer Sigma 2000) with flame ionization detector and porapak-Q80/100 mesh packed column and expressed as nmol ethylene evolved g^{-1}fw h^{-1}. Change in respiration was traced to track ripening process in both control and MCP treated fruits. Respiration was measured with help of Infra Red Gas Analyzer (IRGA; Model ADC, LCA2-6606) using specially designed chamber and expressed as µmolCO_2 released g^{-1}fw h^{-1}.

1-Methylcyclopropene is a new chemical that is attracting the attention of scientists and the horticultural industry world wide. The scientific research on this compound has shown it as a powerful inhibitor of ethylene action and capable of maintaining post harvest quality in many fresh horticultural products (Robert and John 2003). Inhibitory effect of 1-MCP on ethylene evolution was evident from the observation that autocatalytic evolution of endogenous ethylene is completely inhibited for 8 days in 1-MCP treated mature unripe fruits of mango supporting the finding of Golding *et al.* (1998) and Nakatsuka *et al*. (1997). Rise of endogenous ethylene was resumed after 8 days after treatment of 1-MCP and there after the trend was same as exhibited by the control set of fruits. In contrast ethylene evolution started 1 day after harvest (DAH) in control set of fruits not treated with 1-MCP and exhibited climacteric peak at 4 DAH. (Table 1).

Table 1. Ethylene evolution (nmol g^{-1}fw h^{-1}) during ripening in control and 1-MCP (1 ppm) treated fruits of mango cv. Amrapali

Days after harvest	Ethylene evolution (nmol g^{-1}fw h^{-1})	
	Control	Treated
0	1.20	0
2	2.46	0
4	3.10	0
6	2.58	0
8	2.30	0
10	-	0.98
12	-	2.51
14	-	3.19
16	-	2.46

There was a steady rise in malic enzyme activity in CaC_2 treated control set of fruits 1DAH (Table 2). On the other hand with 1-MCP treatment, malic enzyme could not be activated for a period during which ethylene production was inhibited. Malic enzyme activity increased with resumption of autocatalytic evolution of endogenous

Table 2. Malic enzyme activity during ripening in control and 1-MCP (1 ppm) treated fruits of mango cv. Amrapali

Days after harvest	Malic enzyme activity (nmol of NADPH formed mg^{-1} protein min^{-1})	
	Control	1-MCP Treated
0	0	0
2	2.5	0
4	9.4	0
6	8.0	0
8	5.7	1.6
10	-	3.9
12	-	9.1
14	-	8.4
16	-	6.2

ethylene in 1-MCP treated fruits. These results suggest the participation of ethylene in activation of malic enzyme as there is a parallel relation between the two processes. These results are in line with the observations reported earlier by Srivastava *et al.* (1996). Application of 1-MCP could prolong the shelf life of mango by 8 days.

A substantial reduction in respiration was detected within a day of treatment, after which the rate began to increase but remain considerably low in comparison to the control sets. With 1-MCP treatment, the respiration rate was typically halved in mango cv. Amrapali when compared with the control sets and respiratory burst appeared only after 8 days of 1-MCP treatment indicating the inhibitory effect of 1-MCP on respiratory climacteric (Table 3). These results clearly indicate that 1-MCP mediated inhibition of endogenous ethylene, is directly causing inhibition of respiratory climacteric by 2-folds in mango fruits.

Table 3. Respiration rate (µmol CO_2 g^{-1}fw h^{-1}) during ripening in control and 1-MCP (1 ppm) treated fruits of mango cv. Amrapali

Days after harvest	Respiration rate (µmol CO_2 g^{-1} fw h^{-1})	
	Control	Treated
0	1.90	0.66
2	2.24	1.12
4	6.47	2.29
6	10.59	3.34
8	7.48	4.42
10	3.96	4.98
12	-	5.57
14	-	7.49
16	-	9.97
18	-	7.12
20	-	4.43

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