Plant Physiology and Biochemistry 69 (2013) 34–38

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Low temperature stress ethylene and not *Fusarium*, might be responsible for mango malformation

Mohammad Wahid Ansari [a,b], Gurdeep Bains [a], Alok Shukla [a], Ramesh Chandra Pant [a,*], Narendra Tuteja [b,*]

[a] Department of Plant Physiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, Uttarakhand, India

[b] Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, 110 067 New Delhi, India

ARTICLE INFO

Article history:
Received 4 February 2013
Accepted 22 April 2013
Available online 9 May 2013

Keywords:
Colony growth
Conidia germination
Fusarium mangiferae
Mango malformation
Stress ethylene
Low temperature stress

ABSTRACT

Malformation is arguably the most crucial disease of mango (*Mangifera indica* L.). The etiology of the disease has not yet been successfully resolved. Here, we quantified the endogenous ethylene content in malformed and healthy vegetative and floral tissues of mango cultivars *viz.*, Amrapali, Bombay green, Chausa, Dushehri and Mallika. Levels of ethylene were higher in malformed vegetative and floral tissues as compared with that of healthy tissues at both prior to full bloom and full bloom stages. The study also revealed that isolates of *Fusarium* dissected from mango exhibited most morphological similarities to the accepted standard features of *Fusarium mangiferae*. The growth dynamic of *F. mangiferae* were evaluated with varying temperatures ranging from 5 to 40 °C. Temperatures of 25 °C, 30 °C and 35 °C were better suited for growth of *F. mangiferae* than temperatures of 20 °C or 40 °C. Conidium germination of *F. mangiferae* was maximum at 30 °C and minimum at <15 °C. World-wide occurrence of mango malformation showed its most severity at 10–15 °C temperature range. Stress ethylene level is higher in diseased tissue at the same temperature range where growth of *Fusaria* is found to be completely restricted. The present study provides direct evidence that low temperature induced 'stress ethylene' is potentially responsible for the disease while on the other hand *Fusarium* role in the disease either through toxic principle or malformation inducing principle is not conclusive at <15 °C and is rather out of question.

1. Introduction

Malformation is arguably the most crucial disease of mango (*Mangifera indica* L.) at present. It is receiving great attention not only because of its widespread and destructive nature but also because of its etiology and control is not absolutely understood. Malformation causes gross deformations of vegetative and floral tissues in mango [1]. Since its recognition in India [2], disease has also been confirmed in most mango growing countries such as Pakistan, the Middle East, Egypt, South Africa, Brazil, Sudan, Central America, Mexico and USA, Cuba, Malaysia, Australia, Israel, UAE, Bangladesh and Sultanate of Oman. The malady has been variously ascribed to be acarological, viral, fungal and physiological in nature [3]. It was proposed that the disorder may be due to the production of 'stress ethylene' by mango plants [4]. The occurrence of hypertrophy of lenticels [5], leaf epinasty and disturbance in the natural orientation of shoots and panicles in malformed trees, suppression of apical dominance, existence of vegetative and floral malformation in the same tree, decayed root system, flower drop from panicles, and increased gummosis, necrosis etc have been attributed to ethylene effect in malformed trees [6]. The methionine-ACC pathway has been established to operate in plants for ethylene biosynthesis [7]. The key regulatory enzymes in the pathway are ACC synthase and ACC oxidase which are positively and negatively modulated by a number of factors such as chilling injury, physical injury flooding, certain chemicals, pathogen infestation. Hydrogen cyanide, formed in the last step of ethylene synthesis, is detoxified by β-CAS [7–9]. The severity of disease is correlated with the seasonal variation in temperature at the time of flowering. The incidence of disease is most severe in North-West region of India where mean temperature preceding flowering remains between 10 and 15 °C. It is mild where the corresponding temperature is 15–20 °C,

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; β-CAS, β-cyanoalanine synthase; CDA, Czepeck's dox agar; CLA, carnation leaf agar CLA; d, day(s); FW, fresh weight; $HgCl_2$, mercuric chloride; MIP, malformation inducing principle; $(NH_4)SO_4$, ammonium sulfate; PDA, potato dextrose agar; pM, pico mole; TP, toxic principle.

* Corresponding authors. Tel.: +91 11 26742357; fax: +91 11 26742316.
E-mail addresses: pant_rc@yahoo.co.uk (R.C. Pant), narendra@icgeb.res.in (N. Tuteja).

0981-9428/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.plaphy.2013.04.019

sporadic at 20–25 °C and nil over 25 °C. The similar trend is also reflected with the world distribution of the disease and its incidence is mainly recorded where the mean temperature during winter is less than 16 °C [10]. The various *Fusaria*, including *Fusarium mangiferae*, were found to be associated with the disease [11,12]. There was no growth of *Fusaria* at temperatures below 10 and above 40 °C. The temperatures 27 °C followed by 25 °C with 65% relative humidity were found optimum for better growth and sporulation of *F. mangiferae* [13]. There are a multiple reports over the last thirty years which support that ethylene production is induced in response to various environmental stresses [14]. Besides, ethylene produced by *F. mangiferae* itself is assumed to be contributed to stress-induced ethylene pool [15].

The present study is aimed to investigate the low temperature induced 'stress ethylene' production in mango and growth dynamics of *F. mangiferae* under different temperature regime. Here, we have observed that low temperature induced 'stress ethylene' is responsible for mango malformation while on the other hand *Fusarium* role may not be authentic in the disease due to its restricted growth at <15 °C in functioning either through toxic principle (TP) or malformation inducing principle (MIP).

2. Results

2.1. Endogenous ethylene levels in vegetative tissues of mango cultivars

The ethylene levels (pM g^{-1} FW min^{-2}) of malformed vegetative tissues mango cultivars, namely, Amrapali, Bombay green, Chausa, Dashehri and Mallika were higher as compared to that of healthy tissues (Table 1). The ethylene content of malformed vegetative tissue of Bombay Green, Mallika, Chausa, Amrapali and Dashehri cultivars was detected at the level of 60.48, 76.02, 81.90, 50.86, 147.00 pM g^{-1} FW min^{-2} as opposed to healthy tissues 28.56, 18.18, 25.20, 18.48, 41.37 pM g^{-1} FW min^{-2}, respectively. The fold induction in ethylene production was higher in malformed vegetative tissue of Mallika (4.18) cultivar which followed by Dashehri, Chausa, Amrapali (3.55, 3.25, and 2.75, respectively) and was lower in Bombay Green (2.11).

2.2. Endogenous ethylene levels in floral tissues of mango cultivars

At prior to full bloom stage of flower bud, ethylene level was higher in malformed tissues in contrast to analyze in health floral tissues of tested mango cultivars. Malformed floral tissues of Bombay green cultivars exhibited highest level of ethylene 114.03 pM g^{-1} FW min^{-2} followed by Mallika, Chausa, Amrapali (77.53, 50.82, 27.72 pM g^{-1} FW min^{-2}, respectively) while Dashehri showed lowest (23.94 pM g^{-1} FW min^{-2}). In contrast, healthy tissues depicted 46.20, 55.23, 27.30, 9.66, 11.76 ethylene pM g^{-1} FW min^{-2} in Bombay green, Mallika, Chausa, Amrapali and Dashehri cultivars, respectively. Fold induction of ethylene level in malformed over healthy was maximum 2.86 in Amrapali while it was minimum in Mallika 1.40 (Table 2). At full bloom stage of flower bud, ethylene level was detected 103.32 pM g^{-1} FW min^{-2} in malformed floral tissues of Mallika which gradually decreased in Bombay green, Amrapali Dashehri, Chausa 97.44, 88.59, 88.26, 59.58 pM g^{-1} FW min^{-2}, respectively. In contrast, 39.90, 28.14, 17.85, 17.22, 10.92 ethylene pM g^{-1} FW min^{-2} were quantified in healthy floral tissues of Amrapali, Chausa, Bombay green, Dashehri, Mallika, cultivars, respectively. Fold induction of ethylene in malformed over healthy were reflected with same trend (Table 3).

Table 1
Levels of endogenous ethylene content in healthy and malformed vegetative tissues of mango (*Mangifera indica* L.) cultivars. Results are the means of two independent experiments each with three replicates.

Mango cultivars	Ethylene (pM g^{-1} FW min^{-2})		Malformed/healthy	**/ns
	Malformed	Healthy		
Bombay green	60.48	28.56	2.11	**
Mallika	76.02	18.18	4.18	**
Chausa	81.90	25.20	3.25	**
Amrapali	50.86	18.48	2.75	**
Dushehri	147.00	41.37	3.55	**

SEM = ±2.22, cd at 1% = 9.90, cd at 5% = 6.97, ** = significant, ns = non significant.

Table 2
Levels of endogenous ethylene content in healthy and malformed floral tissues of mango (*Mangifera indica* L.) cultivars at prior to full bloom stage of flower bud. Results are the means of two independent experiments each with three replicates.

Mango cultivars	Ethylene (pM g^{-1} FW min^{-2})		Malformed/healthy	**/ns
	Malformed	Healthy		
Bombay green	114.03	46.20	2.46	**
Mallika	77.53	55.23	1.40	**
Chausa	50.82	27.30	1.86	**
Amrapali	27.72	9.66	2.86	**
Dushehri	23.94	11.76	2.03	**

SEM = ±2.78, cd at 1% = 12.44, cd at 5% = 8.75, ** = significant, ns = non significant.

2.3. Effect of temperature on radial growth and conidia germination of F. mangiferae

The isolates of *F. mangiferae* from Amrapali, Bombay green, Chausa, Dashehri and Mallika mango cultivars revealed morphological features in a similar fashion with accepted standard features of *F. mangiferae* [16]. At each of 7, 14, and 24 d of incubation the average growth of *F. mangiferae* was significantly greater at 25 °C, 30 °C, 35 °C than at 20 °C and 40 °C (Fig. 1A). The temperature of 30 °C consistently displayed the highest growth rate at all incubation periods (Fig. 1B–D). Below 15 °C and above 35 °C temperature colony growth was dramatically reduced and beyond this value growth of *F. mangiferae* was completely ceased. At most temperatures, germination was observed to start at 6 h or with a maximum number germinating at all temperature after 24 h. The maximum germination of conidia was recorded at 30 °C followed by 35 °C and 40 °C (Fig. 2A, B–D). Below 25 °C, conidia germination was dramatically reduced. No conidia germinated at 5 °C, 10 °C or 15 °C even after 24 h (Fig. 2E).

3. Discussion

The basic phenomenon of increased ethylene production in response to stress is commonly called 'stress ethylene'. Ethylene is a

Table 3
Levels of endogenous ethylene content in healthy and malformed floral tissues of mango (*Mangifera indica* L.) cultivars at full bloom stage of flower bud. Results are the means of two independent experiments each with three replicates.

Mango cultivars	Ethylene (pM g^{-1} FW min^{-2})		Malformed/healthy	**/ns
	Malformed	Healthy		
Bombay green	97.44	17.85	5.45	**
Mallika	103.32	10.92	9.46	**
Chausa	59.58	28.14	2.12	**
Amrapali	88.59	39.90	2.22	**
Dushehri	88.26	17.22	4.95	**

SEM = ±3.39, cd at 1% = 15.18, cd at 5% = 10.68, ** = significant, ns = non significant.