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Changes in catalase enzyme expression profile during malformation disorder of mango (*Mangifera indica* L.)

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Abstract
Mango holds a significant place in the tropical and subtropical areas of the world. Its fruit are widely appreciated for unique aroma and flavor. But this crop is afflicted by a disorder/disease known as mango malformation. Mango malformation imposes a serious restriction on production of mango in India and other mango cultivating provinces of the world bringing in heavy economic losses. The disease/disorder results in alteration of vegetative and floral tissues of the crop. A broad range of biotic and abiotic factors have been assigned as the etiology of malformation. Several evidences have been put forth to justify the involvement of stress ethylene synthesized in response to low temperature, high relative humidity and a soil borne fungal pathogen during bud inception stage. These stresses induce the accumulation of reactive oxygen species (ROS) in the cells, which can cause severe oxidative damage to the plants, thus inhibiting growth and yield. The equilibrium between the production and scavenging of ROS is commonly known as redox homeostasis. However, when ROS production overwhelms the cellular scavenging capacity, thus unbalancing the cellular redox homeostasis, the result is a rapid and transient excess of ROS, known as oxidative stress. Plants have antioxidant mechanisms for scavenging the excess ROS and prevent damages to cells. It has been shown that ROS have a direct antimicrobial effect on the pathogen also. They are involved in the cross-linking of cell walls around the site of infection and also, activate both local programmed cell death and systemic increase in stress-induced pathogen resistance. The present study compares the expression profile of catalase in normal and afflicted mango leaf tissues. The study revealed the over-expression of catalase in malformed leaves as compared to healthy ones. The results clearly demonstrated that tissues from infected leaves accumulated ROS while their scavenging capacities are decreased or even absent. The suppression of ROS detoxifying mechanisms can be involved in the induction of the disorder.

Keywords: Catalase profile, mango malformation, stress ethylene, weather variables

Introduction
Mangoes are aromatic fruits of an evergreen tree, which are thought to have originated over 5000 years in the Indo-Burma region. Mango trees thrive in tropical and subtropical climates. Exposure to temperature below 30 ^{0} F can kill or severely damage a mango tree. Low temperature along with high relative humidity during bud inception stage trigger the synthesis of stress ethylene in the plant leading to development of disorder known as malformation.

This disorder causes gross deformation in vegetative and floral tissues (Ploetz, 2001). Vegetative malformation leads to hypertrophied growth of vegetative buds leading to a condition referred to as "Bunchy top" or "Witches Broom" (Bhatnagar and Beniwal, 1977; Kanwar and Nijjar, 1979; Ploetz, 2004). Floral malformation affects panicles leading to production of diseased hermaphrodite flowers having enlarged and non-functional ovary (Mallik, 1963; Shawky *et al*., 1980; Pleotz, 2004). The stigma of hermaphrodite flower is hooked and lacks broad landing platform required for pollen reception (Singh *et al.*, 1963 and Rani *et al.*, 2012). Malformed bisexual flowers bear abnormal fused lobed anthers with scanty pollen grains insufficient for pollination; also malformed pollens are sunken with villi like abnormal structure. Such abnormal pollens are supposed to affect fertilization and thus fruit set (Rani *et al.*, 2012). The deformation of vegetative and floral tissues caused severe reduction in production and productivity leading to negative growth rate of mango based trade. Biotic and abiotic stress induces the accumulation of reactive oxygen species (ROS) in the cells, which can cause severe oxidative damage to the plants, thus inhibiting growth and yield. ROS scavenging enzymes are expressed in plants which continuously scavenge ROS under normal physiological condition. The equilibrium between the production and scavenging of ROS is commonly known as redox homeostasis. However, when ROS production overwhelms the cellular scavenging capacity, thus unbalancing the cellular redox homeostasis, the result is a rapid and transient excess of ROS, known as oxidative stress (Mullineaux and Baker, 2010;

Sharma *et al*., 2012). Plants have antioxidant mechanisms for scavenging the ROS excess and prevent damages to cells. This equilibrium between the production and detoxification of ROS is sustained by enzymatic and non-enzymatic antioxidants (Mittler, 2002; Mittler *et al*., 2004).

The presently study is therefore undertaken to study the response of catalase enzyme in malformed as well as in normal condition

Material and Methods

Growing conditions of plant and collection of plant material.

Five commercially recognized varieties of mango namely Amrapali, Dasheri, Langra, Chausa and Bombay Green growing under natural condition in orchard were selected. The experimental materials used were healthy and malformed vegetative tissues which were collected from experimental and commercial mango orchards of Uttarakhand, Jharkhand, New Delhi, Uttar Pradesh and Bihar. Weather parameters like maximal and minimal temperature, RH and Wind velocity prevailing in these locations were obtained from Indian Meteorological Department Database, 2016 for the months of February and March (during flower initiation stage to flowering stage), Activity of scavenging enzyme i.e. Catalase was estimated for the experimental samples of different varieties obtained from different pockets of northern India.

Extract preparation

Fresh leaf samples were collected from each five varieties, were washed throughly with tap water followed by distilled water. Cleaned sample weighing one gram was homogenized in pre-chilled mortar and pestle by adding 5ml of chilled phosphate buffer (50mM; P^H 7.0). The homogenate was centrifuged for 20 min at 15,000 rpm at 4^0C. Two layer muslin cloths were used to sieve the supernatant. It was then used as extract for the estimation of catalase activity.

Catalase assay

Catalase activity was estimated by the method given by Luck (1965). In this the residual hydrogen peroxide was estimated via oxidation with potassium permanganate through titration. One gram of leaf sample was homogenized to prepare the enzyme extract. The preparation of reaction mixture consists of the addition of 3 mL of phosphate buffer (0.1M, PH 7.0), 2 mL of hydrogen peroxide (5mM) and 1 mL of enzyme extract. In order to prevent the reaction, the reaction mixture was incubated for 1 min at 20 ^{0} C followed by the addition of 10 mL of H_2SO_4 (0.35M). For blank, an enzyme extract was prepared and added to an acidified solution of reaction mixture at zero time. The reaction mixture was titrated in opposition to KMnO4 (0.01M) till the faint pink color persists for at the least 15 s to estimate the residual H_2O_2.

Statistical analysis

The data were analyzed statistically by two factor Randomized Block Design (RBD).

Result and Discussion

The combined effect of environmental stress, stress ethylene and pathogen attack on the expression of catalase in healthy and malformed tissues of mango is shown in Fig 1 and Table 1.

In the present study the expression profile of catalase was analyzed in normal and malformed tissues in several varieties of mango in five states of northern India during flower initiation stage up to flowering stage, when cold waves prevails in this region along with high soil humidity. Our result suggests that expression pattern of catalase was significantly higher in normal tissues of all the varieties as compared to malformed ones. Catalase catalyzes the disproportion of H_2O_2 in water and molecular oxygen, due to which malformation symptoms did not appear in normal tissues. The expression of catalase in malformed tissues was found to be significantly lower and the generation of ROS was relatively higher, due to which the symptoms of malformation appeared. The present result provides an insight that stress tolerance in crops can be enhanced by manipulating ROS levels by expression of catalase.

Table 1: Catalase activity (μ mol H_2O_2 mg^{-1} fresh weight min^{-1}) in malformed and healthy leaf tissues of different mango varieties from different states

S. No.	Variety	Catalase activity (μ mol H_2O_2 mg^{-1} fw min^{-1})									
		Bihar		Jharkhand		Uttar Pradesh		Uttarakhand		Delhi	
		Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy
1.	Amrapali	22.63 ±1.12	29.62 ±1.57	21.59 ±0.67	28.35 ±1.12	21.60 ±0.97	27.60 ±1.77	22.50 ±1.36	26.20 ±1.57	22.50 ±1.36	25.02 ±1.36
2.	Dasheri	21.13 ±1.39	30.30 ±0.63	22.78 ±1.22	30.41 ±0.63	20.81 ±0.78	29.41 ±0.63	21.75 ±0.81	27.90 ±1.07	23.75 ±1.39	26.75 ±1.52
3.	Langra	23.32 ±0.75	28.75 ±1.08	24.10 ±0.75	29.72 ±1.34	22.10 ±1.65	29.61 ±1.55	22.25 ±1.43	29.42 ±1.55	21.25 ±1.43	29.35 ±1.55
4.	Chausa	21.33 ±1.01	29.32 ±0.94	21.37 ±0.69	29.75 ±0.94	24.56 ±1.38	29.75 ±1.23	24.56 ±1.16	29.76 ±1.39	24.75 ±1.38	29.72 ±1.59
5.	Bombay Green	22.70 ±0.85	30.50 ±1.21	22.60 ±0.85	30.20 ±1.21	22.15 ±1.55	30.25 ±1.21	21.53 ±1.14	30.75 ±1.65	22.02 ±1.74	30.75 ±1.43
	Mean	22.22	29.70	22.49	29.69	22.24	29.32	22.52	28.81	22.85	28.32
		Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)
	S.Em. ±	0.17	0.27	0.19	0.31	0.50	0.79	0.43	0.68	0.24	0.38
	CD at 5%	0.51	0.80	0.59	0.93	1.50	2.37	1.28	2.03	0.72	1.15

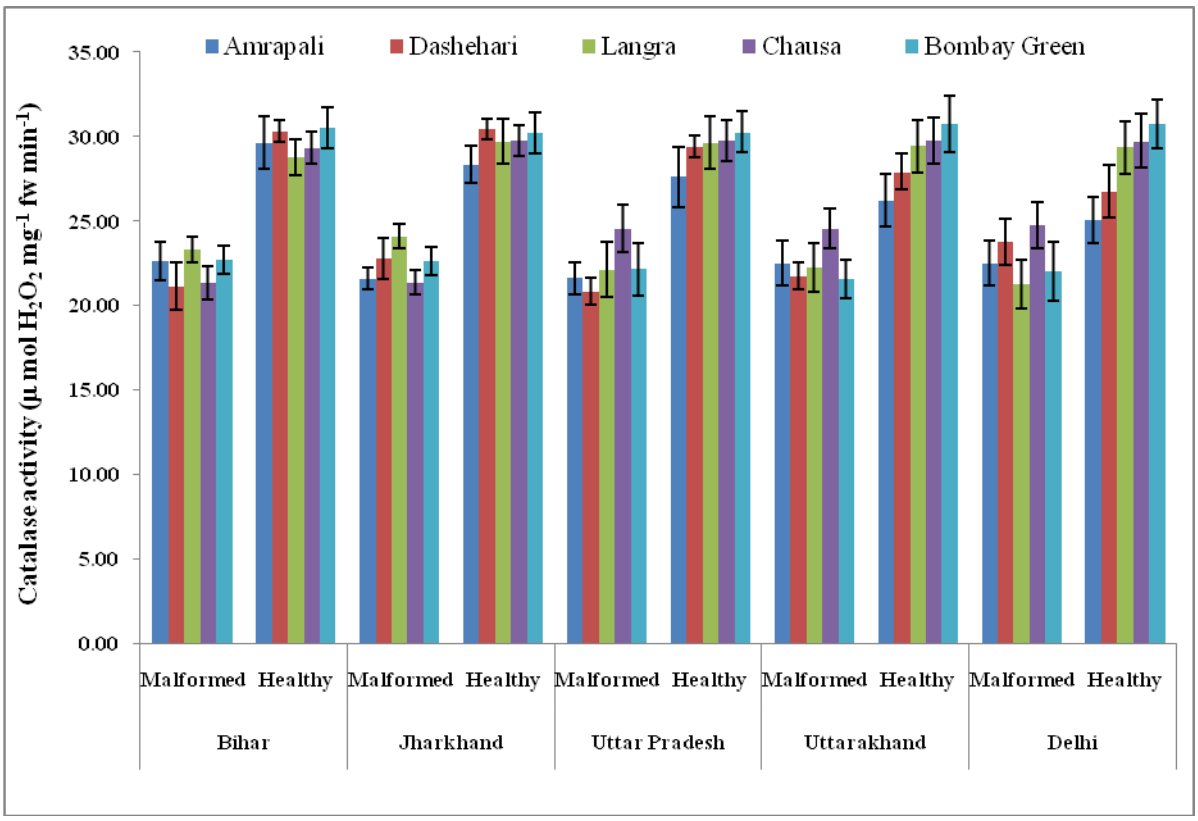

Fig 1: Catalase activity (µ mol H_2O_2 mg^{-1} fw min^{-1}) in malformed and healthy leaf tissues of different mango varieties from different states

References

1. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* 2004; 84:551-562.
2. Ansari MW, Bains G, Shukla A, Pant RC, Tuteja N. Low temperature stress ethylene and not *Fusarium*, might be responsible for mango malformation. *Plant physiol. and Biochem.*, 2013, 1-5.
3. Ansari MW, Shukla A, Pant RC, Tuteja N. First evidence of ethylene production by *Fusarium mangiferae* associated with mango malformation. *Plant Signal Behav*, 2012; 8(1):e22673. doi: 10.4161/psb.22673
4. Ansari MW, Shukla A, Pant RC, Tuteja N. First evidence of ethylene production by *Fusarium mangiferae* associated with mango malformation. *Plant Signal Behav.*, 2012; 8(1):e22673. doi: 10.4161/psb.22673
5. Asada K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. 2006; 141:391-396.
6. Bains G, Pant RC. Mango malformation: etiology and preventive measures. *Physiol. Mol. Biol. Plants.* 2003; 9:41-61
7. Murata N, Allakhverdiev SI, Nishiyama Y. The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, atocopherol, non-photochemical quenching, and electron transport. Biochim. Biophys. Acta 2012; 1817(8):1127-1133.
8. Weidner S, Karolak M, Karamać M, Kosińska A, Amarowicz R. Phenolic compounds and properties of antioxidants in grapevine roots (*Vitis vinifera*) under drought stress followed by regeneration. *Acta Soc. Bot. Pol.* 2009; 78:97-103.