Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 3225-3227

Enhanced tolerance to malformation in tissues of mango (*Mangifera indica* L.) expressing higher level of Peroxidase enzyme

V Rani, G Bains, MW Ansari, AK Tiwary, S Verma, Ashisan Tuti, Rajesh Kumar Mishra and SM Prasad

V Rani
Department of Crop Physiology, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

G Bains
Department of Plant Physiology, College of Basic science and Humanities, G.B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

MW Ansari
Department of Botany, Zakir Husain Delhi College, University of Delhi, Jawaharlal Nehru Marg, New Delhi, India

AK Tiwary
Department of Horticulture, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India.

S Verma
Department of Horticulture, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India.

Ashisan Tuti
Department of Genetics and Plant Breeding, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Rajesh Kumar Mishra
Department of Genetics and Plant Breeding, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

SM Prasad
Department of Plant Pathology, Faculty of Agriculture, Jharkhand Rai University, Ranchi, Jharkhand, India

Correspondence
V Rani
Department of Crop Physiology, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Abstract
Mangoes originated in India and have been cultivated for more than 5000 years. They have got popularity throughout the globe for its unique taste, aroma and color. Mango is afflicted by several diseases and disorder during its life cycle. Among all the known diseases/disorders malformation is the most destructive in nature. Mango is the only known host of this disorder. For vegetative malformation, the affected leaves become significantly smaller in size and curve towards the stem giving a bunchy top appearance. In case of floral malformation, the malformed hermaphrodite flowers bear hooked stigma and stigmatic surface meant for pollen reception is lacking. Moreover such flowers bear fused lobed anthers with scanty pollens which are unable to facilitate proper pollination, fertilization and fruit set. This physio-biochemical and morpho-anatomical alteration is thought to be brought about by the interplay of low temperature, high R.H, fungal pathogen and endogenous stress ethylene. When plants are exposed to such multiple stress, the production of ROS increases and can cause significant damage to the cells. A major detoxifying antioxidant enzyme is Peroxidase which play a key role in scavenging ROS. The focus of the present study is to compare the level of expression of peroxidase in normal and diseased vegetative tissues of mango plants exposed to stress during bud inception stage. The study revealed substantial differences in the level of this antioxidant enzyme in normal and afflicted tissues. Increased expression of peroxidase in normal tissues resulted in enhanced tolerance towards malformation.

Keywords: Peroxidase, Malformation, Mango, Reactive oxygen species

Introduction
Mango (*Mangiferae indica)* belongs to order Sapindales, family Anacardiacea and genus *Mangiferae*. It is innate to S. Asia, particularly east India, Burma and Andaman Islands. Its production is prominent in tropical lowland areas 23° 26° north and south of the equator in the Indian subcontinent, south East Asia and Central and S. America. The Mango tree crops can survive the ambient temperature up to 2°F (-39°C) for some hours, but the trees growing in early stage of life cycle may die off at 29° to 30°F (-1.7 to 1.1°C). Temperature less than 40°F (4.4°C) for some hours, may kill the fruits and flowers (Crane and Campbell, 1994). India holds first rank in area and production of mango in the world (Negi, 2000). In spite of the highest area and production, the productivity of mango is very low due to several diseases and disorders. Amongst the several known diseases, malformation of mango is the most threatening. In vegetative malformation a number of vegetative buds emerge with hypertrophied growth, this is called the vegetative malformation. The condition of multiple branching of apical shoots along with scaly leaves and shortened internodes is referred to as "Bunchy top", also known as "Witches' Broom" (Bhatnagar and Beniwal, 1977; Kanwar and Nijjar, 1979, Ploetz, 2004). Malformed panicles produce large number of unopened flowers that are mainly staminate and rarely hermaphrodite (Singh *et al.*, 1961; Schlosser, 1971; Hiffny *et al.,* 1978). The diseased hermaphrodite flower has enlarged and non functional ovary and has poor pollen viability or if fertilized eventually abort (Mallik, 1963; Shawky *et al.,* 1980; Pleotz, 2004). The stigma of malformed 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). Being a mysterious disease its etiology is still disputed (Rani and Bains, 2012) and to some extent it has now been accepted among scientific communities that biotic (*fusarium mangiferae*) and abiotic (low temperature, high relative humidity) stresses trigger the synthesis

of stress ethylene in plant. Exposure of plants to stressed conditions, lead to the production of Reactive Oxygen Species (ROS) which can cause significant damage to the cells. Plants are equipped with antioxidant defense system, which can detoxify ROS (Caverzan, 2012). A major hydrogen peroxide detoxifying system in plant cells is the peroxidase enzyme which play a key role catalyzing the conversion of H2O2 into H2O. The present study is therefore undertaken to study the induction and activity of Peroxidase in malformed as well as in normal vegetative tissues of five commercially important varieties in different states of northern India, during flower initiation stage up to flowering stage, when the environmental conditions like low temperature, high relative humidity and feeble wind remains quite conducive for malformation.

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.

Peroxidase assay

The peroxidase activity was determined with the aid of the technique given by Thomas *et al*. (2006). Guaiacol was used as the enzyme substrate. One gram of clean leaf tissue was homogenized to prepare enzyme extract. The reaction mixture was prepared by including 2.87 mL phosphate buffer (0.1M; PH 7.0), 30 micro litre of H_2O_2 (20 mM), 50 micro litre of guaiacol (20 mM) as enzyme substrate and 50 micro litre of enzyme extract. The reaction mixture was kept for 10 min at room temperature for incubation. The absorbance was recorded at 436 nm wavelength on a UV-Vis spectrophotometer. The activity of peroxidase was expressed as the number of absorbance units per gram fresh weight of leaf.

Statistical analysis

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

Result and Discussion

The combined effect of biotic and abiotic stress on the activity of peroxidase activity in healthy and malformed tissues of mango is shown in Fig 1 and Table 1.

In the present study it was observed that expression of peroxidase enzyme was higher in normal tissues of mango as compared to malformed ones of all the five cultivars of mango, growing in five states of northern India. It is suggested that stress causes disturbances in antioxidant gene expression by producing alterations in the transcriptional pattern in the malformed tissues. While the over expression of peroxidase in normal tissues of mango plant enhanced the tolerance to malformation. Ascorbate peroxidase is a key enzyme regulating ROS levels acting in different subcellular compartments. It plays important and direct roles as protective elements against adverse environmental conditions. Similar finding shows that the expression of peroxidase genes is regulated in response to biotic and abiotic stresses which are directly involved in the protection of plant cells against adverse environmental condition (Caverzan *et al.,* 2012).

Table 1: Peroxidase activity (units g^{-1} fw) in malformed and healthy leaf tissues of different mango varieties from different states.

S. No.	Variety	Peroxidase activity (units g^{-1} fw)									
		Bihar		Jharkhand		Uttar Pradesh		Uttarakhand		Delhi	
		Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy
1.	Amrapali	1.47 ±0.10	3.07 ±0.05	2.47 ±0.01	3.75 ±0.03	1.38 ±0.10	3.68 ±0.17	2.37 ±0.14	3.95 ±0.11	2.06 ±0.05	3.80 ±0.12
2.	Dashehari	1.67 ±0.06	4.95 ±0.02	1.36 ±0.11	4.25 ±0.11	2.27 ±0.13	4.25 ±0.11	1.26 ±0.06	4.70 ±0.04	2.73 ±0.15	4.75 ±0.32
3.	Langra	2.57 ±0.12	5.65 ±0.14	1.71 ±0.03	5.61 ±0.14	1.75 ±0.15	4.60 ±0.09	1.50 ±0.11	5.65 ±0.09	1.52 ±0.12	5.60 ±0.14
4.	Chausa	2.71 ±0.00	4.93 ±0.21	2.15 ±0.08	4.30 ±0.03	2.50 ±0.09	5.71 ±0.15	2.75 ±0.05	4.38 ±0.12	1.50 ±0.04	4.35 ±0.12
5.	Bombay Green	2.96 ±0.14	5.72 ±0.20	2.91 ±0.10	4.78 ±0.16	2.85 ±0.10	4.92 ±0.17	2.82 ±0.09	4.92 ±0.10	2.25 ±0.02	4.70 ±0.16
	Mean	2.28	4.86	2.12	4.54	2.15	4.63	2.14	4.72	2.01	4.64
		Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)
	S.Em. ±	0.04	0.07	0.03	0.04	0.06	0.10	0.04	0.07	0.06	0.10
	CD at 5%	0.13	0.21	0.09	0.14	0.19	0.30	0.14	0.23	0.19	0.30

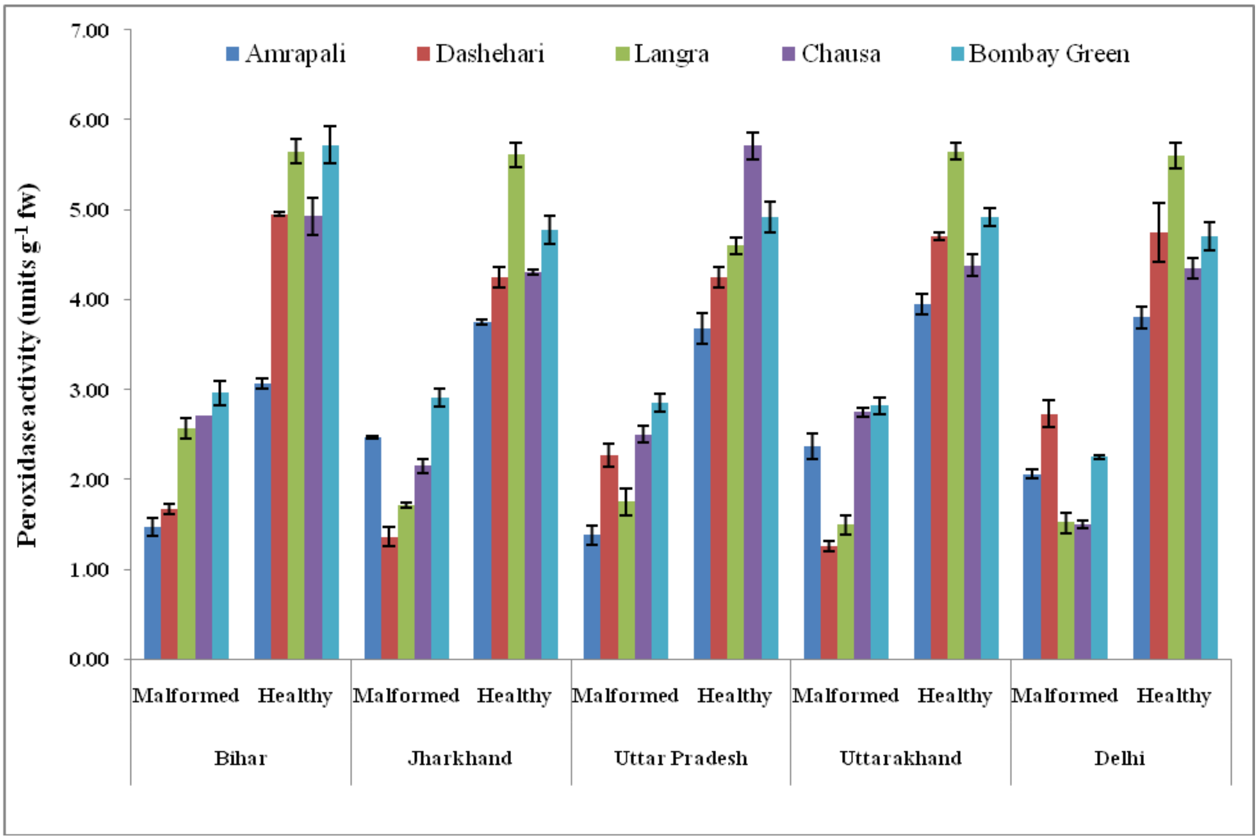

Fig 1: Peroxidase activity (units g^{-1} fw) in malformed and healthy leaf tissues of different mango varieties from different states

References

1. Beniwal SPS, Bhatnagar SS. Annual Research Report. GBPUAT, Pantnagar, 1975, 1974-1975.
2. Bhatnagar SS, Beniwal SS. Involvement of *Fusarium oxysporum* in causation of mango malformation. Plant Dis Rep 1977; 61:894-898.
3. Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis Pnheiro M. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biology* 2012; 35:1011-1019.
4. Rani V, Ansari MW, Shukla A, Tuteja N, Bains G. Fused lobed anther and hooked stigma affect pollination, fertilization and fruit set in mango: a scanning electron microscopy study. *Plant Signal Behav.* 2012; 8(3):e231671-5.
5. Thomas G, Claude P, Federico J, Castillo HG. A two-step control of basic and acidic peroxidases and its significance for growth and development. *Physiol Plant*. 2006; 64:418-423.