academicJournals

Vol. 13(18), pp. 1876-1882, 30 April, 2014
DOI: 10.5897/AJB11.2093
Article Number: 6B9E23F44434
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Certain growth related attributes of bunchy top virus infected banana under *ex-vitro* conditions

Ikram-ul-Haq[1]*, Shahrukh[1], Um-E-Aiman[1], Saifullah Khan[2], Nazia Parveen[3], Madiha Fatima[1] and Muhammad Umar Dahot[1]

[1]Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro-76080, Pakistan.
[2]Plant Tissue Culture and Biotechnology Wing, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.
[3]Department of Statistics, University of Sindh, Jamshoro-76080, Pakistan.

Received 07 July, 2011; Accepted 14 November, 2011

Effect of banana bunchy top virus (BBTV) on morpho-physiological characteristics of banana (*Musa* sp.) cv., Basrai plants was assessed. Healthy and BBTV infected samples of banana were collected from its open fields and micro-propagated aseptically. These plantlets were established in wire-house for three months. Presence of BBTV was confirmed by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). Micro-propagation efficiency of BBTV-infected plants was observed in less than healthy plants under *in-vitro* as well as *in-vivo*. Significant reduction in plant height, fresh weight (F wt) and dry weight (D wt) of leaf was observed in BBTV infected plants. A co-relation of peroxidase (POX) activity was observed with total carotenoids that increased in BBTV infected plants, while chlorophyll contents decreased significantly. Nitrate reductase activity also decreased with increase in proline contents in BBTV-stresses plants ($p<0.05$). Meanwhile, reducing sugars also increased but not-significantly. Bunchy top virus infection in banana therefore resulted in alteration of growth related physiological traits that led to retardation of plant growth.

Key words: *Ex-vitro*, *Musa* sp., micro-propagation, photosynthetic pigments, BBTV, peroxidases, total proteins, reducing sugars, cell size.

INTRODUCTION

In plants, a number of environmental stresses (biotic and abiotic) produce characteristically identifiable symptoms because of deleterious impacts on different physiological processes. Each stress has developed a number of bio-chemical changes (Miteva et al., 2005). Viral pathogens (biotic stress) have also caused severe damage in many

*Corresponding author. E-mail: rao.ikram@yahoo.com. Tel: +92-345-2914291.

Abbreviations: MS, Murashige and Skoog; **BAP,** benzyleaminopurine; **PCR,** polymerase chain reaction; **ELISA**, enzyme-linked immunosorbent assay; **BBTV,** banana bunchy top virus; **RWC,** relative water contents; **F Wt,** fresh weight; **D Wt,** dry weight; **NRA,** nitrate reductase activity; **POX,** peroxidases; **Na_2CO_3,** sodium carbonate.

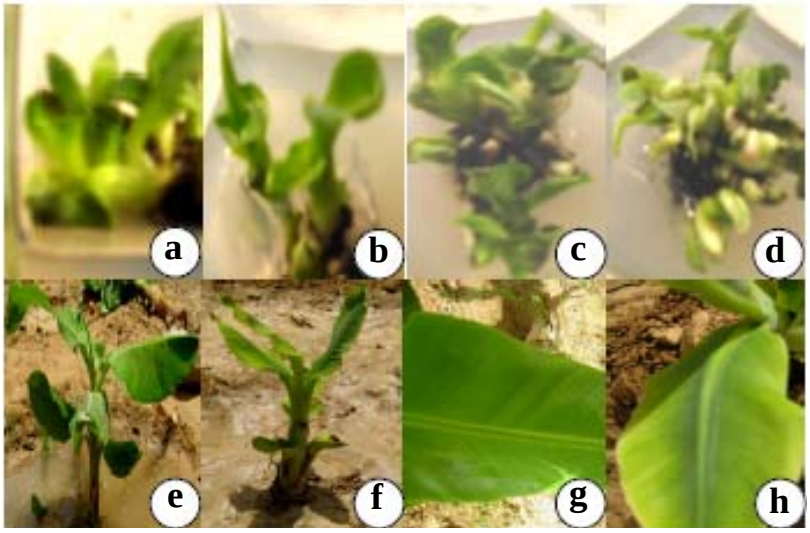

Figure 1. *In-vitro* and *ex-vitro* multiplying BBTV-infected and healthy banana (*Musa* sp.) cv., Basrai plants under experimental conditions. a and b, 1st micro-propagation culture of the non-infected and BBTV infected suckers; c and d, 3rd sub-cultures respectively; e and f, six weeks old healthy and infected banana plants growing in wire-house, respectively; g and h, 3rd leaf from top to bottom of three-months old healthy and infected plants, respectively. BBTV, Banana bunchy top virus.

major crops either by reducing yields or quality of plants as a whole (Kang et al., 2005). Presently, banana is affected by five growth limiting viruses (Stover, 1972), with the most destructive being the banana bunchy top virus (BBTV) transmitted through planting infected banana nursery and by banana aphids (*Pentalonia nigronervosa*) to healthy plants. This virus is observed around the world, especially in Asia and the Pacific regions (Magee, 1940; Wu, 1978; Dale, 1987; Su, 1990; Harding et al., 1991; Moffat, 2001). Symptoms of this viral infection become visible when virus severity in infected tissues reaches to critical point. The BBTV infected plants have been noted with stunted growth, deformation of young leaves and bunched at top of plant (Chia et al., 1992, 1995; Hendry, 1987).

Virus-free plants show relatively better vegetative growth in field, but reduce with viral infection due to decreased photosynthetic surface, as well as respiration rate (Balachandran et al., 1997; Guo et al., 2005). In general, the plant growth of virus infected plants is limited because of inhibitions of biological system in communication to growth related metabolic processes. Similarly, POX enzymes play an integral role in plant metabolism and immune system from seed germination to maturity and become highly active during senescence (Siegel, 1993). The plants immune system is primarily dependent on POX and show hypersensitivity response. Systemic viral infection may lead to increase in defense activity, as well as wound repair by POX (Ye et al., 1990; Candela et al., 1994). Meanwhile, necrosis or chlorosis appears by virus infection (Wood, 1990). In higher plants, both biotic and abiotic stresses have produced a number of characteristic changes in plant morphs and metabolic processes. For example, tissues' POX activity increases because of toxic elements present in cells (Espelie et al., 1986; Stroinski, 1995; Miteva et al., 2005).

In the present experiment, morpho-metabolic attributes were quantified in BBTV infected and virus-free banana plants (*Musa* sp.) comparatively. BBTV infection developed a number of specific abnormal traits in multiplied banana plants under *in-vitro* and *ex-vitro* also. Identification of these characters may be helpful to the studies of future amelioration for BBTV resistance in banana crop.

MATERIALS AND METHODS

The BBTV infected and healthy suckers of banana (*Musa* sp.) cv., Basrai were collected from different open field banana farms. Inner meristematic regions were excised from suckers and used as explants for micro-propagation (Haq and Dahot, 2007a, b). Micro-propagated plantlets were propagated for three sub-cultures and rooted. These plantlets were screened for presence of BBTV through enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Figure 2) (Ennos, 2000; Haq et al., 2009) and shifted to wire-house for further development. When plants were of three months old, they were subjected to different morpho-physiological studies (Figure 1). The location of this experiment was far from the nearest banana field about 60 km. *Ex-vitro* propagating plants were sprayed twice (one-month interval) with imidacloprid (Provado®) at 60 ppm to control banana aphids by using small farmer's hand-pump (Robson and Wright, 2007).

Number of leaves was counted and plant height was taken from the sucker to petiole of fully emerged uppermost leaf and pseudo-

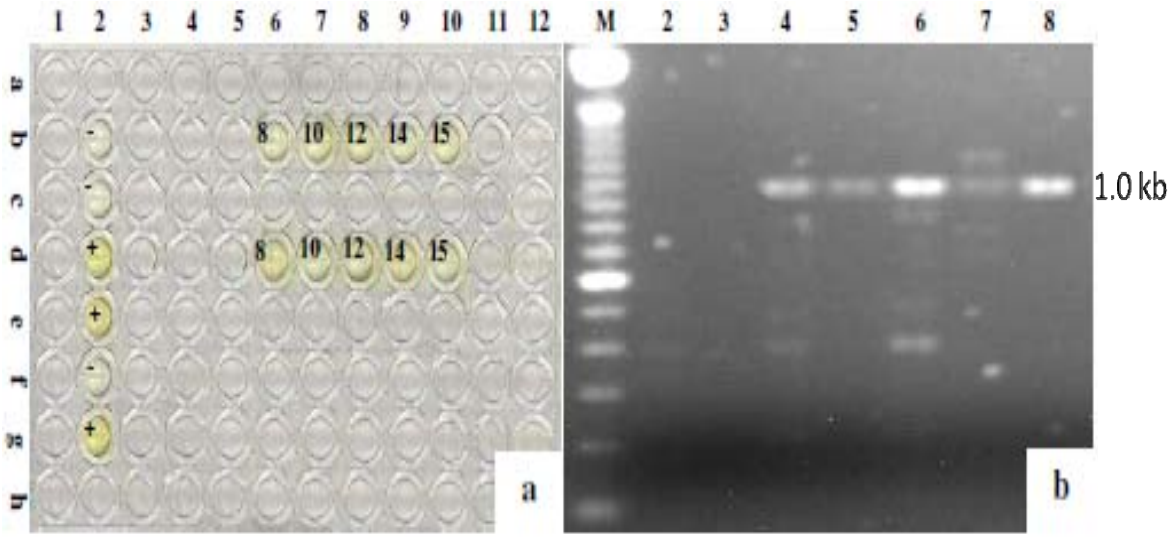

Figure 2. Detection of BBTV in aseptically micro-propagated banana (*Musa* sp.) cv. Basrai plantlets before establishing in wire-house. a, ELISA of five-BBTV infected plants in comparison to controls [positive (+ve) and negative (–ve)]; b, PCR based detection of BBTV infection in same 5 BBTV infected plants in comparison to controls (+ve and –ve); lane M, 100 bp DNA marker; lane 2, water; lane 3, -ve plant control; lanes 4 to 8, represent the five samples (8, 10, 12, 14 and 15) respectively. BBTV, Banana bunchy top virus.

stem diameter at centered point of plant height (Smith et al., 2000). Petiole related parameters were quantified from base of leaf blade to its attachment-point with pseudostem (Ennos, 2000). Leaf area (3rd leaf from top) was measured by multiplying, maximum width of leaf with length and conversion factor (0.83). Fresh weight (F Wt) and dry weight (D Wt) of 100 leaf disks (6.3 mm) were taken and relative water contents (RWC) also calculated (Robinson and Neil, 1985; Conroy et al., 1988). Moreover, the third leaf from top of developing banana plants was used for different bio-chemical and anatomical studies. It was chopped into small fragments (2 to 3 mm in length) and fixed in fixation buffer [3.5% glutaraldehyde, 0.1 M phosphate buffer (pH 7.0)] for 10 h at room temperature. Mixture was washed with 0.1 M phosphate buffer twice and again fixed in osmium tetra-oxide (2.5%) overnight (Gielwanowska et al., 2005). Free hand sections were cut from the material for further analysis (Johansen, 1940).

In addition, chlorophyll contents were determined by using Arnon's method (1949), while for determination of POX activity, 1.0 g plant material was homogenized with 3.0 ml sodium phosphate buffer (66 mM; pH 6.1) according to the method of Ranieri et al. (1995) and analyzed as by Curtis (1971) and Bergmeyer et al. (1974). The nitrate reductase activity (NRA) was determined as 100 mg young leaf tissue in phosphate buffer. A reaction mixture was raised by mixing 1.0 ml of 0.1 mM potassium phosphate, 0.5 ml of 0.05 M potassium nitrate (KNO_3) and 1% isopropanol (v/v) with pH 7.5 at 30°C in dark. After 1 h, 1.0 ml sulfanilamide (1%) and 1.0 ml naphthyl ethylenediamine dihydrochloride (0.02%) were also added. The nitrate contents were quantified at 540 nm (Klepper et al., 1971).

More also, proline (Bates et al., 1973), total carbohydrates (Dubois et al., 1956; Ciha and Brun, 1978), reducing sugar (Miller, 1959), protein (Bradford, 1976) and nitrate (Morris and Riley, 1963) were determined. For phenol determination, 0.1 g young leaf tissues were homogenized in 25 ml commercial ethanol (95%) according to Ozyigit et al. (2007) and was kept at 0°C for 48 h. Absorbance was taken after making its complex by adding 0.5ml Folin-Ciocalteu (50%) and 1.0 ml sodium carbonate [Na_2CO_3 (5%)] at 760 nm against 95% ethanol. Furthermore, the cations [sodium ion (Na^+), potassium ion (K^+) and calcium ion (Ca^{2+})] were determined in leaf midrib by acidic digestion (Wolf, 1982; Malavolta et al., 1989), while chloride contents was measured following the manual of chlorometer.

Statistical analysis

Collected data was computed for significant measurements using COSTAT computer package (CoHort Software, Berkeley, USA) at 5% level. Each of the two types of banana plants, healthy (control) and BBTV (infected), had at least seven replicates.

RESULTS AND DISCUSSION

During this experiment it was observed that BBTV had differential effects on plant growth of banana from early stages to its maturity (Figure 1). Severity of symptoms increased with the increase in age of plant due to the decreased growth potential of developing plants under viral stress. Of course, leaf morphology was affected significantly. Petiole distance decreased ($p<0.05$), while its width and length also decreased but non-significantly. With the decrease in canopy size (non-significantly), leaf production rate was also low in the BBTV infected plants. Meanwhile, size of the structural cells also decreased (non-significantly), while leaf area decreased in BBTV infected plants significantly (Figure 3 and Table 1). Collectively, all of these characters resulted in the decrease in plant height of the BBTV infected plants (Ennos et al., 2000; Kang et al., 2006).

Imbalance ionic toxicity causes abnormalities in combination with all metabolic functions of plants. A

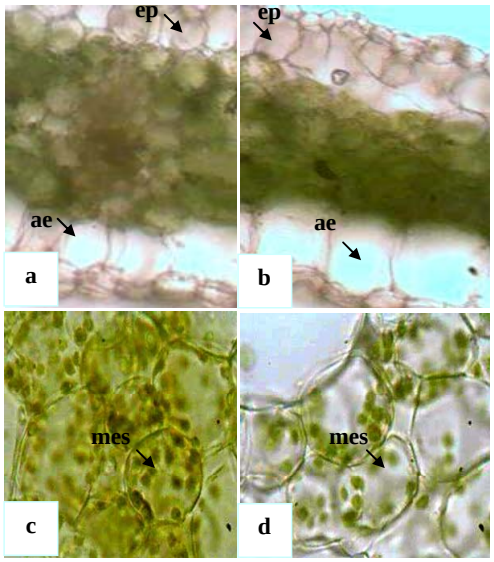

Figure 3. Different structural cells, ep, epidermal; mes, mesophyll; ae, aerenchyma; from three- months old BBTV infected plants of banana (*Musa* sp.) cv., Basrai. a and b, The structural cells of healthy and infected banana leaves; c and d, mesophyll cells of healthy and infected banana leaves respectively.

Table 1. Certain comparative morpho-physio-anatomical attributes of three-months old BBTV infected banana (*Musa* sp.) cv., Basrai plants.

Parameter/character		Control	BBTV Infected
A. Morphological parameter			
1	No. of plantlets/plant	3.95 ± 0.075	1.38 ± 0.144**
2	Plant height (cm)	69.52 ± 2.65	51.49 ± 1.63**
3	3rd leaf area (cm^2)	1513.17 ± 14.9	1267.24 ± 27.89**
4	Fresh weight (g)	1.892 ± 0.06	1.561 ± 0.04**
5	Dry weight (g)	0.538 ± 0.03	0.469 ± 0.03^{ns}
6	Pseudostem diameter (cm)	8.24 ± 0.275	7.95 ± 0.268^{ns}
7	Canopy size (cm)	140.21 ± 4.39	129.25 ± 4.18^{ns}
8	Leaf production rate	1.42 ± 0.05	1.35 ± 0.05^{ns}
B. Leaf petiole measurements			
I	Petiole length (cm)	105 ± 5.60	90.21 ± 2.79^{ns}
II	Petiole distance (cm)	10.12 ± 0.12	8.59 ± 0.06***
III	Petiole width (cm)	5.24 ± 0.12	4.89 ± 0.27^{ns}
C. Chlorophyll contents (mg g^{-1} F Wt)			
a	Chlorophyll a	0.123 ± 0.006	0.066 ± 0.001*
b	Chlorophyll b	0.112 ± 0.002	0.072 ± 0.002**
c	Chlorophyll ab	0.235 ± 0.015	0.138 ± 0.013**
d	Total carotenoids	1.625 ± 0.013	1.72 ± 0.006*
D. Cell size (µM)			
i	Epidemal cells	0.257 ± 0.008	0.245 ± 0.002^{ns}
ii	Mesophyll cells	1.612 ± 0.007	1.45 ± 0.113^{ns}
iii	Aerenchyme calls	3.252 ± 0.005	2.50 ± 0.34^{ns}

^{ns}Non-significant; *,**,***statistically significant. BBTV, Banana bunchy top virus; F Wt, fresh weight

Table 2. Some enzymic and bio-chemical characters in three- months old BBTV infected banana (*Musa* sp.) cv., Basrai plants.

Parameter/character		Control	BBTV Infected
A. Organics (mg g^{-1})			
a	Protein contents (mg g^{-1})	0.423 ± 0.011	0.537 ± 0.006**
b	Reducing sugars (mg g^{-1})	0.045 ± 0.005	0.063 ± 0.003^{ns}
c	Total sugars (mg g^{-1})	0.135 ± 0.002	0.152 ± 0.004*
d	Proline (mg g^{-1})	0.245 ± 0.003	0.272 ± 0.01*
e	Phenol (mM g^{-1})	0.584 ± 0.035	0.602 ± 0.069^{ns}
f	ELISA complex (mg g^{-1})	0.0	0.365
g	RWC (%)	55.72 ± 2.85	53.79 ± 2.71^{ns}
B. Enzymes activity			
1.	**POX activity** (U min^{-1} mg $protein^{-1}$)		
	a. Soluble	4.67 ± 0.02	7.98 ± 0.38*
	b. Ionicaly-bounded	2.25 ± 0.76	6.25 ± 0.41**
	c. Covalently bounded	6.93 ± 0.46	11.78 ± 0.51**
2.	NRA (mg NO_2 g^{-1})	0.962 ± 0.01	0.841 ± 0.05***
C. Inorganics (mg g^{-1} D Wt)			
i	Na^+	2.92 ± 0.05	3.03 ± 0.321^{ns}
ii	K^+	7.39 ± 0.04	6.658 ± 0.348^{ns}
iii	Ca^{2+}	6.11 ± 0.059	5.356 ± 0.325^{ns}
iv	Cl^-	3.25 ± 0.035	3.447 ± 0.134^{ns}
v	NO_3^-	0.105 ± 0.003	0.094 ± 0.006^{ns}

^{ns}Non-significant; *,**,***statistically significant. BBTV, Banana bunchy top virus; RWC, relative water contents; D Wt, dry weight; NRA, nitrate reductase activity; Na^+, sodium ion; K^+, potassium ion; Ca^{2+}, calcium ion; D Wt, dry weight; POX, peroxidase.

decreased order of K^+, Ca^{2+} and nitrate (NO_3^-) was observed to be interlinked with Na^+ and Cl^- inverse proportionally (Table 2). Decrease in relative water contents (RWC) was also observed (Table 2). Deficiency in water contents causes a decrease in leaf turgor or hydraulic pressure on which various physiological processes and morphological traits depend like as stomatal opening and growth of leaves (Tecsi et al., 1996; Balachandran et al., 1997). Furthermore, the BBTV infection in banana caused a decrease in green pigments such as chlorophyll (Chl) a, b and ab. Systemic chlorosis due to BBTV infection causes a decrease in rate of photosynthetic processes in leaves. The Chl a have been considered as more sensitive than Chl b in BBTV infected plants (Table 1). The systemic infection caused by plant viruses may be acting as inhibitors for certain enzymes involved in biosynthesis of chlorophyll contents (Goodwin and Britton, 1988; Tecsi et al., 1996). BBTV continuously spread within vascular system and reduces plant growth or accelerate senescence. Moreover, total carotenoid contents increased significantly, which was a typical sign of senescence (Sutic and Sinclair, 1990; Valjakka et al., 1999).

In addition, accumulation of reducing sugars in infected plants was higher than healthy plants as had been widely assumed to be a response of pathogen stress (Table 2). Similarly, significant increase in proline contents in the BBTV infected plants is also a well known indicator of environmental stress (Table 2). Increased proline content may ameliorate the impact of certain stresses (Dorffling et al., 1990; Csonka and Hanson, 1991). Generally, it has been considered that accumulation of proline content is a typical plant osmotic stress response marker, as well as against biotic stress (Table 2). Increase in free proline and protein contents in leaves plant have been observed to be activated naturally under specific hypersensitive response against microbes, including viruses (Goodman et al., 1986; Shalitin and Wolf, 2000). A relationship between reducing sugars, proline contents and total carotenoids was observed as each was increased in infected plants than healthy plants. Phenolic contents in infected plants were also higher than control plants (Table 2).

Moreover, plants are able to tolerate certain environmental stresses to an extent because of accumulation of

either inorganic or low molecular weight organic compounds. Their relative contribution varies from plant to plant, but they play a crucial rule in the plants growing under various stresses (Ashraf and Harris, 2004). For example, POX in healthy plants were observed to remain inactive (covalent-bonding forms), but active (soluble and ionic-bonding forms) in BBTV-infected plants (Table 2). The POX activity was higher in infected plants, while nitrate reductase activity was low in BBTV infected plants. POX therefore remained active and developed a relationship with the chlorophyll contents. It is observed that when chlorophyll content decreases, POX activity increases in virus infected plants but proportioned to total carotenoids (Tables 1 and 2). These alterations are involved in developing pathogen resistance mechanisms (Kuroda et al., 1990; Wood, 1990; Chittoor et al., 1999; Milavec et al., 2001; Wang et al., 2010).

According to Kuroda et al. (1990), POX is responsible for chlorophyll degradation during senescence. Similarly, Smart (1994) reported that chlorophyll degradation occurs due to increase in hydrogen peroxide (H_2O_2) and phenolic contents in leaves (Table 2). The horseradish POX is responsible for the catalyses of magnesium ion (Mg^{2+}) removal from the precursor of chlorophyll a and chlorophyllin (Azuma et al., 1999). Still, the breakdown of chlorophyll contents through POX pathways in the senescent leaves is dubious (Matile and Hörtensteiner, 1999). In senescent leaves, a variant POX activity has been observed in different plant species during plant growth. Johnson-Flanagan and Spencer (1996) also reported that at maturity stage, plant de-greening occurs. At that time the degradation of the green pigments was associated with increased activity of POX, while low chlorophyllase activity in case of canola has been measured. However, POX and an oxidase in thylakoids of barley leaf are activated for degradation of chlorophyll contents during senescence (Kuroda et al., 1990).

Conclusion

In this experiment, plant growth decreased in BBTV infected plants due to reduction in synthesis of photosynthetic pigments. Rate of photosynthesis was associated directly or indirectly with a series of metabolic activities. Systematic viral infection caused inhibition of photosynthetic related processes. Meanwhile, significant increase in total carotenoids occurred with the increase in severity of BBTV infection. Accumulation of carotenoids and activation of POX are dependent on the inhibition of different physiological processes due to viral infection. Such loss of biological systems therefore triggers the visibility of specific coloring and become first symptom of BBTV infection than loss of yield and death of plants.

REFERENCES

Arnon, DT (1949). Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris.* Plant Physiol. 24: 1-15.

Ashraf MP, Harris JC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Azuma R, Takahashi Y, Kurata H, Kawano T, Shimokawa K, Adachi M (1999). Does *peroxidase* act as a Mg-*dechelatase*. Plant Perox. Newslett. 13: 145-151.

Balachandran S, Hull RJ, Martins RA, Vaadia Y, Lucas WJ (1997). Influence of environmental stress on biomass partitioning in transgenic tobacco plants expressing the movement protein of tobacco mosaic virus. Plant Physiol. 114: 475-481.

Bates LS, Waldren RP, Tear ID (1973). Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.

Bergmeyer HU, Gawehn K, Grassl M (1974) Enzymes as Biochemical Reagents, Acid Phosphatase. In: Bergmeyer HU (ed) Methods of enzymatic analysis, (2nd ed), pp 495-496, Academic Press, New York San Francisco London.

Bradford MN (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann. Biochem. 72: 248-254.

Candela ME, Munoz R, Alcazar MD, Espin A (1994). *Isoperoxidase* involvement in the resistance of *Capsicum annuum* to infection by cucumber mosaic virus. Plant Physiol. 143: 213-217.

Chittoor JM, Leach JE, White FF (1999). Induction of *peroxidase* during defense against pathogens. In: Datta SK and Muthukrishmnan S, Editors, *Pathogenesis Related Proteins in Plants*, CRC Press, Boca Raton, F. L. pp. 171-193.

Ciha AJ, Brun WA (1978). Effect of pod removal on nonstructural carbohydrate concentration in soybean tissue. Crop Sci. 18: 773-776.

Conroy JP, Virgona JM, Smillie RM, Barlow EW (1988). Influence of drought acclimation and CO_2 enrichment on osmotic adjustment and chlorophyll a fluorescence of sunflower during drought. Plant Physiol. 86: 1108-1115.

Csonka LN, Hanson AD (1991). Prokaryotic osmoregulation: genetics and physiology. Ann Rev. Microbiol. 45: 569-606.

Curtis CR (1971). Disc electrophoretic comparisons of proteins and *peroxidases* from *Phaseolus vulgaris* leaves infected with *Agrobacterium tumefaciens*. Can. J. Bot. 49: 333-337.

Dale JL (1987). Banana bunchy top: an economically important tropical plant virus disease. Adv. Virus Res. 33: 301-325.

Dorffling K, Schulenburg S, Lesselih G, Dörffling H (1990). Abscisic acid and proline levels in cold hardened winter wheat leaves in relation to variety specific differences in freezing resistance. J. Agric. Crop Sci. 165: 230-239.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Analyt. Chem. 28: 350-356.

Ennos AR, Spatz H-C, Speck T (2000). The functional morphology of the petioles of the banana, Musa textiles. J. Exp. Bot. 51: 2085-2093.

Espelie K, Franceschi V, Koattukudy P (1986). Imunocytochemical localization and time course of appearance of an anionic *peroxidase* associated with suberization in wound healing potato tube tissue. Plant Physiol. 81: 487-492.

Gielwanowska I, Szczuka E, Bednara J, Gorecki R (2005). Anatomical features and ultrastructure of *Deschampsia antarctica* (*Poaceae*) leaves from different growing habitates. Ann. Bot. 96: 1109-1119.

Goodman RN, Kiraly Z, Wood KR (1986). The Biochemistry and Physiology of Plant Disease. University of Missouri Press, Columbia.

Goodwin TW, Britton G (1988). Distribution and analysis of carotenoids". In: T.W. Goodwin, Editor, *Plant Pigments*, Academic Press, London. pp. 61-132.

Guo D, Guo Y, Zhao J, Liu H, Peng Y, Wang Q (2005). Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. tsatsai) after turnip mosaic virus infection. Plant Sci. 168: 57-63.

Haq I, Dahot MU (2007a). Effect of permanent and temporary immersion systems on banana micro-propagation. Pak. J. Bot. 39: 1763-1772.

Haq I, Dahot MU (2007b) Micro-propagation efficiency in banana (*Musa* spp) under different immersion systems. Pak. J. Biol. Sci. 10: 726-733.

Haq I, Khan S, Dahot MU, Naheed K (2009). Screening of banana bunchy top diseased plants: A way to control its spreading. Plant Omics J. 2: 175-180.
Harding RM, Burns TM, Dale JL (1991). Virus-like particles associated with banana bunchy top disease contain small single-stranded DNA. J. Gen. Virol. 72: 225-230.
Johansen DA (1940). Plant microtechnique. New York, USA: McGraw-Hill.
Johnson-Flanagan AM, Spencer MS (1996). *Chlorophyllase* and *Peroxidase* activity during degreening of maturing canola (*Brassica napus*) and mustard (*Brassica juncea*) seed. Physiol. Plant. 97: 353-359.
Kang S, Blair JE, Geiser DM, Khang C, Park S, Gahegan M, O'Donnell K, Luster DG, Kim SH, Ivors KL, Lee Y, Lee Y, Grunwald N, Martin FM, Coffey MD, Veeraraghavan N, Makaloska I (2006). Plant Pathogen culture collections: It takes a village to preserve these resources vital to the advancement of agricultural security and plant pathology. Phytopathol. 96: 920-925.
Klepper L, Flesher D, Hageman RH (1971). Generation of reduced Nicotinamide Adenine Dinucleotide for nitrate reduction in green leaves. Plant Physiol. 20: 580-590.
Kuroda M, Ozawa T, Imagava H (1990). Changes in chloroplast
peroxidase activities in relation to chlorophyll loss in barley leaf segments. Physiol. Plant. 80: 555-560.
Magee CJP (1940). Transmission studies on the banana bunchy top virus. J. Inst. Agric. Sci. 6: 109-110.
Malavolta E, Vitti GC, Oliveira SA (1989). de Avaliação do Estado Nutricional das Plantas. Piracicaba: Associação Brasileira para Pesquisa do Potássio e do Fosfato, p. 201.
Matile P, Hortensteiner S (1999). Chloropyll degradation, Annu. Rev. Plant Physiol. 50: 67-95.
Milavec M, Ravnikar M, Kovac M (2001) *Peroxidases* and photosynthetic pigments in susceptible potato infected with potato virus YNTN. Plant Physiol. Biochem. 39: 891-898.
Miller GL (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 31: 426-429.
Miteva E, Hristova D, Nenova V, Maneva S (2005). Arsenic as a factor affecting virus infection in tomato plants: changes in plant growth, *peroxidase* activity and chloroplast pigments. Sci. Hort. 105: 343-358.
Moffat AS (2001). Finding new ways to fight plant diseases. Science, 292: 2270-2273.
Morris AW, Riley JP (1963). The determination of nitrate in sea water. Anal. Chem. Acta. 29: 272-279.
Ozyigit II, Kahraman MV, Ercan O (2007). Relation between explant age, total phenols and regeneration response in tissue cultured cotton (*Gossypium hirsutum* L.). Afr. J. Biotechnol. 6: 3-8.
Ranieri A, Nali C, D'Urso G (1995). *Peroxidase* activity in *Cucurbita pepo* L. leaves exposed to ozone. Agric. Med. pp. 47-54.
Robinson JC, Neil DJ (1985). Comparative morphology, phenology and production potential of banana cultivars 'Dwarf Cavendish' and 'Williams' in the Eastern Transvaal Lowveld. Sci. Hortic. 25: 149-161.
Robson JD, Wright MG (2007). Effect of imidacloprid foliar treatment and banana leaf age on *Pentalonia nigronervosa* (Hemiptera, Aphididae) survival. New Zealand J. Crop Hortic. Sci. 35: 415-422.
Shalitin D, Wolf S (2000). Cucumber mosaic virus infection affects sugar transport in melon plants. Plant Physiol. 123: 597-604.
Siegel BZ (1993). Plant *peroxidases* - an organismic perspective. Plant Growth Regul. 12: 303-312.
Smart CM (1994). Gene expression during leaf senescence. New Phytopathol. 126: 419-448.
Smith MK, Searle C, Langdon PW, Schaffer B, Whiley AW (2000). Comparison between micropropagated banana (*Musa* AAA; 'Williams') and conventional planting material during the first 12 months of development. J. Hort. Sci. Biotechnol. 76: 83-87.
Stover RG (1972). Banana, Plantain and Abaca Diseases. Commonwealth Mycological Institute, Kew.
Stroinski A (1995). Effect of cadmium and Phytophtora infestans on *peroxidase* activity in potato leaves and tubers. Roczniki, A. and R. Poznaniu, CCLXXVI, Ogrodn. 23: 53-61.
Sutic DD, Sinclair JB (1990). Physiology of diseased plants, In: D. D. Sutic and J.B. Sinclair, Editors. Anat Physiol Diseased Plants 2, CRC Press, Boca Raton. pp. 157-221.
Tecsi L, Smith AM, Maule AJ, Richard C (1996). A spatial analysis of physiological changes associated with infection of cotyledons of marrow plants with cucumber mosaic virus. Plant Physiol. 111: 975-985.
Valjakka M, Luomala EM, Kangasjärvi J, Vapaavuori E (1999). Expression of photosynthesis- and senescencerelated genes during leaf development and senescence in silver birch (*Betula pendula*) seedlings. Physiol. Plant. 1060: 302-310.
Wang Q, Zhang L, Wang B, Yin Z, Feng C, Wang Q (2010). Sweetpotato viruses in China. Crop Prot. 29: 110-114.
Wolf B (1982) A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Comm. Soil Sci. Plant Anal. 13: 1035-1059.
Wood KR (1990). Pathophysiological alternations", in: Mandahar C. L. (Ed.), Plant Viruses, vol. 2, Pathology, CRC Press, Boca Raton. pp. 30-52.
Ye XS, Pan SO, Kuc J (1990). Activity, isozyme pattern, and cellular localization of *peroxidase* as related to systemic resistance of tobacco to blue mould (*Peronospora tabacina*) and tobacco mosaic virus. Physiol. Biochem. 80: 1295-1299.