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Assessing Banana Varieties for their Response to Banana Bunchy Top Virus (BBTV) Infection

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ABSTRACT: The Banana Bunchy Top Virus (BBTV) causes severe disease development in bananas resulting in a considerable reduction in yield. This study aimed to investigate the response of commercial banana cultivars, *viz*. Red Banana, Grand naine, Safed Velchi, and Rasabale, to BBTV. Ensuring consistent evaluation across diverse banana types, managing subjective symptom identification, and conducting extended observations demand meticulous resources. Integrating genomic analysis, ethical concerns, and real-world adaptability further complicates the implementation of resistant sources in sustainable agriculture. Pentalonia nigronervosa, an insect vector that feeds on banana plants, was used to transmit the virus. During the experiment, ten viruliferous adult aphids were allowed to feed on each healthy plant, with an acquisition feeding period of 24 hrs. and 48 hours of inoculation feeding period. Banana plants were then observed for three months to determine the incubation period and intensity. At the end of the observation period, all cultivars showed typical symptoms of BBTV. The disease incidence rate was 73.33%, 83.33%, 26.66%, and 16.66% for Red Banana, Grand Naine, Safed Velchi, and Rasabale, respectively, at the three-month growth stage. At the six-month growth stage, the incidence of BBTV was 66.33%, 80.00%, 36.66%, and 13.33%, respectively. At the nine-month growth stage, the incidence of BBTV was 60.00%, 66.66%, 30.00%, and 13.33%, respectively. This paper highlights the importance of exploring banana germplasm to find a resistant BBTV source.

Keywords: Banana bunchy top virus (BBTV), Incubation period, Germplasm, Insect vector, Cultivars, Acquisition feeding period.

INTRODUCTION

Banana is one of the most economically important fruit crops globally, contributing to the livelihoods and nutrition of millions of people in tropical and subtropical regions. However, banana production faces numerous challenges, including the threat of viral diseases. One such viral pathogen that has emerged as a significant concern for the banana industry is the Banana Bunchy Top Virus (BBTV). BBTV is a destructive and devastating virus that primarily affects banana plants, leading to severe yield losses and economic repercussions for growers.

The transmission of BBTV primarily occurs through the banana aphid (*Pentalonia nigronervosa*), making it a persistent threat in regions where the aphid vector is prevalent (Hu *et al*., 2007; Allen, 1983; Magee, 1940). Once infected, banana plants exhibit symptoms such as dark green streaks of variable length on the leaf midribs and petioles, progressive dwarfing of leaves and development of marginal chlorosis, and upright and bunched-up leaves at the apex of the plant, hence the name of the disease. In addition, a plant may not produce any fruit if the infection occurs at an early stage of growth (Su *et al*., 2003; Dale, 1987).

Efforts to manage BBTV have involved various strategies, including the use of resistant or tolerant banana genotypes. Identifying banana varieties that exhibit resistance or tolerance to BBTV is a critical step in mitigating the impact of this virus on banana production. Screening different banana genotypes for their reaction to BBTV is a proactive approach to developing resilient and sustainable banana cultivation systems. This screening process not only aids in understanding the genetic diversity of banana types in

response to BBTV but also paves the way for the development of BBTV-resistant banana cultivars through breeding programs.

This study aims to assess the susceptibility or resistance of four banana genotypes commonly cultivated in the Konkan region to BBTV. By understanding how different banana types respond to BBTV, we can not only contribute to the development of disease-resistant cultivars but also provide valuable insights for implementing effective management strategies that can safeguard banana production. The results of this screening will serve as a foundation for future research and practical applications in the battle against BBTV, ultimately benefiting banana growers.

MATERIALS AND METHOD

A. Source of genotypes

Four banana genotypes *viz.*, Rasbale (AAB), Grand naine (AAA), Safed velchi (AB), and Red Banana (AAA) were obtained from the Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, for this investigation. The response of these four banana genotypes to Banana Bunchy Top Virus (BBTV) infection was investigated in the field, during 2021-22. In the experiment, a total of 90 plants of each genotype were planted in the field. Of these plants, 30 plants of each genotype were inoculated with viruliferous aphids at three different growth stages. One plant in each row was maintained as a non-inoculated control.

Table 1: Details of genotypes used in the study.

Sr. No.	Name of Genotypes	Total no. of Control Plants	Total no. of Test Plants	Time Interval Between Aphid Inoculation (Month after planting)		
				3	6	9
1.	Red banana (AAA)	09	90	30	30	30
2.	Grand naine (AAA)	09	90	30	30	30
3.	Safed velchi (AB)	09	90	30	30	30
4.	Rasabale (AAB)	09	90	30	30	30

B. Rearing of insect vectors

The transmission of BBTV is carried out by a specific type of aphid called the black aphid (*P. nigronervosa*). This transmission is persistent, circulative, and non-propagative. The culture of *Pentalonia nigronervosa* was obtained from virus-infected banana plants located in the Palghar district. Banana black aphids are present on the outer sheath that covers the basal portion of the pseudostem, as well as on the lower side of the leaf midrib. To collect them, a hairbrush that had been moistened with water was used to gently remove aphids from the infected plants. These aphids were then released on potted tissue cultured banana plants, for maintenance and multiplication. Symptoms of bunchy top were observed three six and nine months after inoculation on these healthy plants. Progeny of such viruliferous aphids was used for future inoculation.

C. Virus transmission using insect vector

Banana plants having 2-3 leaves were planted in the field and each plant was covered with a plastic cage of 60 cm × 60 cm × 120 cm (Fig. 1). Banana aphids were given a 24-hour acquisition feeding period on infected banana plants as a source of BBTV. The aphids were then transferred to test plants for a 48-hour inoculation feeding period. A total of 10 adult aphids were placed at the base of the midrib of the youngest leaf of banana seedlings for the inoculation feeding period. Inoculation of viruliferous aphids was done at the three-month, six-month, and nine-month growth stages. At the end of the inoculation feeding period, the aphids were sprayed with Dimethoate 30% EC @ 0.03% to kill them. As many as 30 plants for each cultivar were used as test plants and one plant in each row was maintained as control.

D. Observations to be recorded

The time between BBTV inoculation by aphids and the onset of disease symptoms (i.e., incubation period) was determined. Expression of symptoms, disease incidence (DI), and the reaction of genotype were calculated according to the following formula (Gibbs1983; Allen *et al*., 1983) given below.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{The total number of plants observed}} \times 100$$

The designation of the reaction of the different cultivars was based on the percent Disease incidence it was determined by a Scale developed by Espino *et al.* (1993) which is as follows.

Table 2: Scale used to determine the percent Disease incidence of BBTV.

Percent disease incidence	Reaction
0	Resistant
1-25	Moderately resistant
26-50	Moderately susceptible
51-75	Susceptible
76-100	Highly susceptible

RESULTS AND DISCUSSION

Four banana varieties commonly cultivated in Konkan *viz.* Grandnaine, Safed velchi, red banana, and Rasbale were artificially inoculated for screening purposes. During the three-month growth stage, [screened plants from four banana cultivars for Banana Bunchy Top Virus (BBTV) by inoculating them with a viruliferous aphid vector *P. nigronervosa* (Fig. 1)]. After the inoculation, several plants showed symptoms of BBTV infection. The initial symptoms involved dark green stripes and dots on some parts of the leaf blade and petiole which then spread to other parts of the leaf (resembling green J-rocks and morse code). More severe symptoms caused the plant to become stunted, the leaves to narrow and become more erect, the edges to curl up and appear yellowed, the chlorosis lines to be visible on the edges of the leaf bones, and the leaves to become more easily broken. The incubation period of BBTV in cv. Red Bananas ranged from 50-55, in Safed velchi 50-55, Rasabale 80-85, and Grand, naine ranged from 25-30 days after inoculation, which is faster than other cultivars. The incidence of BBTV infection varied between cultivars, with 73.33% in red banana, 83.33% in Grand naine, 26.66% in Safed velchi, and 16.66% in Rasabale. Among the four cultivars, Rasabale showed a moderately resistant response while Grand naine showed a highly susceptible response.

During the six-month development stage, after aphid inoculation, numerous plants showed symptoms of BBTV infection that were similar to those observed at the three-month growth stage. The incubation period of BBTV in cv. Grand naine ranged from 25-30 days after inoculation and 80-85 days after inoculation in Rasabale (Table 3). The incidence of BBTV infection varied between cultivars; 63.33% in red banana, 80.00% in Grand naine, 36.66% in Safed velchi, and 13.33% in Rasabale. Among the four cultivars, Rasabale showed a moderately resistance response, while Grand naine showed a highly susceptible response.

At the nine-month stage of growth, typical symptoms of BBTV, include dark green stripes and dots on some parts of the leaf blade and petiole, a bunchy appearance, stunted growth, and no flowering. On the other hand, the healthy, non-inoculated plants showed good flowering and fruiting. The incubation period of BBTV in cv. Grand nine were found to be between 25-30 days, which was faster than in other cultivars (Red banana50-55, Rasable 80-85, and Safed velchi 50-55 DAI) (as shown in Table 4). The incidence of BBTV infection varied between cultivars with a rate of 60.00% in red banana, 66.66% in Grand naine, 30.00% in Safed velchi, and 13.33% in Rasabale. Among the four cultivars, Rasabale displayed a moderately resistant response, while Grand naine showed a susceptible response to BBTV. This indicated that Rasabale is moderately resistant to BBTV followed by Safed velchi.

Similar results were recorded by Arubi *et al.* (2021) reported that the initial symptoms of BBTV are generally dark green stripes and dots on some parts of the leaf blade and petiole which then spread to other parts of the leaf (green J-rocks and morse code). More severe symptoms cause the plant to become stunted, and the leaves are narrowed. The incidence of the disease varies between cultivars, i.e. 80% in CV. Cavendish, 60% in cv. Bebek, 20% in CV. Goroho, Barangan Merah and Halabanensis. The incubation period of BBTV in CV. Cavendish ranged from 25-40 days after inoculation, faster than the incubation period in other cultivars (Tables 2-5). Therefore, wild banana accessions have a fairly high resistance to BBTD. In contrast, symptoms were easily observed in commercial banana cultivars. Symptoms appeared in cultivars with AA (Bebek) and AAA (Barangan Merah, Cavendish and Goroho) genomes but did not appear in cultivars with AAB genomes (Tanduk). According to Widyastuti and Hidayat (2005). This study echoes C. Niyongere E. *et al.'s* (2011) findings on Banana Bunchy Top Virus (BBTV) and *Fusarium* wilt in various banana genotypes. Among 40 genotypes, 'Poyo' (AAA) showed the highest BBTV susceptibility, while those with one or two B genomes exhibited resistance. This aligns with dela Cruz *et al.'s* observations (2016). 'Highgate' (AAA), with a purely A genome, displayed susceptibility. *Fusarium* wilt symptoms appeared in B genome-containing genotypes, notably 'Highgate' and 'Nakitembe.' This reinforces genomic influence on disease susceptibility.

Table 3: Reaction of different Banana Genotypes against Banana Bunchy Top Virus (BBTV) at three-month growth stage.

Sr. No.	Cultivars/ Genotypes	Genome	No. of Plant		Disease incidence	Response of Genotypes	Incubation Period (DAI)	Expression of symptoms
			Inoculated	Infected				
1.	Red banana	(AAA)	30	22	73.33%	Susceptible	50-55	SG, BT, MC and LB
2.	Grand naine	(AAA)	30	25	83.33%	Highly susceptible	25-30	SG, BT, LB and RLS
3.	Safed velchi	(AB)	30	8	26.66%	Moderatelysusceptible	50-55	SG, BT, and LB
4.	Rasabale	(AAB)	30	5	16.66%	Moderately resistant	80-85	SG, BT, MC and LB

Table 4: Reaction of different Banana Genotypes against Banana Bunchy Top Virus (BBTV) at the six-month growth stage.

Sr. No.	Cultivars/ Genotypes	Genome	No. of Plant		Disease incidence	Response of Genotypes	Incubation Period (DAI)	Expression of symptoms
			Inoculated	Infected				
1.	Red banana	(AAA)	30	19	63.33%	Susceptible	50-55	SG, BT, MC, RLS, LB and NSt
2.	Grand naine	(AAA)	30	24	80.00%	Highly susceptible	25-30	SG, BT, LB and RLS
3.	Safed velchi	(AB)	30	11	36.66%	Moderately susceptible	50-55	SG, RLS, BT, and LB
4.	Rasabale	(AAB)	30	4	13.33%	Moderately resistant	80-85	SG, BT, MC, LB and NSt

Table 5: Reaction of different Banana Genotypes against Banana Bunchy Top Virus (BBTV) at nine-month growth stage.

Sr. No.	Cultivars/ Genotypes	Genome	No. of Plant		Disease incidence	Response of Genotypes	Incubation Period (DAI)	Expression of symptoms
			Inoculated	Infected				
1.	Red banana	(AAA)	30	18	60.00%	Susceptible	50-55	BT, MC, LB, NF and NSt
2.	Grand naine	(AAA)	30	20	66.66%	Susceptible	25-30	SG, BT, LB, NF and RLS
3.	Safed velchi	(AB)	30	9	30%	Moderately susceptible	50-55	SG, BT, NSt, NF and LB
4.	Rasabale	(AAB)	30	4	13.33%	Moderately resistant	80-85	SG, BT, MC, NF, LB and NSt

SG- Stunted growth; LB- Leaf brittleness; MC- Marginal chlorosis; NSt- Necrotic streaks; BT - Bunchy top; NF- Non-flowering of the plant RLS- Reduction in leaf size

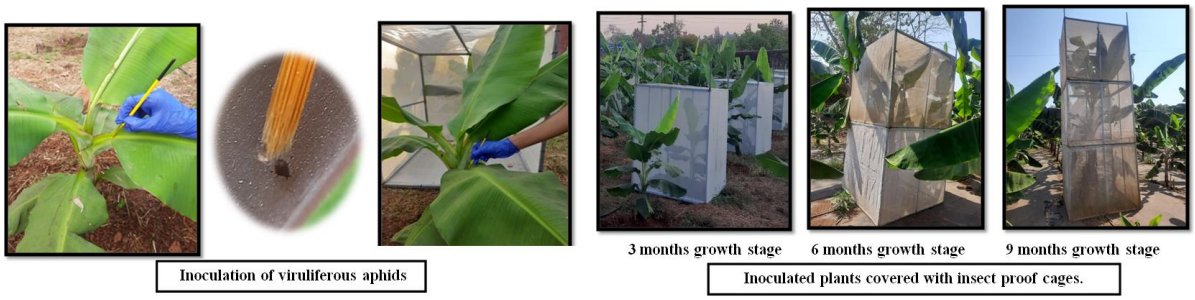

Fig. 1. Virus transmission by using insect vector.

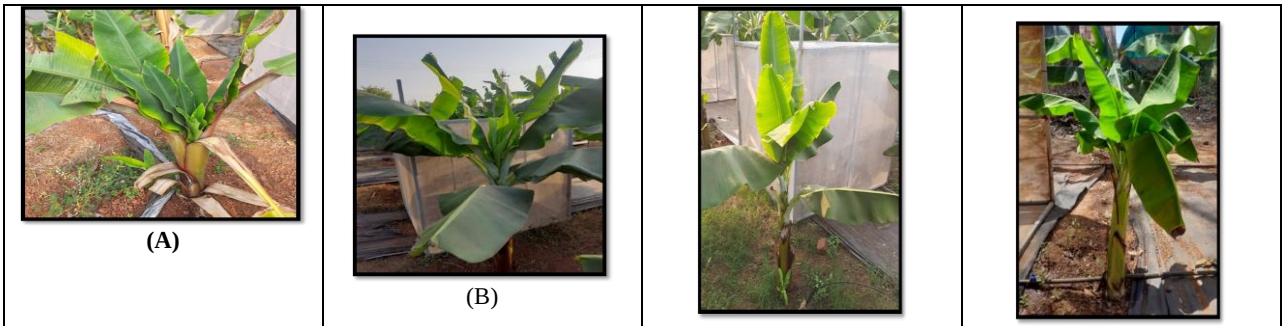

Fig. 2. Response of banana genotypes after inoculation of Banana bunchy top virus using banana aphid, *Pentalonia nigronervosa* (A) Red Banana, (B) Grand naine, (C) Safed velchi and (D) Rasabale.

CONCLUSIONS

This study aimed to assess various banana varieties for their response to Banana Bunchy Top Virus (BBTV) infection, and our findings have provided valuable insights into the susceptibility and resistance of different banana cultivars to this devastating viral disease. Through rigorous experimentation and data analysis, we observed significant variations in the response of the tested banana varieties to BBTV infection. Some varieties displayed a high degree of susceptibility, showing severe symptoms and rapid

spread of the virus throughout the plants. In contrast, Rasabale and Safed velchi varieties exhibited a remarkable level of resistance, with limited symptom development and restricted virus proliferation. These findings have important implications for banana cultivation and management strategies, especially, in regions where BBTV poses a significant threat to banana production. Farmers and stakeholders can make informed decisions regarding which banana varieties to cultivate based on their susceptibility or resistance to BBTV. By choosing resistant varieties, they can potentially reduce the impact of the disease and minimize crop losses with reduced use of Pesticides.

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