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Studies on Lipoxygenase activity levels in seed and during seed germination in different genotypes of tobacco (Nicotiana tabacum L.)

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ABSTRACT: Lipoxygenase activity was measured in seed and germinating seeds of different genotypes of tobacco with varied reaction to the pathogen Tobacco Mosaic Virus. Lipoxygenase enzyme activity level in seeds of resistant genotype Va 770 was double than that in the susceptible (FCV Spl.). The trend of results was validated with a series of genotypes with varied levels of resistance/susceptibility. The susceptible genotypes recorded the lowest activity. The level of enzyme during sprouting in tobacco mosaic virus resistant genotype was 60% higher as compared to that in susceptible variety. The tolerant variety showed only 20% higher activity than the susceptible. Higher lipoxygenase activity may have a protective function in resistant genotype at the initial stage of germination (sprout) which is vulnerable to infection. Extent of activation of enzyme during seed germination and seedling growth varied with the variety. Initial activation of lipoxygenase followed by a decrease in enzyme activity was observed in all the three varieties tested. The higher titers of enzyme at seedling stage or in seed provide a scope to use lipoxygenase as possible biochemical marker in Tobacco to screen out genotypes with resistance to Tobacco Mosaic Virus disease.

Key Words: Lipoxygenase, Enzyme, Seed, Tobacco Mosaic Virus, Resistance, Tobacco

INTRODUCTION

Lipoxygenases (LOX) (E.C. 1.13.11.12) catalyse the hydroperoxydation of polyunsaturated fatty acids and esters containing cis, cis - 1, 4-pentadiene system.

OOH LOX R-CH - CH=CH - CH=CH-R' $R - CH = CHCH_2CH = CH-R'$ -----> OOH O_2 R – CH =CH - CH=CH-CH-R'

They also catalyse co-oxidation reactions with chlorophylls and carotenoids (Axelrod et al., 1981). These reactions lead to the formation of compounds such as jasmonic acid, having growth regulatory activity (Vic and Zimmerman, 1987). Lipoxygenases are known to occur in legumes notably soybean, in some cereal grains and oil seeds. Their presence in various other plant tissues, including leaves, has also been documented by Galliard and Chan (1980). LOX is believed to play a major role in plant growth and development. In a wide variety of plant species, increase in LOX activity during early seedling growth has been reported (Siedow, 1991). Increase in LOX activity due to pathogen attack in resistant genotypes is known in many plants including tobacco (Somnath and Mahesh, 2013; Ruzicska et al., 1983). In the present study lipoxygenase levels were measured in seed and during seed germination in tobacco (Nicotiana tabacum L.) genotypes susceptible, tolerant and resistant to tobacco mosaic virus (TMV) disease to ascertain the role of LOX in virus disease resistance.

MATERIALS AND METHODS

Seeds of 17 tobacco genotypes with varied reactions viz., susceptibility, tolerance and resistance to TMV were collected from CTRI Research Station, Hunsur. Surface sterilized seeds were spread on moist blotting paper held in petridishes and incubated in seed germinator at 25±1°C under darkness for required number of days (Nagarathna et al., 1992).

A. Enzyme extraction and assay

At an interval two days, germinating seed/seedling (0.5g) samples were drawn in triplicate and macerated with an equal quantity of acid washed sand and 5 ml of 0.05M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 900xg for 5 min. The whole process of extraction was carried out at 4 °C and the supernatant was used as the enzyme source (Nagarathna *et al.*, 1992). Spectrophotometric method (Borthakur *et al.*, 1987) was followed by recording the increase in absorbency at 234nm as the substrate linoleic acid is catalysed to hydroperoxides.

The reaction mixture contained 2.7ml of 0.2 M sodium phosphate buffer (pH 6.5) and 0.3ml of 10mM linoleic acid in 0.28% (w/v) between 20. The reaction was initiated by adding 25µl of enzyme extract and change in absorbance was recorded for 3min using Shimadzu UV-160A, UV-Visible recording spectrophotometer. Enzyme activity was expressed as change in absorbance per mg protein per min.

The protein content was estimated using Coomassie Brillient Blue G 250, (Bradford, 1976). The experiment was repeated three times and the data were subjected to statistical analysis.

RESULTS AND DISCUSSION

A. Lipoxygenase activity in seed

Lipoxygenase (LOX) was assayed in seeds of TMV susceptible (FCV Special), tolerant (FCH 6248) and resistant (Va 770) varieties (Table 1). The activity was 0.46 units (absorbance 234 nm / mg protein / min) in susceptible variety and lowest among the three. In tolerant and resistant varieties, the activity measured in control samples were 67% &107% higher over that of susceptible. The levels were measured in water soaked seeds as well as in powdered & defatted seed, since the fat content was suspected to interfere with enzyme extraction and assay. There was no appreciable increase in activity by imposing treatments. However, the difference among varieties was maintained in both the treatments.

Tabl	e 1.	Lipoxygenase	e activity in a	seeds of	different A	Vicotiana tabacum	cultivars.

	Lipoxygenase activity**			
Variety	Control	Soaked	Defatted	
FCV Special (S)	0.4615 ± 0.06	0.4709 ± 0.10	0.5130 ± 0.04	
FCH 6248 (T)	0.7705 ± 0.05	0.7970 ± 0.01	0.8195 ± 0.06	
Va.770 (R)	0.9575 ± 0.04	0.9562 ± 0.04	1.0057 ± 0.05	
CD at P= 0.05		0.0940		

(R) - Resistant; (T) - Tolerant; (S) - Susceptible

**Change in absorbance at 234 nm mg⁻¹ protein min⁻¹

Varieties having resistance as well as tolerance to tobacco mosaic virus showed around 2 times higher LOX activity than that in susceptible variety. Tobacco seed is known to contain more than 40% oil. Maestri and Guzman (1993) have reported predominance of linoleic acid constituting more than 70% of total lipids in tobacco seed oil. Linoleic acid being a good substrate in LOX pathway, the enzyme may use linoleic acid leading to the production of compounds like jasmonates (Koda, 1992). Farmer and Ryan, (1992); Creelman et al., (1992) have attributed jasmonates to carryout signal transduction in plants on wounding and pathogen attack. Tobacco seeds are also known to contain many defense-related proteins to counter the attack by pathogens. (Bowels, 1990). Hence, higher level of LOX in TMV resistant variety may be indicative of its incompatible interaction with virus.

To confirm this hypothesis, LOX activity level in seeds of several other genotypes both susceptible and resistant to TMV were measured. All the resistant varieties recorded higher LOX activity than that of susceptible. The activity in resistant varieties ranged from 0.86 to 2.07 units (Fig. 1), while in susceptible varieties it was in the range of 0.40 to 0.68 units.

However, the levels of LOX activity in all the 13 genotypes were higher than that recorded in susceptible varieties FCV Special, Bhavya and Swarna. The activity in tolerant variety (FCH 6248) was below the level of variety Va 770. These results are in agreement with the findings of Nagarathna *et al.*, (1992) wherein, higher activity in sorghum seeds was positively correlated to the increase in powdery mildew resistance level of a variety.



Fig. 1. Lipoxygenase levels in seeds of different genotypes.

B. Lipoxygenase activity during seed germination

Lipoxygenase activity was measured during the seed germination and seedling growth at an interval of 2 days up to 14 days from seeding. In all the varieties tested, enzyme level increased during germination process reaching a maximum at 12 days after plating. Level of increase in the activity during germination and seedling growth varied with variety (Table 2). In TMV susceptible variety FCV Special, the increase in activity from day 2 to day 12 was 10 fold. While, increase in tolerant (FCH 6248) and resistant (Va.770) varieties were 8 and 4 fold respectively. LOX activity showed a distinct decrease on the 14th day after plating as compared to day 12. The susceptible variety showed 58% fall in enzyme level, followed by tolerant variety (56%) and resistant variety (35%). This confirms the earlier reports in different crops. The number of days taken to reach activity peak varied with the crop; in rice

it was 3 days, in corn and sunflower 4 days and in watermelon 6 days (Siedow, 1991), Altschuler *et al.* (1989) as well as Park and Polacco (1989) attributed these increases in the enzyme levels, to the synthesis of new lipoxygenase isoenymes. Lipoxygenase possibly plays a major role in mediating scavenging of oxygen during germination of seeds (Mathur and Sharma, 1989).

Tobacco seed is known to contain more than 40% oil. Maestri and Guzman (1993) have reported predominance of linoleic acid constituting >70% of total lipids in tobacco seed oil. Linoeic acid being a good substrate in lipoxygenase pathway, the enzyme may use linoleic acid to produce compounds like traumatic and jasmonic acid which have growth regulatory roles (Seidow, 1991; Anderson, 1989).

Table 2. L	ipoxygenase	activity levels a	t different stages of	f seed germination in	n Nicotiana tabacum	genotypes
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Variety		Lipoxygenase activity **				
Days	FCV Special (S)	FCH 6248 (T)	Va.770 (R)			
2	0.5552 ± 0.001	0.6660 ± 0.011	0.8869 ± 0.015			
4	0.6076 ± 0.029	0.6220 ± 0.017	0.6621 ± 0.080			
6	1.7079 ± 0.017	0.9482 ± 0.037	1.1517 ± 0.049			
8	1.2994 ± 0.019	1.1097 ± 0.109	1.1653 ± 0.042			
10	3.6111 ± 0.187	2.1387 ± 0.055	3.0359 ± 0.209			
12	5.5336 ± 0.064	5.3419 ± 0.154	3.8731 ± 0.307			
14	2.3268 ± 0.086	2.3579 ± 0.089	2.5126 ± 0.285			
CD at $P=0.05$	0.2032					

(R) – Resistant; (T) – Tolerant; (S) - Susceptible

**Change in absorbance at 234 nm mg⁻¹ protein min⁻¹

Interestingly, 2 days after plating i.e., at the sprout stage, lipoxygenase activity was highest in TMV resistant variety (Va. 770) followed by tolerant and least in susceptible variety (Table 2). LOX activity was 60% higher in resistant variety as compared to the activity in susceptible variety (FCV Special). The increase was found to be significant. But, tolerant variety showed only 20% higher activity than the susceptible. During sprouting there is possibility of infection to many pathogens including tobacco mosaic virus. Ruzicska et al., (1983) reported the increase of LOX activity in resistant genotype after TMV infection. Kunstler et.al., (2007) have attributed early and greater induction of LOX activity in the TMV inoculated leaves of genotype resistant to TMV than in susceptible to disease resistance. Production of jasmonates through lipoxygenase pathway (Koda, 1992) and attribution of jasmonate to carryout signal transduction in plants on wounding and pathogen attack (Farmer and Ryan, 1992; Creelman et al., 1992) possibly explain the present findings, where high LOX activity is recorded in resistant and tolerant genotypes as compared to susceptible. This high activity may help the resistant genotype to ward off the pathogen to avoid infection.

Hence, level of LOX activity in seed may serve as a marker to identify resistant genotypes. In addition, the biochemical marker was found to be useful not only in seeds but also at seedling stage to enable nondestructive assay supporting advancement of progeny in breeding programmes.

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