

Estimation of DNA From Healthy and *Alternaria* Blight Infected Leaves of Sunflower

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Abstract

The present investigation is undertaken to study the amount of DNA in variously infected and healthy leaves of sunflower of different varieties. The changes in DNA contents as analyzed at different incubation periods (zero-day, fifth day, tenth day, fifteenth day, twentieth day and twenty fifth day) on different isolates of sunflower, it was found that DNA content ($\mu\text{g/gm}$) increased as incubation period increases from zero to twenty fifth day. From results it is evident that DNA content was maximum in PKVSH-27 followed by LS-11, LSH-1, LSH-2 and minimum in PC-1091 and SCH-35. Among six isolates of sunflower, in all isolates, the DNA content was increased but it was maximum in PKVSH-27 and minimum in SCH-35 and PC-1091, hence the variety PKVSH-27 is highly susceptible followed by LS-11, LSH-1, LSH-2 and the varieties SCH-35 and PC-1091 were found resistant to *Alternaria* blight.

Key Words: Sunflower, DNA, LSH-1, LSH-2, LSH-11, SCH-35, PKVSH-27 and PC-109.

Introduction

Oilseed crops have been a vital foundation of India's agricultural economy since ancient times. India is one of the world's largest producers of oilseeds, and these crops play a critical role in rural livelihoods, food security, and industrial growth. Sunflower was introduced commercially to Indian agriculture in 1969 as an alternative to meet the rising demand for edible oils. Sunflower is a nutrient-rich crop that serves as a vital resource for both human nutrition and livestock feed across India. Traditionally grown in Karnataka, Maharashtra, and Andhra Pradesh, India has expanded cultivation to states like Haryana, Punjab, and Rajasthan, with Karnataka leading via 9.85 lakh hectares of land. (Shrine, 2024).

The different varieties cultivated in Maharashtra are LSH-1, LSH-2, LSH-11, SCH-35, PKVSH-27 and PC-1091 (Deshmukh, 2009).

Sunflower cultivation faces major challenges from various pathogens and insect pests that cause significant yield losses. Fungal, bacterial, viral, and nematode agents attack seedlings, leaves, stems, and heads under favorable weather conditions (Kothakota *et al.*, 2020). Among fungal diseases, *Alternaria* blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is very severe in Maharashtra and yield losses up to 11.3 to 73.5 per cent (Deshmukh, 2009).

Alternaria helianthi grows parasitically and cause heavy damage to plants. The infection caused due to this pathogen may lead to pronounced changes in nucleic acid content and composition during pathogenesis (Ekbote, 1988). Therefore, the present investigation is undertaken to study the amount of DNA in variously infected and healthy leaves of sunflower of different varieties.

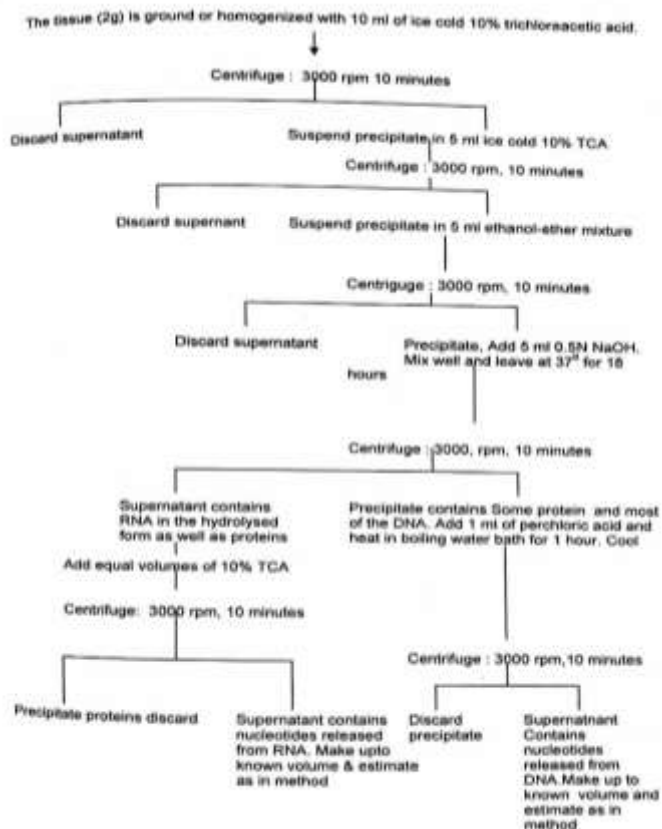
Materials and Methods

- 1) **Sample collection:** The *Alternaria* blight infected leaves of sunflower were collected from fields of sunflower and brought to laboratory. The infected leaves show typical circular, dark brown coloured, unlimited killing of the host tissue.
- 2) **Isolation and Purification of *Alternaria helianthi*:** For isolation and purification of *Alternaria helianthi*, infected patches from leaves were cut with the help of sterilized blade into pieces. These pieces were surface sterilized with 0.1 per cent Mercuric chloride and washed with sterilized distilled water for three times. These leaves were incubated at room temperature at 28°C to 30°C . After seven days of incubation, the young growing hyphal tips were transferred to freshly prepared PDA plates by hyphal tip method given by Aneja (1996).
- 3) **Pathogenicity of *Alternaria helianthi*:** In order to prove pathogenicity, simple detached leaf (SDL) technique was used as described by Mayee *et al.*, (1979).

4) **Disease severity index:** Disease severity index of six varieties of sunflower leaves infected by *Alternaria helianthi* was carried out by five-point scale as given by Mayee *et al.*, (1983). DSI was calculated by using following formula-

$$DSI = \frac{\sum \text{ of All Readings}}{\text{No. of Observations} \times (\text{All Ratings}-1)} \times 100$$

5) **DNA estimation:** Estimation of DNA from healthy and *Alternaria* blight infected leaves of sunflower at different incubation periods was carried out by the method proposed by Jayaraman (1996).



Flow Chart for Extraction and Estimation of DNA

Results

The *Alternaria* blight infected leaves of sunflower were collected from field showed dark brown spots on leaves; spots were irregular in shape and size with a very dark border and grey centre. The spots on young plants may have a yellow halo. Leaf lesions may coalesce causing leaves to wither. Stem lesions begin as dark flecks which enlarge to form long, narrow lesions. Lesions may form on the receptacle.

The *Alternaria* fungi overwinter on diseased stalks. The fungi may be seed borne at low levels. Seedling blights caused by *Alternaria* may develop when sunflower plants emerge in rainy weather on *Alternaria* infested land. However, plants at the flowering to maturing stage are more susceptible the plants in the vegetative or budding stage. The infection is responsible for biochemical changes in leaf contents.

The studies on disease severity index (DSI) of *Alternaria* blight of sunflower of different varieties revealed that DSI in *kharif* season was highest in PKVSH-27 i.e. 43.25 % and lowest in PC-1091 i.e. 24.90 % while in *rabbi* and *summer* seasons, DSI was maximum in PKVSH-27 i.e. 35.18 % and 18.90 % respectively and minimum in PC-1091 i.e. 20.18 % and 12.30 % respectively (Table 1).

Table 1: DSI of *Alternaria* blight of sunflower of different varieties

Sr. No.	Varieties	DSI %		
		Kharif	Rabbi	Summer
1	LSH-1	32.75	25.17	15.18
2	LSH-2	33.20	24.29	14.99
3	LS-11	40.70	36.10	18.15
4	SCH-35	25.75	21.12	12.50
5	PKVSH-27	43.25	35.18	18.90
6	PC-1091	24.90	20.16	12.30

The DNA content of the tissues in various varieties was estimated and the results are given in Table 2 and Figure 1 and 2.

The changes in DNA contents as analyzed at different incubation periods (zero-day, fifth day, tenth day, fifteenth day, twentieth day and twenty fifth day) on different isolates of sunflower, it was found that DNA content ($\mu\text{g/gm}$) increased as incubation period increases from zero to twenty fifth day. From Table 2 it is evident that DNA content was maximum in PKVSH-27 followed by LS-11, LSH-1, LSH-2 and minimum in PC-1091 and SCH-35.

From those observations, it can be concluded that among six isolates of sunflower, in all isolates, the DNA content was increased but it was maximum in PKVSH-27 and minimum in SCH-35 and PC-1091, hence the variety PKVSH-27 is highly susceptible followed by LS-11, LSH-1, LSH-2 and the varieties SCH-35 and PC-1091 were found resistant to *Alternaria* blight.

Table 2: Changes in DNA content ($\mu\text{g/gm}$) of sunflower due to *Alternaria* blight

Incubation period (Days)	DNA Content ($\mu\text{g/gm}$)					
	LSH-1	LSH-2	LS-11	SCH-35	PKVSH-27	PC-1091
00	62	62	60	55	62	55
05	64	65	63	57	65	58
10	67	68	73	65	72	66
15	75	74	81	70	80	71
25	81	82	92	76	93	75
Mean	69.8	68.2	73.8	64.6	74	65.2

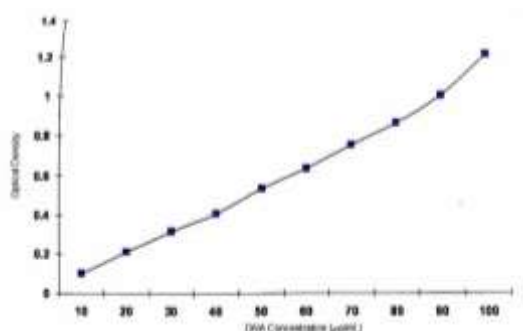
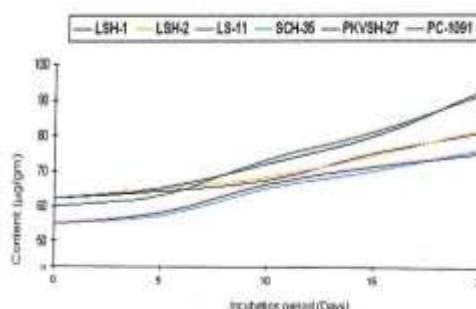


Figure1: Standard DNA Graph


Figure2: Changes in DNA content ($\mu\text{g/gm}$) of sunflower due to *Alternaria* blight

Discussion:

These observations are supported by Ekbote (1982), whose studies suggested that the infection of plants by different parasites may lead to pronounced changes in nucleic acid content and composition during pathogenesis. Specifically, he noted that the DNA content in sunflower plants infected by *Alternaria* blight initially increased, but began to decline after a certain period (25 to 30 days). Estimating DNA from infected versus healthy sunflower leaves reveals critical changes in plant-pathogen interactions. When leaf spot or blight pathogens infect a tissue, the absolute

composition of the extracted DNA shifts dynamically. By comparing DNA from healthy and infected leaves, this research allows us to detect the disease at a molecular level before the visible damage ruins the crop. Understanding these genetic differences helps scientists trace how the pathogen grows and spreads inside the plant. Ultimately, this work gives the agricultural community the tools needed to protect sunflower yields, save farmers money, and secure the supply of sunflower oil and seeds.

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