https://doi.org/10.48047/AFJBS.4.4.2022.920-931

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

BRASSICA SPECIES' BIOACTIVITY AND USE OF MOLECULAR MARKER TECHNIQUES

Hiteksha , Dr. Anubha Joshi

Department of Botany , Kalinga University , Kotni, Near Mantralaya ,Naya Raipur Chhattisgarh ,India

Volume 4, Issue 4 , July 2022

Received: 15 May 2022

Accepted: 05 Jun 2022

Published: 09 July 2022

doi:10.48047/AFJBS.4.4.2022.920-931

ABSTRACT

The term "molecular markers" refers to certain DNA sequences that are connected to genes or phenotypes that researchers are interested in examining. There is a greater likelihood that these sequences are connected to certain genes due to the fact that they are readily detectable. In order to fulfill their function as chromosomal genetic landmarks, these markers contribute to the identification of certain regions. The dicotyledonous plants that are classified under the genus Brassica are all members of the same family, which is known as the Brassicaceae . A reduced degree of evergreenness may be seen in them. Plants that belong to the cruciferae family are easily identifiable by the cross-shaped flowers that they produce. Genetic variation, we are talking to the changes that occur within the genes that make up an individual of a species. These changes may be found in the constitutions of heredity. For the sake of preserving the developmental stability and biological potential of plant species, this variety is absolutely necessary. The detection of a wide range of polymorphism and genetic distance brought to light the presence of a huge range of variety across Brassica species. This diversity occurred throughout a wide range of genetic distances. The variants of BS-10 and BS-14 have the largest amount of genetic variety with regard to the variations that were used in this study.

Keywords: *Brassica, Species' , Bioactivity Molecular , Marker , Techniques*

INTRODUCTION

Molecular Marker Techniques: Concept

As a consequence of the incorporation of cutting-edge biotechnological techniques into plant breeding and modern agriculture, there has been a dramatic shift in the application of conventional methods to crop improvement. This transformation has happened as a result of the integration of these methodologies. As a result of this change, a great number of benefits have already been accumulated. As a direct result of this revolution, there has been a significant change in the way that crop development is approached. One of these methods, known as molecular marker technology, stands out as a game-changer that has accelerated the process of plant breeding. The approach that is being considered here is one of many that has been praised as an innovation that has significantly advanced the field. Breeders now have the ability to choose desirable characteristics in a manner that is both more efficient and accurate as a result of this technological advancement. Through the use of this technology, it is now possible to study the genetic composition of plants with more accuracy and convenience than ever before. The term "molecular markers" refers to certain DNA sequences that are connected to genes or phenotypes that researchers are interested in examining. There is a greater likelihood that these sequences are connected to certain genes due to the fact that they are readily detectable. In order to fulfill their function as chromosomal genetic landmarks, these markers contribute to the identification of certain regions. These regions are connected to characteristics that are desired. In addition to the nutritional content, disease resistance, prospective yield, and environmental adaptability, these features also include the potential yield.

Brassica species' bioactivity with the use of molecular marker techniques

The dicotyledonous plants that are classified under the genus Brassica are all members of the same family, which is known as the Brassicaceae. The more than forty species that make up this group display a diverse variety of traits; the group is endemic to Europe and the Mediterranean. Herbaceous plants are the most common kind of plants, and they typically only bloom once per year or every two years. A reduced degree of evergreenness may be seen in them. Plants that belong to the cruciferae family are easily identifiable by the cross-shaped flowers that they produce. There is a broad variety of morphological changes that may occur in farmed species as a result of domestication for the purpose of harvesting leaves, petioles, buds, flowers, roots, or seeds. The wide variety of plant morphologies that may be seen in cultivated species is the explanation for this phenomenon. This is due to the fact that animals that are kept in captivity exhibit a diverse spectrum of morphologies. There are a wide variety of applications for these crops, some of which include the production of medicinal herbs, sauces, oilseeds, and vegetables. Brassica oleracea, also known as different varieties of cole, Brassica nigra, also known as black mustard, Brassica napus, and Brassica rapa, also known as turnip, rape, or Chinese cabbage, are the four most prevalent species of Brassica. These species are vital for human consumption.

There are just two Brassica oleracea crops that are grown because of their hypertrophy reproductive organs. Brassica oleracea L. var. italica, also known as broccoli, and Brassica oleracea L. var. botrytis L., also known as cauliflower, are both examples of this. The vegetative organs of all other Brassica oleracea crops, on the other hand, have been genetically engineered to represent the products of the agricultural system. Both broccoli and cauliflower are thought to have been domesticated for the first time by the Romans. In spite of this, people have been perplexed about which descriptors to use for these two crops since the structures of their edible components are so similar to one another. This is because of a number of different variables.

The similarities between the inflorescences of broccoli and cauliflower, in particular the hypertrophy of the inflorescences that are identical to one another, may have had a role in the development of the scientific and colloquial names, which are often used interchangeably in certain situations. The fact that broccoli and cauliflower seem to have inflorescences that are relatively comparable is the reason why this is the case is. There is a significant degree of antioxidant activity in the vegetable products that include B. oleracea because of the high concentration of bioactive compounds that they contain. This observed behavior may be attributed to the very high concentrations of glycosinolates, isothiocyanates, and polyphenols that are present in the edible section of the plant.

Molecular Markers

Every single marker has the potential to be regarded as an ideal genetic marker due to the significant number of distinctive qualities that it has. When it comes to expression, it should be co-dominant, have a single copy, be economical to employ, highly polymorphic, readily assayable, multifunctional, and highly accessible (which means that its use should not be restricted). Additionally, it should not have any negative consequences on the phenotypic in any way. It is feasible to automate and multiplex activities that are unique to the genome, particularly when working with polyploids. This is a very important point to keep in mind while dealing with polyploids.

1. Abundance of genes

The number of markers that may be developed is mostly determined by the frequency with which the locations of interest are located across the genome. This is the key criterion that determines potential markers. The quantity of markers that are generated by RFLPs and AFLPs is influenced by a number of variables, including the prevalence of restriction enzymes and the frequency of their recognition sites in genomes. It is important to keep in mind that both of these components are not difficult to acquire. There is a significant amount of microsatellites present in the genomes of eukaryotic creatures, as has been shown. The RAPD marker is yet another kind of marker that is easily available in regular situations. It is possible to conduct primer synthesis by making use of a vast number of random sequences, which is the explanation behind this innovation. Furthermore, if genome coverage and genomic abundance are simultaneously needed, it is of the utmost

importance to take caution while picking markers. In spite of the fact that it is common knowledge that some markers have a tendency to congregate in particular regions of the genome, it is also common knowledge that other markers, such as the AFLP markers, have a tendency to be dispersed rather widely throughout individual genomes.

2. Degree of polymorphism

The degree to which genetic markers are able to resolve genetic differences is determined by the polymorphism level, which is a function of the mutation rate in the genomic regions that are important. According to the information that we are able to glean from the figures, the frequency of mutations that occur at microsatellite and minisatellite loci is rather high. The roots of this mutation may be traced back to changes in the repeat unit count of the core sequence. When there is a larger degree of similarity between the samples, it is necessary to have a better resolving power.

OBJECTIVES OF THE STUDY

1. To study on Brassica species' bioactivity with the use of molecular marker techniques
2. To study on Molecular Markers

RESEARCH METHOD

Collecting and Storing Isolates of Alternaria Brassicae from Leaf Samples Damaged By Black Spots

In this study, the following Brassica crop hosts were utilized: mustard (Brassica juncea), cauliflower (Brassica oleracea L. var. botrytis L.), cabbage (B. oleracea var. capitata), and rape (B. campestris). Fields in Uttar Pradesh, India, located at a latitude of 26.01 degrees North and a longitude of 83.28 degrees East, were the locations from where the samples were gathered and then transported to the laboratory. After the collection of leaf samples, the procedure of isolating the various species of Alternaria was started.

Two millimeter diameter blighted leaf and stem sections were surface sterilized with 0.1% Mercuric Chloride ($HgCl_2$) for one minute in order to collect isolates from sick cauliflower and mustard leaves as well as other plant tissues. This was done in order to get isolates from sick plant tissues. Obtaining plant tissue isolates that were devoid of disease was the objective of this project. Following that, the pieces were submerged in sterile water for ten minutes during each rinse, and then they were placed on Potato Dextrose Agar (PDA) plates with a pH of 5.6.

Morphological Characters Evaluation

In order to determine the identities of each of the isolates, a light microscope (made by Carl Zeiss in Germany) and readily available literature (Ellis, 1971) were used. Evaluation of the morphology

of the PDA isolates was the objective of this endeavour. According to what was discussed earlier, single-spored colonies were used in order to ascertain the physical characteristics of the colony as well as the apparatus that was utilized for the sporulation process for each individual isolate. We took note of the characteristics of the mycelia that were proliferating and observed the emergence of the conidia. The ocular and stage micrometers were used in order to determine the size of the conidia, namely their longitudinal and transverse dimensions. Additionally, there were counts of septa that were recorded beside it.

All of the Alternaria species that were being cultured were maintained on PDA, which is an abbreviation that stands for potato dextrose agar. In a volume of water that was one thousand milliliters, twenty grams of dextrose, fifteen grams of agar, and two hundred grams of potato infusion were dissolved with the water. The content from Himedia was compiled into a synthesis by the personal digital assistant that was used. Following the autoclaving of the agar plates at 121 degrees Celsius for fifteen minutes, a sample of about twenty milliliters was transferred to sterile Petri dishes that had dimensions of ninety millimeters by two millimeters.

Characterization of Molecules

Following the extraction of DNA from each sample, the polymerase chain reaction (PCR) amplification was performed on the DNA in a 96-well microtiter plate. The plate was then put inside of a G-Storm heat cycler. For the purpose of conducting a single reaction with a volume of 25 microliters, the PCR mix was composed of the following components (Table one). The reaction mixture used for the amplification was the same as the one used for the evaluation of the negative controls; however, template DNA was not present. The circumstances were the same as before. We did this to ensure that the results were accurate and reliable. Following the completion of the amplification procedure, the product of the polymerase chain reaction (PCR) was prepared for gel electrophoresis.

Table 1: Components of the PCR master mix

Component	Stock concentration	Volume
Sterile distilled water	-	17.2 µl
PCR Buffer	10X	1.4 µl
dNTP mix	10mM	1.4 µl
Primer	10pmol	0.5 µl

Taq polymerase	3 units /µl	0.3 µl
DNA	25 ng /µl	2.5 µl
Total		**25 µl**

DNA Extraction:

The cultivation of all Alternaria species took place over a period of ten to twelve days using an agar medium that included potato dextrose. The biological oxygen demand (BOD) was measured at fifty milliliters, and the temperature was set at 25 degrees Celsius with a standard deviation of ±1). Yadav (2003) conducted an experiment in which five grams of blot dried mycelium was exposed to the modified CTAB procedure for the purpose of extracting genomic DNA. Consequently, DNA was successfully retrieved as a result of this. The mycelium was collected with the use of sterile filter paper, and it was then thoroughly cleaned with distilled water using the same method. In the last step, the mycelium was ground into a powder by utilizing mortars and pestles that had been pre-cooled with liquid nitrogen. On three separate occasions, each of these stages was carried out.

To proceed with the following step, a sterile spatula was used to transfer the finely powdered mycelium to centrifuge tubes with a capacity of fifty milliliters that had previously been sterilized. These tubes accommodated fifteen milliliters of DNA extraction buffer that had been heated to 650 degrees Celsius before being put inside. Furthermore, during the process of transferring the powdered mycelium to the DNA extraction solution, a total of 300 microliters of α-mercaptoethanol was added to the extraction buffer. A hundred milliliters of a variety of substances were included in the extraction buffer. These substances included twenty milliliters of 10% CTAB, thirty-five milliliters of 4.0M sodium chloride, four milliliters of 0.5 M EDTA, ten milliliters of 1 M Tris HCl, and two milliliters of v-mercaptoethanol. The powdered samples were well mixed with a pre-sterilized spatula before being heated to 650 degrees Celsius for one hour in a water bath. This was done before the samples were heated. After this, the mixture was stirred for a period of two minutes using a vortex. At least three times every fifteen minutes, the samples were turned over in order to ensure that they were well mixed.

Analysis of the internal transcribed spacer (ITS) region:

For the purpose of rDNA repeat amplification, the 3' end of the 18s gene and the 5' end of the 28s gene were selected as the particular targets for the PCR conditions. The two universal primers that were used were an ITS1-3' TCC GTA GGT GAA CCT GCG G 5' and an ITS4-3' TCC TCC GCT TAT TGA TAT GC 5'. For the purpose of producing these primers, researchers searched for sections of the eukaryotic rRNA gene that have been conserved (White et al., 1990; Jasalavich et al., 1995). The synthesis of primers such as these took place. Within the scope of this study, thirty distinct A. brassicae isolates were analyzed for their characteristics. For the purposes of the

polymerase chain reaction (PCR) amplification operations, a total volume of fifty microliters was used.

Table 2: Internal transcribed spacer (ITS) primer DNA sequences

Target sequences	Primer name	Sequence	Fragment size (bp)
Eucaryotic DNA	ITS1	5'-TCC GTA GGT GAA CCT GCGG-3'	500-600
	ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	

DATA ANALYSIS

Study and Isolation of Alternaria Brassicae From Uttar Pradesh's Brassica-Growing Areas

From diseased leaves of Brassica juncea and B. rapa var. toria that displayed characteristic signs of Alternaria leaf spot produced by A. brassicae, a total of thirty isolates of Alternaria brassicae were recovered. These isolates were collected from the leaves of the two species. The collection of these isolates took place at a number of different areas around the state of Uttar Pradesh. These locations include Kanpur, Allahabad, Varanasi, Moradabad, Mau, Ballia, Basti, Ghazipur, Lucknow, and sonbhadra. Between January 25, 2012 and March 25, 2013, these isolates were collected.

The collection took occurred between those two dates. After being cleansed three to four times in distilled water that had been sterilized, the surface was then sterilized by immersing it in a solution of 4% sodium hydroxide for one minute, and then washing it three to four times with sterilized water. This process was repeated three to four times. The leaf spot pieces were placed into 9 cm Petri plates that contained Potato Dextrose Agar (PDA) after the surface sterilization procedure was completed.

A. Brassicae: A Morphological Characterization and Identification

It was found that there were differences in the rates of linear growth and the color of the colony among the thirty isolates of A. brassicae that were recovered from the infected mustard leaves. These were revealed via the process of microbiological analysis. As can be seen in Figure 5, there was not a great deal of variation in the color of the colonies and the conidia that were produced on PDA. From a light olive gray to an olivaceous black spectrum, the color of the A. brassicae isolates may be described as light olive gray. There are two conceivable colors for the mycelia: brown and golden. Both of these colors are possible. Furthermore, the characteristics of the conidia were consistent across all of the isolates when compared. As an example, the conidia had a surface that was mostly smooth and had a color that was either golden or brown. The vast majority of the

conidia were fashioned like obpyriform projections and had a significant beak that was somewhat long.

In accordance with the data shown in Table 2, the thirty single-spore cultures of A. brassicae displayed morphological variability in terms of the length of the conidia, the width of the conidia, the length of the beak, and the number of septa. On average, the length of the conidial region varied between 31.2 and 51.4 µm, with a range that included 24.4–54.4 µm. In terms of conidial length, the Mau isolate (AB-16) was found to have the greatest length, measuring 51.4 µm. On the other hand, the Kanpur isolate (AB-15) had the smallest conidial length, measuring 31.2 µm. The average width of the conidial region, which varied from 6.2 to 9.5 µm (with a range of 5.0 to 12.0 µm), was 9.4 µm in the isolates obtained from Kanpur (AB-03), Ballia (AB-07), Kanpur (AB-15), Ghazipur (AB-18), Etawah (AB-25), and Ghaziabad (AB-30). On the contrary, the breadth of the isolates obtained from Lucknow (AB-02), Ballia (AB-07), Kanpur (AB-15), Ghazipur (AB-18), Etawah (AB-25), and Ghaziabad was measured to be 6.8 µm, respectively. The Mau isolate (AB-28) had the greatest beak length, measuring 20.6 µm, while the Kanpur isolate (AB-15) had the smallest beak length, measuring 10.2 µm. This was due to the fact that the average beak length varied from 10.2 to 20.6 µm, with a range that extended from 7.2 to 28.0 µm. It was found that the isolates from Anpara (AB-05) and Moradabad (AB-12) had the highest average number of transverse septa, which was 7.2. On the other hand, the isolate from Kanpur (AB-15) had the lowest average number, which was 3.5.

Fig 1: Alternaria brassicae symptoms on oilseed rape

Table 3: Internal transcribed spacer (ITS) and species-specific primer DNA sequences utilized in this investigation

Target	Primer	Sequence	Fragment size

sequences	name		(bp)
Eucaryotic DNA	ITS1	5'-TCC GTA GGT GAA CCT GCG G-3'	500-600
	ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	
Alternaria spp.	AS-F	5'-ACACTGCTTCAGCATTTTTCTTCATAG-3'	226 bp
	AS-R	5'-TTGTCCAATTCATGGTATAGCACTCA-3'	
A.brassicae specific	ABCsens	5'-CTGGTGAAAAGGTTGCGATCGT-3'	780bp
	ABCrev	5'-GTGACTTTCATGAAATGACATTGATG-3'	
ERIC	ERIC1	5'- ATGTAAGCTCCTGGGGATTCAC-3'	
	ERIC2	5'-AAGTAAGTGACTGGCGTGAGCG	
RAPD fungal half set primers			

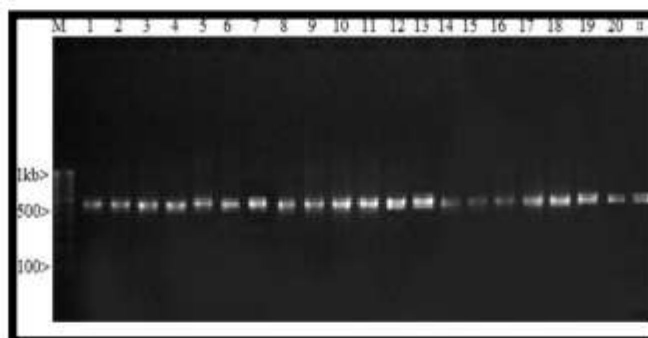

Fig 2: ITS region amplification using ITS 1 and ITS4 primers from several Alternaria brassicae isolates. Lane M: 100 bp ladder; Lanes 1–21 show several Alternaria brassicae isolates

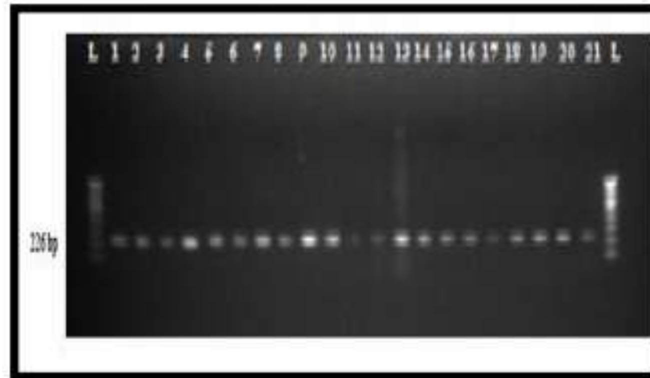

Fig 3: Amplification of several Alternaria brassicae isolates using a primer unique to the genus. Lane L: 100 bp ladder; Lanes 1–21 show several Alternaria brassicae isolates

Fig 4: Amplification of several Alternaria brassicae isolates using a species-specific primer. Lane L: 100 bp ladder; Lanes 1–21 show several Alternaria brassicae isolates

Submicridal Variability

Polymorphic DNA amplification at random (RAPD):

For the purpose of this work, the random amplified polymorphic DNA (RAPD) techniques were used to explore the genetic diversity of twenty-one Alternaria brassicae isolates. These isolates were collected from a variety of places within the state of Uttar Pradesh in India. During the preliminary investigations, a fungal primer-half set was tested with five distinct isolates, one of each species of Alternaria. The purpose of these tests was to find primers that not only indicated great amplification but also exhibited a consistent band pattern.

Through the use of the RAPD-PCR technology, a fingerprinting pattern was generated that was extremely distinctive, indicating that the many Alternaria isolates that were created exhibited a considerable level of variation. The amount of bands that were amplified did not stay consistent throughout the experiment since it was dependent on the primers or the isolates that were used.

Only five of the ten primers (RFu-C1, RFu-C2, RFu-C4, RFu-C9, and RFu-C10) were able to establish a fingerprint pattern that was both unique and reproducible. Furthermore, there was a high degree of diversity across the isolates. Figure 14 illustrates that each of the isolates produced a different quantity of fragments with their own unique characteristics.

Our ability to establish the degree of relatedness that existed between the isolates was made possible by the use of polymorphic DNA bands that were able to be scored. RAPD was also used in order to determine the degree of genetic similarity between the isolates, and Jaccard's coefficient was utilized for this purpose. In Figure 14, a dendogram that was developed is presented, and in Figure 15, a three-dimensional distribution of the isolates that was based on RAPD is displayed. Both figures are given in the document. The replicable DNA fragments that were scored for the development of the Dendogram revealed that there was a wide range of variation among the isolates, with 0.70-0.94% of genetic similarities. This was shown by the fact that the Dendogram was constructed.

CONCLUSION

Genetic variation, we are talking to the changes that occur within the genes that make up an individual of a species. These changes may be found in the constitutions of heredity. For the sake of preserving the developmental stability and biological potential of plant species, this variety is absolutely necessary. It is possible to determine whether or not a species has an adequate quantity of genetic resources by observing the degree of genetic diversity that exists within variations and the degree of differentiation that exists between variants. In order to differentiate the Brassica variants that were explored, the findings of our present inquiry indicated that there were changes in the position, amount, and staining intensity of protein bands among the variants that were investigated. This was done in order to differentiate the versions of Brassica that were investigated. This is an example of the use of polyacrylamide gel electrophoresis, often known as PAGE. There was a wide range of genetic distance detected across the thirteen distinct varieties of Brassica, in addition to a high frequency of polymorphisms (89.47%) that was found. The detection of a wide range of polymorphism and genetic distance brought to light the presence of a huge range of variety across Brassica species. This diversity occurred throughout a wide range of genetic distances. The variants of BS-10 and BS-14 have the largest amount of genetic variety with regard to the variations that were used in this study. On the other hand, the variants of BS-9 and BS-12 contain the least amount of genetic diversity. The potential exists that variations that are found in close vicinity to one another in terms of their origin, physical traits, and breeding technique are less genetically distant from one another. This is because of the near closeness of these variants. The results of this inquiry revealed that the variations of BS-10, BS-9, BS-12, and BS-14 may give the facilities essential for selection as parental source in the subsequent breeding program to develop Brassica variants in India. This was the conclusion that was reached as a consequence of the findings included in this investigation.

REFERENCES

[1.] Jain KK. (2002). Current trends in molecular diagnostics. Med. Device Technol. 13: 14-18.

[2.] Jasalavich CA, Morales VM, Pelcher LE and Seguin Swartz G. (1995). Comparision of nuclear ribosomal DNA sequences from Alternaria species pathogenic to crucifers. Mycological Research 99: 604-614.

[3.] Jhanji, Shalini & Kaur, Gurpreet & Kaur, Ravijot & Dhatt, Ujjalpreet. (2022). Physiological and biochemical changes during flower development and senescence in Chrysanthemum and Gladiolus. Acta Physiologiae Plantarum. 45. 10.1007/s11738-022-03486-4.

[4.] Kashyap PL and Dhiman JS. (2010). Eco-friendly strategies to suppress the development of Alternaria Blight and black rot of cauliflower. World Applied Science Journal. 9 (4): 345–350.

[5.] Kaur S, Singh G and Banga SS. (2007). Documenting variation in Alternaria brassicae isolates based on conidial morphology, fungicidal sensitivity and molecular profile. (in) Proceeding of the 12th International Rapeseed Congress, 26–30 March, Wuhan, China 4: 87–89.

[6.] Labuda R, Jr PE, Sert H and Sterflinger K. (2008). Alternaria jesenskae sp. nov., a new species from Slovakia on Fumana procumbens (Cistaceae). Microbiol. Res. 163: 208-214.

[7.] Laura S and Juha H. (2004). Do inspection practices in organic agriculture serve organic values? A case study from Finland. Agriculture Human Values 21: 1-13.

[8.] Laxminarayana P and Reddy SM. (1976). Post harvest diseases of some cucurbitaceous vegetables from Andhra Pradesh. Ind. Phytopathol. 29: 57-59.

[9.] Maude RB, Humpherson-Jones FM. (1980). Studies on the seed-borne phases of dark leaf spot (Alternaria brassicicola) and gray leaf spot (Alternaria brassicae) of brassicas. Annals of Applied Biology 95: 311-319. [DOI: 10.1111/j.1744-7348. 1980.tb04752.x]

[10.] McKay GJ, Brown AE, Bjourson AJ and Mercer PC. (1999). Molecular characterisation of Alternaria linicola and its detection in linseed. European J. of Plant Path. 105: 157-166.