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RESEARCH ARTICLE

ROLE OF BIOTECHNOLOGY IN DISEASE MANAGEMENT OF OIL SEEDS (MUSTARD).

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Abstract

Indian mustard is one of the major *rabi* oilseed crop in India. Continuous improvement in rapeseed-mustard has resulted in nutritionally superior edible oil, and meal as an important source of protein in animal feed. The crop is affected by various biotic and abiotic stresses. There are many biotic stresses which affect the production of Mustard .Many attempts have been made by the scientists to improve Brassica using molecular markers like Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Single Nucleotide Polymorphism (SNP) based maps (Ramen *et al.*, 2014) etc. Somaclonal variation as a tool for creating in vitro variability offers a unique opportunity for desirable attributes. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), AFLP and SSR have been used for improving selection efficiency and selecting plant genotypes with the desired combinations of traits. Transgenic approaches have been followed to develop the transgenic for aphid resistance, male sterility, AB tolerance, herbicide resistance and drought tolerance. Bar, Barnase and Barstar based herbicide resistance and genetic male sterility have been used in the development of experimental hybrids.

Introduction:-

Brasssica juncea (L.) Czern & Coss., also known by the name of Indian mustard, belongs to the family *Brassicaceae* (Syn. *Cruciferae*). The family currently includes 3709 species and 338 genera (Warwick et al., 2006) and is one of the ten most economically important plant families (Rich, 1991). *Brassica juncea* (2n=36) is an amphidiploid species derived from interspecific cross between *Brassica nigra* (2n=18) and *B. rapa* (2n=20). Over the past couple of decades, these crops have become one of the most important sources of vegetable oil in the world. Continuous improvement in rapeseed-mustard has resulted in nutritionally superior edible oil, and meal as an important source of protein in animal feed. Rapeseed mustard crops are commercially cultivated in more than 60 countries and major produces include China, Canada, India, Australia, France, Germany, United Kingdom, Poland, Ukraine, Russia, USA and Czech Republic. The crop is affected by various biotic and abiotic tresses. There are many biotic stresses which affect the production of Mustard.

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Major Diseases, Causal Agents and their Control in Managed Ecosystem

Among various diseases, 4 diseases viz; Alternaria blight (*Alternaria brassicae*), White rust + Downy mildew complex (*Albugo candida* + *Hyaloperonospora brassicae*), white rot (*Sclerotinia sclerotiorum*) and Powdery mildew (*Erysiphe cruciferarum*) are of great economic importance. Among a number of other relatively less important diseases, seedling blights/ Damping-off *(Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium solani*), Phyllody (caused by *Sesame Phytoplasma*), Bacterial rot (*Xanthomonas campestris pv campestris*), Club root (*Plasmodiophora brassicae*) and Mosaic (*Turnip Mosaic Virus*) appear to become important only under specific agro-ecological conditions in certain geographical areas and hence are assumed to be of regional and sporadic importance (Kolte, 1985).

Damping off and Seedling Blight

Many fungal species such as *Alternaria, Fusarium, Phoma*, and *Rhizoctania solani* are involved in causing seed rot and seedling blight. Among them, Rhizopus stolonifer is reported to be more important (Petrie, 1973). Disease occurs all around the world in Rapeseed-mustard. Damping-off can produce many symptoms ranging from pre-emergence rot (failure of plants to emerge) to post emergence damping-off (plants emerge and collapse at ground level). The pathogens involved in India, cause 6-15% incidence (Khan and Kolte, 2002).

Alternaria Blight:

Alternaria blight or black spot, the most common, widespread and destructive disease is caused by *Alternaria brassicae* (Berk.) Sacc. infecting all above ground parts of the plant. In India, the disease is severe mainly in the states of Himachal Pradesh, Haryana, Rajasthan, Uttar Pradesh, Uttara Khand, Bihar and Madhya Pradesh, but appears in almost all the parts of the country. The symptoms of disease are formation of brown to black spots with concentric rings on leaves, stem and siliquae (Meena et al., 2010a).

White Rust

White rust, caused by *Albugo candida* (Pers. Ex Fr.) Kuntz. is an obligate pathogen of all cruciferous crops and reported to be infected by *A. candida* (Biga, 1955) all over the world.

(1) **(2)** **(3)**

Fig1:-Showing the disease symptoms of (1) Alternaria Blight (2) White Rust (3) White Rot

Disease appearing on leaves is characterized by the appearance of white or creamy yellow raised pustules up to 2 mm in diameter, which later coalesce to form patches (Verma, 2012). The result in yield loss up to 47% (Kolte, 1985).

Sclerotinia Rot or White Rot:

Rot of mustard caused by *Sclerotinia sclerotiorum*. The pathogen is *Sclerotinia sclerotiorum* (Lib.) de Bary. Mycelium is thin, 9-18µm in diameter with lateral branches of smaller diameter than the main hyphae. The sclerotia are black, round or semi spherical in shape measuring 3-10 µm. One to several apothecia may grow from a single sclerotium. Ascospores discharged from the apothecia at the base of the plants in soil constitute important primary sources of infection.

Powdery Mildew:

Occurrence of powdery mildew on Rapeseed-mustard is reported from various parts of the world. Recent reports on the occurrence of powdery mildew of Rapeseed mustard deal with *Erysiphe cruciferarum* (Sharma, 1979). The symptoms appear in the form of dirty-white, circular, floury patches on both sides of lower leaves of the infected plants. The floury patches increase in size and coalesce to cover the entire stem and leaves under environmental conditions favourable to the pathogen.

(4) (5) (6)

Fig 2:-Showing the disease symptoms of (4) Powdery Mildew (5) Club Root(6) Bacterial rot

Club Root:

Club root disease of the Brassicaceae has been a major threat to the crop caused by *Plasmodiophora brassicae* Woronin. Incidence and severity are greater in regions of extreme winters. The disease is reported to occur in East Germany, Malaya, New Zealand, Poland, Sweden, United Kingdom and the U. S. In India, the disease has been reported from hills of Darjeeling Nilgiri (Rajappan et al., 1999) on vegetable Brassicas. The disease has been reported from West Bengal and Orissa, respectively with losses in yield being up to 50% (Laha et al., 1985; Chattopadhyay, 1991).

Fusarium Wilt :

Rapeseed-mustard is affected by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *conglutinans* (Wr.) Snyder and Hansen. Distribution: The first authentic report of *F. oxysporum* f. sp. *conglutinans* as the cause of the disease in B. juncea was made from India by Rai and Singh (1973). Yield losses of greater than 30% are common. The affected plants show drooping, vein clearing and chlorosis of leaves, followed by wilting and drying, resulting in the death of the plant.

Bacterial Stalk Rot

The occurrence of stalk rot caused by *Erwinia carotovora* (Jones) Holland was reported first by Bhowmik and Trivedi (1980). On an average, about 60-80% of plants were affected by the disease in a farmer's field in Mahua Simpani village of Bharatpur district of Rajasthan (Meena et al., 2010b). The bacterium can infect *Brassica oleracea* var. *Botrytis, Daucus carota, Lycopersicon esculentum* and *Nicotiana tabacum*. Disease symptom: The disease is characterized by the appearance of water-soaked lesions on the collar region of plants, which are usually accompanied by a white frothing.

Bacterial rot:

The pathogen is *Xanthomonas campestris pv. campestris* (Pammel) Dowson.The black rot symptom on *B. juncea* was first observed in India by Patel et al. (1949). The disease is now reported to occur in a severe form in the State of Haryana (Vir et al., 1973; Gandhi and Parashar, 1978). Disease appears when the plants are two-months old. In the initial stages, dark streaks of varying length are observed either near the base of the stems or 8 to 10 cm above the ground level. These streaks gradually enlarge and girdle the stem.

Application of Biotechnological tools in management of disease in Brassica:

Biotechnological interventions have also been made in Indian mustard breeding programme. Many attempts have been made by the scientists to improve *Brassica* using molecular markers like Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Single Nucleotide Polymorphism (SNP) based maps (Ramen et al., 2014) etc. Abraham et al. (2000) at BARC Mumbai, reported somaclonal variants from mesophyll protoplast in *B. juncea* cv Rai 5 which showed 3-5 days early flowering. Commonly, some techniques e.g. tissue culture techniques to create somaclonal variation, anther culture to produce haploids and homozygous lines, protoplast culture and somatic hybridization, marker- assisted selection, development of transgenics for biotic and abiotic stresses have yielded promising results. Optimization of regeneration protocols have been 9. Biotechnological developments in Brassica achieved for most of the *Brassica species* using different explants such as cotyledons, hypocotyls, leaf segments and protoplasts, cotyledonary petiole and shoot apex (Verma and Singh 2007).

Management of diseases of Mustard using tissue culture techniques:
Somaclonal variation as a tool for creating in vitro variability offers a unique opportunity for desirable attributes. A somaclone of Varuna, BIO-902, has been released as a variety which possesses shattering resistance along with high yield (Katiyar and Chopra, 1995). Prakash et al., (2004) reported regeneration of normal plants by culturing anthers of CMS line of *B. juncea* carrying Diplotaxis erucoides cytoplasm. Somatic cell fusion of sexually incompatible species has also been made possible through production of somatic hybrids which have been utilized for transfer of desirable traits from parents to hybrids. Inter-specific hybrids were produced by fusing mesophyll protoplast of B. juncea and *B. spinesceens* (Kirti et al., 1991). Prakash et al. (1998) developed a male sterility and fertility restoration system in *B.juncea* by protoplast fusion with Moricandia arvensis.) These CMS lines were found to be chlorotic. Protoplast fusion of chlorotic male sterile *B. juncea* with green male sterile *B. juncea* resulted in green male sterile plants (Kirti et al., 1998).

Management of diseases of Mustard using molecular marker technology and transgenic Approaches:
Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), AFLP and SSR have been used for improving selection efficiency and selecting plant genotypes with the desired combinations of traits. Markers linked with white rust resistance (Prabhu et al., 1997), fatty acids, oil content, yellow seed colour and fertility restoration have been reported. Transgenic approaches have been followed to develop the transgenic for aphid resistance, male sterility, AB tolerance, herbicide resistance and drought tolerance. Bar, Barnase and Barstar based herbicide resistance and genetic male sterility have been used in the development of experimental hybrids (Jagannath et al,. 2002). Transgenics expressing Cod A (from A. glabiformis) gene for tolerance against abiotic stresses (salt and drought) have also been reported (Singh, 2003). Lectin gene for aphid resistance and DREB gene construct for drought tolerance are being used. Osmotin (from tobacco) for drought and salt tolerance, annexin gene for stress tolerance, Chitinase and Glucanase (from *Arabidopsis*) for tolerance to Alternaria blight disease and FAE1 gene for low erucic acid mustard cultivar are other transgenes being used in Rapeseed-mustard.

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