Rice is the principal food crop of more than half of mankind. It provides 20% of global human per capita energy and 15% of per capita protein (IRRI Rice Almanac, 1993). Global rice production needs to be raised by another 300 million tons in the next 30 years to meet the increasing food requirements. The area cultivated under rice is so large (about 56 million hectares worldwide) that any marginal increase in yield would substantially influence the total rice production (Manibhushanrao, 1995). The grain yield in rice is influenced by a number of environmental factors like high and low temperatures, salinity etc., and biological factors like diseases, pests etc. Among the fungal diseases, sheathblight is considered as one of the major production constraints for rice. The principal components of the green revolution namely, intensive cultural practices, especially, close planting and heavier application of nitrogenous fertilisers on improved varieties, leading to the change of the microclimate of the crop favour the disease incidence. Further, the farmers also create an ideal situation for the spread of the disease by using a single glamorous variety and also by applying high doses of Nitrogen fertiliser (about 200 kg/ha and above). It is an excellent example to illustrate how a minor disease could become a major one due to the shifting in varietal pattern coupled with modification of agronomic practices.

In this chapter, an attempt has been made to review information on causal organism, disease development, pathogenic variability and characterization of rice sheathblight fungus.

CAUSAL ORGANISM

Rice sheathblight disease is caused by the basidiomycetous fungus anamorph or imperfect sclerotial stage *Rhizoctonia solani* with the teleomorph or perfect basidial stage *Thanatephorus cucumeris* (Frank.) Donk. (Parmeter, 1970). It infects all parts of rice plant (except roots in submerged condition) at all stages of growth (Ou, 1985). *R. solani* is both a pathogen and a saprophyte; it aggressively colonises organic debris and is one of the fastest growing fungi. The fungus is multinucleate and the cytoplasm is interconnected through a septal pore (doliopore) that
is characteristic of *basidiomycetes*. The fungus forms *sclerotia* and basidiospores. The basidiospores are white and powdery and are found on the host plants only around the booting stage of rice plants. The sclerotia form as soil borne propagules, which are knots of undifferentiated, intertwined, pigmented, moniloid cells.

**ANASTOMOSIS GROUPING (AG)**

On the basis of different anastomosis reactions, the isolates of *Rhizoctonia* are classified into 12 Anastomosis Groups (AGs) (designated as AG1 through AG 11 and AG B1 (Carling and Kuninaga, 1990). The grouping of isolates is based on their ability to anastomose. Hyphae of the isolates belonging to a common group make contact and their cell walls fuse and plasmogamy occurs. Plasmogamy between isolates belonging to different groups is followed by lysis of several cells on each side of the fusion, such that their contents are visibly vacuolated. Each Anastomosis Group (AG) represents a non-interbreeding population and a genetically independent entity. Characterisation and grouping of isolates using the AG system represents a valuable approach for understanding variations within the species as well as identifying characteristics of the various groups inciting plant diseases. Again within these AGs, by pathogenic variations, DNA-DNA homology, serological studies, fatty acid analysis, protein electrophoresis and nutrient utilisation, **Intra Specific** Groups (ISG) are recognised (Kuninaga and Yokosawa, 1982-85). A more recent concept has been to classify isolates on the basis of the pectic enzymes produced during growth on pectin (Sweetingham *et al.*, 1986), according to which isolates are divided into pectic Zymogram Groups (ZG).

The sheathblight fungus belongs to AG I having 3 to 16 nuclei (Ahuja and Payak, 1985). Based on morphological and pathogenic characters, AG 1 can be divided into three **Intra Specific** Groups, viz., IA, IB and IC. The rice sheathblight pathogen was placed in sasaki type IA (Watanabe and Matsuda, 1966).
OCCURRENCE AND ECONOMIC IMPORTANCE

The disease now widespread in the East and South East Asian countries, was first recorded from Japan by Miyake (1910). It was first reported in India by Paracer and Chahal (1963). Besides the Oriental countries, sheathblight now occurs in Brazil, Venezuela, Surinam, Madagascar, USA, Fiji, Papua, New Guinea, Sub-Saharan Africa, Nigeria and Iran (Ou, 1985).

The disease is considered second in importance after blast disease. Various estimates of crop losses due to sheathblight have been made and losses usually vary from negligible to 50% (Lee and Rush, 1983). The pathogen R. solani is a soil borne saprophyte with a wide host range and world wide distribution (Ogoshi, 1987). Kozaka (1965) recorded 188 species in 32 families from Japan. Dath (1990) listed plant species recorded as hosts from India. The pathogen invades all parts of the crop plants at all age levels in different climatic conditions (Baker, 1970).

DISEASE DEVELOPMENT

The primary infection of ShB comes from sclerotia. Although basidiospores of this fungus have been reported from India, their role in pathogenesis is negligible (Singh and Pavgi, 1969). Basidiospores can initiate infection, but do not play important role in the epidemiology of this disease (Kozaka 1970, 1975; Chin, 1976).

Two types of mycelia-straight and lobate are present, of which only the latter type is infectious. Lesion is covered by lobate mycelium while the straight type may extend beyond it without causing infection. Infection pegs develop from lobate mycelium forming infection cushion which penetrates through cuticle or stomata. Lesions in the initial stages appear as circular, oblong or ellipsoidal, greenish gray, water-soaked spots about 1 cm, which are later enlarged to 2 - 3 cm in length, with irregular dark brown margins, while the center is bleached to grayish white. Soon after the primary lesions are formed, mycelia raising from the lesions spread rapidly upwards or towards the lateral sides directly through air spaces or interstices between the sheaths and along the outer
surface of the plants (Yoshimura, 1954; Kozaka, 1961). Under favorable microclimatic conditions, the lesions coalesce and encompass the entire leaf sheaths and stems and reach to panicles (Ou et al, 1973). Severely diseased plants often snap at lesion sites on upper plant or lodge entirely on the ground.

Sheathblight is usually severe on cultivars that are short, high tillering and responsive to high fertiliser in comparison to tall cultivars with fewer tillers (Lee and Rush, 1983). The intensity of primary infection rests mostly on the number of sclerotia which are in contact with the plant at the water surface. Subsequent disease spread is governed by the favourable environmental conditions as well as susceptibility of the host plant. Under the conditions of high humidity (ca. 95% RH) and high temperature (25 - 32° C) runner hyphae spread rapidly to the upper parts of the plant and to adjacent plants. Kozaka (1961) reported two distinct phases of disease development, the vertical i.e. upward spreading and the horizontal i.e., to the neighboring plants and fields. Both these developments occur rapidly under favorable conditions, while the vertical spread generally occurs only after heading stage. R. solani can attack rice plants at any growth stage right from seedling. Yoshimura and Nishizawa (1954) observed that maximum tillering stage is the optimum for varietal testing and Roy (1979) recorded active tillering as the most susceptible stage.

**HOST - PLANT RESISTANCE**

Studies on the mechanisms of host resistance are hindered due to the lack of rice cultivars resistant to tf.w/am( Marshall and Rush, 1980). Crill et al (1981) tested more than 20,000 selections from the IRRI rice germ plasm to evaluate their reaction to ShB disease and Tapoochoz, Bahagia and Laka were least affected and cultivar IR 1487-372-1-1 was considered as the most susceptible. Among 6000 rice cultivars collected from more than 40 countries of the world, no rice cultivar exhibited clear resistance to rice sheathblight (Hashiba, 1984). It has been shown that susceptibility of rice to this disease is greatly influenced by physiological, morphological and ecological factors.
Knowledge of the inherent level of resistance in rice cultivars to sheath blight disease is far from adequate. Resistance may be dominant and monogenic (Marshall and Rush, 1980; Ou, 1985), governed by two pairs of complementary genes and modified by epistasis, (Goita, 1985) or, most possibly and nearly universally, incompletely dominant due to multiple genes. Studies on the inheritance reactions with virulent isolate RH-9 using Tetep, IET 4699, Jawn no. 14 and Yedao as resistant donors and IR 9752-72-3-2 as susceptible parent revealed that the F1 of four combinations of moderately resistant donors and susceptible parent showed intermediate reactions to inoculation and the F2 distributions tend to be continuous implying that resistance to ShB is controlled by multiple genes (Sha and Zhu, 1989). Attempts have been made to locate ShB resistance in wild rice by Amante et al (1990). Accessions from Oryza minuta, O.rufipogon and O.eichengeri may serve as donors of ShB resistance for rice improvement. There is a great need for more work to identify sources of resistance genes from primary and secondary gene pool. Wax thickness is correlated with resistance (Ou, 1985).

Indica cultivars are usually more tolerant than Japonica cultivars (Roy, 1979). Short culmed and high tillering rice cultivars provide a favorable microclimate for disease development at early stages of plant growth than tall varieties with few tillers (Lee and Rush, 1983; Marchetti, 1983).

MANAGEMENT

Current control methods of sheathblight in rice depend upon chemicals, cultural management, biological control and use of so-called tolerant cultivars, all of which may be used in an integrated manner. Chemical control is the most effective counter measure against sheathblight. Organo-synthetic chemicals such as validamycin, mepronil, diclomezine etc are being applied. However, the use of chemicals is expensive and potentially damaging to the environment and over time chemicals become less efficient due to adaptation of the pathogen. Cultural manipulations are not an ideal solution to sheathblight control in rice as the measures such as crop rotation involve
hidden losses that are unacceptable to specialist growers or those with few alternative crops. Cultural operations such as destruction or burning of infected crop residue and destruction of collateral hosts were suggested as a part of integrated management. Cultural methods can control the disease to some extent, but, as the only means of control, these methods are usually ineffective. Various fungi such as *Trichoderma* sp., *Aspergillus* sp., *Neurospora crassa* can inhibit mycelial growth, cause hyperparasitism and lysis and exhaust substrates early and rapid competitive saprophytic colonization (Gangopadhyay and Chakrabarti, 1982; Mew and Rosales, 1984; Manibhushanrao et al., 1989). Vasanthadevi et al. (1989) reported the loss of viability of sclerotia in soil by various bacteria like fluorescent and non-fluorescent pseudomonads, enterobacteria like *Bacillus subtilis* etc. Though the use of resistant cultivars has been one of the most effective and economic means of combating the disease, no commercially acceptable level of resistance to sheathblight pathogen has been identified in rice so far.

**CHARACTERS ASSOCIATED WITH PATHOGENECITY**

Although the AG concept correlates to some extent with pathogenecity, evidence from several studies suggests that there is considerable variation between isolates from the same AG and the pathogenecity of *R. solani* cannot be explained solely in terms of AG or ZG (Ogoshi, 1987; Jabaji-Hare et al., 1990; Vilgalys and Gonzalez, 1990). Size of sclerotia is related to pathogenecity. A positive correlation between number of sclerotia and disease severity was found, but not between plant density and sclerotia on soil surface (IRRI, 1986). Newton and Mayers (1935) were the first to detect a toxic principle in the culture filtrates of *R. solani*. The pathogen was reported to produce several low molecular non-enzymatic, extracellular, phytotoxic metabolites namely phenyl acetic acid (PAA) and its hydroxy (meta, ortho and para) derivatives (Ramalingam, 1981). Manibhushanrao and Ramalingam (1993) obtained a positive correlation between toxin production and the relative virulence of the ShB fungus isolates (R1 to R5). However, the quantitative correlation of the virulence of the isolates with toxin production is not easily interpreted since toxin production *in vitro* is
influenced by the composition of the medium and the physical environment Pectin transeliminase (Lin, 1976) and Phosphatidase (Kuan and Kuo, 1974) have been implicated with pathogenesis. In studies with two \textit{R.solani} isolates differing in virulence, Pectin lyase production was detected to take place earlier than Pectin \textit{Methyl} esterase and was found to be greater in more virulent isolate (Ramalingam, 1983).

In studies on electrophoretic patterns of mycelial proteins and \textit{isozyme} patterns of \textit{poly} phenol oxidase, peroxidase, acetyl esterase and acid and alkaline phosphatase of differentially virulent isolates of \textit{R.solani}, no relation could be obtained with enzyme patterns and virulence (Zuber and Manibhushanrao, 1982). Further, aminoacid composition of differentially virulent isolates of \textit{R.solani} could not reveal any specific correlation with their relative virulence rating (ManibhushanRao \textit{et al}, 1987).

Recent evidence shows that cytoplasmic determinant is required for virulence in \textit{R.solani} (Finkler \textit{et al}, 1985). All virulent isolates examined so far contained several double stranded (ds) RNA segments, at least some of which were encapsulated in \textit{isometric} particles, whereas hypovirulent isolates had either no ds RNA or fewer ds RNA segments. The virulent factor could be transferred along with the ds RNA segments, from a virulent strain (resistant to \textit{benomyl}, sensitive to BTN fungicide, melanin producing and with large \textit{sclerotia}) to hypovirulent strain (sensitive to benomyl, resistant to BTN, no melanin produced, small hyaline sclerotia) by hyphal anastomosis, giving a virulent strain possessing all the nuclear markers of the hypovirulent strain. Hence virulence factor was reasoned to be cytoplasmic. Proof of the role of one or more specific segments of ds RNA in virulence will require infection and/or transformation of protoplasts with isolated ds RNA. In this respect it is noteworthy that Martini \textit{et al} (1978) have suggested a DNA plasmid which may be required for the pathogenicity of \textit{R.solani} and Hashiba \textit{et al} (1984) reported the presence of a plasmid DNA designated as pRS64 in \textit{R.solani} which was weakly pathogenic. These results do suggest that there is a possible relationship between extrachromosomal DNA and pathogenicity.
Three extra chromosomal elements were described that putatively diminish virulence in *R. solani*. The first was a double stranded RNA in AG 1, Castanho *et al* (1978) reported that a degenerative decline occurred in a severely diseased isolate of *R. solani* that has been associated with an assortment of double stranded (ds RNA) segments of three different sizes. The second was a covalently closed circular DNA plasmid (Martini *et al.*, 1978). The third was a linear DNA plasmid in AG 4. A double stranded DNA plasmid, designated pRS 64, was detected in three isolates of AG 4 by biophysical methods. Weakly pathogenic isolates contained the plasmids, but pathogenic isolates contained no detectable plasmid DNA (Hashiba *et al.*, 1984), and the plasmids were isolate-specific and could not be transmitted to other isolates by hyphal anastomosis. Out of 114 field isolates of *R. solani*, 48 isolates showed plasmid like DNA. These 48 isolates were distributed among AGs and ISGs recognized among Japanese isolates of *R. solani*. Each isolate carried one, two or three plasmid DNAs identified by gel electrophoresis (Miyasaka *et al.*, 1990).

**PATHOGENIC VARIABILITY**

The fungus is well known for its versatility as plant pathogen, a parasite, a symbiont or a facultative saprophyte and has a wide host range from semiarid plants to aquatic plants. Isolates of *R. solani* show tremendous variation in characteristics, such as morphology, pathogenecity and host specificity. There is need for more genetic information on the pathogen to provide a basis for understanding the high degree of pathogenic variability. The fungal isolates of sheath blight are highly variable in their aggressiveness (*Amaral et al.*, 1979; Zuber and Manibhushanrao, 1982; Dath, 1985). Many isolates from a wide variety of plants are pathogenic on rice. Chien and Chung (1963) grouped 300 isolates into 7 cultural types and 6 physiological races. Twenty strains were identified in Taiwan according to their pathogenecity (Ou and Bandong, 1976). Isolates differing in virulence were also reported by Tsai (1973) among 40 single spore cultures and by Haque (1975) among 25 field isolates.

Knowledge of pathogen population can contribute both to resistance breeding efforts and to the development of strategies for the deployment of resistance (Leung *et al.*, 1993). An improved
understanding of pathogen populations also allows researchers to determine which resistance genes or gene combinations will be most effective in protecting the crop from pests or diseases, by reducing the destructive potential of pathogen evolution. Information regarding the variability within fungal populations is important for better understanding of disease outbreaks and for prediction of future disease development (Stakman and Christensen, 1953).

The genetic variability of the pathogen increases the difficulty of breeding for resistance and deploying available cultivars effectively. A quick method for characterizing isolates within the pathogen population would aid the researchers not only to provide up-to-date information on the genetic diversity of the pathogen, but also to facilitate the study of the effect of various cultivars on the population structure of the pathogen. By regularly sampling diseased tissue at a number of fixed sites, researchers can follow the shifts in the genetic makeup of the pathogen population, which could provide a dynamic picture of the interactions between host and pathogen.

Development of suitable markers should be very useful for analysing the genetic structure of populations. In the absence of stable genetic markers it is very difficult to assess the level of variation within the different groups, and to identify the mechanisms responsible for this variation. For this, markers will need to be developed specifically for each ISG. The markers often used to study pathogen variability are virulence, proteins, isozymes, Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD)-Polymerase Chain Reaction (PCR) etc.

VIRULENCE ANALYSIS

The most widely used method for characterizing pathogens is the determination of the virulence spectrum on a set of differential varieties carrying different resistant factors. This measure of genetic variability provides information which is of direct interest to farmers, breeders and pathologists. Differences in virulence have been widely used as phenotypic and genotypic markers (Al-Kherb et al 1987; Linde et al, 1990), but have not been fully investigated on all anastomosis groups of R. solani (Bolkan and Butler, 1974; Puhalla and Carter, 1976; Anderson and Stretton, 1978).
There are, however, several limitations to virulence analysis. Although it is technically simple, pathotyping is a laborious and time-consuming process. Scoring of disease phenotypes is influenced by environmental variation and the subjective judgment of the investigator. Even with well characterised differentials, new virulence in the pathogen population may not be detected immediately, simply because the available series cannot differentiate the new virulence. Perhaps the most serious disadvantage of relying on virulence data to infer population structure is that genes involved in host-specificity represent a very small fraction of genes in the pathogen and may be subjected to strong selection by the host (Leung et al., 1993).

MOLECULAR ANALYSIS

Molecular approaches have been very fruitful for answering basic questions about genetic variation and systematic relations in many groups of phytopathogenic fungi. One direct approach for assessing genetic variation in fungi is by comparative study of molecular markers (Michelmore and Hulbert, 1987). A large number of genetic loci from the genome should be assayed so that genetic differences detected will reflect the variability of the genome as a whole. Molecular markers that are presumably selectively neutral satisfy such criteria. Many problems associated with studying differed levels of diversity in *Rhizoctonia* are best addressed through the use of molecular genetic markers.

PROTEINS

Gel electrophoresis of fungal proteins has been used as an adjunct to morphologic criteria for taxonomy. The use of electrophoresis in fungal taxonomy has been reviewed by Hall (1973) for fungi in general and by Snider (1973) for phytopathogenic fungi. Electrophoretic poly pep tide patterns of total soluble proteins have been found to be valuable in fungal taxonomy, because they express the genetic constitution of the cell. Clare et al (1968) demonstrated that total soluble protein patterns revealed on starch gel were variable within isolates of *R. solani* from crucifers and cereals and that those isolates could be grouped based on the similarity of their protein patterns. The results of
gel electrophoresis of soluble proteins of *R. solani* indicated that there was considerable variation in the banding patterns from isolate to isolate and this was interpreted as evidence that *R. solani* is a collective species. Welch (1976) surveyed the proteins from representative isolates of five AG and concluded that electrophoresis could prove useful in identifying the various groups. Reynolds *et al* (1983) were able to distinguish five different AGs (AGs 1 through 5) from each other based on distinct protein patterns. The total soluble protein patterns of AGs 1 to 7 and B1, were not only different among AGs but distinct patterns were recognizable among homologous groups within an AG (Kuninaga, 1986). Analysis of 117 AG identified isolates of East China, from 11 anastomosis groups showed that the same AG or sub AG shared the same bands even when they came from different areas or host plants or diverse virulence (Liu and Ge, 1988).

Comparative analyses of the soluble proteins from the mycelia of 45 isolates of 5 AG (AG 1-AG 5) and subgroups of *Rhizoctonia* from Japan, USA and China using IEF electrophoresis showed that the protein patterns differed significantly. Groups AG 3, AG 4, AG 5 and subgroups AG 1-1 A, AG 1-1C, AG 2-1, and AG 2-2 had distinct protein patterns with most significant differences between subgroups AG 1 and AG 2 (Cehn *et al*, 1991).

ISOZYMES

The study of protein polymorphisms through isozyme analysis provides a powerful tool for assessing and characterizing the genetic variability in plant pathogen populations. The term isozyme describes different molecular forms of enzyme with the same substrate specificity. An isozyme is a direct expression of genotype and can be used as an indicator of genetic relationships within related populations (Ayala, 1983; Burdon and Marshall, 1983, Newton, 1987, Micales *et al*, 1988 b). They are usually coded by different alleles or separate genetic loci and appear on a gel as multiple bands due to their different electrophoretic mobilities (Micales *et al*, 1988 b). The technique
relies on the existence of genetic polymorphisms resulting in aminoacid substitutions that cause differences in relative mobilities among allelic forms on an electrophoretic gel (Lewontin, 1974).

Isozyme analysis has been used for several purposes, to clarify taxonomic relationships among organisms that are presumably closely related, but that are difficult or impossible to distinguish morphologically (Burdon and Marshall, 1981; Beakes and Ford, 1983; Leung and Williams, 1986; Micales et al, 1988 b), to study phylogenetic relationships among organisms (Bonde et al, 1984; Maghrabi and Kish, 1987; Micales et al, 1987) to study the genetics of organisms within the same biological species (Avise and Saunders, 1984. Old et al, 1984, Burdon and Roelfs, 1985, Tooley et al, 1985, Michaelis et al, 1987) and to develop a rapid electrophoretic technique for routine identification / diagnostic markers (Chen et al, 1992).

Isozyme electrophoresis data are increasingly used to describe inter or intraspecific variation in various fungal groups (May and Royse, 1988; Stasz et al, 1989, Oudemans and Coffey, 1991). Detailed studies have also shown that isozyme analysis can be a powerful method for genetic and population studies (Leuchtmann and Clay, 1989; Zambino and Harrington, 1989).

Isozyme analysis in taxonomy and genetics of fungi was reviewed by Micales et al (1988b). Species-specific isozyme banding patterns were reported for species of Erysiphe (Clark et al, 1989), Perenosclerospora (Bonde et al, 1984; Micales et al, 1988 a) and Phytophthora (Nygaard et al, 1989).

Leung and Williams (1986) used enzyme polymorphisms and genetic distance to examine the degree of similarity between isolates of Magnaporthe grisea. Lu and Groth (1987) used isozyme data to calculate the percentage similarity between isolates of the bean rust fungus. Linde et al (1990) constructed dendrograms with cluster analysis to examine relationships between isozyme phenotypes and virulence for Uromyces appendiculatus.
As sexual state is difficult to obtain in *R. solani*, hybridization methods of studying its genetic variability are not possible. Isozyme analysis provides an ideal method of examining the level of genetic variability. In *R. solani*, isozyme electrophoretic profiles provided a good indication of the genetic diversity among selected AGs and reconfirmed the genetic basis of the AG concept (Liu *et al.*, 1990; Laroche *et al.*, 1992). Zuber and Manibushanrao (1982) studied electrophoretic patterns for five isozymes of five isolates of rice sheathblight fungus and no correlation was obtained with the enzyme patterns and virulence of the isolates examined.

Studies on genetic relationships among 14 isolates of *R. solani* anastomosis group AG 2 by evaluating electrophoretic data derived from 11 enzyme systems revealed variability across the isolates. Three groups were differentiated by isozyme analysis which were congruent with previous grouping of AG 2 isolates based on studies of anastomosis, morphology, physiology and DNA / DNA hybridization (Liu *et al.*, 1990). Isozyme studies have also revealed evidence for at least five different ISG within AG 2 (Liu *et al.*, 1990; Liu and Sinclair, 1992).

Laroche *et al.* (1992) demonstrated the utility of isozyme data for discrimination between 48 isolates from potato fields belonging to AG 3 and AG 9. Based on variation in isozyme patterns of seven enzymes, three sub groups within AG 3 were identified indicating that AG 3 is a heterogeneous group. Based on isoenzyme profiles derived from 10 enzyme systems, several genetically distinct subgroups (6 within AG 1 and 5 within AG 2) were identified (Liu and Sinclair, 1993). Kaufman and Rothrock (1995) distinguished the isolates of AG 11 from Australia and Arkansas, USA through the studies of isozyme polymorphisms. The above studies demonstrate that isozyme electrophoresis is a promising means for studying relationships among groups and it has special merits and is well suited to the study of genetic diversity because isozymes normally show high levels of variation within species, they are encoded by numerous loci scattered over the genome and some of these loci could provide valuable genetic markers for population studies and the analysis is relatively easy to apply to large number of individuals.
Another technique which allows the detection of isozymes of specific enzymes such as pectic enzymes, has been found useful in identifying groups within *R. solani*. This method is based on the detection of various pectinases (e.g., pectin esterases and polygalacturonases) in pectic acid acrylamide gels by staining with ruthenium red (Cruickshank and Wade, 1986). Sweetingham *et al* (1986) separated 140 isolates of *Rhizoctonia* from Western Australia into distinct pectic zymogram pattern groups (ZGs) and further demonstrated that individual pectic zymograms contained cultural characteristics and pathogenic capabilities which are similar. Using the same technique, five distinct ZGs within AG 8 have been established (Neate *et al*, 1988, MacNish *et al*, 1993) that also corresponded with results based on random amplified polymorphic DNA (RAPD) markers (Duncan *et al*, 1993). Schneider *et al* (1995) demonstrated the utility of pectic zymogram patterns for discriminating between AG 2 isolates pathogenic to tulips from AG 2-1 isolates, while Balali *et al*, (1995) separated field isolates of *R. solani* AG 3 into three distinct pectic zymograms and suggested that zymogram group variation is related to the morphology of tuber sclerotia and disease symptoms.

Other studies with members of *Rhizoctonia* complex from Australia have also shown that different ISGs can be differentiated on the basis of their pectic zymogram patterns (Sweetingham *et al*, 1986, Neate *et al*, 1988, MacNish *et al* 1993).

**DNA / ONA HYBRIDIZATION**

Molecular studies on DNA base sequence complementarity have provided convincing evidence for the possibility to demonstrate genetic relatedness between the species, by measuring the degree of reassociation (Hybridisation) between DNA molecules.

Genetic relatedness was estimated using spectrophotometric measurement of DNA/DNA hybridisation by Kuninaga and Yokosawa (1985) clearly demonstrated that different ISGs are genetically isolated. Vilgalys (1988) also demonstrated that genetic differences among ISG from four different AG using the measurement of the extent of hybridization based on isotope labeled DNA. The
relatively low DNA relatedness (47 - 87%) between certain isolates within each of these AGs corresponded well with previously defined sub-groups based on cultural morphology and pathogenicity. Kinetic analyses of whole-cell DNA reassociation estimate a haploid genome size of *R. solani* to be around 2.8 x 10^9 nucleotide base pairs (Kuninaga, unpublished). As the evolution of fungi is considered to be accompanied by a progressive decrease in the G-C content of DNA, AG I-IA with its GC content of 49.1 moles of DNA could be considered as the most highly evolved. The percentage of DNA that hybridizes among AGs is typically around 15%. Thus the limited resolution afforded by DNA-DNA hybridization of the whole genome does not provide sufficient understanding of the evolutionary relationships in *R. solani* (Kuninaga, 1986).

**RFLP**

RFLP analyses in which mitochondrial, ribosomal or random cloned fragments were used as probes would result in DNA polymorphisms which in turn can be used as genetic markers. RFLP approach has been successful with other species of phytopathogenic fungi (Levy *et al*., 1990; Manicom *et al*., 1990).

Using Southern blot techniques with anonymous fragments of nuclear DNA cloned from an isolate of AG 3, Jabaji-Hare *et al*., (1990) demonstrated that group-specific RFLP could be detected, in which cloned DNA probes hybridize only with members of their own ISC. RFLP of ribosomal DNA (rDNA) studies of 87 isolates of *R. solani* from 15 ISGs indicated divergent variations among isolates (Vilgalys and Gonzalez, 1990). Liu *et al* (1993) demonstrated based on restriction analysis of ITS sequences from a large sample of field isolates, patterns of rDNA variation revealed at least six ISGs within AG 1 and five ISGs within AG 2 (Liu and Sinclair, 1992; Liu and Sinclair, 1993). Evidence for the five ISG groupings detected within AG 2 is also supported by data from a portion of the mitochondrial rDNA gene (Liu and Sinclair, 1992) and also by isozyme data (Liu and Sinclair, 1992; Liu *et al*, 1993). Detailed restriction analyses of DNA fragments amplified by the PCR (Polymerase
Chain Reaction) provide even greater resolution of genetic differences within and among ISGs. Similar patterns of group specific hybridizations were seen in one of the three ISGs classified within AG 4 using random nuclear probes (Cubeta et al., 1991).

RFLP studies of isolates of *R. solani* from Japan and Australia, using clones of 18s ribosomal RNA (rRNA) gene or random cloned fragments of *R. solani* demonstrated that group specific patterns could not be identified for all groups with rRNA probe, whereas group specific patterns could be identified with random cloned DNA fragments (O'Brien, 1994). Development of a DNA probe specific to AG 8 showed that the variation occurs within AG 8 (Matthew et al., 1995).

**RAPD / PCR**

PCR (Polymerase Chain Reaction) provides amplified portions of DNA of specific sequence, which are defined by primers that match the ends of DNA to be amplified. The method of RAPD (Random Amplified Polymorphic DNA), a modification of PCR procedure, employs a single primer (decanter) to amplify, under low stringency, any genomic region that happens to be flanked by a pair of 10 base priming sites in opposite orientations within 5000bp of each other, and yields fingerprint like patterns upon gel electrophoresis (Williams et al., 1990; Welsh and McClelland, 1990). The development of RAPD markers provide another powerful method for investigation of intraspecific genetic variation.

The presence of amplified DNA fragment in one individual and the absence of the same molecular weight fragment in another gives rise to polymorphism. The unique fragment constitutes a genetic marker. Ideally each band amplified by the use of random primers would represent a specific locus in the genome and alleles would produce easily identified bands of a different size. Polymorphisms may be because of fragment length or presence or absence of a band. Length polymorphisms are the result of insertion into or deletion from the amplified fragment between the primer binding sites, whereas presence-absence polymorphisms are caused by the destruction or
creation of one of a pair of the primer binding sites. Many areas in the genome contain a non-binding sequence that would, with a little alteration, serve as a primer-binding site. Mutation of, insertion into, or deletion from this site may convert the sequence into a valid primer-binding site. In this case, a new band would appear (if the criteria of proximity and suitable orientation of the corresponding primer-binding site were satisfied). Conversely, changes of sufficient magnitude to a functioning primer-binding site would lead to the disappearance of a band, because the fragment that has that site as one of its ends would no longer amplify exponentially (Guthrie et al., 1992).

As long as a PCR amplified segment of a defined size produced by a particular primer represents a specific locus in the genome of the test species, RAPD markers have great potential for use in population analysis even when alleles cannot be identified. In fact 'null alleles' (i.e. the failure to detect a band at all) may be more common for changes at a specific locus.

ADVANTAGES OF RAPD / PCR

1. The amplified DNA fragments behave as simple Mendelian markers that can be used for characterising a population.

2. The main advantage of RAPD analysis is that no prior knowledge of DNA sequences is necessary to design the primers. Hence abundant genetic markers can be generated for any organism of interest.

3. Methods involving Southern Hybridisations and/or cloning are, however, relatively labour intensive and costly.

The reliability and reproducibility of RAPD techniques are at times questioned because results obtained from different labs were not always comparable. But the reproducibility of fingerprint patterns achieved in a standard reaction mixture with different thermocycler programs and in
different thermocyclers, suggests that the RAPD procedure can be reliable when the temperature profile was properly maintained (Tommerup et al, 1995).

RAPD-PCR has been successfully used to study populations of plant pathogenic fungi (Goodwin and Annis, 1991; Moller et al, 1995, Pipes et al, 1995). RAPD markers have been successfully used to differentiate among isolates of *Colletotrichum graminicola* (Guthrie et al, 1992), pathogenic races of *Fusarium oxysporum* (Grajal-Martin et al, 1993), to separate species within the genus *Hypoxylon* (Yoon and Glawe, 1993), to study intraspecific genetic diversity of *Chaunopycnis alba* (Moller et al., 1995), to characterize pathotype of *Fusarium oxysporum f.sp. ciceris* (Kelly et al., 1994). Duncan et al (1993) found that RAPD markers were able to discriminate between Australian 1SG in the *R.solani* complex, as well as between different strains with same ISG. Cubeta et al., (1991), were able to identify many AGs of binucleate *Rhizoctonia* species based on PCR amplification of region of DNA coding for the 25s rRNA. As RAPD-PCR is simple and easy to perform, careful studies using this method are very useful for characterising variation in rice sheath blight fungus *R.solani*.

It is a challenge for scientists to evolve strategies for resistance to diseases either by conventional breeding or genetic engineering techniques. Any such endeavour needs a thorough understanding of the variability of the pathogen. Though it is quite apparent from the preceding discussion that the progress has been made in understanding the molecular aspects of genetic variability of different anastomosis groups, several lacunae are still left to be removed. There are only a limited instances in which variability of AG 1 was investigated. The identification and characterization of the rice sheathblight fungal isolates can be useful in understanding the variability of the pathogen, thereby improving the effectiveness of breeding for resistance.