Review of Literature

Oryza sativa L. - nature's gift to humanity

Rice, a member of the grass family, is one of the three cereals on which the human species largely subsists, along with wheat and maize. In the developing world as a whole, rice provides 27 percent of dietary energy supply and 20 percent of dietary protein intake. Rice apparently was domesticated in Arunachal Pradesh in the north-east India, bordering China in Asia (Muralidharan and Siddiq 2000). Rice is produced over a wide range of locations and climatic conditions. In India, rice is grown under diverse ecosystems as rainfed uplands, rainfed shallow, semideep and deepwater lowlands, irrigated lands and hills. These are the major rice ecosystems found world over. No other plant species can grow under such wide range of conditions and produce grains to feed the population in Asia. Annually, 148 million hectares (m ha) are sown to rice in the world. This includes 79 m ha (53%) in irrigated ecosystem, 17 m ha (12%) in rainfed uplands 41 m ha (27%) in rainfed lowlands and 11 m ha (8%) in floodprone ecosystem (Prasad et al 1996; FAO 1995; Muralidharan et al 2002).

The bulk of rice area is subjected to an alternating wet and dry seasonal cycle, and contains many of the world's major rivers, each with its own vast delta. Here enormous flat, low-lying agricultural lands are inundated and flooded annually during the crop-growing wet season. Only rice adapts readily to production under these conditions of humid air, saturated soil and high temperatures (Prasad et al 2001). In India, rice is cultivated in 42 m ha under four major ecosystems, *viz.,* irrigated (19 m ha), rainfed

lowland (14 m ha), flood prone (3 m ha) and rainfed upland (6 m ha) ecosystems (Muralidharan et al 2002). Rice ecosystems in India represent 24% of irrigated areas, 34% of rainfed lowlands, 26% of flood-prone areas and 37% of rainfed uplands cultivated to rice in the entire world. No other country in the world has such diversity in rice ecosystems. Therefore, Indian rice research program is the principal moving force in the world (Prasad et al 1996; Muralidharan et al 1996, 2002; Prasad et al 2001).

India has a rich diversity in rice germplasm with more than 30,000 accessions. Diversity exists in the plant type, panicle size and weight, grain type, weight and color, maturity duration, and resistance to biotic and abiotic stresses. Using this germplasm and exploiting the advantage of multi-location or multi-environment tests (METs) spanning four decades, a number of varieties were released (Muralidharan and Siddiq 1997). Over 1035 rice varieties with varying maturity duration (early, mid-early, medium, and late) were also developed (Rani et al 2011). In these released varieties, maturity duration, grain size, appearance, scent and quality, resistance to biotic and abiotic stresses could be tailored to suit consumer preferences and needs.

Blast pathogen and the disease

The fungus *Pyricularia oryzae* (telomorph *Magnaporthe oryzae* (Hebert) Brarr) Couch and Kohn) is the causal agent of rice blast. It is a haploid filamentous Ascomycete with a relatively small genome of ~40 Mb divided into seven chromosomes (Dean et al 2005). *M. oryzae* is becoming an excellent model organism for studying fungal phytopathogenicity, inheritance of resistance and host-parasite interactions (Muralidharan

and Raghu 2012). It occurs at all stages of crop growth in rainfed, irrigated and hill rice, and severe incidence results in heavy or total loss in yield. Farmers in Andhra Pradesh, Himachal Pradesh, Tamil Nadu and in many other parts of India, had to abandon popular but susceptible cultivars because of the loss caused by blast (Muralidharan and Reddy 2005). The Centers for Disease Control and Prevention has recently recognized and listed rice blast as a potential biological weapon (Madden and Wheelis 2003). Thus, no part of the world is now safe from this disease.

Unlike many phytopathogenic fungi such as the mildews and rusts, the rice blast fungus can easily be cultured on defined media, facilitating biochemical and molecular analyses. Early stages of the infection process, including germination, appressorium formation and penetration can be studied *explanta*. Tools for molecular genetic manipulation have been well-developed in the last decade. Many genomic resources such as expressed sequence tag (EST), bacterial artificial chromosome (BAC), genetic methodology, a physical map and the draft sequence are now publicly accessible. One of the big issues resulting from the prediction of the genes encoded in the *M. oryzae* genome was, this pathogen contains more genes than its non-pathogenic cousins, *Neurospora crassa* and *Aspergillus nidulans* (Dean et al 2005). The sexual phase of blast pathogen has not been detected in nature and only asexual phase, *Pyricularia oryzae* causes blast epidemics.

Infection by *P. oryzae* leads to formation of spindle-shaped lesions with brownish margin and grayish center. The spots usually begin as small water-soaked, whitish,

greyish or bluish dots. Fully developed lesions in 2-3 days reach 1.5 cm in length, and 0.5 cm in breadth. Adjacent lesions often coalesce under favorable conditions turning a major part or the entire leaf to produce a burnt appearance. Brown or black patches may appear around nodes and the nodes so infected often break apart. At a later stage, the fungus attacks the base of panicle at neck region and this infection is known as panicle blast or neck blast or spikelet blast. Neck infection causes the panicles to break and fall over, resulting in the loss of grains (Muralidharan and Venkatarao 1987). Small brown to black spots formed by *P.oryzae* may also be seen on the glumes.

The factors that influence blast epidemics are the susceptible variety, availability of inoculum to initiate the disease, excessive application of nitrogen fertilizer, low night temperature, high humidity, cloudy and drizzle weather or dew resulting in leaf wetness. When conditions are conducive, the pathogen multiplies rapidly to produce abundant conidia from lesions (Dinaker and Muralidharan 2006). The disease moves quickly from field to field by producing myriad number of spores that are disseminated by wind in all directions. These spores upon falling on rice plant, initiate further disease to progress rapidly through the entire field. The repeated cycles of spore production and infection continues throughout the crop growth. Under favorable conditions, the green lush crop growth is turned into burnt up appearance (Muralidharan and Venkatarao 1987). During mid-1980s, some farmers in Andhra Pradesh and Tamil Nadu committed suicide as they lost everything in rice crop failure following blast epidemics on IR 50 (Muralidharan 2000).

Blast continues to be a threat to realizing yield potential in cultivars in all the rice ecosystems (DRR 1975-2014). The methodology for uniform blast nursery (UBN) evaluations of germplasm accessions was developed by Padmanabhan (1975) at Central Rice Research Institute, Cuttack. Studies indicated that resistance in rice cultivars to blast disease was governed by genes that were either monogenic dominant or monogenic recessive or digenic or polygenic (Padmanabhan 1979). In Japan, Kiyosawa (1981) made gene analysis using seven stable blast isolates. Thirteen dominant resistance genes were identified at eight loci. Diseases in plants are caused by an interaction between host, pathogen and environment. Evidence on co-evolution of host and pathogen suggest for the presence of large variability in both host and pathogen. This variability must be dynamic i.e., continuously undergoing change, if the two organisms are to exist without eliminating each other (Muralidharan 2005). Results from investigations on rice and *P. oryzae* demonstrate variability as a dynamic weapon for the survival of the combatants in the environment. This variability can be equally used as a tool for humans to tilt the balance in the contest to favour the crop species. Based on such an understanding, pathologists and breeders used multi-environment tests (METs) in India to successfully develop many varieties with durable resistance to blast (Dinaker and Muralidharan 2007).

Many reports on the resistance genes for rice blast have been published. More than 70 genes and 347 quantitative trait loci (QTLs) have been detected (Ballini et al 2008) and 96 genes or major QTLs have been reported (Koide et al 2009). Among the reported resistance genes, several gene symbols are synonymously used for the following two reasons. The first is that gene symbols were revised in accordance with the international

committee on gene symbolization in 1995 (e.g., *Pib* and *Pis*, *Pita* and *Pi4, Piz* and *Pi2*, and, *Pi11* and *Pizh*). And the second is that genes are suggested to be identical to each other based on their reaction pattern to blast isolates and/or linkage analysis (e.g. *Pi3*(t) and *Pi5*(t), and *Pi1* and *Pi7*(t)). In addition, several genes are suggested to be allelic or tightly linked; e.g., *Pi2/Piz, Piz-t,* and *Piz-5* on chromosome 6, *Pik, Pik-s, Pik-p, Pik-m, Pik-h*, and *Pik-g* on chromosome 11, and *Pita* and *Pita-2* on chromosome 12.

Extensive METs were made with near-isogenic lines (NILs) carrying different blast resistance genes across the country. Tadukan carrying resistance gene *Pi-ta* showed small lesions infecting < 2% leaf area indicating a very high level of durable resistance to blast disease. The expression of a high degree of resistance was clearly demonstrated in A57 carrying three resistance genes (*Pi-1, Pi-2* and *Pi-4*). The performance of BL 245 with two resistance genes (*Pi-2* and *Pi-4*) and C101LAC (*Pi-1*) was comparable to A57 (Muralidharan et al 2003a; Muralidharan et al 2004a). The performance of these NILs was marginally superior to the resistant checks (Tadukan, Rasi, Tetep and IR 64) and commercial cultivars like Tulasi, Vikas, Vivekdhan 62, VL Dhan-221, VL Dhan-61 and VL Dhan-81. Different state variety release committees have so far released over 250 cultivars resistant to *P. oryzae* (Rani et al 2011; Prasad et al 2011).

Sheath blight pathogen and the disease

Sheath blight disease is caused by the fungus *Rhizoctonia solani* Kuhn (telomorph *Thanetophorus cucumeris* Frank Donk.). In nature, *R. solani* occurs as an aggregate of strains that differ in cultural appearance, virulence, and physiology (Sherwood 1969;

Parmenter and Whitney 1970). Likewise, morphology and virulence among *R. solani* isolates vary greatly within the same and among different groups. Rice sheath blight is cosmopolitan with a very wide host range and attacks a number of crop plants and weeds (Stroube 1954; Adams 1988; Sharma and Singh 2003). At one stage, it was claimed that there is hardly any plant species, which cannot be infected by *R. solani* (Singh et al 1999). The fungus has a worldwide distribution (Ogoshi 1987) and isolates of *R. solani* are highly variable in aggressiveness. *R. solani* complex is a taxonomic entity composed of morphologically similar groups that share characteristics like multinucleate cells with dolipores, production of sclerotia and lack of conidia (Parameter and Whitney 1970). It is widely prevalent, particularly in irrigated rice ecosystem but seldom assumes epidemic proportions. The asexual phase of the pathogen actually incites the disease in plants just above the water line in fields to cause damage to yields. The disease appears in moderate to severe intensities in a few states like Andhra Pradesh, Assam, Kerala, Orissa and West Bengal. Although sheath blight occurs, its impact on rice yields is demonstrated to be relatively less in the country when judged against blast. Losses from sheath blight in irrigated ecosystem may be around one tonne per hectare (Muralidharan et al 2003c, 2004c). *R.solani* as a pathogen causes lesions and necrosis in plant tissues. The profuse saprophytic external growth visually appears to be more threatening due to thick mycelia and numerous sclerotia. The pathogen affects all plant parts *viz*., sheaths, internodes, upper leaves and panicles. On sheaths, *R.solani* infection leads to appearance of spots that are grayish in colour and ellipsoid or ovoid in shape. The leaf of the affected sheath dries up. In humid weather, white threads of fungal body can be seen all over the surface of

leaf sheaths. The infected leaves and internodes turn grey to straw colour with lateral brown bands resembling snakeskin.

On the infected portions, initially white mycelial knots (1-2 mm) appear which later turn to brown and dark brown flattened visible structures called sclerotia. Many such sclerotia aggregate and appear in a large compact mass with dark brown colour. They adhere to plants, but are easily detached even by gentle wind. Once detached, the sclerotia are carried by water stream, and upon coming in contact with plants get adhered to the sheath and initiate the disease at the point of attachment. The infection then ascends to the upper part of the foliage and to flag leaves. Plants become more susceptible with increasing age apparently due to loosening of leaf sheath from the culm. Early sowing, early planting and dense plant populations encourage disease development. Application of nitrogenous fertilizers at high doses increases the severity of sheath blight disease. The progress of the disease is governed by ambient humidity and temperature. Sheath blight epidemics occur in highly humid conditions with a daily mean temperatures hovering at 30^{o}C. Under favorable conditions, the disease spreads to top portions of the plant and chokes the emergence of panicles. *R.solani* invades spikelets causing sterility or improper grain filling. In southern parts of India, this pathogen does not produce any propagative air-borne spores. However, in cooler conditions, air-borne sexual spores are produced (Muralidharan and Reddy 2005).

The pathogen has a wide host range and occurs on all grasses and broad-leaved weeds that grow on rice bunds. Similar symptoms and sclerotia are produced on all these

hosts. The sclerotia fall into water in fields and initiate infections on rice crop. Even if leaves of rice plants come in contact with infected weeds on bunds, they pick-up infection and spread the disease. The nutritional requirement for the mycelial growth in *R.solani* is so minimal that virtually on the surface of water, the mycelia grow and descend from bunds on to fields. Hence, keeping bunds clean of weeds will help in checking the disease spread from primary sources (Muralidharan and Reddy 2005).

Sheath blight spreads very slowly and a change in environment from humid to dry weather will stop the disease progress. In addition to keeping bunds clean, soil solarization helps to reduce sheath blight incidence. Only T 141, OS 4, BCP 3, Saibham, Buhjan, Saduwee, Remadja, Ta-Poo-Cho-Z, Nangmons 4, Athebu, Phoure and ARC 15368 have been identified as donors expressing moderate resistance to sheath blight. So far no rice culture has been found to be resistant to *R.solani* infection. Pankaj, Swarnadhan and Vikramarya exhibit a good degree of tolerance to sheath blight (Prakasam et al 2013). Transgenic IR 64 rice plants over-expressing rice chitinase, a pathogen-related protein, have been generated (Datta et al 2001). The performance of these transgenic plants is yet to be tested in India.

Bacterial leaf blight pathogen and the disease

Symptoms of bacterial leaf blight disease appear from the tip or edges of leaves as yellow water soaked and undulating lesions parallel to the veins that later turn to straw yellow colour. The disease initially starts from either one or both sides of leaf margin. As the disease progresses, the drying spreads downwards and inwards of leaf blade to cause

drying and death of the leaf. Often amber coloured bead-like bacterial exudates are present on lesions. In systemic infection, seedlings wilt and die. Grains are either partially filled or become chaffy. Bacterial blight is caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swigs *et al.* The rod-shaped bacterium is gram negative, 1.75 x 0.60 μ in size, motile with a polar flagellum and is without spore forming capacity. Bacterial cells are surrounded by mucus capsules and are joined to form aggregated and stable mass. After the introduction of dwarf, high-yielding TN1, which was known for susceptibility to bacterial blight, the disease assumed greater importance in India.

Bacterial blight is associated with cyclonic storms during monsoon periods. Its incidence and severity is very much influenced by rainfall, winds, rainy days, susceptibility of the cultivar and nitrogen fertilizer application. The yield losses have been reported to vary from 2-74% in such epidemics (Rao and Kauffman 1977; Muralidharan and Venkatarao 1979). Severe epidemics of bacterial leaf blight in two consecutive wet seasons (1979 and 1980) in northwestern India reduced the grain yields drastically. Every year, bacterial leaf blight causes some damage to rice crop in several districts in India (DRR 1965-2014, DRR 1975-2014). The disease occurs in two phases. *Kresek* or wilt phase usually occurs during active tillering stage and results in the death of tillers. Seedlings are killed by *kresek* if inoculum pressure is very high (Srivastava 1972; Rangareddy 1987). The leaf blight phase is common in all the rice growing areas and appears from maximum tillering to heading stages. Sometimes, farmers mistakenly identify the disease as crop drying due to unfavorable conditions; they expect the crop to recover. But under favorable conditions, the disease spreads quickly to devastate the

entire crop. In diseased plants, panicle emergence is poor and spikelets are partially filled and discolored. Bacterial leaf blight causes more damage in rice fields where high doses of nitrogen fertilizers are used as in Punjab and Haryana. Epidemics of bacterial leaf blight occur frequently all along the Indian coast exposed to cyclonic storms and intense monsoon rains. Injuries to rice leaves caused by rain storms, strong winds, sap sucking insects and intercultural operations form entry points for the pathogen (Muralidharan and Venkatarao 1979) besides other natural pores present in rice leaves. Once inside the leaf, the bacteria multiply quickly to produce millions of bacterial cells and cause blight. Milky dewdrops ooze out of the affected leaf lesions containing numerous bacterial cells.

The studies on pathogenic variation from India deal with the leaf blight phase only, as this is the major problem encountered in rice fields. Gupta et al (1986) reported the presence of 11 virulence genotypes in *X. campestris* pv. *oryzae* populations from northwestern India based on differentials. Reddy and Reddy (1992) collected 150 isolates from 25 locations in India and classified them into two pathotypes: pathotype I was avirulent on DV85 but virulent on Cemposelak and Java 14, and pathotype II was virulent on DV85 but avirulent on Cemposelak and Java 14. Pathotype I was further divided into two sub-groups *i.e.,* pathotype Ia and Ib based on the avirulence or virulence, respectively on another differential, IR20. They reported that pathotype Ia was prevalent in Punjab, East Uttar Pradesh, Tamil Nadu and Kerala, while pathotype II was limited to West Bengal. DNA finger printing of 67 isolates of *X. oryzae* pv. *oryzae* collected during 1994 and 1995 from 18 locations in India belonged to a single lineage representing pathoype Ib (Yashitola et al 1997). The resistance to bacterial leaf blight disease in some

cultivars was considered to be due to a combination of two or more genes or to new genes that were often described as dominant, recessive, inhibitory, complementary or polygenic.

Rice genotypes carrying resistance genes to bacterial blight disease were evaluated in multi-environment tests (METs). Differential cultivars like Java 14 (*Xa1, Xa3* and *Xa12*) or DV 85 (*xa5* and *Xa7*), and cultivars IET 8320 and IET 8585 carrying several unknown resistance genes showed resistance at many of the test locations (DRR 1965-2014). Their stable performance over decades indicated durable resistance to bacterial blight. Near-isogenic lines (NILs) carrying different single genes for resistance to bacterial leaf blight *viz*., *Xa1, Xa3, Xa4, xa5, Xa7, xa8, Xa10, Xa11, xa13, Xa14, Xa18,* and *Xa21,* and two genes *viz., Xa4 + xa5,* and *Xa4 + xa13* were shown to be susceptible at 7-12 locations in the country (Muralidharan et al 2003b). Yet, resistance genes *viz., Xa4, xa5, xa13,* and *Xa21* in other two-, three- or four-gene combinations were found to exhibit a high level of field resistance. This study also brought out the futility of identifying and classifying pathotypes based on the reaction of differentials to *X. oryzae* pv. *oryzae* (Muralidharan et al 2004b, d).

The options for control of bacterial blight include use of resistant cultivars and judicious nitrogen management. If favorable weather persists and disease is already incident on crop, it is advisable to withdraw application of nitrogen fertilizer. Some of the commercially cultivated resistant cultivars are Ajaya, PR 4141, PR 114, Swarna, MTU 4870, HKR 120, IR 36, IR 64 and Saket 4. Donors identified for resistance to

bacterial leaf blight are BJ 1, TKM 6, Lacrose-Zenith-Nira, Java 14, Wase-aikoku and Sayaphal (Laha et al 2009).

Tungro virus pathogen and the disease

Tungro virus disease occurs if a susceptible crop, virus inoculum and green leafhoppers (*Nephotettix virescens* Distant.) to carry virus as vectors, are available in a rice field. The disease attracted the public attention for the first time following an epidemic outbreak in the eastern parts of Uttar Pradesh, Bihar, and West Bengal (Raychaudhuri et al 1967). Later, the disease apparently moved towards peninsular India during 1977. Severe tungro virus disease outbreaks threatened rice production in Andhra Pradesh and Tamil Nadu during 1984. Regular production oriented survey records clearly established the discontinuous occurrence of tungro in the country (DRR 1975-2014).

Stunting of infected plants and turning leaf colour from green to yellow to orange red characterize tungro disease incidence. Newly emerging leaves of infected plants are often pale with chlorotic intervenial areas. The leaf lamina is often twisted following virus attack. While orange-yellow colouration of the foliage is characteristic, variations often exist ranging from green to pale, or intensely orange, red and sometimes with brown spots. If the plants are infected in early growth stages, there is no flowering. If plants are infected late, there is a delayed and uneven flowering (Muralidharan et al 2003b). Tungro virus reduces the number of spikelets in panicles and yield; it also decreases filling, weight and starch content in grains (Chowdhary and Mukhopadhyay 1975).

Two viral particles namely spherical (RTSV – an RNA virus) and bacilliform (RTBV- a DNA para retrovirus) particles were considered to be associated with the tungro disease (Saito et al 1976; Hibino et al 1978). Recent studies, however, raised the question of involvement of bacilliform virus as a pathogen in tungro disease (Muralidharan et al 2003b). Viruliferous green leafhoppers *Nephotettix virescens* Distant., *N. nigropictus* Stahl. and *Recilia dorsalis* Motsch., introduce the virus into rice leaves when they probe to suck nutrients. Thus, a tungro-infected plant suffers from damage caused by both virus and the insect vector. The disease can occur at any stage from nursery onwards. The initial disease on rice crop is seen along the weedy border of rice fields which later spreads into the main field. The self-sown of seedlings in infected IR 64 in fields in coastal Andhra Pradesh led to an outbreak of tungro in the ensuing crop also during 1990. Tungro is found only in irrigated and rainfed lowland rice ecosystems. Applying any chemical cannot directly control tungro virus disease. But, the spread of tungro disease can be checked indirectly by controlling the vector with a pesticide application. A low dose application of imidachloprid 200 SL (100 ml/ha) in nurseries after a reported outbreak in the earlier crop can effectively control tungro from affecting the new crop. Practicing a fallow or introducing a pulse or oilseed crop can also break the continuous availability of virus inoculum in fields. Many other rice cultivars possessing resistance or tolerance to tungro virus in fields have been released by different states for commercial cultivation (Muralidharan et al 2003b). Alternatively introduction of resistant cultivars like Nidhi, Vikramarya, IET 8565 and IET 8902 will prevent yield losses from tungro (Krihsnaveni et al 2009).

Plant pathogens and yield losses

Potato blight, caused by *Phytophthora infestans*, struck Europe like "a bolt from the blue" in the 1840s. In Ireland, about a million people died of starvation and more than a million attempted to emigrate (Large 1940; Strange 2003). The reasons for this calamity were the arrival in Europe of a virulent strain of the pathogen, the high dependence of much of the Irish population on potato for sustenance, the lack of resistance in the plant to the pathogen, and weather conditions favorable to epidemic development. There have been other disasters caused by plant diseases such as the Great Bengal Famine of 1943 (Padmanabhan 1973) and the southern corn leaf blight epidemic of 1970–1971 in the USA (Ullstrup 1972), to name but two. In the former, an estimated 2 million people died owing to the high dependence of most of the population on a single crop, rice, which was attacked by the fungus *Drechslera oryzae* (Breda de Haan) Subram. & Jain (*Cochliobolus miyabeanus*). In the USA, by contrast, although in some areas the maize crop was completely destroyed by another fungus from the same genus, *Cochliobolus heterostrophus*, alternative sources of nutrition were plentiful so no one died, although the effect on the agricultural economy was severe. The first two of these painful examples demonstrate with brutal clarity that in areas of the world where a large proportion of the population is dependent on a single crop or a few crops, they are at risk should that crop fail owing to one or more devastating diseases. At the present time, the threat is particularly great in developing countries, where populations are growing fastest, poverty is endemic and the population depends on locally produced staples (Strange and Scott 2005). More than 700 ha of rice of diverse genotypes with varying levels of

resistance in Bhutan were affected in 1995, resulting in losses of 1090 tonnes (Thinlay et al 2000).

In 1970 the corn leaf blight swept through fields of Texas T cytoplasm corn and yield was reduced by approximately 710 billion bushels (~18 billion tones). The cost to farmers was about $1 billion (Ullstrup 1972). Browning (1988) argued that the epidemic was the greatest biomass loss of any biological catastrophe and that it was a man-made epidemic caused by excessive homogeneity of the USA's tremendous maize hectarage. The catastrophic outbreak of coffee rust in 1970 caused great losses in Brazil with higher coffee world market prices as a consequence. In 1916, a rust fungus destroyed about 3 million bushels of wheat in the United States, roughly one-third of the crop. Other examples include the coffee rust epidemic in Ceylon in the 1870s, the tropical maize rust epidemic in Africa in the 1950s and the blue mould epidemic on tobacco in the USA and Europe in the 1960s (Marshall 1977).

Losses from rice pathogens

i) Blast

Blast is probably the most devastating disease as it causes considerable yield losses (Rajarajeswari and Muralidharan 2002, 2006). The damage from natural epidemics of blast of rice caused by *Pyricularia oryzae* was examined in 300 farmers' fields during wet seasons from 1995-97. A new modification was made to consider the disease prevalence as percentage of farms with disease above economic threshold level in a district. This adjustment enabled the production loss estimate to be tailored to the actual

area damaged by the pathogen. By multiplying district production estimates by the disease prevalence (%), production loss in a district was precisely derived. In blast epidemics, the most conservative estimates for the production yield losses in actual districts were 8 to 13%. These production losses during the three epidemics were considerably different, ranging from 69,245 to 77,072 tonnes in Karnal, 49,765 tonnes in Nellore, and 89,111 to 106,901 tonnes in Ranga Reddy district (Rajarajeswari and Muralidharan 2006).

ii) Sheath blight

Sheath blight is a soil-borne pathogen and is widely prevalent. Sheath blight disease may be identified by the detection of profusely growing mycelia on the surface of water, leaf or plant. Therefore, it often gives an impression of causing severe yield losses. Sheath blight disease is generally not incident on the entire field, but is restricted to border or shaded areas or isolated patches. The sclerotia produced by the sheath blight pathogen remain viable in the soil for several years (Rajan et al 1988). Although sheath blight causes yield losses in rice, in comparison, it is only one-thirds the level of yield losses caused by blast (Muralidharan et al 2003a).

iii) Bacterial leaf blight

The yield losses have been reported to vary from 2-74% in such epidemics (Rao and Kauffman 1977; Muralidharan and Venkatarao 1979). The damage from natural epidemics of bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* was examined in 400 farmers' fields in four rice-growing districts in India during

wet seasons (Rajarajeswari and Muralidharan 2005). By multiplying district production estimates by the disease prevalence (%), production loss in a district was precisely derived. In BLB epidemics, the most conservative estimates for the production yield losses in districts were 3 to 16%. The production losses during the four epidemics were considerably different ranging from 92,000 to 105,000 tonnes in Nellore, 30,000 to 36,000 tonnes in West Godavari, 46,000 tonnes in Karnal and 22,000 tonnes in Rangareddy district (Rajarajeswari and Muralidharan 2005).

iv) Rice tungro virus

Tungro outbreaks are discontinuous within a district, state and country over the years. The outbreaks of this virus disease are restricted to irrigated and rainfed shallow lowlands. Three major epidemics in farmers' fields (1984, 1988 and 1990) caused severe quantitative and monetary losses. Each of the other two epidemics (1987 and 1998) led to a similar loss of about a million tonnes in rice production but showed a steady increase in loss in terms of real value. An epidemic outbreak of tungro during 2001 in only three districts of West Bengal caused a paddy or unmilled rice production loss of 500,000 tonnes that amounted to Rs. 2911 million at current prices. This study clearly demonstrated that tungro epidemics could cause a maximum yield loss of 53% in a district, 23% in any one state and 2% in all-India rice production (Muralidharan et al 2003a).

Developing resistance in plants

One of the most effective approaches to disease management is the use of genetically resistant plants. Although the genetic basis for resistance to a plant disease was first

elucidated only in 1905 (Biffen 1905) selection for disease resistance has probably been practiced by farmers and later by plant breeders since the beginning of agriculture. Spontaneous variation in disease resistance can be selected, and genes for resistance can be introduced by hybridization. Genetic resistance to disease, once secured, is a low-cost method of control. Like other approaches to disease management, it need not be complete to be useful. Rather, it is more important that it should have the characteristic of durability, a concept proposed by Johnson (1984). Durable resistance is resistance that remains effective over many years, can only be recognized retrospectively.

Phenotypic variance is a composite of two variables, genetic (G), environment (E) and their interaction. For various cereals (both temperate and tropical), past selection has seemingly evoked responsive varieties, and GE effects constitute about one-third of the total estimated yield increase. It is a common practice in trials involving varieties and breeding lines to grow a series of genotype in a range of different environments. If all the genotypes respond similarly to the entire environment tested, their relative performance in other environments may be predicted with some confidence. A genotype x environment interaction (GEI) exists where the relative performance of varieties changes from environment to environment (Kang 2004). More stable (less responsive) varieties, less dependent upon good environments that do well, have been prominent. Historically, responses have been largely due to unconscious selection, but GE effects could be deliberately manipulated by breeding if it were desirable to do so, for example, to produce varieties adapted to low-input agriculture that is already an object of breeding research in a few crops (Simmonds 1981).

Stability of crop varieties

The term phenotypic stability is often used for yield stability and adaptation of a crop variety or a cultivar. However, stability analysis is not often used in analysis for disease resistance. Different concepts and definitions of stability have been described over the years (Lin et al 1986; Becker and Leon 1988). According to Lin et al (1986), a genotype is considered to be stable if its among-environment variance is small. Becker and Leon (1988) called this stability a static, or a biological concept of stability. Accordingly, a stable genotype possesses an unchanged performance regardless of any variation of the environmental conditions. This concept of stability is useful for quality traits, disease resistance, or for stress characters like winter hardiness. Parameters used to describe this type of stability are coefficient of variability (CV) used by Francis and Kannenburg (1978) for each genotype as a stability parameter.

Plant genetic resistance to pathogens offers an interesting alternative to disease control methods based on the use of pesticides, but lacks durability due to the rapid evolution of pathogen populations. Pathogen populations are able to adapt, frequently resulting in the breakdown of the resistance in the case of major resistance genes faced with the qualitative component of pathogenicity, i.e., the ability of a pathogen to infect a plant. Quantitative resistance based on quantitative trait loci (QTL) induces a partial reduction of pathogen development. Various mechanisms may underlie quantitative resistance including basal defence, chemical warfare, defence signal transduction or weaker forms of major resistance genes (Poland et al 2009), and quantitative resistance may either be specific to some isolates of a pathogen or be broad-spectrum (i.e., efficient

against all isolates of a pathogen). Quantitative resistance affects quantitative components of pathogenicity (e.g., rate of infection, latent period, rate of sporulation), which are dependent on the host, the pathogen and the interaction between host and pathogen (Lannou 2012). Quantitative resistance is frequently assumed to be more durable than qualitative resistance (Parlevliet 2002), which has been experimentally shown, for example, in the case of wheat leaf rust on the cultivar, Apache (Papaix et al 2011). However, isolates sampled from partial resistant cultivars can induce a higher amount of disease on partial resistant cultivars than isolates sampled on susceptible ones, as observed for *Mycosphaerella graminicola* on wheat (Cowger and Mundt 2002), *Phytophthora infestans* on potato (Flier et al 2003; Andrivon et al 2007; Montarry et al 2008) and *Plasmopara viticola* on grapevine (Delmotte et al 2013). Over several generations, quantitative resistance can select for isolates with an increased rate of infection, a shortened latent period or an increased size of lesions, as shown for *Blumeria graminis* f.sp. *hordei* on barley (Kolmer and Leonard 1986), *Puccinia recondita* f.sp. *tritici* on wheat (Newton and McGurk 1991), and *Cochliobolus heterostrophus* on maize (Lehman and Shaner 1997, 2007). Muralidharan et al (2004d) searched in a farmers' field planted to resistant rice variety Ajaya for lesion caused by *Xanthomonas oryzae* pv. *oryzae* and obtained an isolate of the pathogen. They demonstrated the increased virulence of the pathogen which in subsequent inoculation tests nearly killed Ajaya. Therefore, super virulences do occur but in nature leading rarely to generalist populations, however, they do not dominate.

Even if differential efficiencies of quantitative resistance have been shown among sites (Andrivon et al 2007) and among pathogen isolates (Talukder et al 2004; Le Guen et al 2007; Marcel et al 2008; Delmotte et al 2013), the erosion of quantitative resistance at one site over time has rarely been demonstrated (Mundt et al 2002). It is indeed difficult to detect gradual changes in efficiencies of quantitative resistance (McDonald and Linde 2002), since these efficiencies are influenced by environmental conditions (Young 1996; Pariaud et al 2009; Lannou 2012).

The quantitative resistance may be subject to erosion and, apparently, even complete breakdown. The isolates of *Venturia inaequalis* can combine a high level of pathogenicity both on apple genotypes with quantitative resistance and on susceptible genotypes (Caffier et al 2014). As a consequence, there may be a risk of emergence of a generalist pathogen population. Since diverse mechanisms have been proposed to explain quantitative resistance (Poland et al 2009: Kou and Wang 2010: Vergne et al 2010), different corresponding adaptation process becomes a necessity for the pathogen with only a few leading to the emergence of generalist pathogen populations.

The results obtained by Caffier et al (2014) stress the need to look for diversified quantitative resistance factors that combine complementary modes of action on the pathogen, resulting in trade-offs between quantitative components of pathogenicity (Lannou 2012; Azzimonti et al 2013; Pariaud et al 2013), and to optimize the management of cultivar distribution in space and time (Sapoukhina et al 2013) to limit the possibilities of step-by-step evolution in pathogen populations (Bourget et al 2013).

Many rust resistance genes are known in wheat but only a few provide durable, race non-specific resistance to multiple pathogens (McIntosh et al 1995). The most widely deployed and best characterized of these genes, *Lr34*, confers durable, adult plant resistance to leaf rust caused by *Puccinia triticina*, stripe rust caused by *Puccinia striiformis* f.sp. *tritici* and powdery mildew caused by *Blumeria graminis* (Dyck 1987; McIntosh 1992; Morgounov et al 2012; Spielmeyer et al 2005). Adult plant rust resistance genes *Lr67* and *Lr34* confer race non-specific resistance to multiple fungal pathogens of wheat (Spielmeyer et al 2013).

Cotton leaf curl disease (CLCuD) is a serious disease of cotton which has characteristic symptoms, the most unusual of which is the formation of leaf-like enations on the undersides of leaves. The disease is caused by whitefly-transmitted geminiviruses (family Geminiviridae, genus *Begomovirus*) in association with specific, symptom-modulating satellites (betasatellites). India and Pakisatan have experienced two epidemics of the disease, the most recent of which involved a virus and satellite that overcome or break resistance. Loss of this conventional host–plant resistance led to extensive losses from the disease. The epidemic of CLCuD in Pakistan and north-eastern India during the 1990s involved multiple monopartite begomoviruses, with many plants containing more than one species, a single species of betasatellite and various alphasatellites. All previously resistant cotton varieties started to show symptoms of CLCuD from 2001 onwards. The begomovirus associated with this ability to overcome resistance was also found to be a recombinant (Sattar et al 2013).

Theoretical approaches predict that host quantitative resistance selects for pathogens with a high level of pathogenicity, leading to erosion of the resistance. This process of erosion has, however, rarely been experimentally demonstrated (Caffier et al 2014). Genetic diversity as observed in landraces of rice and its wild relatives enables the plants to evolve and differentiate into various cultivars to adapt to different environments (Morishima and Oka 1995).

Breeders of crop species need to select plants with adequate resistance to all significant diseases in the region for which seed of new cultivars is to be sold. They must also combine disease resistance with other commercially important traits, such as yield (Summers and Brown 2013). A major success in plant breeding for disease resistance is the broad-spectrum, durable control of powdery mildew (*Blumeria graminis* f. sp. hordei) of barley conferred by recessive alleles of *Mlo*. The widespread use of *mlo* resistance to control mildew may have inadvertently stimulated the emergence of *Ramularia* leaf spot (RLS) as a major disease of barley (McGrann et al 2014).

During 2005-2007, stem rust became a major threat to wheat production in the highlands of Bale and other wheat growing areas in Ethiopia. This was due to the fact that most resistance genes incorporated in wheat genotypes were *Sr2* and *Sr31* in which these genes were overcome due to appearance of new race of the pathogen. Currently, the disease is mainly controlled by the use of resistance varieties. However, varieties with acceptable level of resistance often rapidly succumb to the disease soon after release.

This scenario is especially more severe in bread wheat than in durum wheat (Letta and Tilahun 2007).

The leaf spot diseases, caused by early leaf spot (*Mycosphaerella arachidis* Deighton) and late leaf spot (*Mycosphaerella berkeleyi* Jenkins (Kirk 2004), are economically the most important fungal diseases of groundnut. In most areas, both diseases occur together but the incidence and severity of each disease vary with environment and cultivars (Pande and Rao 2001). The rosette is another devastating disease for the productivity of groundnut. Groundnut rosette is caused by the *Groundnut rosette virus* (GRV) and the *Groundnut rosette assistor virus* (GRAV) (Reddy et al 1995; Murant et al 1998). The disease is transmitted by *Aphis craccivora* Koch. Twenty-three advanced groundnut lines were evaluated for yield and resistance to early leaf spot (*M. arachidis*), late leaf spot (*M. berkeleyi*) and rosette virus. Most of the early groundnut lines were relatively resistant to rosette virus, early leaf spot and late leaf spot. The medium maturing lines showed mostly higher levels of diseases. The late groundnut lines were mostly susceptible to one or more of the diseases (Iwo and Olorunju 2009)

Plant pathogens are continually evolving to survive. Plants have developed a set of mechanisms to face the challenge of foreign pathogens through a long history of co-evolution. Among these mechanisms, maintaining allele (or ortholog) variation or diversity, either at the gene structure level or the expression level, is an important way for plants to protect themselves from pathogen attack. The *OsWRKY45* alleles, encoding different proteins, positively regulate rice resistance against the fungal pathogen *M.*

grisea. This pair of alleles regulates rice resistance to the same pathogen via different signaling pathways. A candidate gene (*OsWRKY45-2*), which regulates a race-nonspecific disease resistance in rice, is useful for breeding programs (Tao et al 2009).

M. oryzae and *X. oryzae* pv. *oryzae* are considered to be hemi-biotrophic pathogens that parasitize living tissues for a period and can continue life cycle on dead tissues. In the absence of adaptive immunity displayed by animals, plants fend off microbial pathogens via complex resistance mechanisms providing several layers of constitutive and inducible defences. Many of these defences are controlled by a series of signaling pathways within which plant hormones play central roles. Intimately connected to each other via a network of positive and negative interactions, hormones are thought to provide flexibility to the defence signalling network by enabling plants to adaptively tailor their immune system to the type of attacker encountered (Pieterse et al 2009; Robert-Seilaniantz et al 2007).

Sheath blight, caused by *R. solani* is a necrotrophic pathogen that may kill part or all of host plant before deriving nutrients from it. Among 33 rice accessions, mainly from National Institute of Agrobiological Sciences (NIAS) Core Collection, three landraces from the Himalayas—Jarjan, Nepal 555 and Nepal 8 were found to have resistance to sheath blight (Taguchi-Shiobara et al 2013). Rice genetic resources have not been comprehensively exploited for improvement of sheath blight resistance, although many cultivars and lines have been reported as promising sources of resistance (Srinivasachary et al 2011). Jasmine 85 is an *indica* cultivar that has proven to have a

high level of resistance to this pathogen and multiple QTLs are involved in resistance (Liu et al 2009).

Application of host resistance to bacterial blight caused by *X. oryzae* pv. *oryzae* and fungal blast caused by *M. grisea* pathogens is the most economical and environment-friendly approach to solve this problem. Quantitative trait loci (QTLs) controlling quantitative resistance are valuable sources for broad-spectrum and durable disease resistance. Although large numbers of QTLs for bacterial blight and blast resistance have been identified, these sources have not been used effectively in rice improvement because of the complex genetic control of quantitative resistance and because the genes underlying resistance QTLs are unknown (Hu et al 2008).

Li et al (1999) showed most resistance QTLs mapped to genomic locations where major resistance genes and/or QTLs for resistance to *X. oryzae* pv. *oryzae*, blast and sheath blight were identified in the same cross. Most QTLs showed consistent levels of resistance against all three Xoo strains. They concluded that a high level of durable resistance to *X. oryzae* pv. *oryzae* may be achieved by the cumulative effects of multiple QTLs, including the residual effects of "defeated" major resistance genes.

Rice tungro virus disease is a serious constraint to rice production in South and Southeast Asia. Rice cultivar Utri Merah is resistant to *Rice tungro spherical virus* (RTSV). Genetic analysis revealed that resistance to RTSV in Utri Merah was controlled by a single recessive gene (*tsv1*) mapped within an approximately 200-kb region between

22.05 and 22.25 Mb of chromosome 7. A gene for putative translation initiation factor 4G (eIF4G (*tsv1*)) was found in the *tsv1* region. Comparison of eIF4G (*tsv1*) gene sequences among susceptible and resistant plants suggested the association of RTSV resistance with one of the single nucleotide polymorphism (SNP) sites found in exon 9 of the gene. Examination of the SNP site in the eIF4G (*tsv1*) gene among various rice plants resistant and susceptible to RTSV corroborated the association of SNP or deletions in codons for Val (1060-1061) of the predicted eIF4G (*tsv1*) with RTSV resistance in rice (Lee et al 2010).

Genetics of resistance to rice tungro virus disease was studied systematically (Prasad et al 2004). Later, from the sequence information (www.tigr.org), Neeraja et al (2007) have deduced the putative candidate genes for resistance in the mapped regions. For Utri Rajapan, genes in chromosome 7 between RM21576 and RM21640 comprised of two NB-ARC domains, one LRR domain and one protease inhibitor (~1.4 Mb), and in chromosome 2 between RM13530 and RM13608 comprised of domains - one NBS LRR, six LRR and one serine threonine kinase. For Vikramarya, genes in chromosome 7 comprised of one LRR domain, one NB-ARC domain and two protease inhibitors (~1.3Mb) between RM21135 and RM21205, and in chromosome 1 between RM10123 and RM10133 comprised of three LRR and one NB-ARC genes (~0.3 Mb).

Increasing the durability of crop resistance to plant pathogens is one of the key goals of virulence management. Despite the recognition of the importance of demographic and environmental stochasticity on the dynamics of an epidemic, their effects on the evolution of the pathogen and durability of resistance have not received attention (Lo Iacono et al 2013).

Genetic uniformity results in crop failure

Internationally coordinated public wheat breeding efforts have focused in recent decades on increasing resistance to disease and abiotic stress (Reynolds and Borlaug 2006; Braun et al 2010). Genetic uniformity invites disaster because it makes a crop vulnerable to attack by a pest or disease that strikes one plant quickly spreads throughout the crop. Genetic diversity is the basic factor of evolution in species. It is the foundation of sustainability because it provides raw material for adaptation, evolution, and survival of species and individuals, especially under changed environmental, disease and social conditions (Hammer 2004), and it will allow them to respond to the challenges of the next century. Genetic diversity gives species the ability to adapt to changing environments, including new pests and diseases and new climatic conditions. In India rice cultivars have declined from an estimated 40,000 before colonialism to 30,000 in the mid-19th century with several thousand more lost after the green revolution in the 1960s. Erosion of crop genetic resources could pose a severe threat to the world's food security in the long term since loss of genetic variation may decrease the potential for a species to persist in the face of abiotic and biotic environmental change as well alter the ability of a population to cope with short-term challenges such as pathogens (Hammer and Teklu 2008).

Re-testing on performance of old commercial varieties

The continuous technological and environmental changes are reasons for periodical re-evaluation of variety performances and adaptation. Bridge et al (1971) evaluated 13 obsolete cotton varieties and three current commercial varieties for yield, agronomic, and

fiber properties over a period to determine what genetic improvements the new varieties had overcome of the older ones. The current commercial varieties yielded approximately 112 kg/ha more lint than the best obsolete variety. Most obsolete varieties yielded 124-448 kg/ha less lint than the commercial check varieties. These data indicated that varieties exhibiting increased yield potential had higher lint percentages, smaller bolls, smaller seed, and higher micronaire values. Increases in lint percentage have played a major role in increasing yield and fiber properties of varieties currently grown were better than those of some old varieties, but inferior to those of others. Advances in fiber properties have not been as rapid as advances in yield (Bridge et al 1971).

Nsarellah et al. (2011) re-assessed the adaptation of the main registered durum wheat (*Triticum turgidum* var. L. *durum*) varieties in Morocco. The varieties from the medium breeding era were widely adapted and possessed a better ability to exploit favorable environments. The oldest varieties were widely adapted but without a high yield potential in the more favorable environments.

Twelve genotypes of winter wheat were tested (Austin et al 1980). Eight were varieties which formed a chronological series beginning with Little Joss, introduced in 1908. The average yield of the 12 varieties and lines tested was 3·96 t/ha in Paternoster Field and 6·40 t/ha in Camp Field. In both fields the two highest yielding entries, depended on high soil fertility. The newer, high yielding, varieties were shorter and reached anthesis earlier than the older varieties. They had lower stem weights/m^2 than the older varieties but similar maximum leaf area indices and leaf weights/m^2. Within each

experiment the total dry-matter production of the varieties was similar, the increase in grain yield due to variety improvement being associated mainly with greater harvest index (ratio of grain yield to grain + straw yield (Austin et al 1980).

Detached plant-part assays for test of resistance in host plants

Typical techniques of screening for resistance germplasm in most crop plants involve field ratings for the presence of lesions and other damage caused by a pathogen. A disadvantage of this method is dependence on favourable environmental conditions or controlled facilities in a greenhouse for the pathogen development before rating can be taken. Variation in virulence of pathogen populations also will likely influence these tests, producing differences in observed cultivar susceptibility between locations. To bypass these inherent difficulties in conducting field tests, a detached leaf method is used for assessing a crop variety for resistance.

Several techniques using detached leaves have been developed to study host–pathogen interactions, including maize rust (Kushalappa and Hegde 1971), *Fusarium* head blight of wheat (Browne and Cooke 2004), *Septoria tritici* blotch (Arraiano et al 2001), yellow rust of barley (Osman-ghani 1982), and powdery mildew of barley (Brown and Wolfe 1990). These methods were successfully focused on host resistance. Most detached-leaf studies have utilized the cytokinin kinetin and benzimidazole to delay senescence. Two potential problems with the use of these chemicals could be enhancement of senescence in oat by L-serine and L-cysteine in the presence of kinetin (Martin and Thimann 1972; Shibaoka and Thimann 1970) and, because benzimidazole is

structurally related to the fungicide carbendazim, it may affect the production and viability of *P. oryzae* spores.

Dolar (1997) tested successfully resistance of detached chickpea leaflets to two different races of *Ascochyta rabiei* (Pass.) Labr. Detached leaf system was found to be useful for the propagation of numerous single-race cultures of *Puccinia coronata* as well as evaluation of host resistance under highly controlled conditions (Jackson et al 2008). Oat (*Avena sativa*) leaf sections (10 cm each) were harvested, disinfested, and suspended in sterile plastic boxes by enclosing 3.5-cm linear sections of each leaf end between 4% agar blocks amended with various chemical constituents. The exposed sections (approximately 3 cm) were inoculated with *P. coronata* urediniospores suspended in water. Boxes were sealed and incubated in a lighted growth cabinet until the pathogen sporulated. Viable spores were produced on leaves in all treatments including benzimidazole and kinetin (100 mg/l). Detached leaves of differential oat cultivars produced the same reactions as whole plants screened under standard conditions in a growth chamber.

Detached leaves or leaf disks floated on water are characterized by drifts in the activities of various enzymes (De Leo and Sacher 1970; Farkas et al 1964; Kar and Mishra 1975; Kisban et al 1964; Parish 1968) and an increase in the respiratory rate. Manifold increase in the activities of several oxidative enzymes during detached leaf senescence has been reported (Farkas et al 1964; Kar and Mishra 1975; Parish 1968). The majority of the observations support the idea that senescence and higher levels of

oxidative enzymes are closely associated phenomena, but contradictory reports have also appeared. For example, a group of Hungarian workers (Farkas et al 1964; Kisban et al 1964) reported that the catalase (EC 1.11. 1.6) activity in tobacco leaves decreased but in wheat and barley leaves it increased upon detachment. Parish (1968) reported a decrease in the activity of catalase in tobacco leaves during senescence and maturation. There are several reports that peroxidase (EC 1.11.1.7) activity increases during senescence of detached leaves or leaf disks (Farkas et al 1964; Parish 1968). Increase in the activity of this enzyme with the physiological age of the leaves has also been reported. Parish (1968) also suggested that the increase in the activity of peroxidase is one of the most reliable indicators of maturity and senescence. But Ford and Simon (1972) contradicted Parish's (1968) suggestion because peroxidase activity increased several-fold when senescence was delayed and chlorophyll and protein levels increased in the cotyledons of de-topped cucumber seedlings. They concluded from these observations that at least in this particular case, the rise in peroxidase activity cannot be taken as a reliable indicator of senescence. Polyphenoloxidase (EC 1.10.3.1) activity increases during senescence of detached leaves or leaf disks (Farkas et al 1964; Kar and Mishra 1975) as well as with the physiological age of the attached leaves (Maraite 1973).

A detached pod or leaf assay conducted in vitro is only of benefit to the plant breeder if it correlates well to field responses (Irwin et al 2003). Detached plant-part screening assays are useful because they enable assessments to be made under highly-controlled conditions, serve as rapid screening techniques that can be adopted by

breeding programs and can assist our understanding of host-pathogen interactions (Huang et al 2005; Bradley et al 2006; Ergon and Tronsmo 2006).

Study of genetic diversity of released old varieties

Ever since the domestication of crop plants, man has been improving them giving selection emphasis to traits that suit his agro-ecological and socioeconomic needs. In rice, like many crops, selection preference has been for improvement of yield enhancing traits like compact panicle with more grains/panicle, large seed size, non-shattering habit etc. The selection process continued for centuries result in cultivars far different from the wild/weedy progenitor species in their habit and potential. Since the advent of the short statured high yielding varieties in the mid-1960s, the selection priority of breeders has been for higher stability or performance, need based crop duration, tolerance to various stresses and consumer preferred grain quality. Excessive breeding emphasis in this direction given during the last 50 years knowingly or unknowingly has led to some sort of genetic uniformity among the currently cultivated high yielding varieties. The improved germplasm is being excessively depended on for needed variability for progressive improvement of the crop with no precise knowledge of extent of exploitable variability beyond simply inherited traits. Also it is not clear to what extent breeding strategies in vogue have been facilitating to broaden or narrow the genetic diversity in the breeding nurseries of rice.

Many recent scientific papers tracing the trend of genetic diversity in crop varieties released over the years reveal the diversity levels to often fluctuate strongly

from one time period to the successive periods (Christiansen et al 2002; Tian et al 2005; White et al 2007) and sometimes show conflicting results (Huang et al 2007; Roussel et al 2004). By and large no clear pattern has emerged from such studies in the past, as both increasing and decreasing trends in diversity have been observed (Fu et al 2006; Hazen et al 2002; Hysing et al 2008). Diversity loss in rice during the last few centuries is obvious from the rapid decline of rice varieties from 400,000 before colonialism to 30,000 by mid-19th Century. This number has further come down during the era of the Green revolution as few high yielding varieties replaced thousands of native varieties (Heal et al 2004). Estimation of genetic diversity in varieties released in different crops during the last century reveals no clear trends (Wouw et al 2010). In rice, both decreased (Wei et al 2009) as well as increased trend of diversity (Muralidharan et al 1996; Prasad et al 2001; Mantegazza et al 2008) has been reported.

Reddy et al (2009) determined genetic relationship among 12 rice varieties including 9 tolerant to drought, flood, or salinity using 17 different inter-simple sequence repeat (ISSR) markers. Based on all markers, the nine tolerant varieties formed one cluster distinct from the cluster of three control varieties. The salt-tolerant varieties were closest to two flood-tolerant varieties, and together they were distinct from the drought-tolerant varieties. The most informative primer was (GA)8YG that showed the highest polymorphic information content (PIC) and resolving power (Rp). The drought-, flood-, and salt-tolerant varieties were grouped in three distinct clusters within the group of tolerant varieties, when (GA)8YG was used. Sabita was the only exception. The two aus varieties, Nagina22 and FR13A, were separated and grouped with the drought- and flood-

tolerant varieties, respectively, but they were together in dendrograms based on other primers. The results show that markers associated with (GA)8YG (ISSR 842) delineated the three groups of stress-tolerant varieties from each other and can be used to identify genes/new alleles associated with the three abiotic stresses in rice germplasm.

Genetic diversity in representative sets of high yielding varieties of rice released in India between 1970 and 2010 was studied at molecular level employing hypervariable microsatellite markers (Choudhary et al 2013). Of 64 rice SSR primer pairs studied, 52 showed polymorphism, when screened in 100 rice genotypes. A total of 184 alleles were identified averaging 3.63 alleles per locus. Cluster analysis clearly grouped the 100 genotypes into their respective decadal periods i.e., 1970s, 1980s, 1990s and 2000s. The trend of diversity over the decadal periods estimated based on the number of alleles (Na), allelic richness (Rs), Nei's genetic diversity index (He), observed heterozygosity (Ho) and polymorphism information content (PIC) revealed increase of diversity over the periods in year of release-wise and longevity-wise classification of rice varieties. Analysis of molecular variance (AMOVA) suggested more variation in within the decadal periods than among the decades. Pairwise comparison of population differentiation (Fst) among decadal periods showed significant difference between all the pairs except a few. Analysis of trends of appearing and disappearing alleles over decadal periods showed an increase in the appearance of alleles and decrease in disappearance in both the categories of varieties. It was obvious from the present findings that genetic diversity was progressively on the rise in the varieties released during the decadal periods, between 1970s and 2000s (Choudhary et al 2013).