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RESEARCH ARTICLE

Strategies for management of bacterial blight disease of rice caused by *Xanthomonas oryzae* pv. Oryzae (small oryzae): an overview.

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Abstract

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..... Rice is the most widely cultivated food crop of the world. Asia alone accounts for 90 per cent of the world's production coupled with consumption of rice also because of favorable hot and humid climate. Rice accounts for 35-75 per cent of the calories consumed by more than 3 billion Asian (Khush, 2004). It is also expected that by the year 2050, 90 per cent of world's projected 11 billion people will reside in the developing countries (Krattigar, 1996). The production is constrained mainly due to biotic and abiotic stress. Major advances have occurred in food production during the last seven decades due to adoption of improved techniques including high yielding varieties. This has also lead to emergence of new pests and diseases simultaneously. The loss of the yield in all crops due to biotic stress is approximately 500 billion. It has been estimated that in bacterial blight of rice the yield losses are as high as 6 to 60 per cent depending upon location, season, weather condition and cultivars (Srivastava, 1967). Bacterial blight (BB) of rice, caused by Xanthomonas oryzae pv. oryzae (Xoo) reported as economically important disease in tropical Asia also (Mew, 1987 and 1989). The disease was known to occur in epidemic proportions in many parts of the world, incurring severe crop loss of up to 50 per cent. Crop loss assessment studies have revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar susceptibility and to a great extent, the conduciveness of the environment in which it occurs. Bacterial blight of rice is difficult to control through chemicals. Varietal resistance is considered the most practical and economic way of keeping the

disease below the economic injury level. Variability in pathogenicity have now been fully recognized. Resistant varieties development has been targeted in almost all the breeding programmes of varietal improvement. In this context number of isogenic lines, differential having known genes and pyramids with more than two resistant genes have been also developed by different agencies. The literature pertaining to plant resistance and its nature of response in rice to *Xanthomonas oryzae* pv. *oryzae* has been reviewed under following heads:

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Introduction:-

Mohanty *et al.* (1996) tested during the 1994 wet season, 60 elite deep-water rice entries for their reaction to bacterial blight caused by *Xanthomonas oryzae* of the 60, 4 were highly resistant; 12 and 35 were moderately resistant and moderately susceptible and 8 were susceptible to the disease during *rainy* season. Chang *et al.* (2000) indicated that researchers in different IRRI departments have made a number of limited evaluations of the *O. glaberrima* cultivars and also the strains of various wild species. The African rice and the wild taxa appeared to

offer an array of resistances or tolerances to some of the biotic and environmental stress factors, especially the leaf hoppers, plant hoppers, and drought. Tiwari and Kumar (2000) evaluated a set of 80 new rice breeding lines based on low tillering and heavy panicle eco-ideotype for irrigated eco-systems for their reaction to bacterial blight (Xanthomonas oryzae pv. oryzae), sheath blight (Rhizoctonia solani f.sp. sasakii) and gall midge (Oreseolia oryzae) during Kharif 1996. They observed that three of these breeding lines, NPT 57K-11-104 NPT 63 K-2-109 and NPT 57-K-11-16 were resistant against all five isolates of Xanthomonas and 31 entries showed less than 30 per cent disease index against R. solani f.sp. sasakii, two breeding lines, NPT 63K-2-109 and NPT 57-K-11-104, were not only resistant against all the five isolates of Xanthomonas but also showed a very low per cent disease index against R. solani f.sp. sasakii. However, all the entries were susceptible to gall midge. Zeng et al. (2002) developed first time for cold-tolerance at booting stage in japonica rice through genetic analysis and Chinese near isogenic lines (NILs). Genetic analysis showed that cold tolerance of two pairs of NILs belongs to the major genes and one pair for QTL-NILs. Three pairs of NILs for cold tolerance at boot stage had been bred by using backcrossing under low temperature condition, and had very similar morphological characters, but very different cold tolerance between NILs and Towada. Therefore, NILs and Towada were ideal materials for cold gene location and cloning. Chen et al. (2006) examined 14-3-3 functions of the rice 14-3-3/GF14 proteins in defense biotic and abiotic stress responses. The phylogenetic comparison with the Arabidopsis 14-3-3 family revealed that the majority of rice GF14s might have evolved as an independent branch. At least four rice GF14 genes, GF14b, GF14c, GF14e and Gf14f were differentially regulated in the interactions of rice Magnaporthe grisea and rice Xanthomonas oryzae pv. oryzae and the incompatible interactions stronger induced the genes than the compatible interactions. These GF14 genes were also induced by the defense compounds, benzothiadiazole, methyl jasmonate, ethephon and hydrogen peroxide. Similarly, they were differentially regulated by salinity, drought, wounding and abscisic acid. Magarey et al. (2007) observed that many of the temperate and irrigated areas (described above) do not have Xoo or remains fewer days suitable for infection. It might be possible that these areas do not had the disease because of the unfavorable climate. The opinion that the validation map was not perfect since some areas that had Xoo (such as Ecuador) also had few days favorable for infection. They believed these differences were due to the difficulties with estimating relative humidity in countries with sparse weather observations. Relative humidity was a difficult variable to estimate because it was dependent upon both rainfall and air temperature. Stress related gene induction response was also studied by Jung et al. (2007). They identified 365 genes that showed significant 8-fold or greater induction in the light relative to dark conditions. Screened collections of rice T-DNA insertional mutants to identify rice lines with mutations in the strongly light-induced genes. This analysis effectively provided candidate functions for two genes of previously unknown function and for one gene not directly linked to the tested biochemical pathways. Ndjiondjop et al. (2009) screened that a set of 47 Oryza glaberrima accessions under controlled conditions (28°C and 80% of relative humidity) in a greenhouse with four Xanthomonas oryzae pv. oryzae (Xoo) strains: 3 African strains and 1 Asian, as control. BAI3 (race 1) and BAI4 (race 2) were from Burkina Faso, MAI1 (race 3) from Mali and PXO86 (race2) was from Philippines. Disease scoring was assessed by the measurement of the length of the lesion 21 days after inoculation. Based on the length of the lesion, 9 accessions were identified as resistant to MAI1 while PXO86 induced 12 resistance reactions. None of the accessions tested were resistant to BAI3 and BAI4. They also showed that comparison of grain yield under drought and control treatment, some of the accessions (named in the graphs below) were able to maintain a high yield under drought conditions. Webb et al. (2010) observed that increasing environmental temperatures may complicate R-gene-mediated disease control because high temperatures often promote disease development and reduce R gene effectiveness. Disease severity and virulence of Xanthomonas oryzae pv. oryzae (Xoo) populations was monitored in field plots planted rice with and without the bacterial blight R gene Xa7 over 11 years. The performance of Xa7 was determined in high and low-temperature regimes and growth chambers. Rice with Xa7 exhibited less disease than lines without Xa7 over 11 years, even though virulence of Xoo field populations increased. Xa7 restricted disease more effectively at high than at low temperatures. Other R genes were less effective at high temperatures. They also indicated that Xa7 restricts disease and Xoo population size more efficiently in high temperature cropping seasons compared with cool seasons creating fluctuating selection, thereby positively impacting durability of Xa7. Similarly in other crops also the host-pathogen interaction were studied. Avasthi and Raghavendra (2008) observed that marked and mutual interaction between temperature and light has been during the modulation of the activity and regulatory properties of phosphoenolpyruvate carboxylase (PEPC) in leaf discs as well as leaves of Amaranthus hypochondriacus. They concluded that temperature and light could modulate activity and regulatory properties of PEPC not only individually but also in a synergistic manner.

Phenotypic characters of the genotypes and influence on the resistance:-

Kiryu and Mizuta (1955) found that cultivars with few, short, narrow and erect leaves had low infection than those having luxuriant growth and spreading leaves. Cultivars with hairy leaves showed maximum disease while, the disease was very low in glabrous leaves due to retention of more inoculums by the hairy cultivars (Premlatha Dath *et*

al., 1977; Raju Philip and Devadath, 1980). Resistant variety posessed lower stomatal index than susceptible ones (Shukla and Gangopadhyay, 1981). A negative correlation has been observed between disease development and frequency of distribution of silicate cells in coastal region (Kaul and Sharma, 1987). However, some workers did not find any correlation between posture, colour, length and thickness of leaves, number of tillers/ hill and duration of cultivars with bacterial blight incidence (Singh and Rao, 1971). A high genotypic coefficient of variation coupled with high heritability and genetic gain was found for lesion size and the area under disease progress curve, indicating the predominance of additive gene effects in eight rice cultivars inoculated with Xoo. There was a strong association among the components at genotypic and phenotypic levels. Further, it was noticed that genotypic correlations were higher than phenotypic, clearly indicating the modifying effect of the environment on association of the components (Nayak et al., 1987). Century et al. (1999) studied developmental expression of Xa21 resistance that depends on the unambiguous determination of the physiological stage of leaves. Each sequential leaf just at full expansion was more resistant than the preceding leaf, which means that acquiring the resistance phenotype was a gradual process from fully susceptible leaf two to leaf five with 75 per cent resistance. Leaves 9 and 10 were fully resistant. Koch and Parlevliet (2004) measured lesion length, leaf length and leaf width on infected leaves two weeks after clip inoculation of 64 rice cultivars with two virulent isolates of Xanthomonas campestris pv. oryzae (X. c. pv. oryzae). They found no significant correlation between the lesion length and leaf dimensions, indicating that physical leaf size does not affect the spread of the bacteria once they entered the leaf. Lesion length was therefore an acceptable parameter for assessing resistance to X. c. pv. oryzae, and it was preferred to be above the parameter per cent diseased leaf area (% DLA), specially when small differences between genotypes are to be assessed. The confounding influence of differences in leaf length could cause large changes in the ranking order of cultivars when assessed by the per cent DLA. For this reason lesion length was a better assessor of the value of a quantitative resistance for breeding and research purposes than per cent DLA. De Cleene (2008) observed that scanning electronmicroscopy of the leaves of Italian ryegrass and maize, inoculated with Xanthomonas campestris pvs. graminis, oryzae and oryzicola clearly showed a difference in the distribution pattern among the different pathovars tested. Pathovars *oryzae* and *oryzicola* could be detected on the leaf trichomes of rye grass, while py. *graminis* was not.

Response of different genes to stimulants in imparting resistance:-

Stimulants / catalase, being an antioxidant enzyme, play a major role in combating the toxic effect of reactive oxygen species (ROS) in plant cells. Effect of different stimulant in imparting resistance against Xanthomonas oryzae pv. oryzae were studied by few research workers- Plant growth regulators were known to influence the biochemical processes of higher plants. The 2, 4-dichlorophenoxy acetic acid (2, 4-D); α -napthalene acetic acid (NAA); 2, 3, 5- triiodobenzoic acid; β-naphthoxy acetic acid (NBA) and indole-3, acetic acid were found to bring a change in disease susceptibility through altered metabolism in plants including decrease in plant weight, reducing and non-reducing sugars, proteins and most of the amino acids, accumulation of nitrogen of host tissues and morphological changes. However, there were no significant charges in starch, polysaccharides, crude fibre, ash, ether extract, unsaponifiable material and fatty acids (Davis and Dimond, 1953; Luecke et al., 1949). Pal and Singh (1977) studied the systemic bactericidal property of brestanal against Xathomonas oryzae (Uyeda and Ishiyama) Dowson in rice seedlings and found that it could be translocated from root to leaf through stem and leaf sheath. The quantity of the compound absorbed increased with concentration and exposure time in different parts of the seedling. Maximum amount of the chemical remained in the root, but it was translocated in the leaves in sufficient quantity to inhibit the growth of the test pathogen. Ye et al. (1996) mentioned that the changes in activity of this enzymes (PPO [catechol oxidase]) in 11 rice cultivars infected by Xanthomonas campestris (X. oryzae) pv. oryzae and pv. oryzicola. They observed that PPO activity in healthy leaves showed no relationship with plant resistance In infected leaves, PPO activity was nearly the same as that in healthy leaves in some resistant cultivars and greater in others, but activity decreased in susceptible cultivars. Changes in activity in seedling leaves cross-infected by different virulent strains of X. oryzae pv oryzae or X. oryzae pv. oryzae + X. oryzae pv. oryzicola were related to cultivar resistance. Babu et al. (2003 a) determined the biochemical response of the bacterial blight susceptible rice cv. IR 50 inoculated with 10⁹ cfu Xanthomonas oryzae py. oryzae (Xoo)/ ml 30 days after sowing in pot experiments. They observed clip inoculation with Xoo significantly controlled bacterial blight in IR 50 under greenhouse conditions. The highest phenylalanine ammonia-lyase activity (110 nmol/ minute/ g fresh weight), phenolic content (275 micro g/ fresh weight) and chitinase activity (7.5 n mol/ minute/ g fresh weight) were observed 4, 3 and 5 days after the treatment. Western blot analysis showed the induction of a 25 KDa thaumatin-like protein and a 35 KDa chitinase. The 35 KDa chitinase started accumulating one day after the treatment and the intensity of the protein bands increased throughout the duration of the experiment. Babu et al. (2005) studied the systemic acquired resistance induced by benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) in rice against bacterial blight (BB) caused by Xanthomonas oryzae pv. oryzae. Rice plants (IR 50) pre-treated with BTH showed resistance to a challenge infection with Xanthomonas oryzae pv. oryzae. About 50% reduction in disease intensity was observed in

plants treated with BTH at 100 µg a.i./ml. Immunoblot analysis using barley chitinase antiserum revealed the induction of a 35 kDa chitinase in rice in response to treatment with BTH. The results indicated that the BB resistance induced even in genetically susceptible cultivar through application of BTH. Nishioka et al. (2005) studied the effect of CMPA (Pyrazole-derivatives-3, chloro-1-methyl-1-H-pyrazole-5-carboxylic acid) which reduced the disease symptoms in a particular dose, but it did not exhibit any direct antibacterial activity against Xanthomonas oryzae at concentrations up to 1 mg/ml. The treatment of CMPA induced the expression of PBZ1, a defense-related gene, which was evoked by several plant activators. Mahmood et al. (2006) studied that compatible and incompatible interaction between rice and bacteria. Rice cv. Jawa 14 seedlings were inoculated with compatible (Xo7435) and incompatible (T7174) races of Xanthomonas oryzae pv. oryzae (Xoo). Cytosolic and membrane proteins were fractionated from the leaf blades and separated by 2-D PAGE. From 366 proteins analyzed, 20 were differentially expressed in response to bacterial inoculation. These proteins were categorized into classes related to energy (30%), metabolism (20%), and defense (20%). Among the 20 proteins, ribulose-1, 5-bisphosphate carboxylase / oxygenase large subunit (RuBisCO LSU) was fragmented into two smaller proteins by T7174 and Xo7435 inoculation. Treatment with jasmonic acid (JA), a signaling molecule in plant defense responses, changed the level of protein accumulation for 5 of the 20 proteins. Thaumatin-like protein and probenazole-inducible protein (PBZ) were commonly up-regulated by T7174 and Xo7435 inoculation and JA treatment. These results suggest that synthesis of the defense-related thaumatin-like protein and PBZ were stimulated by JA in the defense response pathway of rice against bacterial blight. Mahmood et al. (2007) studied that role of jasmonic acid (JA) in the rice self-defense mechanism, a proteomic approach was applied. When 3 week old rice cv. Jawa-14 was treated with 100 micro M JA for 3 days, numerous necrotic brown spots were observed on the leaf blade. Three week old rice was treated with JA and proteins from cytosolic and membrane fractions of leaf blade were separated by twodimensional polyacrylamide gel electrophoresis. A total of 305 proteins were detected in both cytosolic and membrane fractions. When rice plant was treated with 100 micro M JA for 2 days, 12 proteins were up-regulated and 2 proteins were down-regulated. Out of them, 8 proteins were changed in dose dependence manner, while 4 proteins were changed in a time course manner. Among them, pathogenesis-related protein 5 (PR5) and probenazole inducible protein 1 (PBZ1) were significantly induced by 100 micro M JA for 2 days. These results suggested that PR5 and PBZ1 were important proteins expressed down-stream of JA signals in rice cv. Jawa-14. Ding et al. (2008) suggested that the bacterial infection induced the accumulation of indole-3-acetic acid (IAA), the major type of auxin, in rice (Oryza sativa). IAA induced the expression of expansins, proteins that loosen the cell wall. Loosening the cell wall was key for plant growth, but may also make the plant vulnerable to biotic intruders. They further reported that rice GH3-8, an auxin-responsive gene functioning in auxin-dependent development, activates disease resistance in a salicylic acid signaling- and jasmonic acid signaling-independent pathway. GH3-8 encodes an IAAamino synthetase that prevents free IAA accumulation. Over-expression of GH3-8 resulted in enhanced disease resistance to the rice pathogen Xanthomonas oryzae py oryzae. This resistance was independent of jasmonic acid and salicylic acid signaling. Over-expression of GH3-8 also caused abnormal plant morphology and retarded growth and development. Both enhanced resistance and abnormal development might be caused by inhibition of the expression of expansins via suppressed auxin signaling. Nishioka et al. (2005) assessed a pyrazole derivative, 3chloro-1-methyl-1H-pyrazole-5-carboxylic acid (CMPA) on rice bacterial blight caused by Xanthomonas oryzae pv. oryzae and the expression of a defense-related gene. The treatment of CMPA reduced the disease symptoms in a dose-dependent manner, but CMPA did not exhibit any direct antibacterial activity against X. oryzae at concentrations up to 1 mg/ml. The treatment of CMPA induced the expression of PBZ1, a defense-related gene, which was evoked by several plant activators. This ability to induce PBZ1 expression and enhance disease resistance without antimicrobial activity suggests that CMPA could activate systemic acquired resistance in rice as well as in tobacco. Babu et al. (2008) studied that role of the plant defense activator, acibenzolar-S-methyl (ASM), in inducing resistance in rice against bacterial blight caused by Xanthomonas oryzae pv. oryzae (Xoo). However, in the rice plants pretreated with ASM, infection was significantly reduced. Induced systemic resistance was found to persist for up to 3 days in the pretreated rice plants. Increased phenolic content and accumulation of pathogenesis-related (PR) proteins, viz. chitinase, β -1, 3-glucanase and thaumatin-like protein (TLP; PR 5) were observed in rice plants pretreated with ASM followed by inoculation with Xoo. Immuno blot analysis using rice TLP and tobacco chitinase antiserum revealed rapid induction and over-expression of 25 and 35 kDa TLP and chitinase, respectively, in rice in response to pretreatment with ASM followed by Xoo inoculation. Based on these experiments, it was evident that induction of disease resistance in rice was accelerated following treatment with ASM. Choodamani et al. (2008) investigated the role of catalase in determining the virulence of Xanthomonas oryzae pv. oryzae isolates and the reaction of different rice cultivars to virulent isolates. Among the 11 isolates studied, a variable level of catalase activity and differential expression of isoforms in different isolates were recorded. The maximum level of catalase activity was found in isolate Xoo2 followed by Xoo4, and the minimum level was recorded in isolate Xoo3. A virulence assay conducted on the susceptible rice cultivar Jawa-14 revealed that the bacterial isolate with the highest

level of catalase activity caused the maximum lesion length and vice versa. Among 31 cultivars screened, cv. CTH-1 showed the lowest incidence of disease, and lesion length was almost nil. The cultivars Thanu, Rasi and CTH-3 were found to be highly susceptible to *X. oryzae* pv. *oryzae* infection. They further ascribed that the possible use of catalase enzyme as a biochemical marker in studying the virulence of *X. oryzae* pv. *oryzae* isolates. Yang (2009) analyzed that bacterial blight (BLB) losses by hyperspectral canopy reflectance spectra of two rice cultivars with different susceptibilities to BB to establish spectral models for assessing disease severity for future site-specific management. The results indicated that wavebands from 757 to 1039 nm were the most sensitive region of the spectrum for the moderately susceptible cultivar TNG 67, whereas most narrow bands showed a significant relationship for the highly susceptible cultivar TCS 10. All the spectral indices (SI's) calculated had significant relationships with proportions of infested area in cultivar TCS 10, but only two SI's correlated significantly with cultivar TNG 67 could be improved by using a multiple linear regression approach. Similarly in other crops also the host-pathogen interaction was studied. These results were consistent with the hypothesis that sensing jasmonic acid by this bacterium helped the pathogen to ingress inside plant tissues.

Systemic Acquired Resistance:-

Many findings from researchers revealed the importance of some chemicals in imparting acquired resistance prior to the infection at the site of activity in host pathogen interaction system. The SAR with reference to *Xanthomonas* oryzae pv. oryzae was also reported by several workers.

Dempsely and Klessing (1995) reported that salicylic acid played an important role in inducing signal for disease resistance and development of systemic acquired resistance. It appears to transude this signal by inhibiting catalase activity and generating increase level of reactive oxygen species. Silverman et al. (1995) found that rice seedling had the highest level of salicylic acid among all plants tested for salicylic acid content. To investigate the role of salicylic acid in rice disease resistance, the level of salicylic acid in rice cultivar M-201 after inoculation with bacteria and fungal pathogen were examined and found that the second leaf of rice seedling had slightly lower level than any younger leaves. Xiao et al. (1996) observed that when seedling roots were soaked in salicylic acid, the malondialdehyde content and permeability of Plasma membranes dropped but Phenylalanine Ammonia Lyase activity, chlorophyll content and IUFA value increased. It was concluded that exogenous salicylic acid might increased the resistance to bacterial blight. Salicylic acid, occurring naturally in rice plants, was synthesized from Cinnamic acid via benzoic acid. Its accumulation was proposed to activate the low level expression of SAR genes throughout the plant and the newly synthezied salicylic acid was stored as glycoside (Gaffney et al., 1993). Salicylic acid was known to bind and inhibit the activity of catalase that might serve as a second messenger for the induction of defense response (Durner et al., 1997). Liu and Wang (2000) investigated the role of salicylic acid in defense mechanism and found that it induced resistance in rice plants against leaf blight at a concentration range of 5-50 µg/ml. They further observed that foliar spraying of salicylic acid 2 or 5 days after root soaking could increase the resistance induced and elongated the sustained resistance. When rice seedlings were treated by root-soaking (5 µg/ ml solution) or by foliar spraying (10 μ g/ ml solution) and inoculated with a spore suspension after 1 or 2 days, the disease incidences of leaf blight was reduced by 50.8 per cent and 50.4 per cent, respectively compared with that treated with water as a control. Wang et al. (2000) revealed that examination of the genes involved in the initial steps of oxylipin synthesis abrogation of the PLD α attenuated the wound-induced expression of lipoxygenase 2 (LOX_{2}) but had no effect on allene oxide synthase (AOS) or hydroperoxide lyase in wounded leaves. The systemic induction of LOX₂, AOS, and vegetative storage protein was lower in the PLD α -suppressed plants than in wild-type plants, with AOS exhibiting a distinct pattern. These results indicated that activation of PLD mediated wound induction of JA and that LOX₂ was probably a downstream target through which PLD promotes the production of JA. Rohilla et al. (2001) reported that SA spray did not reduce bacterial blight severity significantly when sprayed seven days before and seven days after inoculation @ 1 g/l. Salicylic acid (SA) mediated resistance in generally restricted to treated tissue indicated that it did not translocated efficiency. Exogenous SA was rapidly conjugated mostly into b-glycoside and these conjugates lack the phloem mobility of free salicylate (Iwata et al., 1980; Malamy et al., 1992). Song et al. (2001) investigated the suppression of the bacterial blight disease (caused by X. oryzae pv. oryzae) in rice seedlings pretreated with benzothiadiazole (BTH, 0.5 m mole/liter), salicylic acid (1 m mol/liter), nickel nitrate (0.5 m mol/liter), paclobutrazol (0.3 g/ liter) or uniconazole (0.04 g/ liter) 3 days before bacterial inoculation. The inhibitory effect of BTH (1 m mol/liter) on the growth of X. oryzae py. oryzae in vitro was not significant. The optimum concentration of BTH for resistance induction was 0.5-1.0 m mol/liter. An interval of at least one day between BTH induction and challenge inoculation was required for the establishment of induced resistance. A seven day interval gave the highest protection. The BTH induced resistance resulted in protection from

disease for at least 15 days. Babu et al. (2003 b) investigated the effect of salicylic acid (SA) and hypothesized, to be as a natural signal that triggers the systemic induction of phenolics, pathogenesis-related proteins and disease resistance in rice against the bacterial blight pathogen Xanthomonas oryzae pv. oryzae. A three-fold increase in the endogenous SA levels was observed in the rice tissues pretreated with 1000 µ mol/l SA and the resistance persisted for at least 3 days after SA treatment prior to inoculation with Xoo. Increasing the endogenous level of SA in rice leaves to those naturally observed during systemic acquired resistance resulted in increased resistance to Xanthomonas oryzae pv. oryzae, expressed as a reduction in leaf blight lesion length. Immunoblot analysis revealed an induction of a 25 kDa protein cross-reacting with rice thaumatin-like protein (TLP) antiserum in response to SApretreated and SA non-pretreated rice plants followed by pathogen inoculation. A significant increase in the induction of TLPs 3 days after Xoo inoculation in the tissues pretreated with SA was observed when compared with the 2 days and 1 day after Xoo inoculation in SA-treated plants. Increased phenolics content and enhanced activities of some pathogenesis-related (PR) proteins, viz., TLP, chitinase and β -1,3-glucanase were observed in rice plants treated with SA. Based on these experiments, it was inferred that the defense responses are induced locally at the infection site only after pathogen attack and was augmented when the rice tissue has been pretreated with SA. Koch and Parlevliet (2004) assessed the lesion length, leaf length and leaf width on infected leaves two weeks after clip inoculation of 64 rice cultivars with two virulent isolates of Xanthomonas campestris pv. oryzae (X.c. pv. oryzae). No significant correlation was found between the lesion length and the leaf dimensions, indicated that physical leaf size does not affect the spread of the bacteria once these has entered the leaf. Lesion length was therefore an acceptable parameter for assessing resistance to (X. c. pv. oryzae), and was to be preferred above the parameter per cent diseased leaf area (% DLA), especially when small differences occurred between genotypes. The confounding influence of differences in leaf length can cause large changes in the ranking order of cultivars when assessed by the % DLA. For this reason, lesion length was a better assessor of the value of a quantitative resistance for breeding and research purposes than % DLA. Mahmood et al. (2006 and 2007) also suggested that synthesis of defense related proteins were stimulated by jasmonic acid in the defense response pathway of rice against bacterial blight. Meirong et al. (2008) sonducted that NPR1 was significantly up-regulated in transgenic rice line, which required for salicylic acid (SA) activation of pathogenesis-related (PR) gene expression, and the expression of the SA-responsive PR genes, PR protein 10 gene and PR Bet vI family protein gene was 5-fold and 4-fold up-regulated, respectively, indicating that the SA-mediated defense pathway played a role. It had been generally shown that SA exerts its inhibitory effects on jasmonic acid (JA) mediated gene expression through the action of the regulatory protein NPR1. Strikingly, they found some JA responsive genes, including vegetative storage protein gene that acts an early step in the stress perception/transduction pathway before JA and ET pathways, 12-oxophytodienoate reductase involved in biosynthesis of JA and RERJ-1 in response to JA expressed at significantly high level, suggesting that both SA and JA dependent signaling pathway were associated with the resistance to Xoo in rice line with Rxol. Ding et al. (2008) also studied that role of IAA in the rice-pathogen interaction (Xanthomonas oryzae pv. oryzae) and reported that this resistance was independent of jasmonic acid and salicylic acid signaling. The data suggest that wound-induced metabolic conversion of JA/JAME into 12-OH-JA altered expression pattern of genes including a switch off in JA signaling for a subset of genes.

Role of bio-agents in imparting resistance:-

Bio-agents were identified as an important component in plant disease management. Their use had been well established especially in integrated way with the management practices being eco-friendly, the research intensification of different bio-agents, their formulations, self-life and mode of application was established in some host-pathogen system. Besides direct inhibition of the pathogen, their role in stimulating the defense mechanisms were also studied by several workers (Van Loon *et al.*, 1988).

Erwinia herbicola and native strain of *Pseudomonas fluorescens* (biotype III) from roots of rice, pearl millet and citrus proved inhibitory to *Xanthomonas campestris* pv. *oryzae* and reduced the disease development substaintially (Anuratha and Gnanmanickam, 1987; Hsieh and Buddenhagen (1974) and Sivamani *et al.* (1987). Seed and seedling bacterization with plant growth promoting rhizobacteria, *Azospirillum brasilense* and *Bacillus polymixa* individually and in mixture proved effective in reducing bacterial blight severity (Islam and Bora, 1998). Higher population of *Azotobacter* (1:1 ratio to the pathogen) and two strains of nitrogen fixing bacteria *Enterobacter cloacae* MR12 and *Alcaligenes paradoxus* R4 suppressed bacterial blight to a considerable extent (Pandey and Iswaran, 1982 and Yang *et al.*, 1999). Santhi *et al.* (1987) evaluated the suppressive effect of the *Erwinia herbicola* on the *Xanthomonas campestris* pv. *oryzae*. The suppressive effect was reported on the development of BB symptoms in rice leaves which received a mixture of *E. herbicola* and *Xanthomonas campestris* pv. *oryzae* cells and not on leaves which were inoculated with the pathogen alone. A massive accumulation of phytoalexin (Van Peer *et al.*, 1991) phenolic compound (M'Piga *et al.*, 1997); PR-proteins (Maurhofer *et al.*, 1994); peroxidase (Zdor and Anderson, 1992);

mRNA's enhanced lignification (Anderson and Guera, 1985) had been reported in plants following treatment with Plant Growth Promoting Rhizobacteria (PGPR) strains. The increase activity of the above said substances in PGPR treated plant might had either direct or indirect role in suppression of pathogen development in the host ultimately protecting the plants from pathogenic microorganism. Saikia and Chowdhary (1993) evaluated all the micro organisms including the heat killed and avirulent cells of pathogen, and reported that antagonistic effect towards Xoo. Erwinia herbicola controlled the pathogen most effectively even at lowest ratio (1:1) and registered more than 90 per cent reduction in disease development at 50:1. Bacillus subtilis also performed better than other antagonists in reducing the build up of the bacterial blight of rice. The phylloplane microorganisms namely Erwinia herbicola, Bacillus subtilis, Serratia sp., Pseudomonas acidovorus, Fusarium pallidoroseum, F. chlamydosporum, Aspergillus spp., Penicillium janthinallum, Streptomyces sp. and Micrococcus sp. suppressed bacterial growth and reduced bacterial blight incidence. The efficacy of biocontrol agents varied with their inoculums level and time of application. The heat killed and avirulent cells of X. oryzae pv. oryzae were also found antagonistic to the pathogen (Sindhan et al., 1997 and Gupta, 1975). Erwinia herbicola and native strains of Pseudomonas fluorescens (biotype III) from roots or rice, pearl millet and citrus proved inhibitory to X campestris py. oryzae and reduced the disease development substantially (Anuratha and Gnanamanickam, 1987 and Gnanamanickam et al., 1999). Vidhyasekaran et al. (2000) studied that rice seeds treated with the formulation of P. fluorescens (P.fl.) and sown, the 30-day-old seedlings showed resistance to X. oryzae py. oryzae and the disease intensity decreased from 6.8 to 1.2. In the induced resistant leaves, a sharp increase in lignification and activities of peroxidase, phenylalanine ammonia-lyase and 4-coumarate: CoA ligase was observed when the leaves were challenge-inoculated with X. oryzae pv. oryzae. An approximately three fold increase in lignin content, peroxidase activity and phenylalanine ammonia-lyase activity and a fivefold increase in 4-coumarate: CoA ligase activity were observed 5 days after challenge inoculation with X. oryzae pv. oryzae in rice leaves pretreated with P. fluorescens for 5 days. A similar increase in defense-related activities was not observed in susceptible interactions or in P. fluorescens treated plants at later stages of interactions when no resistance to the pathogen was observed. Vasudevan and Gnanamanickam (2002) screened 516 bacterial strains isolated from rice root and rhizosphere samples collected and assessed their efficacy in terms of increase in plant height, and grain yield in net house tests and increases in root / shoot length of treated seedlings in laboratory assays. It appeared that mixtures of Bacillus strains did not improve their efficacy over application individually as single strains, although all treatments led to significant levels of disease suppression (up to 55%) when compared with untreated control. However, strain combinations seemed to exert a definite positive impact on growth of rice plants in terms of two-to-threefold increases in plant height, tiller number and rice yield (kg m⁻²). Someya et al. (2002) reported that antagonistic bacterium, Serratia marcescens strain B₂, controlled rice blast after being sprayed onto rice phylloplane, as did the bacterial suspension when applied into rhizosphere soil of rice plants. They observed a week after pathogen inoculation, rice blast was suppressed and lesions caused by the pathogen decreased in size. Rangrajan et al. (2003) tested the antagonists Pseudomonas sp. and reported the suppression of BB by 15 to 74 per cent in an unamended soil. The efficient strains were tested under saline soil conditions and found to suppress disease by 46 to 82 per cent. Kumawat et al. (2008) observed that pre-application of spore suspension of bio-agents protected plant against challenge infection of Drechslera oryzae in paddy from 59.21 to 12.40% reduction in disease severity. Biochemical analysis of treated leaves showed increased level of total soluble protein exhibited 11.19 - 85.21% and -10.06 to 66.57% and total phenol content from -8.62 to 79.31 and 9.43 – 99.23% over diseased and healthy plants respectively. Kumar et al. (2009) evaluated ten botanicals, four bioagents and certain chemicals against pathogen Xanthomonas oryzae pv. oryzae and revealed that among botanicals Curcuma longa and Eucalyptus grandifolia caused maximum inhibition of bacterial growth while bio-agents Pseudomonas fluorescens and Trichoderma harzianum restricted maximum growth of the pathogen. Among the chemicals copper oxychloride (0.25%) + streptomycin sulphate (200ppm) was found most effective as caused maximum per cent inhibition as compared to other chemicals.

Influence of plant nutrition (nitrogen) on resistance genes:-

Nitrogenous fertilizer is conductive to disease development through increased vegetative growth of the plant, influence the micro climate in favour of the pathogen. It was studied by few research workers:

Rao and Devadath (1977) observed that no significant effect of spacing on the incidence of bacterial leaf streak. However, a significant increase in the disease incidence with an increase in the nitrogen level was reported. Reddy *et al.* (1979) revealed that high N levels (> 100 kg/ha) increased disease and reduced yield in BB susceptible IET 2895. BB resistant IET 4141 was least affected by all N levels. The relationships between yield and N level and between BB severity and N level were best described by quadratic and linear functions, respectively. The optimal level of N application (to derive maximum yield with minimum disease effect) was 76 kg/ha for the susceptible cultivar IET 2895. Choi et al. (1980) investigated that doubling the amount of nitrogen application (30 kg) increased the incidence of bacterial blight, regardless of planting density and the incidence of bacterial blight in standard nitrogen application was significantly higher at 15 x 30 cm and 20 x 40 cm planting density. Cha et al. (1982) revealed that disease severity was also different depending upon the maturity of rice cultivars of early maturing group. Such difference was more obvious in the field with higher amount of fertilizer application. The fertilization rate and per centage of ripeness were decreased resulting in heavy loss of yield. Parashar and Sindhan (1986) observed increase in disease intensity and decrease in total phenols, O.D. phenols, total sugars and reducing sugars in plant supplied with nitrogen in both susceptible and resistance cultivars. As the doses of nitrogen increase, there was a significant increase in disease intensity and decrease in phenolic and carbohydrate contents in both resistant and susceptible cultivars. On the other hand, significant decrease in disease intensity and increase in total phenol, O.D. phenol, total sugar and reducing sugar was observed in plants supplied with potassium both in resistant and susceptible cultivars. As the doses of potassium increased, there was a significant decrease in disease intensity and increase in total phenol, O.D. phenol, total sugar and reducing sugar in both the cultivars against Bacterial blight of rice. Avoidance of excessive application of nitrogenous fertilizers particularly in inorganic form at the tillering stage helped minimizing bacterial blight incidence (Devadath, 1969 and Padmanabhan, 1983). Basal application of nitrogen in the form of neem cake coated urea and musoorie rock phosophated urea reduced disease incidence compared to three application of prilled urea (Baruah et al., 1991). A significant relationship of potassium with bacterial blight incidence has been demonstrated (Reddy and Sridhar, 1975). Deficiency of phosphate and potassium, and excess of silicate had been reported to increase the disease (Ou, 1985). Increase in calcium supply significantly reduced the host susceptibility due to inability of the pathogen to disrupt the integrity of the membrane, while abundant supply of magnesium markedly enhanced the susceptibility (Kaul and Sharma, 1987). Mahto et al. (2001) investigated that BB incidence significantly increased with leaf injury together with increasing rates of N. They also observed the rice grain yield was high at high N rates, but yield reduction also increased with increasing N rates due to high disease incidence.

Performance of resistant cultivars:-

Most of the workers have evaluated rice genotypes for resistance to bacterial blight using local bacterial isolate (s) with unknown degree of virulence; however, some researchers have identified bacterial blight donors against known virulence(s) prevalent in the region. Several findings were reported by research workers:

High degree of horizontal resistance had been reported in Nakashim 120, Chinsurah, Boro II, IR 29, Palita 1/1, Jelita, Dora, Remadja, IR-20, IR-22 in Indonesia, Ogyoku, Shimotsuki, Iphonbare, Kunihikari, Yaeho, Satominri, Asominori and Gomashirazu in Japan (Yamada et al., 1979; Yoshida and Yasugi, 1977). Horino and Yamada (1979) evaluated the resistance of IRRI varieties including IR-28 to representative strains of the five Japanese bacterial groups. They found that most IRRI varieties showed qualitative resistance to group I and V and a high degree of qualitative resistance to groups II-IV. Agrawal and Philip (1982) screened 1201 entries against bacterial blight disease under artificial epiphytotic conditions at Rice Research Institute, Raipur during kharif 1980. They observed seven entries namely IR 2798-88-3, IR 442-46-3-3-3, IR 6808-189-2-2-1-3, IR 9801-9-3, RP 1033-43-2, CR 242-43-150 and R8-2535 were highly resistant (score 1), 140 entries were resistant (score 3), 397 entries were moderate (score 5) and remaining 657 entries were in susceptible group (score 7 and above). Reddy and Shukla (1986) found that cultivars Karuna, IR 28 and PR 106 were highly susceptible to both kresek and leaf blight phases, while TN-1, BJ-1 and IR-42 were more susceptible to leaf blight than kresek cv. TKM-6 was least susceptible to both the phases. Goel et al. (1990) found that TN-1 was highly susceptible to all the isolates of X. campestris pv. oryzae. AC 19-1-1, IET-8320, IET 8584 an IET 8585 were resistant (R) to all the seven isolate while five rice lines namely IR 29295-70-1-1, IR 29341-41-1, IR 29341-41-1, IR 29341-85-3-1-3, IR 5853-218-6-1 and Kachamota were resistant to six isolate IR -33356-2-23 and BR 285-5-6-6-2, C 721313 were resistant to five isolates. On the other hand IRAT 109 was susceptible (S) or highly susceptible (HS) to all the isolates. Kotasthane and Agrawal (1991) revealed that out of 278 entries, 33 entries were highly resistant (score 1); 66 entries were resistant with score 3; 82 entries showed moderate resistance with score 5 and the remaining 97 entries were in susceptible group with score 7 and above during their study at Raipur. Raina et al. (1999 b) reported that out of 980 entries, three were resistant (score 3) to both the pathotypes of the BLB pathogen. Agrawal et al. (2000) investigated the field trials resistance to bacterial blight (BB) in six entries RP 2151-33-2, RP 2151-33-2-11-21, RP 2151-40-1-71, IR 54, RP 2151-21-22 and Ruchi which showed consistently low score of BB, the mean of twelve years varying from 2.67 to 3.83 while local resistant check Usha had 5.05 and susceptible T(N)1 check 8.50. the mean yield of these entries varied from 4.8 in RP 2151-21-22 to 5.5 t in Ruchi, while local resistant check had 4.7 and susceptible check 3.7 t/ha. Khan et al. (2000 a) evaluated some 104 local rice varieties/ lines for resistance to bacterial blight pathogen (Xanthomonas campestris [X. oryzae] pv. oryzae) under field conditions at Kala Shah Kaku during 1996-98. They observed none of the varieties/ lines showed complete resistance to bacterial blight and IR 64, IR 8 and Shadab was moderately resistance while 50, 44 and 7 genotypes showed moderately susceptible and highly susceptible reaction, respectively. Khan et al. (2000 b) reported most basmati rice varieties cultivated in Pakistan were susceptible to bacterial blight (BB, Xanthomonas campestris [X. oryzae] pv. oryzae) disease. They observed 38 (in 1998) and 39 (in 1999) entries/ varieties from Rice Research Institute Kala Sahah Kaku and rice breeding group of NIAB, were screened for resistance under field conditions at NIAB. Plants were artificially inoculated. No entry/ variety were found resistant against BB. Only Bas-370 was found moderately resistant. Liu et al. (2001) reported that the varieties 94-44, 96218, Zehndao 272, Shanyoukang 63, Shanyou 084, Jinyou 63, 109, 701, 7057, 9510, 9619, 5-172, 92-133, Yangjing 7057 Sidao 98-3789, Xin 108, Zhendao 99, 44/157 and 9522 had high levels of disease resistance. During the Kharif (wet) season of 2000, 149 entries of NSN-1 rice were evaluated for their response to bacterial blight (caused by Xanthomonas oryzae pv. oryzae) under artificial inoculated condition at Bankura, West Bengal, India. Five entries exhibited a resistant reaction to the disease, while 14 entries showed a moderately resistant reaction (Saha, 2003). Adhikari and Mew (1994) evaluated that resistance of rice cultivars to bacterial blight (BB), caused by X. oryzae py. oryzae, in field and greenhouse experiments during 1987-91. In the field studied, the plants were assessed by measuring lesion length (LL) and disease severity (per cent of leaf diseased). The area under the disease progress curves (AUDPC) and LL were used to compare rice cultivars. Rice cultivars BR-34-13, PAU-50-B-25, Laxmi, Sabitri, BW293-21, IR7167-33, Rodina and Amonghaud had significantly shorter LL and less AUDPC than the susceptible check IR 24. They also observed in the greenhouse studied, highly significant cultivar, strain, dose, cultivar A- strain and cultivar A- dose effects were observed, indicating a differential host-pathogen interaction. Differences in virulence among bacterial strains and resistance among rice cultivars were observed. Inoculum that obtained 10⁹ c.f.u. /ml induced larger differences in LL between resistant and susceptible cultivars. Laxmi consistently exhibited the highest level of resistance, and it was suggested that this cultivar could be a source of resistance to BB in Nepal. Chandrawanshi et al. (2006) screened varieties grade 3 score was designated as resistant and 5 score was moderate resistant. None of the varieties were found resistant in SIET-1, SIET-II and SIET-V of the AICRIP composition. Three varieties viz., Bamleshwari, Swarna and Mahsuri were found resistant from SIET-IV. While, in SIET-III trial, only one variety Bamleshwari was showed resistant reaction. In all twenty two varieties showed moderate resistant reaction and remaining one hundred three varieties exhibited susceptible and highly susceptible reaction against BLB. Noh et al. (2006) investigated that changed virulence of Xoo in the collected 134 strains of Korea. The most resistance gene was IRBB-5 (Xa-5) and the most susceptible gene was IRBB-3 (Xa-3) in test resistance germplasm. They selected five differential varieties and established new differential system to bacterial leaf blight of rice following as Milyang 23 (none), IRBB-1 (Xa 1), Pusangsanbyeo (unknown), IRBB-5 (Xa 5) and IRBB-21 (Xa 21). The new race differential system was able to group 16 race types.

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