Siva Kumar Panguluri · Are Ashok Kumar
Editors

Phenotyping for Plant Breeding

Applications of Phenotyping Methods for Crop Improvement

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Preface

Plant breeding is defined as the art and science of changing genetic architecture of plants for the benefit of mankind and it has been in practice for thousands of years, since the beginning of agriculture. However, it is being practiced more scientifically ever since the rediscovery of Mendel's laws in 1900 and has become increasingly precise by the use of new molecular tools. In addition to simple selection methods, crop improvement involves sexual hybridization of desirable parents followed by selections in the segregating populations so as to select desirable combinations and eliminate the undesirable ones. Even today, this is the predominant practice in crop improvement, although various tools like mutation induction, wide hybridization, exploitation of somaclonal variation, genomic tools, and genetic transformation are also employed. Thus plant breeders have been largely engaged with creation of variation and selection to improve the crop plants over decades.

Plant breeder's task is to select the plants that most likely meet the breeding objectives. Selecting a desirable combination and rejecting the undesirable one remains a challenging task given the fact that selections have to be exercised on a large number of plants/progenies with due consideration to a large number of traits, both qualitative and quantitative. The greatest apprehension haunting a breeder is the loss of superior plant/progeny during selections. As a consequence, the number of selections increased, sometimes by selecting the undesirable combinations, which burdens both time and resources required to handle them. Thus, success of a breeding program largely depends on use of an appropriate phenotyping method enabling a breeder to make judicious selections. Plant breeders have been using new tools like trait selection and use of markers to select gene(s) (marker-assisted selection) and/or genomes (genome-wide selection) to enhance the speed, accuracy, and scope of selection process. These techniques complement the selection process in breeding but cannot replace phenotyping for two reasons: first the design of these tools itself needs high-throughput phenotyping and second the need for the genotyped selections be confirmed by phenotypic data. Thus even the application of new tools essentially requires appropriate phenotyping systems.

A phenotype is any measurable characteristic or trait of a plant and is a result of combination of genes expressing in the plant (referred to as genotype), environmental influence, and their interactions. Phenotyping to a plant breeder means characterizing the performance of the plants for desired trait(s). Phenotyping is central to plant breeding to carry out selections; in addition, it is also done to study genetics of the traits, to associate markers with traits, to understand trait diversity, etc. Although routinely used, it still remains a factor of paramount importance for the success of breeding programs and to derive valid conclusions from genetic studies. In fact generating reliable phenotype data is now considered as a major limiting factor in breeding programs. Even in this era of genomics where state-of-the-art genotyping techniques and bioinformatics tool are available, the progress and validity of the results are largely constrained by the generation of reliable and reproducible phenotype data.

The objectives of a crop-breeding program in general are to develop improved varieties/hybrid parents with specific adaptation, high yield potential possessing pest and disease resistance, abiotic stress tolerance, enhanced nutritional content, high quality, market preferred traits, etc. These additional traits are as important as increasing yield and are often of critical significance as they offer protection from yield losses, improve quality, and thus enhance the economic returns. Plant breeding is often a painstakingly slow process; therefore a breeder often has to look many years ahead of the requirements of farmers and consumers to prioritize crop-breeding objectives. These objectives are location specific and depend on the economic importance of the trait.

We have therefore in this book discussed the phenotyping techniques for prioritized traits in some of the agriculturally important crops. This book broadly discusses various established methods of phenotyping for important biotic and abiotic constraints and other traits of interest. Thus it serves the requirements of a practical plant breeder who is often perplexed with the selection process requiring a good phenotypic method. A large number of reviews and books are now available on the use of molecular and genetics tools in plant breeding, although not many breeders have access to use them in their breeding programs. On contrary, we don't find comprehensive information on phenotyping of plants which indeed can be routinely used in breeding programs, and a large number of breeders even in developing counties can use such phenotyping techniques. A crop breeder has to pull information from many different publications before she/he chooses an appropriate screening method. This book is also important in the context of dwindling numbers of plant breeders who can guide students and younger generations on practical issues of selections, and a majority of students now consider plant breeding an old-fashioned science where modern tools are not applied. While the fact remains that plant breeding has played an important role in increasing the crop production through improved cultivars and will continue to play a key role in future in meeting future food, fodder, fiber, and fuel demands.

This book is intended to serve as a useful guide to practicing plant breeders to use appropriate phenotyping methods for improving the major traits in selective crops. This also helps the teachers and students in plant breeding to better understand the phenotyping and its importance in plant breeding.

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Chapter 1
Phenotyping Rice for Molecular Plant Breeding

M.S. Madhav, G.S. Laha, A.P. Padmakumari, N. Somasekhar, S.K. Mangrauthia, and B.C. Viraktamath

Abstract Rice is an important food crop, has the plasticity in growing in different ecologies in many countries around the world, which makes this crop to expose to many diseases and pests. The recent development in the genomics has led to the intensive efforts in molecular breeding for improvements of some of the qualitative traits. To make the successful molecular breeding programme, accurate phenotyping techniques need to be coupled with high-throughput genotyping. The chapter discusses the various phenotypic methods available for different diseases, pests and abiotic stress like drought.

Keywords Rice • Diseases • Pests • Phenotype

Molecular breeding programmes in most of the crops including rice is on increase day by day and lot of public and private partners are joining hand in this programme to develop varieties through relatively faster technology than the classical plant breeding programme. In recent years there are tremendous improvements in development of markers and genotyping techniques in rice enabling the researchers to genotype rapidly and accurately. But for any successful molecular breeding programme, the precise phenotyping technique needs to be accurate and the standard uniform techniques need to be followed across the environments, since the phenotype is dependent on environment. In this context, we focused on the phenotyping techniques for major diseases, insect pests, nematodes and abiotic stress like drought.

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1.1 Phenotyping of Rice Diseases

Plant pests including diseases are the important production constraints in rice. Rice crop threatened by a number of pests and diseases. Among these, stem borers, brown plant hopper (BPH) and diseases of fungal and bacterial origin were ranked the most dangerous followed by others (Geddes and Iles 1991). Due to change in cultivation practices which are heavily dependent on chemical fertilizers and the apparent changes in the climate, the intensity and scenario of rice pests and diseases has changed over the years. Many diseases which were earlier considered as minor, have assumed the proportion of major ones. For example, false smut of rice, which was earlier considered as a sign of bumper harvest, is appearing in threatening intensity in many rice growing areas in India and other Asian and south Asian countries (Ladhalakshmi 2007; Muthuraman et al. 2007). Many diseases which were earlier restricted to certain parts of the country, have now spread to newer areas. Rice diseases which can cause major economic losses are blast, bacterial blight, sheath blight, rice tungro virus disease and brown spot. The most economic and environmentally safe strategy to manage these diseases is deployment of resistant varieties. Therefore, phenotyping for resistance in different germplasm is an important criterion in disease management. The most ideal method of evaluating resistance against different rice diseases is to grow the germplasm in the fields (in hot spots) and exposing them to natural infection. However, this is labor oriented and results may fluctuate due to inconsistent and uneven degree of natural infection. To obtain certain and uniform occurrence of the disease, artificial inoculation of the plants is required. The following sections describe the methods adopted for artificial inoculation and screening for accurate phenotyping of rice diseases.

1.1.1 Major Rice Diseases

1.1.1.1 Bacterial Blight of Rice

Pathogen and Its Isolation

Bacterial blight of rice is caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al., which is gram negative, non-spore forming and rod shaped bacterium. Bacterial blight is a typical vascular disease and has two distinct phases *i.e.* leaf blight phase and kresek (wilt phase), among these leaf blight phase is most common. One of the most important criteria in artificial inoculation of the pathogen is purity and its multiplication. Before isolation, the infected leaf samples are checked for bacterial ooze under microscope. The positive samples are then surface sterilized with 0.1 % mercuric chloride or 95 % ethanol for 30 s followed by 2–3 times rinsing with sterile distilled water. The infected leaf (preferably the portion with advancing lesion) is then cut into small sections (2–3 mm) and put in a drop of

sterile distilled water on a sterilized glass slide or in a small vial containing sterile water. After about 4–5 min, when the bacterial ooze comes out from the cut ends of infected leaf bits into water, a loop full of water can be streaked on to a suitable medium. The bacterium can be isolated on a number of culture media *viz.*, potato semi-synthetic medium, peptone sucrose agar (PSA) or modified Wakimoto's medium. After 4–5 days of incubation at 28±2 °C, pinhead sized colonies of the bacterium can be observed in culture plates which can be further purified by sub-culturing. The identity of the bacterium can be confirmed through pathogenicity test by inoculating on to susceptible rice varieties like TN1.

Mass Culturing and Artificial Inoculation

The bacterium can be multiplied by streaking on culture plates using any of the above mentioned media. Multiplication in broth culture is not preferred as it is difficult to detect any contamination during culturing. Using 3–4 days old culture, a bacterial suspension (10^8–10^9 cfu/ml) is made with distilled water. This suspension is then used for artificial inoculation. A number of methods have been used for artificial inoculation of bacterial blight pathogen. The methods can be broadly divided into two groups.

Methods for creating leaf blight phase. Reitsma and Schure (1950) used spraying method (spraying the plants with the bacterial suspension) and needle prick or pin prick method (pricking the leaves with a needle dipped in bacterial suspension or putting a drop of bacterial suspension on the leaf and then pricking the leaf with a pin through the bacterial suspension droplet). Needle prick inoculation method is suitable for accurate evaluation of resistance but it is laborious and time-consuming and is not suitable for large scale screening. Several modifications were made to pin prick inoculation. Mukoo and Yoshida (1951) and Yoshida and Muko (1961) developed multi-needle prick inoculation method, which was more convenient and practiced by a number of research workers. Though, the number of needles may vary from 1–100, usually 4–6 needles are sufficient for successful inoculation. Usually, the needles are mounted on a rubber pad and a cotton pad soaked in bacterial suspension provides the inoculum so that in one operation, leaves are punctured and gets inoculated with the bacteria (Ou 1985). Goto et al. (1953) used injection inoculation method where disease was created by injecting the bacterial suspension in the leaf veins. In spraying method, the disease development is generally slower when compared with the pin prick method. This method was slightly modified by Rao and Srivastava (1970) where leaf tips of the seedlings were clipped before spray inoculation to create more disease pressure. The cut-and-spray inoculation method of Ezuka and Horino (1976) was a similar modification in which the leaves of rice plants at maximum tillering stage were clipped with pruning shears followed by immediate spraying with bacterial suspension.

A leaf clipping method was developed at AICRIP (All India Coordinated Rice Improvement Project) wherein the leaves (45–50 days old plants) are cut with scissor dipped in bacterial suspension. This method is very efficient and very

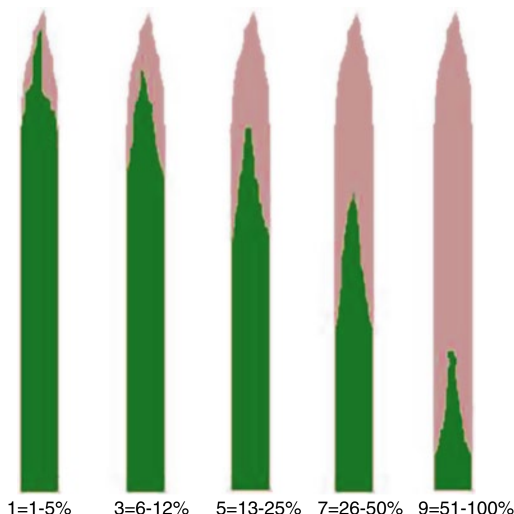

Fig. 1.1 Diagram key for assessment for bacterial leaf blight in field

convenient for inoculation of large number of plants in practical breeding work in the field and glass house (Kauffman et al. 1973). Presently, this method of artificial inoculation is being used by most of the research workers around the world.

Method for creating Kresek phase. Reitsma and Schure (1950) used immersion inoculation (immersing the seedlings in a bacterial suspension) to reproduce kresek phase of the disease. Root dip-inoculation method was developed for mass screening of breeding materials (Yoshimura and Iwata 1965; Yoshimura and Yamamoto 1966). In this method, the rice seedlings are pulled off from the nursery and their roots and crown parts are dipped in the bacterial suspension for 24–48 h before transplanting in the main field. This method is very efficient in creating kresek symptoms. Crown inoculation method for creating kresek symptoms was developed by Durgapal et al. (1979) in which the seedlings were pricked at the crown region and dipped in bacterial suspension for 10 min and then transplanted in pots or fields. They also reported that pricking the crown at 5-leaf stage did not induce any injury and provided most reproducible results.

Observations

Observations are recorded 15 days after inoculation. For assessing resistance, the Standard Evaluation System for Rice (SES) developed at International Rice Research Institute (IRRI), Philippines (Anonymous 1996) is usually followed (Fig. 1.1). Generally, the scores from several plants are averaged and categorized as resistant (mean score below 4), moderately resistant (mean score 4–5) and susceptible (mean score more than 5). Many researchers prefer absolute lesion length as criteria for characterizing host reaction though the length of the lesion for categorizing resistance/susceptibility varied among the research workers. Lee et al. (1999)

categorized the plant reaction according to lesion length as resistant (<3 cm), moderately resistant (3.1–5.0 cm), moderately susceptible (5.1–7.0 cm), and susceptible (>7.1 cm). Chen et al. (2000) classified a plant as resistant if the average lesion length was shorter than 3 cm, moderately resistant if the lesion length was 3–6 cm, moderately susceptible if the lesion length was 6–9 cm and susceptible when lesion length was >9 cm. Shanti et al. (2001) followed lesion length up to 4 cm as resistant and lesion length greater than 4 cm as susceptible while Sanchez et al. (2000) and Chen et al. (2002) recorded plants with lesion length less than 6 cm as resistant and those with lesion length greater than 6 cm as susceptible.

1.1.1.2 Blast

Pathogen and Its Isolation

Blast caused by the fungus *Pyricularia grisea* (Cook) Sacc. [teleomorph: *Magnaporthe oryzae* (Hebert) Barr] is the most widespread and destructive rice disease causing substantial loss in yield both in upland and irrigated rice production system. The fungus affects the leaves, nodes and panicles and produces characteristic symptoms *viz.* leaf blast, node blast and panicle or neck blast, respectively. Commonly used media for culturing rice blast fungus are oat meal agar, rice leaf extract agar, rice polish agar etc. The panicles and leaves showing typical blast symptoms are surface sterilized with 70 % ethyl alcohol for 10 s and then washed repeatedly 3–4 times in sterile distilled water. The portions of infected tissue are then excised with a sterile blade or scalpel and put in a sterile Petri plate lined with filter paper moistened with sterile water. The plates are then incubated for 24–48 h at 25–27 °C temperature to induce sporulation. When the lesions become grey (sporulating lesions), they are held over a plate containing thin layer of water agar and gently tapped to dislodge the spores. The plates are then observed under a dissection microscope and the portions of the agar having single conidia are marked. The portion of agar is then cut with a sterile scalpel and transferred into a culture medium plate by putting the agar bit upside down. The mono-conidial culture can then be sub-cultured in fresh agar plates or tubes. Alternatively, the tissues with active sporulation can be tapped directly onto a culture medium (preferably supplemented with some antibacterial agents). The typical single colony (growing from a single blast spore) can then be further purified by transferring into fresh culture medium. The fungus can be maintained for long term in sterile filter paper discs at −20 °C (Valent et al. 1986).

Mass Culturing and Artificial Inoculation

The fungus can be mass multiplied on a number of natural media. The fungus can be easily cultured on autoclaved sorghum seeds soaked with 0.2 % yeast extract powder and then incubating them at 28 °C for 7 days. Mass production of conidia of

blast pathogen can also be done by growing the fungus on autoclaved barley grains (barley grains: water, 1:1.2 w/w) (Chen et al. 2001). After incubation when the grains are covered with white and grey hyphae, the grains are washed with sterile distilled water to remove the hyphae from the surface of the grains and the washed grains are then put in a sterile Petri plate lines with moistened sterile filter papers and incubated at 28 °C for 48 h under fluorescent light to allow sporulation. The fungus can also be mass multiplied by growing them on rice polish agar or oat meal agar medium and incubating at 25 °C for 7 days in dark after which the plates are placed under continuous fluorescent light at 25 °C for 4 days to induce sporulation (Mekwatanakarn et al. 2000). Sporulation of the fungi can be obtained by scraping the mycelia growth with a sterile rubber spatula and then exposing the plates to fluorescent light at 25–28 °C (Bonman et al. 1987). Conidial suspensions are then made by washing the grains or scrapping the culture plates with sterile distilled water and then filtering the solution through cheese cloth. Tween 20 can be added to the conidial suspension at 0.05 % (v/v). The concentration of the suspension should be adjusted to approximately 10^5 conidia/ml using a hemocytometer before inoculation.

Screening using uniform blast nursery (UBN). Varietal resistance is usually done at the seedling stage. A dry upland nursery bed is more favorable than a flooded field for evaluation of blast resistance. A heavy application of N fertilizer (120–160 kg N/ha) and high humidity (>95 %) should be maintained in the microclimate of the nursery. Temperature for infection and disease development is 24–26 °C. Considering all these parameters, a uniform blast nursery (UBN) method of evaluation of blast resistance was developed at IRRI (Ou 1965). This method can accommodate a large number of entries, requires small quantity of seed, and ensures uniform infection. Briefly in this method, the seedlings will be raised in upland nursery. Test entries are sown in 50–100 cm long rows with a row to row distance of 10 cm. After every 20 test entries, seeds of a highly susceptible variety are sown. The entire nursery should be surrounded on all sides by two rows of susceptible variety to act as spreader/infector rows to ensure heavy disease pressure. Initial inoculum can be introduced by transplanting infected plants or spreading plant parts such as pieces of infected leaves, nodes, or panicles in the spreader rows. Spore suspension of specific isolates can be applied, if necessary. For proper development and spread of the disease, care need to be taken for dense planting, high N fertilizer application, and maintenance of prolonged dew period by covering the plots with plastic film at night and supplemental overhead sprinkling of water 3–4 times a day depending on the weather conditions. Proper check varieties should be kept for comparison of the results. Plants at 15 day old stage are inoculated and observations are taken after 10–15 days of inoculation.

Screening in trays/pots. To determine the phenotypic reaction of rice seedlings to specific isolates of the pathogen, artificial inoculation under controlled condition is essential. Seeds of the test cultivars should be sown in rows in a plastic tray in glass house. Seedlings of 18–20 days age will be the right stage for spraying freshly prepared conidial suspension. Inoculated trays are then incubated at 25 °C temperature and >95 % relative humidity for 7 days in greenhouse (Bonman et al. 1987;

Table 1.1 Descriptive key (SES) for recording leaf blast disease severity (Anonymous 2002)

Score	Description of symptoms
0	No lesions observed
1	Small brown specks of pin-head size or long brown specks without sporulating centre
2	Small roundish to slightly elongated, necrotic grey spots, about 1–2 mm in diameter with a distinct brown margin
3	Lesion type is the same as in scale 2, but significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions, 3 mm or longer, infecting less than 4 % of the leaf area
5	Typical blast lesions infecting 4–10 % of the leaf area
6	Typical blast lesions infecting 11–25 % of the leaf area
7	Typical blast lesions infecting 26–50 % of the leaf area
8	Typical blast lesions infecting 51–75 % of the leaf area and many leaves are dead
9	More than 75 % leaf area affected

Chen et al. 2001). Long et al. (2001) described a method of creating blast disease by growing the fungus on autoclaved rice seeds and then applying the colonized rice grains on the soil in between the rows 10 days after sowing either in fields or in nursery beds. They reported that the disease incidence was high when 25–30 infested grains were applied in an area of 0.1 m^2. Kuribayashi and Terazawa (1953) artificially induced the disease by injecting the spore suspension into the leaf sheaths of rice seedlings. In this method, the lesions appear on the young leaves which unfold in a few days.

Artificial inoculation for neck blast. Inoculations can be done through injection of 1 ml of spore suspension with a syringe into the leaf sheaths of emerging panicles (about half way emerged). This method develops 100 % infection (Ou and Nuque 1963). In another most commonly used method, the neck region (5–6 cm long) can be cut placed in Petriplate having moistened filter paper soaked with benzimidazole solution (Chai and Jin 1995).The necks are then smeared with aqueous solution of conidia containing 2 % carboxymethyl cellulose. The Petri plates are then covered and incubated under light at 28 °C and observations are taken after 10 days of inoculation.

Observations

Disease scoring will be carried out in 10–15 days after inoculation when the disease severity in susceptible control plants has reached to the maximum. For all practical purposes, the observations are recorded following the SES (Anonymous 2002). This scale is mainly used for recording blast reaction in the nursery stage. In general, the average score 3 or below is taken as resistant, 4–5 as moderately resistant and score greater than 5 is taken as susceptible. Sometimes, based on these scores, disease severity index or disease index is calculated (Table 1.1).

Mackill and Bonman (1992) recorded blast reactions after 7 days of inoculation following a 0–5 scale, where 0 = no evidence of infection; 1 = brown specks smaller

Table 1.2 SES scale based on symptoms for measuring neck blast

SCALE (based on symptoms)	
0	No visible lesion observed or lesions on only a few pedicels
1	Lesions on several pedicels or secondary branches
3	Lesions on a few primary branches or the middle part of panicle axis
5	Lesions partially around the base (node) or the uppermost internode or the lower part of panicle axis near the base
7	Lesions completely around panicle base or uppermost internode or panicle axis near base with more than 30 % of filled grains
9	Lesions completely around panicle base or uppermost internode or the panicle axis near the base with less than 30 % of filled grains.

Table 1.3 SES Scale for neck blast based on incidence of severely neck infected panicles

Score	Description
0	No incidence
1	Less than 5 %
3	5–10 %
5	11–25 %
7	26–50 %
9	More than 50 %

than 0.5 mm in diameter; 2 = brown specks about 0.5–1 mm in diameter; 3 = roundish to elliptical lesions about 1–3 mm in diameter with gray centers and brown margins; 4 = typical spindle shaped blast lesions, 3 mm or longer with little or no coalescence of lesions and 5 = same as 4 but half of one or more leaves killed by coalescence of lesions. Plants with score 0–3 are considered resistant and those with scores of 4–5 are considered as susceptible.

Padmanabhan and Ganguly (1959) described a scale for recording blast reaction of germplasm. The scale describes as A = reddish flecks only; B = minute reddish spots showing no differentiation into distinct zones; C = Circular spots about 2–3 mm in diameter with a central ashy zone and a purple brown margin; D = broadly spindle shaped spots, only slightly longer than breadth, 3–5 mm in diameter and E = large, distinct spindle shaped spots with a central ashy zone and marginal zones 3–5 mm broad and up to several cm in length. The cultivars were with class A and B were classified as resistant, those with C as moderately resistant and with D and E as susceptible.

Scale for measuring neck blast is based on the percentage of panicles infected. In addition, girdling of the neck (partial or complete) and site of infection (on the main or the smaller branches) may also be considered (Table 1.2).

However, for mass evaluation of germplasm against panicle blast, the number of severely infected panicles is considered (Anonymous 2002) as follows in Table 1.3.

1.1.1.3 Sheath Blight

Pathogen and Its Isolation

Sheath blight of rice caused by the fungus *Rhizoctonia solani* Kuhn [teleomorph: *Thanetophorus cucumeris* (Frank) Donk] is second most important fungal disease next to blast. The teleomorph belongs to Basidiomycetes. It belongs to anastomosis group 1 IA (AG-1-IA). In addition to *R. solani*, two other species of *Rhizoctonia viz.*, *R. oryzae* causing rice sheath spot and *R. oryzae-sativae* causing aggregate sheath spot have been found to be associated with this disease. All the three pathogens may occur concurrently and sometimes referred to as rice sheath blight disease complex. The fungus can be readily isolated into culture medium. The fungus produces abundant sclerotia (dark compact mass of hyphae capable of surviving under unfavorable environment) in culture media and also on infected plants. The infected sheath/leaf samples are first washed in tap water, cut into small pieces (2–5 mm), washed 2–3 times in sterile distilled water and then dried using sterile blotting papers. These sheath/leaf pieces are then placed on 2 % water agar (WA) plates and incubated at 28 °C for 24–48 h. The sclerotia collected from the infected plant parts can also be used for isolating the fungus following the above method. The emerging hyphal tip of a single mycelium is then transferred to potato dextrose agar medium (PDA) to obtain pure culture of the fungus. The fungus can be maintained in PDA slants at 4 °C.

Mass Culturing and Artificial Inoculation

Several methods for artificially inducing the disease have been used by various workers. Yoshimura and Nishizawa (1954) found that placing sterile straw bits inoculated with the fungus among the tillers in each hill and wrapping them for 1 week was most efficient in inducing the disease. They also found that maximum tillering stage of the plant is most suitable for varietal screening. Amin (1975) described an improved method 'stem-tape-inoculation' for sheath blight disease by placing *R. solani* colonized stem bits on to the non-injured sheath of 6-week old rice plants using a cellotape at about 6–10 cm above the water line. The disease development is faster in this method. Freshly developed sclerotia of the fungus can also be used as inoculum source in this method. However, this method is time consuming and impractical for screening large number of germplasm under field conditions.

Bhaktavatsalam et al. (1978) developed a simple, rapid and mass inoculation technique to induce sheath blight disease in rice and to evaluate germplasm and breeding lines in fields and glass house. The pathogen is multiplied on autoclaved stem pieces (2–3 inches in length) of water sedge (*Typha angustata*) soaked with 1 % peptone solution for 8–10 days. Four to five stem bits colonized with fungal mycelia (and sclerotia) are then placed in between the tillers in the central region of the hill, 5–10 cm above the water line and then tied with a rubber band to maintain high humidity in the micro-climate. In glass house tests, the inoculated plants are

kept in a humid chamber for 4–5 days for rapid pathogen establishment after which the plants are transferred into glass house benches. This method is very easy, less time consuming and highly reproducible. In case of non-availability of Typha plants, the fungus can be multiplied in cut stem pieces of rice plants or very young sorghum plants.

Toothpick method of inoculation of *R. solani* was described by Zou et al. (2000) and Rodrigues et al. (2001). Wooden toothpicks (1 cm in length) are placed in Erlenmeyer flasks containing a shallow layer of potato dextrose broth and autoclaved. Ten to fifteen autoclaved toothpicks are then placed in a PDA plate keeping small gaps in between the toothpicks. The plates are then inoculated with 4–5 mycelial plugs from actively growing culture and incubated for 5–6 days so that the fungus colonizes the toothpicks. Plants at the maximum tillering stage are then inoculated by placing one *R. solani* colonized toothpick into the lowest inner sheath of the main tiller. The plants are then kept in a moist chamber for varying period of time for the development of the disease. This method is highly reproducible and has been used by many workers for artificially inducing the disease.

Singh et al. (2002) described a method of artificially inducing rice sheath blight disease by carefully placing a freshly harvested sclerotium inside the leaf sheath. Adding few drops of water is required to maintain high humidity inside leaf sheath. The plants are then kept in a humid chamber for rapid disease development. This method has also limitations in screening large number of germplasm accessions.

A micro-chamber screening method was described by Jia et al. (2007) to evaluate sheath blight disease resistance under glass house conditions, wherein the rice seedlings are inoculated at 3–4 leaf stage with PDA agar plugs containing mycelium and then covered with a 2- or 3-litre transparent plastic bottle for maintaining high humidity after inoculation. This method can be used to accurately quantify resistance to sheath blight pathogen under controlled greenhouse conditions but has limited application in screening large number of germplasm accessions and in field evaluation. Recently, Ram Singh et al. (2010) standardized inoculation method for evaluating mass screening of for sheath blight resistance in nursery beds. In this method, 30–40 days old seedlings were inoculated by broadcasting *R. solani* inoculum raised on barley grains and *Typha* pieces (1:1 v/v). A positive correlation ($r = 0.931$) between disease score (0–9) in nursery and field screening tests was obtained. Though nursery screening was not found an absolute indication of resistance in the field but it could be utilized in shortlisting of rice genotypes for screening against sheath blight in the field. Park et al. (2008) described a method of inducing rice sheath blight disease. The fungal mycelium grown in liquid culture is harvested and cut into small balls (approximately 0.5 cm in diameter) with forceps. Rice plants at late tillering stage are then inoculated with *R. solani* by placing a mycelial ball beneath leaf sheath and immediately covering with aluminium foil. The plants are then kept in a humid chamber for rapid disease development. This method has also limited application in screening large number of germplasm accessions.

A detached cut-leaf inoculation technique was developed by Dath (1987) for assessing reaction of a large number of varieties in the laboratory to sheath blight. Briefly, this technique involves placing leaf blades cut to 6–8 cm long over a thin