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PEARL MILLET IMPROVEMENT FOR DISEASE RESISTANCE

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SUMMARY

Bajra is the fourth most important crop, whose cultivation mainly confines to dry land regions. Gene manipulation using conventional and advanced approaches as well as genetic resource conservation and evaluation is an activity that supports the national crop improvement programme to cope with the biotic and abiotic stresses while maintaining high level of productivity, profitability and quality. It's wild species are tertiary gene pool species as reservoir of important genes such as apomixis, perenniality, fodder characteristics, stress tolerance thereby pearl millet improvement has potential of utilizing interspecific hybridization. Plant pathological research in pearl millet did not receive adequate attention until the F_1 hybrids, based on cytoplasmic male sterile line, released for commercial cultivation in India in the mid-1960's and become susceptible to downy mildew in early 1970's. The superiority of hybrids over open pollinated varieties for grain yield, uniform growth and shorter duration resulted in substantial increase in area under hybrid cultivation and this favoured incidence of diseases. The crop yield is adversely affected by several biotic factors which cause substantial yield losses and also adversely affected the quality of produce and thus reduced its market value. Among these, downy mildew (*Sclerospora graminicola*), smut (*Tolyposporium penicillariae*) and ergot (*Claviceps fusiformis*) are of economic importance in major pearl-millet growing areas of the country. Disease management using resistant cultivars is the most feasible way in pearl millet production. Attempts have been made in the past to identify sources of resistance to downy mildew in India based on resistant sources, several resistant hybrids and varieties were released for general cultivation in India. However, identification of new sources of resistance is required to find resistance against evolving virulence where pearl millet is widely cultivated. Plants use a wide range of mechanisms to resist infection and disease caused by pathogenic organisms. A common strategy of resistance breeding utilizing major genes involves : an effective screening method, availability of diverse germplasm, confirmed sources of resistance, knowledge of genetics of resistance, information on variability in virulence, effective utilization of resistance in breeding, and deployment and on-farm monitoring of performance of cultivar. Different breeding methods i. e. mutation, recombination/back cross and biotechnological tools are used for incorporation of resistance/ tolerance genes and obtained several interspecific crosses, identified genomic regions. In the light of past studies, it is proposed that future downy mildew and other disease management need-based strategy in pearl millet should be focussed.

Key words : Pearl millet, host resistance, disease management

Gene manipulation using conventional and advanced approaches as well as genetic resource conservation and evaluation is an on-going activity that supports the national crop improvement programme to cope with the biotic and abiotic stresses while maintaining high level of productivity, profitability and quality. *Pennisetum* genus (Poaceae) is one of the largest genus with over 140 species. Among different species *P. glaucum*, *P. purpureum* and *P. pedicellatum* are agriculturally important species, variable ploidy species : 2X-8X basic chromosome numbers : X=5, 7, 8, 9. Bajra is model crop for genetics, cytogenetics and breeding studies. It's wild species are tertiary gene pool species as reservoir of important genes such as apomixis, perenniality, fodder characteristics, stress tolerance thereby pearl millet improvement has potential of utilizing

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interspecific hybridization.

Bajra is the fourth most important crop following rice, wheat and sorghum, whose cultivation mainly confines to dry land regions. In Haryana, it is cultivated on 4.11 lakh ha (Anonymous, 2014) and 90 per cent of its cultivation is mainly in eight districts. As the crop is cultivated on poor sandy soils with low or no input under rainfed conditions and this resulted in wide swings in production and productivity (891-1843 kg/ha). Bajra besides being good source of protein (12%), fat (6%) carbohydrates (65%), is the richest source of minerals (2-3%) and low in crude fiber. It is an important ingredient of both human and cattle population, the bulk of which is consumed at village level. Yet it may be described as a crop of necessity rather than of choice, for it grows in areas too dry for production of other cereals, where nothing else will grow. In spite of availability of hybrids with high production potential there is a wide gap in potential and realized yield. It is an important crop in the semi-arid and tropical part of the world and grown annually on about 25 million ha, 42 per cent of world area is in India. Plant pathological research in pearl millet did not receive adequate attention until the F_1 hybrids, based on cytoplasmic male sterile line, released for commercial cultivation in India in the mid-1960's and become susceptible to downy mildew in early 1970's. The superiority of hybrids over open pollinated varieties for grain yield, uniform growth and shorter duration resulted in substantial increase in area under hybrid cultivation and this favoured incidence of diseases.

Major Diseases Affecting Crop Yield/Quality

The crop yield is adversely affected by several biotic factors. More than 100 diseases caused by fungi, bacteria, viruses and nematodes have been reported and they cause substantial yield losses and also adversely affect the quality of produce and thus reduce its market value. Among these, downy mildew (*Sclerospora graminicola*), smut (*Tolyposporium penicillariae*) and ergot (*Claviceps fusiformis*) are of economic importance in major pearl-millet growing areas of the country.

Downy Mildew

Downy mildew pathogen was first reported during 1907 in India by Butler. Disease remained sporadic until 1970's. The first pearl millet hybrid HB 1 (tift 23A x Bill 3B) was released in India in 1965 closely followed by HB-2 and HB-3. Downy mildew (DM) caused by an obligate parasite *Sclerospora graminicola* is quite widespread and economically most important disease of pearl millet (*Pennisetum glaucum* R. Br) in India. The disease is particularly more serious on single cross F_1 hybrids than on open-pollinated varieties (OPVs). This is due to narrow genetic base and uniformity of the hybrids than those of OPVs that are highly heterogeneous. Currently, about 50 per cent of the 9 million ha under pearl millet cultivation is grown with single-cross hybrids in India (Rai *et al*., 2006). The DM incidence has been quite variable on different hybrids and more than 90 per cent incidence has been recorded on some hybrids in farmers' fields (Thakur *et al*., 2003; Rao *et al*., 2007).

The estimated annual grain yield loss due to DM is approximately 20-40 per cent (Singh, 1995; Hash *et al.*, 1999; Hess *et al*., 2002) but this could be much higher under favourable conditions of disease development (Singh, 1995; Thakur, 1998, 2008). With the increasing area under hybrid cultivation since the 1970s, the disease has become more severe and more widespread (Thakur *et al*., 2006). The most cost effective management of the disease can be obtained by breeding DM resistant pearl millet hybrids. There has been considerable success in breeding for DM resistance using conventional pedigree breeding, and a large number of disease resistant hybrids have been developed and deployed (Khairwal *et al*., 2004). This has contributed in arresting the occurrence of widespread DM epidemics since the 1990s (Thakur *et al*., 2006). Marker-assisted backcross breeding has further enhanced the ability and efficiency of DM resistance breeding (Hospital *et al.*, 1992; Hash *et al.*, 1999). However, in view of the increasing severity of the disease and evolution of new more virulent pathotypes (Thakur *et al*., 2004 a,b), there is a need to develop a long-term strategy for DM resistance breeding in India. In this paper, different aspects of pearl millet downy mildew and strategy for pearl millet genetic improvement are presented. In general, the long-term success of breeding for disease resistance is influenced by several factors that include : (i) The nature of the pathogen and diversity of virulence, (ii) Availability, diversity and type of genetic resistance, (iii) Screening method and selection environment and (iv) Breeding methods, (v) Utilization & deployment and (vi) Monitoring resistance/virulence.

Mode of Injury

Downy mildew

Downy mildew (DM), caused by *Sclerospora graminicola*, continues to be a major biotic constraint to pearl millet [*Pennisetum glaucum* (L.) R. Br.] growing areas in India as it is one of the destructive and widespread diseases. The disease which had remained incipient on local landrace cultivars until the 1960s, became a serious threat to high-yielding, single-cross hybrids introduced into cultivation during the late 1960s. The first epiphytotics of DM occurred during the crop season of 1971 on the first popular hybrid HB 3 and caused substantial yield loss (Nene and Singh 1976).

Symptoms : Two types of symptoms, downy mildew and green year, are produced. Downy mildew symptoms may appear on the first leaf and generally on second and third leaf in the form of chlorosis of leaf lamina beginning of the base of the leaf. It progresses to the subsequently higher leaves under high humidity (>85%) and moderate temperature (20-25°C). Chlorotic areas on leaves produce abundant asexual sporulation generally on lower leaf, giving them a downy appearance. Severely affected plants remain stunted and do not produce panicle. After leaf symptoms develop, all the subsequent leaves and the panicle have symptoms due to systemic nature of the disease except in case of recovery mechanisms. Green ear symptoms appear on panicles due to the transformation of floral parts and leafy structure. The infected leaves produce sexual spores (oospore) in the necrotic leaf tissue late in the season. Infection occurs at the seedling stage from the soil-borne oospores and systemic symptoms as chlorosis generally appear on the second leaf and on all the subsequent leaves. Seedlings die under severe infection and panicles produced on the infected plant develop green ear. Secondary infection occurs by sporangia produced on the previously infected plants. Oospores are produced in the infected necrotic tissue which fail in the soil and serve as source of primary inoculum for the next crop. The pathogen reproduces both by sexual and asexual processes. Sporangia, the asexual spores, produced on the infected leaves, germinate to release motile zoospores. Zoospores germinate and initiate infection. Oospores, the sexual spores, are produced by two compatible mating type thalli in the infected leaf tissue.

Smut

It is more severe in CMS based single cross hybrids than in open pollinated varieties. The extent of losses caused by this disease is quite variable. The estimated grain yield loss due to smut is 5-20 per cent, although it can be higher under favourable conditions of susceptible host, environment and inoculum load.

Symptoms : Smut symptoms initially appear as green sori in the infected florets. These sori are generally larger than grains and appear as oval to conical bodies projecting beyond the glumes. The sori are bright green initially and later they turn brown to black. The sori are covered by a thin membrane which often breaks at maturity to release spore mass. In panicle having poor head exertion the lower portion covered by the sheath of the flag leaf is frequently heavily infected with smut. Infection on a panicle may appear scattered or a bunch of florets get infected at few places. Smut infection begins from soil and seed borne inocula. Teleutospores from previously infected florets are left in the soil and seeds get contaminated at the threshing floor. Under favourable conditions of soil moisture and temperature the teleutospores germinate in soil and produce numerous air-borne sporidia. These sporidia land on the flowering panicles and initiate infection through young emerging stigma. Rapid pollination is known to reduce or even prevent smut infection. Dark green shining sori appear two weeks after infection and sori mature within next two weeks. Mature sori rupture in release masses of spore balls which germinate to produce second crop of sporidia. These sporidia play a major role in secondary spread of the disease. Infection and spread is most favoured by the prevalence of high relative humidity (>80%) and optimal temperature (25-30°C) at the flowering stage of the crop. The spread of the disease from the early planted to the late planted crops through teleutospores has been observed.

Ergot

It is prevalent in large growing areas of Asia and Africa; however, the disease occurrence and spread is largely influenced by weather conditions prevailing during the flowering time of the crop. Like downy mildew and smut it has become more important due to commercial cultivation of genetically uniform single cross F_1 hybrids based on CMS system in India. Disease

is not as severe and widespread as downy mildew but causes substantial loss both for grain yield and quality under favourable weather conditions. The estimated grain yield loss is reported to be 41-70 per cent. Ergot sclerotia which replace grain contain neuro-toxic alkaloids that affect the health of human beings and animals and in extreme cases can result in death. The first epidemic of ergot occurred in 1958 in Satura district of Maharastra.

Symptoms : Ergot disease can easily be identified when cream to pinkish droplets called honey dew ooze out of the infected florets on pearl millet panicles. These droplets contain numerous asexual spores called conidia. Within 10-15 days these droplets dry out and hard dark brown to black structure larger than seed develop in place of normal grains. If the infection is severe then honey dew falls on leaves and some times on the soil giving them a white appearance. During harvesting and threshing, these sclerotia get mixed with grain or fall to the ground and serve as primary source of inoculum for the next crop. Following rains these sclerotia germinate and release numerous ascospores that are carried by air current to flowering pearl millet panicle. These ascospores germinate and infect the florets through stigma. Under relative humidity (80-85%) and moderate temperature (20-30°C) with cool night honey dew symptoms appear within 4-5 days and sclerotia become visible within 10-15 days after inoculation. It also survives on wild grasses (*Conchrus ciliaris*, *Panicum antidotale* and *Seteria verticillata*). Secondary spread through conidia by insects, wind, rain, etc.

Screening Techniques

(a) ***In vitro***
(b) ***In vivo***

Screening Methods for Downy Mildew

Field and greenhouse screening techniques : Some basic studies on biology and epidemiology clearly established the roles of oospores (sexual spores) and sporangia (asexual spores) in primary and secondary infection, and the influence of weather factors particularly temperature and humidity on DM disease development and spread (Safeeulla, 1976; Singh and Williams, 1980). These led to the development of a field screening technique that used the basic 'sick-plot' concept combined with infector-rows to provide a uniform disease spread and indicator rows to provide a measure of the disease pressure in the disease nursery (Williams *et al*. 1981; Singh *et al.,* 1993). This technique is precise, independent of the season, and both time-efficient and cost-effective. The technique has since been further refined to make it more effective, and is currently being used to screen breeding lines against the diverse Indian isolates of downy mildew.

Screening method and selection environment : Two types of screening methods are used : field screening and greenhouse screening. Both these screening methods are well established to identify and select resistance. Field screening is based on : (a) the disease sick-plot that provides primary inoculum as oospores in the soil on plant debris, (b) the use of infector rows of a highly susceptible cultivar that provides sporangia as secondary inoculum for the test lines, and (c) provision of perfo-irrigation system to create high humidity (>80% RH) and leaf wetness necessary for infection and disease development. This method has been refined over time and it is quite effective under proper management conditions. Prevalence of high humidity (>80% RH), leaf wetness and moderate temperature (25-30°C) during the first 2-4 weeks of crop growth is critical for infection, disease development and disease spread. The nursery can be operated on a large scale both during the rainy and post-rainy seasons at locations in southern states of India because of the prevailing moderate temperature, if managed properly. However, in northern and western India, where average winter temperatures are low (<15°C), the disease development is adversely affected and the screening cannot be successful. Greenhouse screening method, in contrast to field screening, can be operated throughout the year. High humidity is maintained by a fogging system in the greenhouse that operates for 15 min at 30-min intervals by automated timer connected to a high pressure pump (3 hp). The entire process of isolates maintenance, inoculum multiplication, inoculation and incubation has been well refined and it has been quite effective in screening large number of breeding lines in a short time period (Singh *et al.,* 1993; Thakur *et al.,* 2006). Major advantages of greenhouse screening include : independent of season, precise inoculation (with little chance of escape of seedling), rapid results (takes two weeks between inoculation and data recording), screening against multiple isolates at one place, highly reproducible and thus reliable, cost-effective and easy selection for

resistance. Downy mildew being a highly weather-sensitive disease, microenvironment in the field screening greatly influences the disease development. Despite adequate care and management of the field nursery, disease incidence is often quite variable across years/seasons and locations. However, the field screening provides important information on the general resistance levels of the lines against the highly heterogeneous pathogen population. In a field screening at a particular location, the known highly susceptible and highly resistant lines generally show disease incidence true to their types, but other lines that are moderately resistant or susceptible provide more variable results and these could not be very well compared with the greenhouse incidence data. Field screening, in contrast to greenhouse screening, provides better opportunity for selection of greater number of resistant plants (to a natural population of the pathogen with moderate inoculum load) that can be selfed and seed obtained in the same season. Greenhouse screening, on the other hand, is more severe with high inoculum pressure, useful to screen large number of lines and discard susceptible lines/plants in a most economical and effective way. Resistant plants from greenhouse screen can also be transplanted in field/pots for generation advance. A large number of hybrid parental lines and progenies are screened against single or multiple isolates in greenhouse and resistant selections are made. In some cases, resistant seedlings from the screening pots are transplanted in the field for advancing generation (Thakur *et al*., 2001; Rai *et al*., 2006). The lines showing resistance (≤10% disease incidence) to at least two isolates are designated and disseminated as seed parents. This breeding strategy has led to the development of more than 125 A-lines, most of which have been resistant to 2-5 isolates in the year they were designated and disseminated.

***In vivo* :** Large numbers of germplasm lines were planted during rainy season in a downy mildew sick plot using infector row system (Williams *et al*., 1981). The infector rows (mixture of 7042 S and local susceptible material) are planted three weeks prior to the test entries for uniform spread of sporangial inoculum to test entries. Highly susceptible line 7042 S was also planted as indicator row after every five test entries to know the spread of disease in the plot. Each line was sown in single row (5 m) with spacing of 50 cm (row to row) and 10 cm (plant to plant) in a sick plot using the field screening technique described above. Sprinkler irrigation was provided as and when needed to maintain high relative humidity and favourable conditions for disease development. The disease is scored at two growth stages, 30 and 60 days after sowing (DAS) by counting the total number of plants and number of infected plants and per cent disease incidence was calculated. Based on two years data at 60 DAS the entries are classified in different categories as highly resistant – 0-5 per cent incidence, resistant – >5-10 per cent incidence, susceptible – >10-25 per cent incidence and highly susceptible – >25 per cent incidence.

Biotic Stress Susceptibility

In each case it is important to state the origin of the infestation or infection i. e. natural, field inoculation and laboratory. These are coded on susceptibility scale from 0 to 9 viz.,

0–No disease
1–Very low sign of susceptibility
3–Low
5–Intermediate
7–High
9–Very high

Management

Disease management using resistant cultivars is the most feasible way in pearl millet production. Attempts have been made in the past to identify sources of resistance to downy mildew in India (Singh *et al*., 1997). Based on resistant sources, several resistant hybrids and varieties were released for general cultivation in India (Khairwal *et al*., 2004). However, identification of new sources of resistance is required to find resistance against evolving virulence where pearl millet is widely cultivated. Various attempts have been made to identify germplasm lines and local landraces resistant to the disease under artificial epiphytotic conditions.

Host Resistance

DM : Use of downy mildew resistant cultivars has been most economical and effective means of managing downy mildew in farmer's field. Well planned intensive research during the past three decades has resulted in the development of highly effective field and lab screening technique, identification of several sources of resistance and development of several downy mildew

resistant cultivars like HHB 67 (improved), HHB 68, HHB 94, HHB 117, HHB 197, HHB 223, HHB 256, HC 4, HC 10, HC 20 and many other at different places.

Smut : Like downy mildew of pearl millet smut can also be best managed through genetic resistance. An effective field screening technique is available to identifying resistance and breed resistance cultivars. Resistance to smut is dominant and simple inherited.

Ergot : Ergot management through genetic means is most effective and economical. Resistant lines may be developed by intermating less susceptible plants and selecting resistant progenies under high disease pressure for several generations following pedigree and recurrent selection procedure. Ergot resistant has been incorporated in both the parent and pollinator using back cross breeding.

The successful operation of field and greenhouse screening techniques resulted in the identification of a good number of germplasm and breeding lines with high levels of downy mildew resistance (Singh *et al*., 1997).

1. Genetic Variability for Resistance to Major Diseases

Nature of the pathogen and diversity of virulence

Sclerospora graminicola reproduces both sexually by producing oospores and asexually by producing sporangia that liberate zoospores at maturity. Oospores are thick-walled structures that can survive for several years on leaf debris and in soil and also contaminate the seed lots and thus could become externally seed-borne. These are primary sources of inoculum in the field through contaminated seed and infested field soil. Once the seedlings are infected, sporangia are produced on the foliage which serves as a source of secondary inoculum for the spread of disease within and between fields. After landing on the young growing foliage, sporangia produce numerous zoospores that swim in the thin film of water on the leaf surface before producing infection hyphae. High humidity (>85% RH) with leaf wetness and moderate temperature range of 20-30°C are congenial for infection and disease development. The infection to pearl millet seedlings is systemic and the disease symptom is expressed from the seedling stage as chlorotic strips to the flowering stage as green-ear in the panicle. The host-pathogen interaction in the pearl millet-DM system is expected to follow the general gene-for-gene concept (Flor, 1971) as is well known in other obligate systems, such as wheat-rusts, wheat-powdery mildew and lettuce-DM. This concept is based on major R-genes for resistance in host and complementary virulence genes in the pathogen. This is a simple concept and easy to explain how resistance genes are defeated and new virulence genes evolve over time and space. The hypothesis states that plant contains a single dominant resistance gene (*R* gene) that specifically recognizes the complementary avirulence gene (*Avr* gene) of the pathogen. Avirulence gene in the pathogen encodes a protein product that is recognized by the complementary *R* gene product of the plant, which results in induction of defence gene expression (hypersensitive reaction) and inhibition of pathogen growth (incompatible reaction). However, if the plants do not contain the *R* gene, the pathogen will be able to grow and infect them (compatible reaction), even though it contains *Avr* gene.

Several *R* genes have been successfully employed through conventional breeding to confer near-complete resistance against specific races of pathogens in major crops (Hovmøller *et al.,* 1997; McDonald and Linde, 2003; Hovmøller, 2007). The modern molecular work is based on this classical concept of gene-for-gene relationship. However, the major drawback of introgression of such *R* genes has been that they have been rendered non-functional when *Avr* genes mutate to virulent forms (McDonald and Linde, 2003). The role of minor resistance genes and other trait genes, such as thick leaf cuticle genes, contributing to resistance cannot be ignored. Under natural ecosystems, the host-pathogen interaction phenomenon is not so simple and several other factors, such as weather variables and agronomic practices greatly influence the interaction and thus the resistance level of the cultivar. Studies conducted at various research centres have shown large pathogenic variability in *S. graminicola* populations from India and other countries (Ball, 1983; Werder and Ball, 1992; Thakur *et al.* 2002., 2004 a,b; Sivaramakrishnan *et al*., 2003). The pathogen is heterothallic, has rapid asexual generation cycles and can produce millions of spores in short time span. These characteristics enable the pathogen to produce new recombinants and rapid build-up of mutants for adapting to the changing host resistance, chemical fungicide and other control methods. Virulence diversity in *S. graminicola* is studied through a collaborative nursery grown annually at 10-12 locations

of the All India Coordinated Pearl Millet Improvement (AICPMIP) centers in well established DM nurseries. Data are recorded twice on disease incidence, first at 30 days and second at 60 days after sowing, compiled, analyzed and the report is presented at the AICPMIP annual group meeting. The results of this multi location testing provide useful information on virulence variability in the pathogen population and on the resistance stability of breeding lines under diverse environmental conditions. Lines showing stable resistance across environments (year/locations) can be useful for understanding the genetic basis of resistance (Thakur *et al.*, 2004 a, b) as well as in resistance breeding. At present various isolates of *S. graminicola* from major pearl millet growing parts of India are being tested for pathogenicity and virulence. Genetic diversity in isolates of *S. graminicola* has been reported using DNA fingerprinting (Sastry *et al*., 1995), and DNA markers, such as random amplified polymorphic DNAs (RAPDs) (Zahid, 1997) and amplified fragment length polymorphism (AFLP) (Singru *et al*., 2003; Sivaramakrishnan *et al.*, 2003; Pushpavathi *et al.*, 2006). Analysis of molecular variance indicated that variation in *S. graminicola* populations was largely due to differences among the isolates within the states. In all these studies, there were no correlations between virulence diversity and genetic diversity. Such genetic diversity studies will not be useful unless genes for avirulence and their markers are identified.

Mechanism of resistance/tolerance

(a) Mechanical
(b) Physiological
(c) Biochemical
(d) Molecular

Plants use a wide range of mechanisms to resist infection and disease caused by pathogenic organisms. Mechanical or chemical barrier present in the epidermal layer of plant tissue prevent the successful establishment and growth of many potential pathogens. Pathogens that make this first line of defence are met by a second battery of defences, ranging from the specific interactions between plant resistance gene and pathogen avirulence gene products to multiple, and far less well understood, non-host and “basal” defence pathways.

Pathogenic variability

With increasing area coming under pearl millet hybrid cultivation in India since the 1970s, downy mildew severity and spread have increased proportionately. The first downy mildew epidemic occurred on the most popular hybrid HB 3 in 1971, and the first report on pathogenic variability appeared in 1973 when NHB 3 was found susceptible at Gulbarga but resistant at Mysore (Bhat, 1973; Shetty and Ahmad, 1981). With increasing reports of pathogenic variability in *S. graminicola* and hybrids succumbing to downy mildew, a systematic study was initiated in the early 1990s. The results of AICPMIP trials during 1995-99 revealed significant differences in *S. graminicola* populations at different locations (Thakur *et al*. 2004a, b). Based on disease incidence, the *S. graminicola* populations have been grouped. The surveys have provided forewarnings on the performance of hybrids and their possible replacement in certain areas. For instance, the surveys indicated the likely resistance breakdown of HHB 67, the most popular hybrid in Haryana and parts of Rajasthan. This led to the development of HHB 67-2, a DM-resistant version of HHB 67, using marker-assisted backcross breeding. It was recommended for release in Haryana in 2005. This was the first successful demonstration of introgression of downy mildew resistance genes into hybrid parental lines using both conventional and DNA marker technologies to produce downy mildew resistant hybrids in pearl millet through partnership research (Hash *et al*., 2003).

Various isolates (oospore inocula) of *S. graminicola* have been drawn from different pearl millet cultivars during the past 10 years of on-farm downy mildew surveys in the major pearl millet growing states of India. Some of the isolates that have caused high disease incidence (>80%) on popular hybrids have been characterized for their virulence and genetic diversity. In a recent study (Thakur *et al.*, 2004b), 15 selected isolates, based on their pathogenic diversity on host differentials, were classified into five major virulence groups. Similarly, based on their genetic diversity using AFLP markers, these isolates were classified into five major clusters (Sivaramakrishnan *et al.*, 2003). However, these two groups were not similar, as genetic diversity was not based on the virulence gene markers. Over the last three decades, there has been a progressive increase in the release of pearl millet hybrids and the spread of downy mildew. However, there have been no widespread epidemics of the disease in the past 10 years.

2. Mode of Inheritance of Resistance/Tolerance Genes

Availability, diversity and type of genetic resistance

Several other lines and germplasm accessions, including P 310-17, P 1449-3, IP 18292, IP 18293, IP 18294, IP 18295 and IP 18298 with high levels of resistance have been identified (Singh *et al*., 1997; Thakur *et al.*, 2004 a, b). Somanth *et al.* (2013) identified male sterile lines ICMA 92777 and ICMA 99666 which contributed the genes for downy mildew resistance in pearl millet hybrids. Though these are useful sources of resistance, high levels of resistance have also been identified in many elite breeding lines and these have been used more extensively in breeding DM resistant hybrid parental lines in India. In general, there is enough geographical, morphological and genetic diversity in germplasm and breeding lines for DM resistance (Singh, 1995; Singh *et al*., 1997). Three types of resistance to DM – incomplete resistance (Singh *et al.*, 1988), complete resistance (Singh, 1995) and recovery resistance (Singh and King, 1988) – have been reported in pearl millet. Incomplete resistance is usually polygenic, but could also be oligogenic. Genes governing this type of resistance confer incomplete resistance, exhibiting variable levels of dominance. Most studies on genetics of DM resistance have shown resistance to be governed by major dominant genes with non-additive gene action (Deswal and Govila, 1994; Singh and Talukdar, 1998; Kumar and Sagar, 2012). Segregation for host plant resistance has generally shown continuous variation (Singh *et al.*, 1980; Basavaraju *et al.*, 1981, Dass *et al*., 1984). However, there is clear evidence that the A_1 cytoplasm is not associated with susceptibility or resistance to pearl millet DM and the nuclear genes are involved in controlling the disease reaction (Anand Kumar *et al.*, 1983; Yadav *et al.*, 1993; Yadav, 1996; Kumar and Sagar, 2009; Kumar 2014). Most of these studies used less defined resistant/susceptible lines and heterogeneous pathogen isolates. Both pearl millet and *S. graminicola* being allogamous and highly variable, and the disease measurement is taken on a continuous 0-100 per cent scale, there is a greater possibility of identifying quantitative resistance with multiple genes involved. Several studies on molecular marker based genetic linkage maps for pearl millet have shown interesting results (Liu *et al.*, 1994; Hash *et al*., 1995, Jones *et al.*, 1995; 2002; Breese *et al.*, 2002; Hash and Witcombe, 2002) that will facilitate genetic manipulation of disease resistance. A number of quantitative trait loci (QTLs) for DM resistance have been identified on different linkage groups, and some of them are specific to different isolates (Hash *et al.*, 1999; Jones *et al*., 2002). DNA markers have been identified for about 60 different putative DM resistance QTLs in pearl millet (Breese *et al*., 2002; Hash and Witcombe, 2002). Using RFLP-based marker-assisted backcross (MAB) method, several mapped QTLs have been transferred to backgrounds of elite inbred parental lines of a popular single-cross hybrid HHB 67 (843A × H 77/833-2). It would be highly useful to strengthen research efforts on understanding the genetic nature of resistance and effectiveness of specific QTLs in different resistant lines for their effective utilization in resistance breeding.

Utilization and deployment of resistance

A common strategy of resistance breeding utilizing major genes involves an effective screening method, availability of diverse germplasm, confirmed sources of resistance, knowledge of genetics of resistance, information on variability in virulence, effective utilization of resistance in breeding, and deployment and on-farm monitoring of performance of cultivar. Genetic diversity and durability should be the main features of a resistance breeding programme. These features are well represented in OPVs (WC-C-75, ICTP 8203 and Raj 171) as they have shown resistance to DM on a large area for more than 20 years. However, the same is not true with hybrid cultivars. During the past 30 years, a number of DM resistant lines have been used in resistance breeding programmes and some of the resultant hybrids have been commercially successful. There has been substantial progress in managing the risk of losses caused by DM epidemic during the past 15 years by diversifying the hybrid cultivars base, monitoring virulence, screening breeding lines to diverse isolates and breeding DM resistant hybrid parental lines that are utilized by private and public organizations for developing hybrids. A study by Mahala *et al.* (2004) showed that more than 80 hybrids (by name) developed by public and private organizations were being grown by farmers in India. There is no systematic and well-organized resistance breeding programme in operation so far at most of the Indian national research centers. The major limitations include : (a) well-established DM sick plots and (b) greenhouse screening facilities. In

addition, there is lack of information on well-defined genetic resistance–Rgenes/QTLs, utilization/ introgression of specific R Rgenes/QTLs and breakdown of specific resistance (Rgene). These aspects have to be addressed by creating research facilities and planning research to generate the information required for proper screening, monitoring virulence and developing a sound science-based DM resistance breeding programme. While utilizing these lines the organizations should keep record of specific DM resistance and monitor its heritability in the hybrids. Such hybrids could be screened for DM resistance to specific isolates in greenhouse and in the DM nursery at key locations before their release and commercialization. Proper monitoring of these hybrids in farmers' fields should be taken up for their resistance stability. There is also a need to strengthen the DM resistance breeding specific to pearl millet adaptation zones provided the existing zones serve the purpose for hybrid breeding programmes.

Monitoring virulence/resistance

Virulence/resistance monitoring is done by a well planned on-farm survey in major pearl millet growing states of India (Thakur *et al*., 2003). These results have benefited the pearl millet researchers in monitoring resistance levels of their hybrids and planning their resistance breeding programmes accordingly. This activity is very critical and needs strengthening through enhanced scientific and financial resources. The short growing period of crop and appropriate time of DM observation (30 to 40 days old crop) and a single survey team put limitation on the areas to be covered during the crop season. An important finding from the surveys was that most of the seed supplied to farmers by private seed companies were treated with the fungicide metalaxyl (Ridomil/Apron). The frequency of treated seed supply has increased over the past five years. This is a matter of great concern in relation to likely evolution of new isolates with higher virulence. The results have indicated that despite fungicide treatments, susceptible hybrids have recorded very high DM incidence (>80%) in some fields. This results in double losses both to seed companies and to farmers. There is a strong need for better understanding of DM isolates and develop a strategy to make the fungicide treatment more cost-effective. It is well known that fungicide seed treatment protects crop only up to 35-40 days, and later the disease appears on the nodal tillers and on panicle on a susceptible hybrid. In case of moderately resistant (10-20% DM incidence) hybrids the fungicide treatment is more effective than in susceptible hybrids. The use of fungicide as seed treatment is considered as a stop-gap arrangement for the replacement of moderately resistant hybrids, and the susceptible hybrids must not be grown at all even with fungicide treatment. This strategy would greatly help in prolonging the commercial life of some popular hybrids and reduce chances of evolution of new virulence in pathogen population. The survey results also indicated that seed supplied to farmers were from 1 to 2 years old stocks and thus the treatment was too old to be effective in controlling DM in the field. Research has shown differential cultivar responses to metalaxyl treatment (Singh and Shetty, 1990). In certain cultivars, metalaxyl treated seed when exposed at 40°C for 30 days loses its effectiveness and storage beyond 60 days prevented germination.

3. Breeding Methods Used for Incorporation of Resistance/Tolerance Genes

(a) Mutation
(b) Recombination/backcross breeding
(c) Biotechnological

Breeding efforts and objectives

- Improving fodder characteristics : yield and quality
- Deciphering genome relationships in the genus
- Tolerance to abiotic and biotic stresses
- Breeding for perenniality and apomixis
- Pearl millet as an excellent system for cytogenetic research

Bottleneck in conventional breeding and promise of biotechnology

- Limited availability of desirable genes in cultivated gene pool
- Incompatibility barriers in crosses between pearl millet and wild species
- Hybrid sterility in potential crosses
- Limited mapping efforts on genes for fodder traits
- Induction of polyploidy to reduce ploidy level differences for obtaining interspecific hybrids in interploidal crosses.
- Utilization of techniques, such as embryo rescue, to overcome post-fertilization incompatibility barriers in interspecific crosses

Breeding methods

Both conventional and molecular breeding methods have successfully been used in DM resistance breeding programme (Hash *et al*., 1999; Hash and Witcombe, 2002). The conventional breeding has mostly been used in pedigree selection for developing hybrid parental lines and recurrent selection for population improvement. In pedigree breeding, eco-region-specific progenies selected for desirable agronomic traits, grain and fodder yields are tested for resistance to the eco-region-specific isolates. During inbreeding selection-generation advance, at least at two inbreeding stages, DM screening for resistance to specific isolates is done under greenhouse condition. In the molecular breeding programme MAB method was used for transferring DM resistant QTLs into the hybrid parental lines (Hash and Witcombe, 2002). Several DM resistant lines, such as IP 18292, 7042R and 700651 have been used in developing hybrid parental lines. A number of DM resistant QTLs effective against diverse Indian isolates of *S. graminicola* have been mapped on the pearl millet linkage groups and some of them have been transferred to the commercial B-lines (843B and 81B) and R-lines (H 77/833-2 and ICMP 451). Development and commercial deployment of DM resistant version of HHB 67 is the first successful story of MAB in field crops in public domain in India (Hash *et al*. 2006). It is believed that both pedigree and MAB breeding methods have been quite effective in transferring DM resistance in advanced breeding lines and should be followed without much problem. Marker-assisted breeding is currently not cost-effective, limiting its application selectively on commercial hybrid parents in few cases. But as more cost-effective tools are developed it could be increasingly used in future. Instead of taking pedigree-derived progenies it may be much effective to begin DM screening from F_2 itself, if DM resistance breeding is the primary focus. Use of doubled-haploid breeding technology (Thomas *et al.*, 2003) could be useful to study inheritance of resistance and genetic diversity in hybrid parental lines and in development of isolate-specific DM resistant inbreds in a short time and in gene pyramiding. It may serve as useful tool for marker-assisted selection (MAS) as well.

Since resistance breakdown is very fast in pearl millet and hybrid replacement rate is also rapid (Thakur *et al.*, 2006), our goal should be to develop more resistant lines in shortest possible time. While MAS can help in improving existing lines, doubled-haploid technology can consistently deliver new resistant lines in a short time.

4. Achievements

Research status (international and national)

- Germplasm evaluated for fodder traits
- Interspecific crossability estimated–several interspecific crosses obtained
- Genomic regions identified–involved in apomixis
- Limited efforts on improving yield and quality of fodder pearl millet utilizing biotechnological approaches
- Several promising lines of *P. pedicellatum* and Napier-bajra hybrids released
- Limited efforts on transferring and study of characters such as perenniality and apomixis for fodder pearl millet improvement.

Success stories

- Production of interspecific hybrids of *P. squamulatum* and *P. orientale* with pearl millet
- Production of trispecific GOS hybrid
- Establishment of protocol for embryo cloning
- Production of perennial, thin stemmed, grassy, high tillering, apomictic fodder pearl millet

Research Efforts Needed for Future Research

Despite the impressive progress on managing downy mildew in pearl millet, the battle is far from over. Given the dynamic nature of the pearl millet-downy mildew pathosystem and the high genetic potential for virulence evolution in the pathogen population in response to genetic changes in the host, an appropriate strategy has to be developed to manage this disease. In the light of past studies, it is proposed that future downy mildew management strategy in pearl millet should focus on : (i) Utilization of tissue culture technique and marker-assisted selection (MAS) for downy mildew, (ii) Identification of sources of multiple disease resistance and their utilization, (iii) Identification of source of different types of resistance (stable, complete and recovery mechanism), (iv) Virulence monitoring through on-farm

downy mildew surveys and multilocation virulence nurseries, (v) Collection and characterization of isolates for virulence, (vi) Breeding hybrid parental lines for resistance to single/multiple isolates and (vii) Developing resistant hybrids for release and commercial cultivation. This paradigm of resistance breeding through monitoring virulence should be a continuous process to effectively manage downy mildew in pearl millet. Other approaches, such as judicious use of seed-treatment fungicides and suitable agronomic practices in combination with host-plant resistance would be desirable to prolong the commercial life of hybrid cultivars.

CONCLUSIONS

Considerable progress has been made in understanding host-pathogen interaction, refining disease screening methods, identifying and utilizing resistant sources and breeding DM resistant parental lines and hybrids. However, the disease is still a major challenge towards realizing the high yield potential of hybrids. Some of the research and development issues that need attention in different time frames can be divided into the following groups. In a short term of 1 to 3 years the focus should be to : (i) Develop well-managed DM nurseries at key locations in each of the hybrid-intensive states under different adaptation zones (A_1, A and B), (ii) Develop greenhouse screening facilities at 2-3 locations, (iii) Conduct well organized on-farm survey, involving pathologists and breeders, and (iv) Minimize the use of fungicide (metalaxyl) for seed treatment. In a medium term of 1-5 years it would be important to : (i) Regularly replace the existing isolates with new more virulent isolates as they occur in different zones/states for greenhouse screening, (ii) Screen breeding lines against representative isolates from each zone (2 isolates/ zone); (iii) Designate hybrid parental lines for resistance to specific DM isolates, (iv) Evaluate hybrids and parental lines to specific isolates in greenhouse and DM nurseries prior to release and commercialization and (v) Provide timely information/feedback among the members on resistance performance of hybrids/parental lines. On a medium to long term of 3-8 years, attention must focus on: (i) Identification of DM resistance genes/QTLs against specific isolate, (ii) Development of genetically diverse and DM resistant parental lines, (iii) Development of near isogenic lines as host-differentials and (iv) Identification of genetic markers for avirulence. Based on the above issues, we propose a protocol of DM resistance breeding in pearl millet for further discussion and refinement. We believe that with cooperation of the organizations involved in DM resistance breeding, we should be able to address the above issues towards developing an efficient and long term DM resistance breeding strategy that would help realize and sustain the high yield productivity of pearl millet hybrids in India and contribute to global food security.

Breeding Point of View

Future thrust

- Production of interspecific hybrids with different species for pearl millet improvement and genome studies
- Identification of genes for apomixis and its components and their utilization
- Genetics and molecular biology of perenniality and its transfer in cultivated pearl millet

Futuristic approach initiatives and results

- Identification of new cytotypes in *P. pedicellatum* and *P. squamulatum* (apomictic, perennial wild species)
- Induced tetraploidy in two male sterile diploids (81A1 and 81A4) and their maintainer 81B.
- Production of interspecific hybrids between pearl millet and wild species viz., *P. squamulatum* and *P. orientale*, and their characterization
- Sequential reduction of *P. squamulatum* genome complement in *P. glaucum* (2*n*=28) × *P. squamulatum* (2*n*=56) hybrids and their progenies revealed its octoploid status
- Partitioning apomixis into its components (apomeiosis, parthenogenesis and functional endosperm development) in F_1s and advanced generation hybrids, thereby revealing possibility of recombination and independent inheritance of these traits.
- Production of population segregating for perenniality, as well as expression of individual apomixis components, from pearl millet × *P. squamulatum* hybrids
- Production of first ever trispecific hybrid in pearl millet involving *P. glaucum*, *P. squamulatum* and *P. orientale* (GOS hybrid) with 2n= 44 (21G+14S+9O)
- Production of hybrids between *P. glaucum* and *P. orientale* with recurrently added *P. glaucum* chromosomes- ploidy series with 2n=16, 23, 30 and

44 chromosomes

- Production of alien chromosome addition lines of *P. squamulatum* in tetraploid pearl millet

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