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Breeding Strategies for Historically Important Plant Pathogens -A Holistic Approach

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A B S T R A C T

Fungi, bacteria, mycoplasma, Spiroplasma, virus, viroid, phanerogamic plant parasites, and other macro pests are all agents, which lead to suffering of plants, this effects the tropic levels above producers who are feeding on them for survival. Science of pathology shouldn't be confined to humans itself (I mean understanding human diseases), it's equally important that the science of plant pathology must be given equal importance as medicine. We have seen many epiphytotic in past, Irish famine which led to death of approx 1 million people and migration of 1.5 million Irish, chestnut blight, Dutch elm disease, lethal yellowing of coconut (in Caribbeans and south America), powdery mildew, downy mildew (Europe especially UK and France), rusts and etc. These incidents in past made us realize how important its to have concern towards crop protection orelse people die out of food or this may disturb ecology by eliminating a plant species which was about to happen in elm and chestnut. Hence its responsibility of plant pathologists to serve humanity the way doctors serve human health. Hence emerged methods to deal with plant pathogens and human being in course of history discovered different methods of controlling pathogens which include agronomic cultural methods, botanical sand etc. and then make chemical method as science advanced, chemistry revolutionized however chemicals were used in ancient antiquity i.e., Homer suggested use of Sulphur far back in 1000 BC and Tillet and Prevost suggested use of copper sulphate for smuts. However, after World War 2 the use of chemicals increased in accelerated rate. Apart from using chemicals (Sulphur for PM, Bordeaux mixture for DM, Copper Sulphate for Smut – which were using in past in history). Keeping all these apart, in 19th century ending till 20th century middle emergence of science of genetics and improvements in plant breeding gave us new technology to make disease resistant plants. And in 20th century ending, improvements in biotechnology, and coming together of plant breeding and biotechnology enabled us further to make disease resistant plants easily. The 5th generation breeding which includes markers and biotechnology as enabled us in pyramiding genes, MABC enabled us to transfer genes governing biotic stress resistance from wild plants into agronomically desirable cultivated plants (introgression), the best classic example being transfer of Xa21, xa5 and xa13 genes into Pusa Basmati – 1 making it Improved Pusa Basmati – 1. Even the conventional breeding methods are still in major use to develop disease resistance plants i.e., selection, introduction, hybridization and etc. The review article is made in very holistic manner which includes all major historic important pathogens and breeding strategies employed to improve them and it includes rusts, Panama wilt, Coffee rust, bacterial blight of rice and etc.

Introduction

Wheat rust, one of most important disease of wheat in fact noteworthy disease of whole plant pathology caused by an obligate pathogen (biotroph) i.e., the pathogen doesn't cause immediate death of plant (i.e., necrosis – blights, spots, cankers, rots, wilts etc.) but will be in long term relation with plant and decrease efficiency of plant in several ways either by decreasing LAI, photosynthetic efficiency or by nutrient diversion (leaching through its haustoria). So, the rust fungi grow intercellularly in leaf, stem hypodermis, cortex insert its haustoria into cell and take nutrients out of cell. And there is no just one rust in wheat and there are multiple rusts with multiple names and are stated below.

Strip/ yellow rust/ early rust is prevalent in north Indian plains such as Punjab, Haryana and Himachal as this rust prefer cold climates. Stem rust/ black rust/ late rust is prevalent in southern peninsular and the brown rust/ leaf rust/ orange rust is prevalent to many regions.

And many economically important crops are infected by rusts of various genera all belonging to Basidiomycota. Rusts of legume crops caused by a pathogen named *Uromyces spp*, Soyabean rust caused by *Phakopsora sp*, Coffee rust by *Hemileia sp*, Rose rust by *Phragmidium sp*, Apple cedar rust by *Gymnosporangium sp*, White pine blister rust and fusiform rust of pines and oaks by *Cronartium spp*. And all these rust pathogens can be microscopically differentiated made of tilial stage.

And rust pathogen spores travel long distances I.e., from Australia to New Zealand (strip rust travelling) over 2000 km in past. Rust was introduced into Australia from Europe was by human aid but introduction of yellow rust into New Zealand was through winds.

The airborne nature of their spores, with the ability to travel long distances, and the rapid evolution of highly virulent races of rust pathogens make their control difficult.

Coming to breeding strategies, selection only enables us to identify rust resistant cultivars, hence we can select plants from wild state by exploration into centers of origin (primary center/ secondary center/ micro center) and in case of wheat its Persian center, wild wheat can be found throughout Persia region i.e., Iran, Afganisthan, Iraq. Bit also in central Asia and Europe. Mere introduction from some other country, works good such as introduction from CYMMIT (International center for maize and wheat improvement, Mexico City, Mexico) in 1960's saved India from severe famines and those introductions from CYMMIT in 1960's also had rust resistant cultivars. Hence Explorations, cataloguing, conservation is foremost important. (Genes related to stress resistance i.e biotic stress or abiotic stress are present in wild relatives, wild forms and landraces) so that we get enough raw materials to continue with breeding strategies.

Hence wheat breeding programs across the world are dependent on a limited crop germplasm and currently, worldwide grown wheat cultivars capture only 10% of existing wheat diversity. However, exploitation of the full range of crop diversity would be beneficial for improving traits such as disease resistance in wheat (Moore 2015, S. Savadi, P. Prasad, P. L Kashyap, S. C Bhardwaj).

Now transferring this into commercial cultivar/ desirable cultivar is important and this can be achieved by back cross breeding, but now this in this era where we are well versed with markers, marker assisted selection, biotechnology, hence marker assisted selection (MAB), marker assisted back crossing (MABS) play a very significant role in disease resistant breeding.

Modern breeding tools include Marker assisted Technology, Targeted induced local lesions in genomes (TILLING), and transgenic technology. Recently, genomic selection (GS), cisgenics, intragenesis, genome editing technology (GET), RNA dependent DNA methylation and reserve breeding have also been added to existing molecular breeding tools.

Conventional breeding methods such as mass selection, pure line selection, bulk method, or recurrent selection (by this we can see if there is any disease resistance cultivar already present in the material which we already have) do exist but they played an important role in past but now platue stagnated their significance as a method for disease breeding hence hybridization especially backcrossing and marker assisted selection play a foremost role now.

From gene for gene hypothesis (proposed by Flor in Linseed rust *(Melampsora lini)*, its understood that if a host to be diseases or to a pathogen to cause disease it depends on genotype of both host as well as pathogen i.e., a pathogen is said to be virulent not only based on its self attributes but also its host attributes and same applied to Host, host is susceptible or resistant depends not only on attributes of host but also of pathogen.

For every resistant gene (R and r) present in host, the pathogen as a gene for virulence (Avr and avr). Susceptible reaction would result only when the pathogen would be able to match each of the resistant gene present in the host with appropriate virulent gene. If one or more virulent genes are not matched by the pathogen with the appropriate virulent gene, resistance reaction will occur. Here resistant gene in dominant and virulent gene being recessive.

Suppose a resistant gene is introduced into a plant through any means (backcross program), when I say resistant gene introduced, I mean to say at that point of time there is no any corresponding allele in pathogen to overcome with resistance but in future it may be possible as pathogen co – evolves (especially because of high fecundity of a pathogen, dikaryon, heterokaryon, parasexual and several such methods which leads to recombination).

And this co evolution is very much common in rusts hence breeder must be always ready to face challenges to deal with evolved pathogens and this happened in past in many instances throughout world which is discussed below. In 1990Yr9 virulence was identified in Syria and in April 1994, the virulence attacking cultivars such as Pak81, Pirsabak 85, Seri 82 possessing Yr9 became susceptible in Pakistan.

In 1996, a virulent pathotype on Yr9 and another virulent on Yr9 and Yr27 in 2002 were identified from the bordering areas of Punjab and subsequently have been identified from Nepal and Bhutan.

A few historic cultivars, such as Thatcher and Hope for black rust, Americano 25, Americano 44d, Surpreza, Frontana and Froteira for brown rust, and Wilhelmina, Capselle – Desprez, Manella, Juliana and Carstens VI for yellow rust, have been maintained some resistance for years.

Ug99 race which evolved in 1998 in Uganda in 1998-1999 was found to be resistant against stem rust gene Sr31 and this has potential to spread throughout the world hence Dr Norman Borlaug led the call for a joint effort to tackle this threat, which led to establishment of the Borlaug Global Rust Initiative (BGRI), in September 9, 2005 at Nairobi, Kenya with the following objectives:

UG99 race is also called TTKSK race.

To monitor the movement of race Ug99.

To evaluate released varieties and germplasm for resistance to Ug99.

To share sources of stem rust resistance worldwide.

To incorporate diverse resistance into high yielding adapting wheat varieties.

Hence whenever a scientist makes a variety with a new resistant gene, pathogen will definitely evolve and breaks the gene. (Scientist vs pathogen). Hence, its better to go with HR (Horizontal resistance) which was actually not much exploited in case of what rust and also in other diseases of plant pathology. And this leads to Boom and Burs cycle,

where a resistant variety gets popular and grown by farmers in wide scale in space and in matter of time the pathogen co – evolves and makes the former susceptible and now the variety suddenly gets unpopular hence no variety is permanent in terms of its popularity and its popularity as a life span.

Let's understand this Boom and Burs cycle with a classical example of Panama wilt (Caused by *Fusarium oxysporium var cubense*). Fusarium oxysporium fsp cubense has many races I.e., Race 1, Race 2, Race 3, and Race 4. In 1950's then dominant Gros Michael variety had to be replaced as it was susceptible to Race 1, hence it was replaced by another variety Cavendish. In 1990's another virulent form of Foc, tropical race 4 (TR4) seriously damaged Cavendish banana production in Asia hence till now popular variety had to now loose its popularity.

However, by every stringent quarantine and help of government we can avoid spread of that particular race (i.e., tropical race 4) so that Cavendish remains in popularity at least in few locations of world.

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Coffee leaf rust, yet another important disease which has caused epidemics in many countries of southern hemisphere, the coffee leaf rust epidemics in Sri – Lanka during 1970's has in fact replaced the farmers choice of growing coffee and let to emergence of tea plantation in Sri lanka.

The disease is however 1st seen in Ethiopia in 1934 but it never reached epidemic level to cause eradication of Arabica coffee but it devastated Arabica coffee plantations in Ceylon at the end of the 19th Century and was responsible for its replacement.

The pathogen prefers temperature of 20 – 28 degree C, needs a leaf wetness period only during spore germination and penetrates with the germination hyphae into the stomata of the host. The fungus tolerates longer seasons without rainfall and spores are wind borne, only attacking leaves and needs no other host for completing the life cycle. Due to the fact that coffee is a perennial host with green leaves all throughout the year, the pathogen produces only uredinio and teliospores with basidiospores is not significant.

Leaf rust affected plants get defoliated, complete defoliation is seen.

Coffee leaf rust resistant genes are 9 in number SH1 to SH9 and pathogen virulence is codified by the genes v1 to v9.

SH1, SH2, SH4e, SH5 could be found in *C arabica* genotype. The genes SH7, SH8 and SH9 and others unknown, were introduced from *C. robusta*, and SH3 from *C.liberica*.

SH3 originated from introgression of *C. liberica* into *C. arabica*.

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Bacterial leaf blight resistant version of Pusa Basmati 1 (PB1), called Improved Pusa Basmati 1 (IPB1) was developed by transferring genes xa13 (located on chromosome 8) and Xa 21 (located on chromosome 11) for bacterial leaf blight resistance from IRBB55 using MAS for foreground selection.

Foreground selection was done for the two genes for resistance (xa13 and Xa21).

Background analysis aimed to recover the recipient parent PB1 genome to the maximum extent, and it's based on 252 (AFLP)polymorphic markers in BC1F3, and 69 STMS markers spread over the entire rice genome i.e., the 12 linkage groups, at an interval of 20cM in BC1F5.

Two genes were transferred into PB – 1 from IRBB55 making IPB- 1 which is the 1st variety in India to be developed using marker assisted back crossing. Subsequently, another variety called Sambha Mahsuri was improved by transferring into 3 bacterial leaf blight resistant genes namely xa5, xa13 and Xa21 and then evolved the variety i.e., Improved Sambha Mahsuri (ISM). In coming paragraphs further details will be explained in depth about molecular strategies in developing Bacterial blight resistance in Rice.

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This is the first variety in India to be developed using marker assisted breeding.

One F1 plant among 20 produced from the cross PB1 x IRBB55, was backcrossed to PB1, and 118 BC1F1 seeds were obtained.

BC1F1 seeds were evaluated for bacterial leaf blight in the field, but the results were inconclusive. Their grains representing BC1F2 generation, were assayed for quality and 23 BC1F1 plants were found to produce seeds of basmati grain quality and they were analyzed for marker specific for xa13 and Xa21, and 9 plants were tested positive for both; seeds from these plants were grown.

Table.1 Foreground selection

xa13	CAPS (Cleaved amplified polymorphic sequence)	RG136	At 3.7 cM from xa13
Xa21	STS (sequence tagged site) Sequence tagged microsatellite marker (STMS)		Gene sequenced based marker waxy gene is located on short arm of 6th chromosome
Restorer gene	STMS		Rf1 restores wild abortive (WA) CMS

Table.2 Background selection

PB1 genome contribution	AFLP (BC1F3) STMS (BC1F5)	252 markers 69 markers	Markers distributed over the entire genome at 20 cM interval

In BC1F2 generation, 513 plants were resistant to leaf blight, but only 57 of them produced grains of Basmati quality. Out of these 7 plants were found to be homozygous for the marker specific for xa13 and Xa21; they were selfed raise 7 BC1F3 families, which were resistant to leaf blight.

From each family 3 phenotypically superior plants were selected to give a population of 21 BC1F3 plants, these plants were homozygous for the markers linked to xa13 and Xa21, and majority of them were agronomically superior to PB1.

All the 21 plants had long panicle than both the parents, and most of them had basmati grain quality.

These 21 plants were subjected to background analysis using 252 AFLP markers; the recovery of PB1 genome ranged from 80.4% to 86.72%.

In BC1F5, 31 elite progenies were evaluated for the foreground selection markers and traits, recipient parent (PB1) genome contribution and agronomic and quality traits. All these 31 plants were homozygous for both xa13 and Xa21, waxy locus and the maintainer (non restorer) specific allele found in PB1.

The genome of BC1F5 lines were surveyed using 69 polymorphic STMS markers.

IPB1 has erect flag lead and sturdy stem from the donor parent IRBB55, which are desirable agronomic features. In addition, its grains are free from chalkiness, which is more desirable than the chalky grains of PB1. Chalkiness is major weakness of PB1, which has chalky grains to the extent of up to 20%. Amylose content of IPB1 was comparable to that of PB1.

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