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A review on gene pyramiding of agronomic, biotic and abiotic traits in rice variety development

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Abstract

Rice *Oryza sativa* L is a staple food crop, and its seeds are the most important component part of the agronomic trait of the cereal crop, rich in nutrient and of economic value to human and even livestock. But, it is often threatened by various abiotic and biotic conditions that reduce the yield, because of high incidences of infectious disease agents and non-pathogenic conditions respectively. Pyramiding of the requisite resistance and tolerance genes into single elite high yielding variety of rice, confers wider spectrum of stress management, resulting to development of single multiline variety of rice. Marker-assisted selection utilizes DNA marker-linked primers for blast resistant gene (RM8225;*Piz,* RM6836;*Piz*, *Pi2*,*Pi9*), bacteria leaf blight (RM224; *Xa-4*, RM122;*xa-5*, RG136; *xa-13,* RM21;*Xa-21*) and drought tolerance (RM236;$qDTY_{2.2}$, RM520;$qDTY_{3.1}$, RM511;$qDTY_{12.1}$) in pedigree, backcross and recurrent selection breeding methods. The objectives are to create awareness on the environmental safety of host-resistance, significance of single multiline resistance variety, effect of the interaction of stress conditions and associated simple sequence repeat (SSR) linked markers.

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Introduction

Rice is an important staple food crop, which forms a major part of the human diet and a good source of carbohydrate for over half of the world's human population. Asia alone produces 90% of the world produced rice, with irrigated lowland providing 75% of the world's production in about 79 million ha of arable land (Bourman et al. 2007; Maclean et al. 2002)

In the year 2010, global statistics showed that, harvested rice was from an estimated cultivated land area covering 154 million ha. 137 million ha of the cultivated land representing 88% was from Asia, while Southeast Asia alone, cultivated 48 million ha, representing 31% production area from the world's harvested areas. Rain-fed lowland accounted for more than 55-60% of the total rice-cultivated land (Redfern et al. 2012; Toojinda et al. 2005). However, by the year 2030 minimum of 40% additional production, from the total global estimate is required to meet the need of the ever increasing population, most especially in Asia (Khush 2005).

The region is always faced with abiotic and biotic stresses that hinder yield and quality of grains of its high yielding varieties. To sustain production, irrigated rice cultivation should be explored, because it offers a better option, as a result of current challenges and effect of climate change, resulting in severe water deficits in the rain-fed lowlands and also frequent flooding. These form part of the major agricultural constraints in these regions since they are unpredictable (Dey and Upadhyaya 1996). Abiotic conditions include; salt, extreme temperature, drought, submergence, mineral deficiency, wounding and oxidative stress among others, while biotic stresses could include, and not restricted to fungal pathogens of blast, bacteria leaf blight and insects, viruses, false smut, sheath blight.

These stress conditions affect leaf length and width (flag-surface area), tillering, length of panicle, filled grains number/panicle, 1000 grain weight (seed quality), thereby resulting in low yield.

The simultaneous assemblage of agronomic traits of yield, biotic and abiotic resistance and tolerance respectively are through pyramiding approach, resulting in development of a multi-line resistance variety. This process employs marker-assisted selection, through; pedigree, backcrossing or recurrent selection breeding method. This is carried out by introgressing the desired multiple quantitative trait loci (QTLs) into a desired background high yielding variety of rice, in the case of pedigree method (Servin et al. 2004). Pradhan et al (2015) reported that, pyramiding of multi-resistance genes into single line often confers wider spectrum of resistance and durability.

The objectives aress to raise awareness on the cost-effect and environmental safety associated to development of host resistance variety of rice, to inform breeders of the significance of multiple disease resistance to blast, bacteria leaf blight diseases (biotic) and drought tolerance (abiotic) conditions, the effect of interaction of these stresses on plant development, and the SSR markers linked to blast, BLB and drought for marker-assisted selection.

Agronomic traits of rice

Seeds are the most valuable component of the agronomic traits of rice, and high yield is the prime goal of crop production. Therefore, to determine rice yield; culm length (CL), leaf length (LL), days to heading (DTH), leaf width (LW), spikelet number per panicle (SN), panicle number per plant (PN), 1000-grain weight (GW), panicle length (PL), fully filled grain (FFG) and flowering time are important parameters for consideration and improvement (Fujita et al. 2014, Xue et al. 2008). These will however, result in increased yield even in limited arable land, with capacity to meet the food demand of the ever increasing world population. Reduction in rice yield is usually connected to increase in the percentage of spikelet sterility and number of spikelets per panicle (Shamsudin et al. 2016). This spikelet encloses the rice grain, borne at its terminal ends. These yield and their related traits are complex and controlled by many quantitative trait loci (QTLs).

Leaves formation is crucial as it forms an integral part of the agronomic trait, it occurs in two positions on the culm, each one at a node. It is made up of sheaths and blades, which basically form the surface area for photosynthetic activities, for manufacture of food substances (rice) borne on the panicles. The wider the surface area (flag leaf surface) and presence of chlorophyll pigmentation (with absence of diseases) on leaves the better, a good trait and sign for higher grain formation. The vegetative and floral organs are often the target of disease pathogens.

Abiotic stresses in rice

These are non-component part of the environment which exerts stress to the rice plant, thereby affecting its productivity. Many of these stress factors have been identified, among which are; mineral deficiency, salinity, drought, wounding, submergence, temperature extremes, oxidative stress and nutrient deficiency. These have quite a lot of impact on agricultural practices globally, with an estimated average yield reduction of major crop plants to more than 50% (Wang et al. 2003).

Drought

It is an important economic challenge for sustainable rice production, and yield in mainly rain-fed growing regions in countries of Asia. Drought stress imposes a great threat on rice cultivation, with recorded effect on over two million (2,000,000) hectares in Asia (Bray et al. 2000, Kumbhar et al. 2015). In almost every cropping year, severe drought affects production of rice in South, Southeast Asia and Africa (Luo and Zhang 2001). 45% of approximate rice growing areas of Asia do not have stable access to irrigation sources and are often subjected to drought (Crosson 1995). All stages of rice growth with even early reproductive stage could be adversely affected by drought, leading to drastic yield reduction. At the meiotic stage, some cultivated variety's yield is reduced (Swamy and Kumar 2013), likewise in cereal crops, like wheat and barley (Barnabas et al. 2008; Boyer and Westgate 2004). The resultant effect of drought to rice yield is often attributed to increased percentage sterility of spikelet (Fukai et al. 1999; Jondee et al. 2002; Liu et al. 2006) and number of spikelets per panicle (Boonjung and Fukai 1996). The time and duration of stress on rice also determines the level of seed yield potential (Garrity and O'Toole 1994).

The unpredicted and unsustainability of rainfall pattern due to climate change, is responsible for severity of drought being experienced, along with source of irrigation, type of soil, availability of water all through and out of seasons, and crop's growth stage (Swamy and Kumar 2013). Fewer water supplies for farming globally informed the need to improve adaptability to drought, and the search for resistance varieties as a crucial necessity (Pandey and Sukla 2015). Many of the genes involved for response of plant to drought stress have been identified (Zhang et al. 2012). This drought stress response result to changes in metabolism, physiology and reduction in growth (Sairam and Srivastava, 2001), it ultimately alters physiological process thereby reducing germination, photosynthesis and seedling vigour (Pandey and Sukla, 2015).

Iron toxicity

This is mostly associated to presence of soils of adjacent slopes, or iron in parent rock from which ground-water flows into lowland as a result of poor drainage (WARDA, 1988). Fe-toxicity can be traced to wide range of soils, such as; peat soils, valley-bottom soils and acid-clay soils receiving interflow water from adjacent slopes and acid sulfate soils (Becker and Asch 2005).

Plants are able to take up Fe in ferocious form, under anaerobic and acidic conditions (pH<5) of water logged soils. Rice is sensitive to Fe toxicity immediately after trans-planting to tillering and also during heading/flowering (Prade et al. 1990). Average yield loss of 30% has been recorded, although it varies depending on variety and intensity of toxicity (Abifarin 1989; Masajo et al. 1986; WARDA 1997).

Salinity

Salinity occurs due to poor irrigation practices and lack of efficient drainage system, which leads to an upsurge in water table and rise in soil salinity and sodium/alkalinity (Bertrand et al. 1993, Miezan and Dingkuhn 2001). Salinity poses stress to rice, which is most sensitive at seedling and reproductive stage (Gregrio et al. 2002). It is more severe in arid climates than humid, which implied environmental variance. 50% yield loss was recorded in humid tropics, at an electric conductivity (EC) of 9.5mS/cm (Flower and Yeo 1981), while at arid, the equivalent yield loss of 50% was recorded at EC of 3.5mS/cm (Dingkuhn et al. 1993). These conditions affect rice yield significantly and therefore, combining tolerant traits to salinity at both seedlings and reproductive stage is essential to develop salt tolerant variety.

Phosphorus deficiency

Weathered soils of humid have a major limitation in crop production due to (P) deficiency. Optimum soil P is generally low, with a total available P of only 2-4% for plants utilization, due to high phosphorus fixing potential of fine textured soils of sub-humid and humid zones (Abekoe and Sahrawat 2001). An estimated 5.7 billion ha globally is deficient of available plant phosphorus (Batjes 1997), while 50% of prospective arable land globally has acid soils, with Sub-Saharan Africa being most widely distributed. Fertilizer application is a corrective measure, and more so, when soluble and absorbable by plant.

Excess water

This could be categorized into two; flash flood of transient nature or submergence that keeps the crop under the water for a short period (≤ 2 weeks), and longer period of flooding, with stagnated water of different depths for up to few months. Depth levels are; partial/stagnant; 30-40cm, semi-deep; >100cm, deep-water; up to 3m, very deep; ≥4m (Mackill et al. 2012). Although rice adapt to water logged conditions, but a situation of complete submergence for a longer than necessary period could be of bad effect. This largely affect usual gas exchange and interception of light, because of flood duration, temperature, turbidity level, flood water turbulence, its depth, although varying in seasons and locations (Das et al. 2009).

Extreme temperature

Rice responds to temperature changes, although its response or sensitivity is dependent on the stage of development; stage of flowering, and 9 to 11 days before flowering are quite sensitive to extreme temperatures of hot or very cold weather, which results in high spikelet sterility (Andaya and Mackill 2003a, Manneh et al. 2007, Yoshida et al. 1981, Zenna et al. 2010). Rice responds to temperatures of less than 15^0C. This implies that crop failure is seen at the onset of low temperatures at different stages of growth like, germination, seedlings, vegetative and reproductive, and to maturity of grains (Andaya and Mackill 2003a). This level of failure depends on water temperature or ambient air, growth stage, air, cropping pattern and variety (Zenna et al. 2010).

Air temperature exceeding 35^0C causes heat injuries to exposed rice plant. 1^0C rise in minimum temperature during the dry season, causes grain yield decline by 10% (Peng et al. 2004), while an insignificant crop yield was recorded, when affected by maximum temperature. Increasing night temperature at a day temperature of 33^0C led to significant decline in grain-filling and gain yield, while at constant day temperature of 29^0C, rising night temperature had not significantly affected growth or yield.

Biotic stresses in rice

When pathogenic agents of rice feed on plant parts, it does that primarily to obtain its' nourishment for survival. Unfortunately, the impact due to feeding becomes deleterious to host plants. These disease agents have quite a wide range; Fungi, Bacteria, Viruses, Insects, false smut, sheath blight, etc.

Leaf blast

Blast is an important fungal pathogen (*Magnaporthe grisea* anamorph; *Pyricularia oryzae*) with deleterious multi-diversity of effect on host plant, whose effect usually leads to poor yield. The infection was noticed and first reported in Asia for more than 300 years ago, and presently wide spread in at least 85 countries of the world. It is able to adapt to several environmental and field conditions of irrigated, low-land rain-fed, upland or deep water rice field (Latif et al. 2011a, Ou 1985).

Rice blast pathogen causes significant yield losses in the whole of Southeast Asia, South America and all major rice producing areas of the world. Its occurrence and severity vary, either by its location, year or and even within field, depending on the condition of the environment and crop management practices (Latif et al. 2011).

The pathogen's long years of existence resulted to adaptability, with sudden changes that are prevalent in the virulence characteristics of the population. The diseases pathogen could not be permanently brought under control, being as a result of either controlled or partial resistance by a single dominant gene, which is mostly not stable to an onslaught by a genetically variable pathogen (McCough et al. 1995). The effect of blast pathogens in Malaysia has been quite alarming. In 2006, an outbreak in Kemubu Agricultural Development Authority Area in Pasit Puteh, Kelantan, Malaysia, was estimated to have destroyed 60% of 4000 ha of cultivated rice field (Rahim 2010). The pathogen's effect on rice crop is at all growth stages (Sharma and Bambawale 2008), and also, has the ability to attack more than fifty other species of grasses (Ou 1985). The world's estimated devastation of the disease resulted in yield losses ranged from 1-50% (Scardaci et al. 2003). Annual yield loss of 10-30% has also been recorded due to blast fungus *M. oryzae* (Skamnioti and Gurr 2009). Xiao et al. (2016) reported frequent and severe incidences of blast on rice in the tropical province of China due to high temperature and abundant rainfall during the growing season, resulting in damage of over 1500 ha of cultivated hybrid rice in 2008. Out of this, 250 ha was totally yield-less due to severe devastation of the panicles.

Magnaporthe grisea affect rice leaves (Fig.1B), the symptoms appear smallish brown or greenish dots. These dots develop to about 1.5cm long and 0.3-0.5cm wide, diamond-shaped lesions with a white or grey center after 2-3 days of infestation. It also affects the panicles (Fig.1D), collar and seeds (Fig.1A, C), and when the collar is unable to endure the weight of the panicle, it eventually leads to the fall of the panicle, as a result, translocation and development is hindered, causing immatured drying, while the effect on the seeds lead to non-viability of seeds as planting material and reduced quality for cooking.

Using durable resistance genes for breeding resistance variety are currently the major and economical methods being used to control the disease. It is also, environmentally safe (Hirano 1994).

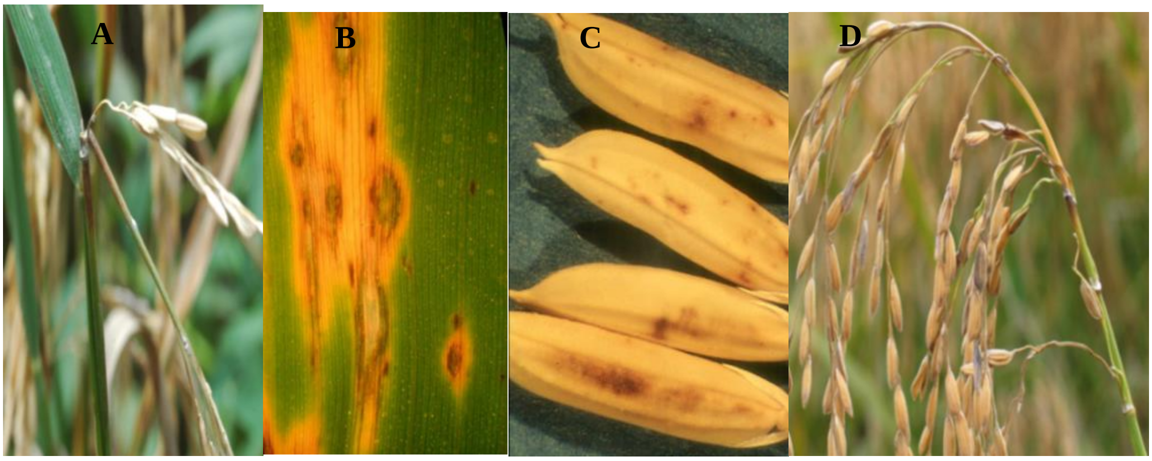


Figure 1: Rice blast symptoms on neck, leaf, seeds and panicle.
[Source: TeBeest et al. 2012]

A. Neck and node blast; the effect of this leads to fall of panicle, and poor seed set or non at all, depending on the development stage of rice in which infection occurs (5.5cm(H)×3.1(W)).
B. Leaf blast; formation of lesions on leaves, which increseases from dots to expanded patches, turning leaves from brownish to dark brown colour. This affects the surface area for photosynthesis (5.5cm (H)×3.0cm (W)).
C. Seeds blast; brownish spots and blotches are symptoms, and the effect is on the pedicels which renders the seed inviable (5.5 cm (H)×3.4 cm (W)).
D. Infected panicles; spikelet are formed on the panicles, this results in poor seed set due to poor translocation of nutrients to other parts of plant (5.5 cm (H) ×4.8 cm (W)).

Bacteria leaf blight

Bacteria leaf blight (BLB) (*Xanthomonas oryzae pv oryzae*) is also important, and one of the oldest known bacterial diseases of rice in Asia (Hassan-Naqvi et al. 2010), and also, one of the most serious bacterial diseases in many of the rice growing regions of the world (Xu et al. 2010). It was first observed in Japanese farms in 1884 (Tagami and Mizukami 1965). It is a rod–shaped bacterium, with leaf blight symptoms which occurs at all growth stages of rice (Fig. 2) plant, with yield losses ranging from 20-30%, and as high as 80% depending on the stage of growth where attack or infestation occurs, the geographic location or seasonal condition (Ou 1985; Singh et al. 1977). Zhai and Zhu (1999) reported 100% loss in severe incidence of BLB infection, it affects both inbred and hybrid rice (Mew 1987). Like blast, the bacteria races are controlled by artificial and natural selection of genes resistant to the BLB pathogens. It is quite necessary to identify the new resistant genes to control the changeful races (Xia et al. 2012), and also utilize pyramiding method, for wider spectrum of host resistance, since thirty five (Shang et al. 2009) BLB resistance genes have been identified, in both cultivated rice and their wild relatives (Nino-Lui et al. 2006; Singh et al. 2007; Wang et al. 2009).

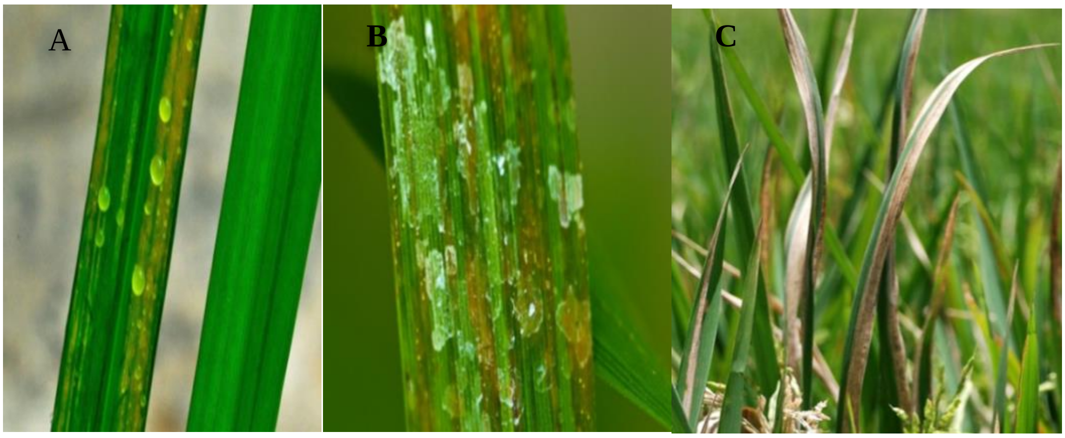


Figure 2: Leaves of rice infected with Bacterial leaf blight
[Source: http://www.knowledgebank.irri.org]

The diagram shows the stages of infection of rice leaves by bacteria leaf blight from early signs to dry leaves;

A. Oozes; these are early symptoms of infection. On young plants, milky-like dew drop are observed usually in the morning (5.5 cm (H)× 4.8 cm (W)).
B. Dry oozes; the oozes dry sticking on the leaves (5.5 cm (H)× 3.8cm (W)).
C. Dry leaves; the dry oozes eventually leads to drying of leaves (5.5 cm (H)× 3.0 cm (W)).

Virus-resistance

The historical view of the effect of viruses on rice spanned several decades, and the devastation impact cut across the entire world with significant yield losses in severe infection. Rice stripe virus (RSV) and rice dwarf virus (RDV) caused greater losses to yield of rice in the 1960s than other viruses (Toriyama 2010). The environmental and cost effect of the use of insecticide in controlling the vector insect, necessitated the need for the development of genetic resistant variety against rice viruses generally or their insect vectors.

Resistant genes against rice plant viruses of RDV (Sasaya et al. 2013; Shimizu et al. 2009) and RSV (Xiong et al. 2009), have been developed and evaluated accordingly.

Tungro disease of rice is a dangerous viral disease, which is caused when infected by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Green leafhopper (*Nephotettix virescens*) is the vector of RTSV, which helps to transmit the virus. Beside the use of marker-assisted selection, a transformation approach has led to the use of coat protein mediated resistance strategy. Transgenic rice plant having RTVS replicate gene produced by Huet et al. (1999) showed moderate resistance to RTSV.

Figure 3: Rice plants infected with tungro

Insect-resistance

Rice has been devastated by insects. Genetically modified rice with expressed gene for *Bacillus thuringiensis* (Bt rice) (High et al. 2004; Tu et al. 2000; Wang et al. 2014) produced resistance to insect attack. Transformed transgenic rice with a synthetic Cry1Ab gene was significantly tolerant to eight lepidopteran insect along with striped stem borer (SSB; *Chilo suppressalis*) and yellow stem borer (YSB; *Scipophaga incertulas*). Field evaluation of improved insect-resistant rice showed high tolerance to leaf folder (RLF; *Cnaphalocrocis medinalis*) and (YSB; *Scipophaga incertulas*) in China (Chen et al. 2011; Deka and Barthakur 2010; Tu et al. 2000), likewise in Pakistan (Mahmood-ur-Rahman et al. 2007), the Mediterranean region (Breitler et al. 2004) and in India (Ramesh et al. 2004). Other categories of insects that infest rice also includes these two species of planthopper; brown planthopper (BPH), *Nilaparvata lugens* (Stal) and whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath). It infests rice in rainfed and irrigated wetland environments. Continuously submerged field, high shade, and humid conditions form a good habitat for it, other thriving conditions are; excessive use of nitrogen, early season insecticide spraying encourages insects to develop, densely seeded crops and closed canopy of rice plant (www.knowlegdebankirri.org).

Figure 4: Rice insect

(a)Rice brown planthopper (b) Rice striped stem borer

False smut

False smut is a disease of rice caused by *Villosiclava virens* and has recorded effect of reducing grain yield and quality globally (Yang et al. 2012; Osada 1995). Increasing incidences of rice false smut has been reported, causing great concerns to rice production. The Asian *V. virens* strains produces mycotoxin which are poisonous to humans and animals (Zhou et al.

2012; Koiso et al. 1994; Nakamura, et al. 1994). *V. virens* is a fungus classified as ascomycete which produces asexual chlamydospores (*Ustilaginoidea virens*) and sexual ascospores (Tanaka et al. 2008). Both have the potential of germination and production of conidia (Wang 1988; Wang 1995). The *V. virens* has an optimum temperature range of 20^0C-30^0C upon which the spores germinate in potato-sugar-agar (PSA). It is quite slow in growth, taking 14 days to achieve about 37mm of colony diameter of mycelium growth (Fu et al. 2013).

Rice spikelets are infected by *V. virens* during booting (Fan et al. 2015; Tang et al. 2013), and the pattern of infection is in two stages: (a) Epiphytic stage. It is a stage where it spores in to an emerging panicle and plunge on the surface of the spikelets, and germinates with the hyphae stretching inside the space of spikelet through the tiny openings in between the lemma and palea (Ashizawa et al. 2012; Song et al. 2016). This phase does not show sites of any infection, probably as a result of epiphytic growth (Fan et al. 2015; Fan et al. 2014; Tanaka et al. 2016). (b) Biotrophic stage. Haphae reaches the inner floral organs at this phase, it primarily infect stamen filaments, stigmata and styles, with occasional infection on the lodicules and ovaries (Tang et al. 2013; Song et al. 2016; Yong et al. 2016. The fungal mycelia of the *V. virens* attaches to the floral organs of rice and extract so much nourishment to develop the false smut ball, maybe by stimulating a system of grain filling intercepting nutrient store (Fan et al. 2015; Song et al. 2016). The false smut disease produces visible false smut ball which is often larger in size to a matured grain of rice, probably so much nutrient required. It could also be formed by utilizing the rice flowers because it has plenty of sugar; glucose, sucrose, oligosaccharides, etc.

Figure 5: False smut infected rice: Spore balls turn greenish black when mature from initially orange colour

Sheath blight

Sheath blight of rice (ShB) is caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk), it is destructive and causes significant yield losses and affects quality (Lee and Rush 1983; Nagarajkumar et al. 2004). It also has the potential of affecting plants like lettuce, tomatoes, maize, barley and sorghum (Zhang et al. 2009). Yield losses ranging from 10-30% (Xie et al. 2008) to even 50% in high incidences has been recorded (Meng et al. 2001). *R. solani* is a global soil saprophytic and plant parasite that manifest when triggered (Anees et al. 2010; Sumner 1996). The pathogen survives in unfavorable condition by forming dormant mycelia or Sclerotia because of restricted movement due to absence of spores (Anees at al. 2010; Summer 1996). Sclerotia have the potential of surviving in soil for up to two years, and could be dispersed at the time of field preparation and on flood irrigation of fields (Webster and Gunnell 1992; Brooks 2007). The Sclerotia could move on continuously flooded irrigation to adjoining fields and attach to available plants, thereby causing ShB disease. And favorable conditions enhance the pathogen’s spread. Early symptoms are found on leaf sheath close to water-line as water-soaked lesions. Advance infection occurs when hyphae grow upwards to parts of plant not earlier infected, thereby developing more lesions and sclerotia on leaf sheath for the cycle to be completed (Webster and Gunnell 1992; Brooks 2007). The flowering stage favours disease development when canopy of rice is dense, creating a micro-climate conducive for pathogen growth and spread (Brooks 2007). The pathogen also infects seed to matured plant, resulting in average to high losses of yield, depending on part of plant infected. Observable symptoms are lesions formation, plant lodging, and lack of grain formation. Extended lesions on lower leaves of rice infected may lead to softened stem, resulting in lodging (Wu et al. 2012). This lodging temper with the normal design of rice canopy, it affects the potential for photosynthesis and overall production of biomass (Wu et al. 2012; Hitaka 1969). The effect on flowering or during panicle initiation leads to loss in total seed weight as a result of low percentage of filled spikelets, which eventually leads to significant yield losses (Nagarajkumar et al. 2004). Severe lodging may result during rice sheath blight epidemic thereby obstructing the transport of nutrients, water, and carbohydrates assimilate via the phloem channels, affecting grain filling (Wu et al. 2012). Other factors that promotes ShB are double cropping, application of high rate of nitrogenous (N) fertilizer, dwarf build, early maturing, high plant density, high tillering, morphological traits of rice plant cultivars due to the height of the plant, because it affects micro-climate and transmission of light due to canopy, supporting increase in disease (Han et al. 2003; Tang et al. 2007) and susceptible variety has been revealed to support ShB severity in many world rice growing environment (Lee and Rush, 1983; Nagarajkumar et al. 2004).

Figure 6: Sheath blight infected rice

Interaction of stress conditions

The simultaneous occurrence of abiotic and biotic stresses is quite complex, hence their response to either stress conditions are mostly controlled by different hormone, whose pathway could interact and inhibit each other (Anderson et al. 2004; Asselbergh et al. 2008b). Most times, when plants are exposed to pest or pathogens, drought stress effect increases (Audebert et al. 2000; EnglishLoeb et al. 1997; Khan and Khan 1996; Smit and Vamerali 1998). Increased abiotic stress is able to reduce the defenses of plants, and thereby strengthen susceptibility to disease (Amtmann et al. 2008; Goel et al. 2008; Mittler and Blumwald 2010).

When stress factors occur simultaneously together, it may be regarded to be additive or interactive (Niinemets 2010). A stress factor can be said to interact, when a stress factor alters or affect the normal response of the other as a result of acclimation response. The effect of interaction between two abiotic stresses on crop was studied, and Mittler and Blumwald (2010) reviewed it, and the stresses were observed to be deleterious. The combination of drought stress and heat in particular, is capable of causing high damage to crops in comparison with each individual stress (Barnabas et al. 2008; Keles and Oncel 2002; Mittler 2006, Rizhsky et al. 2002). Consequently, observations on the simultaneous effect of a biotic impact of herbivore or pathogen and an abiotic stress, were positive and or negative interactions based on the nature, time period and extend of damage of the different stress. Rise in temperature is capable of creating a negative interaction effect, by reducing the strength of resistance to pathogens of viruses, fungi, nematodes and bacteria. Six years experiments on wheat, with higher mean temperature were correlated under the period with increased susceptibility to *Cochliobolus sativus* fungus (Sharma et al. 2007).

Plant resistance to pathogen can be affected by drought stress. Date palms and *Parthenocissus quinquefolia* vine suffers wide spread infestation of bacterial and fungal leaf scorch symptoms, as a result of water deficit (McElrone et al. 2001; Suleman et al. 2001).

In contrast, positive interaction occurs between abiotic and pathogens stress. Many types of bacteria and arbuscular mycorrhizal fungi, have been identified to support stress tolerance in some crop species via production of suppressing ethylene, antioxidants, stabilizing soil structure, improving abscisic acid (ABA) regulation and increasing osmolyte production amongst others (Aroca et al. 2008; Grover et al. 2011; Kohler et al. 2008; Saravanakumar and Samiyappan, 2007).

Infection of soil-borne plant parasitic nematodes worsens or prevents the effect of abiotic stress on plants. Plant water relations are severely disrupted due to their parasitism in roots (Haverkort et al. 1991; Smit and Vamerali 1998). The presence of nematodes ameliorates the intense effect of water deficit. The nematode *Heterodera sacchari* caused increased water deficit related losses in upland variety of rice in Ivory Coast, by supporting the reduction of leaf water potential, leaf and dry weight and stomatal conductance (Audebert et al. 2000).

A good knowledge of the diversity of plant, helps in assessing the behavior of plant and its' adaptation to areas prone to drought (Alonso-Blanco et al. 2009). And also, the knowledge of plant survival, under stress is important in developing an effective strategy for phenotyping (Sarkar et al. 2013), and even in successfully breeding drought tolerance variety. An assessment of genotypic variability of rice under different water deficit conditions is also necessary (Abenavoli et al. 2016; Anower et al. 2017).

Gene pyramiding

This is a breeding method, for the assemblage of gene of desired traits, from different sources into a single elite cultivar. This in turn exhibits the different traits as a single multiple gene variety. It is a host resistant/tolerant variety, with wider broad-spectrum resistance and high yielding potential (Pradhan et al. 2015). Three methods have been identified; pedigree, recurrent selection and backcross methods (Ruengphayak et al. 2015).

Gene pyramiding has been applied to enhance resistance to biotic and abiotic stresses, by selecting for two or more genes at a time. In rice, such approach (pyramiding) has been used to develop a single variety resistance against blast and bacteria blight (Huang et al. 1997, Singh 2001). The use of markers allows selecting for QTL-allele-linked markers that have the same phenotypic effect. For the enhancement and improvement of a quantitatively inherited trait in plant breeding, pyramiding of multiple genes or QTLs is recommended as a potential strategy (Richardson et al. 2006). An appropriate breeding scheme for marker-assisted gene pyramiding (MAGP) depends on the number of genes/QTLs, the heritability of traits of interest, and other factors (Richardson et al. 2006).

Xu et al. (2012) stated that to improve elite variety of rice, pyramiding approach was used to introgress different strains of desired characters of plants, which were successful in exhibiting wider broad spectrum resistance against the invasion of disease agents or tolerance of abiotic conditions. He reported that the introgression of bacteria blight resistance genes, *Xa*7 and *Xa*21 conferred on the variety the ability to withstand all the seven Xoo strains. Blast resistance genes P*i*2 and P*i*54 and, bacterial blight resistance genes *xa*13 and *Xa*21, were introgressed into Pusa Basmatic 6 and were found to be superior hybrids to parents in terms of yield, early maturing and better quality of parameters. The improved varieties conferred resistance to *Magnaporthe oryzae* in South, East and North-West of India (Ellur et al. 2016). Pyramiding backcross method was carried out by introgressing P*i*1; P*iz*-5 and p*i*ta genes to improve resistivity of blast *M. grisea* to leaf blast attack, which were successful and more resistant on trial evaluation carried out in the Philippines and India (Hitalmani et al. 2000). Huang et al. (1997) reported pyramid of four resistant genes of bacteria blight *Xa*-4, *xa*-5, *xa*-13 and *Xa*21 and were listed for resistance against *Xanthomonas oryzae* pv. *oryzae* and found to have higher level of resistance with wide broad spectrum. Three resistance genes *xa*5+*xa*13+*Xa*21 were introgressed, using backcross method into susceptible deep water rice variety popular with Indian farmers, referred to as Jalmagna, from Swarna bacteria blight pyramid line, which produced better yield and characters (Pradhan et al. 2015).

Other multi-traits varieties are; LAC 23, Xiangzi 3150, Gumei 2 (carrying minimum of two R genes each) (Huang et al. 2011; Mackill and Bonman 1992; Wu et al. 2005), Digu, IR64 and Sanhuangzhan 2 (carrying minimum of three R genes each) (Chen et al. 2004, Liu et al. 2004; Sallaud et al. 2003, Shang et al. 2009), Tetep and Moroberekan (carrying a minimum of four R genes each) (Barman et al. 2004; Chen et al. 1999). P*i*ta and P*i*46 were transferred into an elite restorer line HH179 through marker-assisted backcross breeding (MABB). Three categories of improved lines were obtained (i) only harboring P*i*ta, (ii) only harboring P*i*46 and (iii) harboring both P*i*ta and P*i*46.

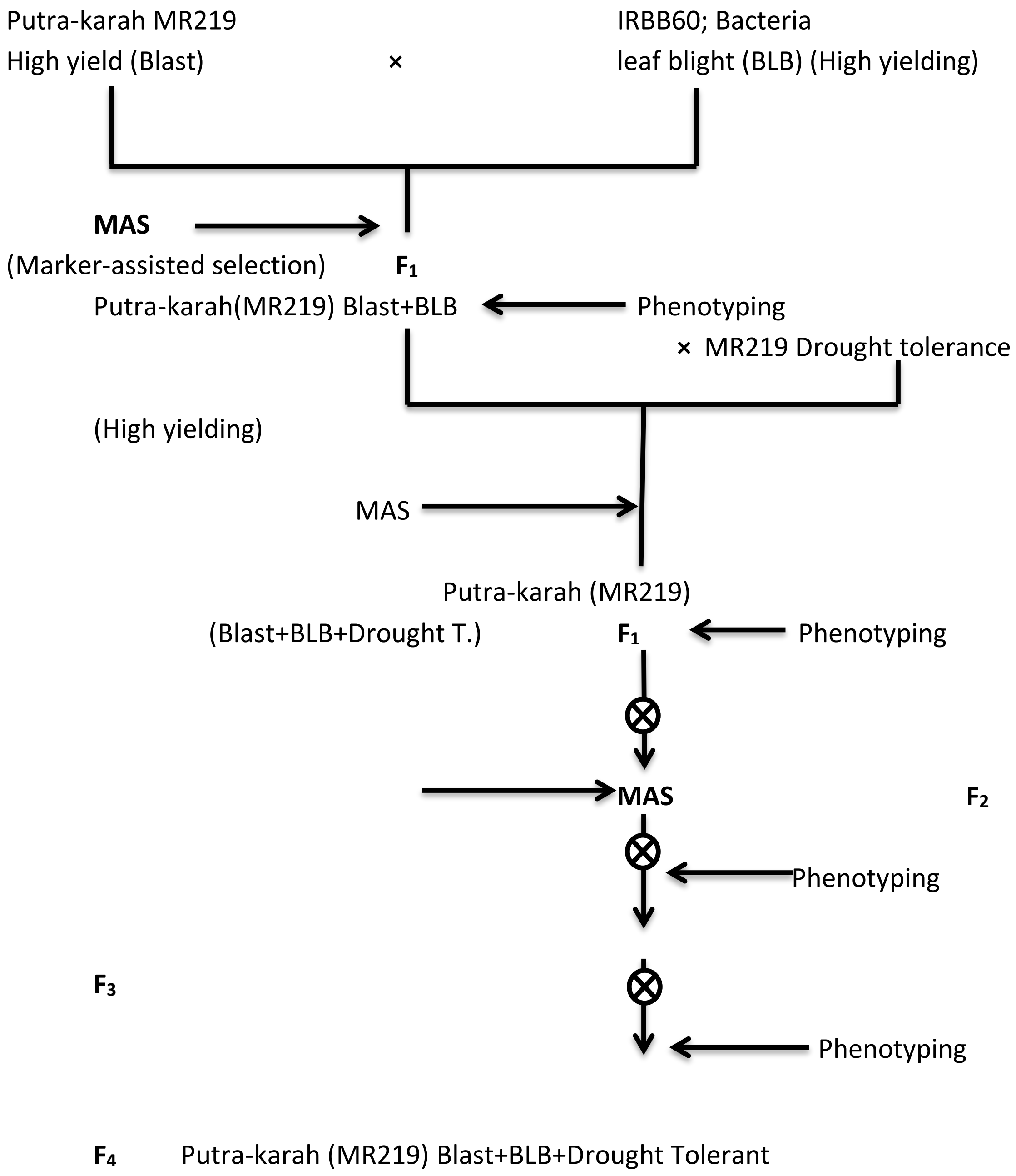
This pyramiding approach strategy should be consistently employed for durable resistance (Jia et al. 2003; Tabien et al. 2002), because the emergence of virulent races has overcome the resistivity of varieties carrying single gene (Koide et al. 2010). The concurrent presence of introgressed multiple major resistant genes often confers lasting broad spectrum resistance (Xiao et al. 2015), and confirmable genotypically through marker-assisted selections. Conventional gene pyramiding takes longer time, and resources to develop and technical expertise in transfer of pollen grains from anthers to stigmas. Length of time or generations is for evaluation trial, to ascertain the success of gene introgression through continued phenotyping, until non-segregating and multiple gene variety exhibiting all the introgressed genes are present in a single line (Miah et al. 2013).

Pedigree method

It is a breeding method that is associated with self-pollinating plants, which mostly have trait for high yield, but lacking in biotic or abiotic traits. It begins with the crossing of two genotypes, with each having desirable traits not present in the other. If the two original parents are not able to provide all desired characters required, a third parent would be introgressed into the hybrid progeny of the first generation (F_1) (Fig. 3). In successive generations, the superior types are selected for and a record is kept of parent to progeny relation (Ruengphayak et al. 2015).

Crossing the progeny of the F_1 individuals which produces F_2 generation, usually offers the opportunity for first selection in pedigree programs. The emphasis here (F_2 generation) is, selection of individuals that carry undesirable major genes for elimination. In successive generations of hybrid, selection leading to natural selection as self-pollinated, pure breeding lines is produced. The families of the hybrid F_2 plant express unique characters. Usually, fewer superior varieties of plant are selected in each of the unique superior family of the hybrid generations. Progressively, at F_5 generation, homozygosity would have been attained and the emphasis shift almost completely to select within families. The pedigree record helps greatly in removal or eliminations of non-superior families with undesirable traits. Now, at this point, any family selected is harvested in mass, so as to obtain larger quantity of seeds required in evaluating families for quantitative traits. Most preferable evaluation, which should encourage or stimulate planting activities for commercial purposes, should be grown in plots within close range. At either F_7 or F_8 generation, usually it is required that visual selection is needed, to reduce the number of families to manageable proportion and for accurate evaluation, for yield performance level and quality (Jiang 2013).

To conclude evaluation of good quality strains, it requires; observation study on multi-locational and time period (years), precise or definite yield testing and, quality testing in multiple locations and over a period of time after which new variety is release for commercial cultivation or production.



⊗ Symbol of selfing or self-pollination

F_1, F_2, F_3, F_4 Filial generations

Figure 7: Pedigree, three-way cross pyramid chart of biotic and abiotic stresses

Figure above shows, a schematic pedigree breeding chart of crosses that leads to development of a single multiline blast and bacteria leaf blight resistance and drought tolerance rice variety, with confirmatory tests through genotyping and phenotyping

Recurrent selection

The selection of certain traits generation after generation in self-pollinated crop plants, the inter-breeding of reselected plants allows the breeder access favourable recombination, as well as stabilized traits within the gene pool. Ideotype in each interbreeding line (IBL) should be selected, but total reliant on the phenotype should be done with aught most caution because, it is not always an indication of actual genotype. Yield and quality trial with test crosses and selection of best ten lines should be done, intercross and repeat (Vales 2010).

After the completion of recurrent selection, new selection of individuals to be the new parents of interbreeding lines (IBL). These are the recurrent selection which has been done in two separate programs. Hybrid (F_1) single cross of the progeny is used as visual indicators or morphological markers of the combining ability that lies in the saved seeds. These specific inbred parental lines are kept in reserve, until the progeny testing of the different (A×B) hybrids have shown which has better selection for combining ability, and will make better hybrid. This strategy is complicated, therefore, good note taking and organization is of immense importance, it includes the following types for cross-pollinated crops; Simple recurrent selection (SRS), Recurrent selection for general combining ability (RSGCA), Recurrent selection for specific combining ability (RSSCA), Reciprocal recurrent selection (RRS) (Singh, 2012, Allard, 2010).

Backcross method

Most times a peculiar variety of plant is improved, by introgressing to it some specific desirable traits that it lacks. The carrier of the trait is required, and then mating the progeny back to a plant having the genotype of the superior parent, a process referred to as backcrossing. After a number of backcrosses, say five or six, in case of conventional approach, the progeny will be hybrid of the character being introgressed (Fig.4), but like the superior parent for all other gene traits. When the last backcross generation is selfed alongside selection, it will give rise to some progeny pure breeding for the genes being introgressed. Backcross methods have predictable outcomes, rapidity and require small number of plants. Conversely, the procedure does not provide opportunity for the occurrence of chance combinations of genes, which could lead to striking improvements in performance (Vales, 2010). Marker assisted backcross has shown that less time was required to recover genes. Suh et al (2013) stated that at least three backcrosses are needed to recover the phenotype of the recurrent parent. While Miah etal (2016) recovered at BC_2F_2 92.7% and 97.7% of lowest and highest recurrent genome recovery respectively, broad spectrum of blast resistance genes into cultivated MR219 rice variety.

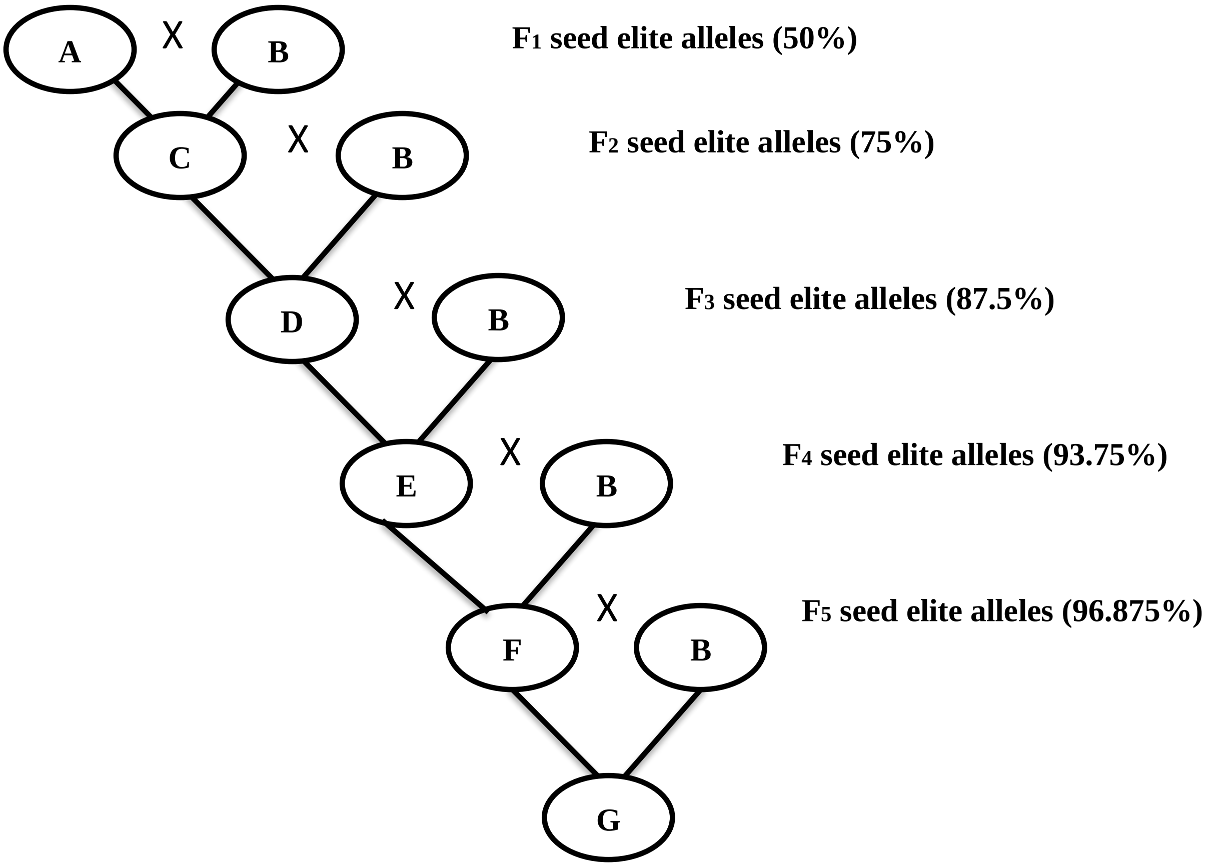


Figure 8: Backcross method of breeding

This is a schematic diagram of a backcross, which is basically recovery of gene of interest from a recurrent parent into a recipient parent lacking in a specific desired trait. The progeny of each successive generation is crossed back to a recipient parent, generation after generation until the trait is fully recovered.

Letters: “**B**” donor parent carrying trait of interest

“**A**”, a desired parent lacking in trait present in B parent

“ ‘**C**’, ‘**D**’, ‘**E**’, ‘**F**’, ‘**G**’ ” are transgenic elite lines in progression.

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Table 1. Some selected SSR (microsatellite) polymorphic and tightly linked markers for blast, bacteria leaf blight resistance and drought tolerant rice

Variety	Genes	Primer sequences (5′ –3′)		Chr. position	Exp. size	References
SSR linked marker		Forward primer	Reverse primer			
Putra 1(Blast resistance)						
RM6836	Piz, Pi2,Pi9	TGTTGCATATGGTGCTATTTGA	GATACGGCTTCTAGGCCAAA	6	240	Askani et al.,2011
M8225	Piz	ATGCGTGTTCAGAAATTAGG	TGTTGTATACCTCATCGACAG	6	221	Miah, et al., 2016
MR219 Drought tolerance						
RM511	qDTY$_{12.1}$	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC	12	130	Shamsudin et al., 2016, www.gramene.com
RM520	qDTY$_{3.1}$	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	3	247	
RM236	qDTY$_{2.2}$	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC	2	174	
RM276	qDTY$_{2.2,3.1}$	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	6	149	
RM1261	qDTY$_{12.1}$	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	12	167	
IRBB60 (Bact. leaf blight)						
RM224	Xa-4	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	11	157	He et al., 2006
RM122	xa-5	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC	5	227	Khan et al., 2015
RM13	xa-5	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	5	141	
RG136	xa-13	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	8	246	Pradhan et al., 2015
Xa13Prom	xa13	GCCATGGCTCAGTGTTTAT	GAGCTCCAGCTCTCCAAATG	8		
RM21	Xa-21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	11	157	
pTA248	Xa-21	AGACGCGGAAGGGTGGTTCCCGGA	AGACGCGGTAATCGAAGATGAAA	11		Chen et al., 1997

(Askani et al. 2011, Miah et al. 2016, Shamsudin et al. 2016, He et al.2006, Khan et al. 2015, Pradhan et al. 2016, www,gramene.com)

Molecular markers in plant breeding

These are biological features determined by the forms of genetic loci or alleles, and are transmittable through generations. They serve the purpose of experimental tags, to keep record of cell, chromosome or a gene, individual tissue and nucleus. This is classified into: classical markers; morphological, cytological and biochemical markers (Collard et al. 2005) and DNA markers (Xu et al. 2010).

Morphological markers

These are simply tools that assist in selection of desired traits carrying plants, which is used right from ancient times in plant breeding. These markers are visible characters of plants such as shape of leaf, colours of flower, pubescence, pod, seed, hilium, flesh, seed shape, fruit shape, awn length and type, exocarp (rind) stripe and colour, length of stem. Generally, these stands for genetic polymorphisms that are easily identified and altered or manipulated, and mostly used to build up of linkage maps by 2 or 3 points classical test. Agronomic traits are linked with these markers which are in use, as criteria in practical breeding for indirect selection.

During green revolution, the choice of semi-dwarf variety of rice was one important selection, which led to the much success of high-yielding cultivars (Liu 1991). These morphological markers have limitations, which are in some situations not linked to traits of economic importance (e.g., yield and quality), which could lead to ineffective and undesirable crop plant growth and development.

Cytological markers

Also known as cell chromosome karyotype and bond, which are invariably the structural features of chromosome. These patterns of banding display of colour, position and width order, divulge the different euchromatin and heterchromatin distributions. For example Q and G bands are products of quinacrine hydrochloride and giemsa respectively, and the reverse G bands are R bands. These landmarks of chromosomes are useful in normal chromosomes characterization, and detection of chromosomal aberration or mutation, as well as in physical mapping and linkage group identification. Using cytological markers directly restrict genetic mapping and breeding, as such, cytological along with physical maps of morphological markers, lay a foundation for gene linked map through molecular techniques.

Biochemical markers

It refers to as protein markers and could be categorized into molecular markers or DNA markers. A substitute structural variant of protein or enzyme, with variation in weight of molecules and electrophoretic mobility, but having same function (catalysis) is also referred to as isozymes (Xu et al. 2010).

DNA markers

They are DNA fragment showing mutation that can be used in detection of polymorphism between genes (alleles) or different genotypes, for certain sequence of DNA in a gene pool or population. In order words, it is little portion of DNA sequence that reveals polymorphism with different individuals (Xu et al. 2010).

In the detection of polymorphism, two important methods in use includes; southern blotting, also referred to as nuclear acid hybridization technique (Southern 1975), and polymerized chain reaction (PCR) technique. Some commonly used DNA markers for plant breeding include; microsatellite or simple sequence repeat (SSR), restrictive fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD), single nucleotide polymorphism (SNP) and amplified fragment length polymorphism (AFLP) (Farooq and Azam 2002, Semagn et al. 2006) (Table 2).

Table 2. Composition of DNA marker system most widely used in plants

Feature and Description	SSR	SNP	AFLP	RAPD	RELP
Types of primers	Sequence specific	Allelic specific PCR primers	Bp Specific sequence	10 random nucleotides	Low copy DNA cDNA clones
Genome coverage	Complete genomes	Complete genomes	Complete genomes	Complete genomes	Low copy coding region
Level of ease of automation	High	High	Moderate-high	Moderate	Low
Loci number	High(1,000s)	Very high	Moderate(1000s)	Small(<1,000)	Small(<1,000)
Ratio of effective complex	High	Moderate-high	High	Moderate	Low
Polymorphism level	High	High	High	High	Moderate
Primary applicability	All purposes	Multipurpose	Diversity and Genetics	Diversity	Genetics
Sequencing and/or cloning	Yes	Yes	None	None	Yes
Amenable to automation	High	High	Moderate	Moderate	Low
Radio-active detection	Usually none	None	Yes or None	None	Yes, usually
Reliability/reproducibility	High	High	High	Low	High
Cost/analysis	Low	Low	Moderate	Low	High
Polymorphism type	Changes in single length of repeat	Single base changes indels	Single base changes indels	Single base changes indels	Single base changes indels
Genotyping through-put	High	High	High	Low	Low
Amount of DNA required	Small(0.05-0.12ug)	Small(≥0.05 ug)	Moderate(0.5-1.0ug)	Small(0.01-0.1ug)	Large(5-50ug)
Quality of DNA required	Moderate-High	High	High	Moderate	High
Technical demand	Low	High	Moderate	Low	Moderate

Time demand/required	Low	Low	Moderate	Low	Moderate
Level of ease of use	Easy	Easy	Moderate	Easy	Not easy
Required DNA(g)	0.05	0.05	0.5-1.0	0.02	10
Inheritance/expression	Codominant	Codominant	Dominant and Codominant	Dominant	Codominant
Start-up cost/Development	High	High	Moderate	Low	Moderate-High
Polymorphic loci per analysis(Number)	1.0-3.0	1	20-100	1.5-5.0	1.0-3.0
Marker index	High	Moderate	Moderate-High	Moderate	Low
PCR-Based	Yes	Yes	Yes	Yes	Usually not
Genome abundance	Moderate-High	Very high	High	High	High

Source: modified (Collard et al. 2010; Korzum 2003; Semagn et al. 2006; Xu et al. 2010)

Conclusion

The economic value of rice as a source of income and staple food crop is of great importance because, increasing human population which invariably requires increase production to meet the projected global ratio, places a demand (Khush 1999) irrespective of devastation by various biotic and abiotic stresses, which could singly or simultaneously affect rice production. This therefore, justifies the review effort towards development of a single multipline resistance and tolerance rice variety through one of the approaches termed pyramiding, and via marker-assisted selection as a check for sustainable yield (Pradhan et al. 2015), as well as, for ease of selection of rice carrying the whole genes introgressed respectively. This approach is proven to confer broad spectrum of resistance and tolerance on crop plant, which is safer compared to chemical sprays which possess a great threat to environment and other important insects and microorganism.

Development of multiline host disease resistance and or non-disease stress variety of rice is always an effort to create a broad spectrum of resistance/tolerance against concurrent attack by stresses of abiotic and biotic, depending on the genes present in a particular plant, and also against the failure of single gene of stress condition, with capacity of susceptibility because of the pathogens evolving nature. This way, yield value is sustained, without cause to environmental pollution via chemical sprays which destroys and contaminates relevant fauna and flora, and aquatic environment.

The interaction of abiotic and biotic resistance and or tolerance genes could hamper or strengthen expression at the site of attack. It therefore implies that, lack of expression of a certain gene may not necessarily be the absence of that gene in the plant, so long as marker-assisted selection was employed in determining the presence of the genes. It simply means a stress condition suppresses the expression. For instance, increased abiotic stress is a potential reducer of the defenses of plants, and thereby strengthens susceptibility to disease (Amtmann et al. 2008; Goel et al. 2008; Mittler and Blumwald 2010).

Marker-assisted selection is a tool that is able to distinctly reveal more than two genes present in a particular plant. This saves times and creates precision in selection of rice lines of choice, either having abiotic, biotic stress resistance/tolerance or both, so long the linked primers are accurately determined.

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