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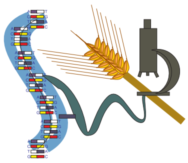

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Review Article

Prospects of climate change effects on crop diseases with particular reference to wheat

Pramod Prasad, Subhash Chander Bhardwaj*, Rajni Kant Thakur, Sneha Adhikari, Om Prakash Gangwar, Charu Lata and Subodh Kumar

ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla, Himachal Pradesh, 171002, India

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***Corresponding author:**
E-mail: scbfdl@hotmail.com

Abstract

Global climate change has considerably threatened wheat production. Rising global temperature is likely to affect wheat productivity directly or indirectly by shifting the dynamics of various abiotic and biotic factors. Shifting diseases and virulence patterns of plant pathogens is assumed to be a significant event for meeting the global food demand in the future, which in turn is expected to make future modifications in disease resistance breeding. Increasing population, industrialization, burning of fossil fuels, and other human activities are going to cause climatic variations. There would be an increased carbon dioxide (CO_2)/ greenhouse gas emissions, temperature, erratic rainfall, and other issues which will have a direct impact on crop production as well as disease and pest situations. In general, the incidence of damping-off, powdery mildew, stem rust, leaf rust, Karnal bunt, Fusarium head blight, and blast on wheat will more likely increase. Stripe rust incidence may decrease on wheat. However, isolates of *Puccinia striiformis tritici* that are adapted to relatively higher temperatures have been observed since 2000 in many countries. From plant disease management perspective, a precise understanding of a particular disease at field level is required, so that the probable effects of different abiotic and biotic factors under climate change situations could be assessed and estimated comprehensibly. Experts working in different areas of agriculture would have to work through a system approach and prioritize the effects of climate change in a broader context, comprising the entire agro-ecosystem. The current article presents the current status of climate changes in relation to the changing wheat disease spectrum and their management strategies.

Keywords: Global warming, wheat, diseases, disease prediction and management

1. Introduction

Climate change is described as the changes in the usual climate with respect to abiotic (temperature, precipitation, wind, and others) and biotic elements that result from various human activities including burning of fossil fuel, deforestation, industrialization, exploitation of natural resources, and others. Such activities increased with the expansion of the industrial revolution after the eighteenth century. The ambient concentration of greenhouse gases (GHGs) including carbon dioxide (CO_2) has increased significantly for more than 650 thousand years (Mohammed *et al*., 2021; Siegenthaler *et al*., 2005). Since the year 2000, the concentration of CO_2 is increasing at a much higher rate than in previous decades (Canadell *et al*., 2007). Same is the case with other GHGs like methane

(CH_4), nitrous oxide (N_2O), ozone (O_3), and others (Song *et al.*, 2014; Spahni *et al.*, 2005). The ambient global temperature on earth was also reported to increase at the rate of 0.2°C per decade during the last few decades (Smith *et al.*, 2015; Hansen *et al.*, 2006), while the mean annual global temperature has increased by 1.0^{0}C since 1881 (IPCC 2019). Changes in the water cycle in the form of erratic rainfall have also been observed. Fluctuations in climate are supposed to occur despite stabilization in GHGs concentrations, due to the thermal inertia of the system and also due to the necessity of an extended time period for the system to achieve a lower equilibrium.

World agriculture is projected to face a significant decline as a consequence of climate change unless and until a substantial reduction in the emissions of GHGs is achieved. As a consequence of global warming, the global agricultural productivity is estimated to drop down from the levels that were otherwise expected to increase by about 3 to 16 percent by 2080s (Cline 2007). Plant diseases, responsible for causing a minimum loss of 10% of global food production, are considered as a major limiting factor in achieving global food security (Strange and Scott, 2005). The role of the different biotic and abiotic environmental factors in the development of a specific plant disease is an acknowledged well-known fact for over a thousand years. These factors can also affect host (growth and resistance), pathogen (reproduction, dispersal, survival and pathogenicity), and their interaction. The dependency of plant diseases on several environmental factors advocates that climate change will force alterations in the current phyto-sanitary setup. Climate change may have positive, neutral, or negative effects on disease development in a specific host plant or region (Ghini *et al.*, 2008). Therefore, the understanding of such effects becomes crucial for implementing improved disease management strategies including disease resistance breeding in plants and thereby avoiding more yield losses (Ghini *et al.*, 2008).

Global warming is one of the serious threats to wheat production mainly in the areas which are vulnerable to soaring air temperature together with reduced rainfall (Wang *et al.*, 2018). Changing climate is expected to influence several abiotic and biotic stresses on wheat. Among the biotic stresses, wheat diseases including rusts, blast, spot blotch, and powdery mildew are the most important limiting factors in achieving the projected target of wheat production in future (Prasad *et al.*, 2020a), and are assumed to be influenced by changing climate variables (Pandey *et al.,* 2019). The occurrence and distribution of these diseases might witness a drastic shift in future due to the direct effect of climate change or indirectly through climate change influence of different abiotic and biotic factors. A simulation study, conducted to predict the effect of climate change on wheat productivity in north-western India, revealed that the rising temperature together with water scarcity will have highly adverse effects on wheat quality, production, and productivity in future and that too under the positive effect of other factors including elevated CO_2 concentration (Kumar *et al.,* 2021; Tripathy *et al.,* 2020; Zaveri and Lobell 2019). In contrast, the increasing temperature would favor wheat production in the regions, where wheat production is not feasible as of now due to a prevailing lower temperature than required for wheat production (Tao *et al.,* 2014). Thus, the estimated climate change effects may be reduced through the sharing of gene pools amongst wheat breeding programs in such situations (Shew *et al.,* 2020). However, temperature rise will also result in early terminal heat, early maturity of wheat that will ultimately cause a yield penalty (Pandey *et al.,* 2019; Singh *et al.,* 2019).

Predicting the probable impact of climate change on global wheat production is extremely complex and challenging due to poor understanding of the interactions among various abiotic factors including temperature, precipitation, ambient concentration of different atmospheric gases such as CO_2, O_3, and others (Asseng *et al.,* 2015). Overall, the future wheat yield forecasts primarily will rely on the use of different wheat yield simulation models, climate change prediction models, emission scenarios, etc. (Ceglar and Kajfez-Bogataj, 2012).

Climate change and crop pests

Besides influencing the crops, the climatic factors also affect their associated pests. The growth, survival, distribution, and multiplication of crop pests are significantly determined by environmental factors. Similarly, pest management strategies including chemical management are also affected by different climatic situations together with the crop type and extent of pest damage or loss. Moreover, the amount, frequency, and timing of rainfall is another important factor that has a direct association with pesticide efficacy, tenacity, and transport. A number of investigations have speculated that the crop pests would become more active and could cause more damage than now under climate change situations, and therefore, could pose a serious threat of monetary loss to growers and global food insecurity (Shew *et al.,* 2020; Coakley *et al.*, 1999).

2. Climate change vis-a-vis crop diseases

Although, substantial success has been accomplished in the management of plant diseases with technological and scientific advancement, yet plant diseases are still posing significant challenges to global crop production. Plant diseases might further impact the range of cultivated crops/cultivars in a specific region based on their adaptability to the changing environmental factors. Some evidences strongly suggest that changing environmental factors such as precipitation, temperature, composition of atmospheric gases etc., would lead to a complex interface among scientific, social, technological, and economic events for plant diseases (Jeger *et al.,* 2021). It is hypothesized that changes in environmental factors possibly will have an insignificant influence on the occurrence of diseases unlike their effect on crop management practices and genetic improvements in wheat (Asseng *et al.,* 2013), potato (Fleisher *et al.,* 2017) rice (Li *et al.,* 2015) and maize (Bassu *et al.,* 2014). The growth, multiplication, pathogenesis, spread, and survival (overwintering or oversummering) of plant pathogens are influenced by several environmental factors comprising temperature, relative humidity, rainfall, photoperiod, wind direction and speed, and other extreme events (Fig 1). Of these, relative humidity, ambient temperature, and precipitation have maximum impact on the outcome of a specific host-pathogen interaction, spread, and survival of pathogens. For instance, moist soil encourages the germination, growth, development, and infectious nature of fungal and bacterial propagules and affects the movement and growth stages of plant pathogenic nematodes (Prank *et al.,* 2019). Conversely, some pathogens thrive poorly under deprived aridity; and some pathogens like *Blumeria graminis tritici* causing powdery mildew in wheat grow well in warm and dry conditions provided the availability of sufficient dew during the night (Te Beest *et al.,* 2008). Predicting the probable effects of climate change on the host, pathogen, their interaction, population dynamics, community structure in agro-ecosystem, and micro-evolutionary developments, etc. is a prerequisite to envisage the effects of changing climate on specific crop disease.

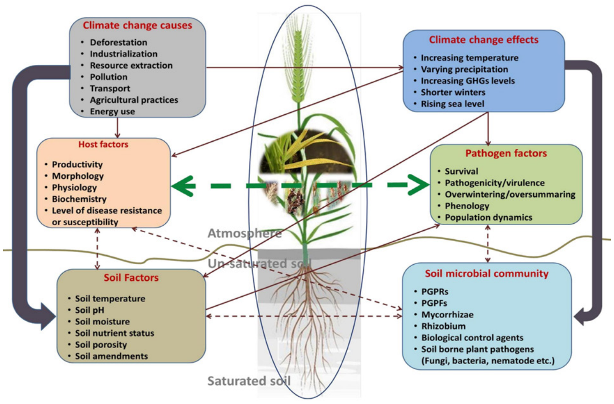

Figure 1. Potential direct and indirect effects of climate change on wheat, its pathogens and their interactions.

The estimation of future losses due to plant diseases under climate change situation would be possible only after analyzing a large number of inter-related abiotic and biotic factors, which will have direct and indirect impacts on plant pathogens and diseases caused by them. A higher CO_2 level is assumed to amend the precipitation and penetration of light through the plant canopy, which will have direct effect on canopy structure and microclimate environment (Sikma *et al.,* 2020). The altered canopy structure and microclimate will change host physiology as well as morphology and therefore, disease epidemiology. We may witness a reduction in plant canopy post-infection by some pathogens even under double ambient CO_2 levels. For instance, as an adaptation of photosynthesis, under increased CO_2 level and powdery mildew disease influenced dropping down in photosynthesis rate, hindered plant growth in barley at higher CO_2 levels is reported (Hibberd *et al.*, 1996). Reduction in growth of diseased plants is observed frequently even if disease severity is reduced under increased CO_2 levels (Chakraborty *et al.*, 1998). For instance, the growth of *Maravalia cryptostegiae*, a fungal biological control agent that causes rust in woody weed rubber vine (*Cryptostegia grandiflora*), is reduced under elevated CO_2 concentration twice to the ambient (Kaukoranta, 1996). A 3-year long controlled environment simulation study was conducted to study the effect of rising temperature on potato yield (Kaukoranta, 1996). The finding of that study has advocated that with 1 to 3°C warming, proficiency of chemical control of potato late blight would extend by 10-20 days per 1°C increase in temperature, and that the extent of yield loss in unprotected potato crops would be of the equal magnitude as the improvement in yield potential, which they estimated about 2 t/ha of dry matter per 1°C temperature increase (Kaukoranta, 1996). The implications of such studies on yield parameters cannot be fully followed unless some field experimentation is conducted, yet these findings suggest that CO_2 levels and improved water use efficiency-related estimation of crop harvest may not be convincing (Asseng *et al.,* 2015).

The indirect effects of environmental factors such as ozone layer transmitted ultraviolet rays (UV-B) may predispose crop plants to a number of plant diseases, which might result in higher yield losses than that caused by a specific disease alone (Manning and Tiedemann, 1995). However, the effects of UV-B are not consistent on plants and their pathogens. Higher disease severity as influenced by changed climate might not constantly result in increased yield losses (Luo *et al.*, 1995). In a comprehensive review on the effect of climate change on plant diseases, Chakraborty *et al.* (1998) listed the probable effect of elevated CO_2 on diseases caused by 10 biotrophic and 15 necrotrophic pathogens. Enhanced disease severity under elevated CO_2 levels was reported in six biotrophic and nine necrotrophic pathogens, while disease severity was reduced in four biotrophic and necrotrophic pathogens each (Chakraborty *et al.*, 1998). Inclusive analysis of potential climate change effects through altered disease severity on yield losses is currently unavailable. Certain assessments of monetary loss or gain due to changed climate effects on diseases of some major crops have been made (Bevitori and Ghini 2014; von Tiedemann, 1996). For example, the effects of climate change on blast disease in rice have been predicted by simulating the changes in temperature and precipitation in five Asian rice-growing countries (Bevitori and Ghini 2014; Luo *et al.*, 1995). There was considerable effect of temperature changes on rice blast severity, whereas rainfall had an insignificant effect on disease in a majority of the locations. However, these effects were inconsistent among different agro-ecological zones. This study also predicted that the future risk of rice blast would be high in currently cooler, subtropical rice-growing zones, for example, Japan; whereas, in the humid tropics and subtropical countries like the Philippines rice blast severity would reduce substantially with rising temperature.

2.1 Shift in infectious plant diseases

Changed climate might modify the structure and dynamics of microbial communities thriving in soil or air to manipulate plant growth and development (Cavicchioli *et al.,* 2019). Altered composition and dynamics of phyllosphere and rhizosphere may affect dynamics of plant pathogens and diseases caused by them under the influence of related microbes with bio-control activities (Fig 1). The soil environment is not likely to be influenced by rising CO_2 levels in the atmosphere since soil microbes are frequently exposed to the CO_2 level that is up to 15 times more than the ambient CO_2 levels in the atmosphere. The establishment of microbes like arbuscular mycorrhizal fungi (AMF) is favored by the plantation of trees in soils with poor nutrient status (Cavicchioli *et al.,* 2019; Klironomos *et al.*, 1997). However, there are contradictory reports on how the soil colonization by AMF is favored by fluctuations in the CO_2 levels and plant and soil nutrient status (Cotton 2018). The AMF could have a positive, negative, or neutral influence on the occurrence of particular plant disease; however, the findings to suggest their conclusive role are poorly documented despite much experimentation in the area (Roger *et al.,* 2013; Pfleger and Linderman, 1994). Accordingly, the understanding of the probable effect of mycorrhizae on the incidence of plant diseases under changed climate needs additional research. The increased CO_2 level in the soil can also influence the effects triggered by other elements for instance nutrient status, nitrogen in particular and water availability. Interaction of these factors and their effect on wheat powdery mildew, caused by *Erysiphe graminis*, was studied by Thompson *et al.* (1993) in England. They found decreased percent shoot nitrogen contents under enhanced CO_2 concentration which resulted in reduced powdery mildew disease. Thompson and Drake (1994) evaluated the effect of water and nitrogen contents on infestation by insects and fungal disease severity in C3 and C4 plants. In elevated concentrations of atmospheric CO_2, there was a significant reduction in shoot nitrogen content and 37% reduction in fungal infection on *Scirpus olneyi* Grey (C3) plants. Whereas, *Spartina patens* (Ait.) Mobl. (C4) plants had unchanged nitrogen (N) concentration and increased severity of fungal infection under elevated CO_2 concentrations. Further to the effect of N and irrigation, the outcome of higher CO_2 levels could also be influenced by the fluctuating concentration of other gases. For instance, wheat leaf rust (*Puccinia triticina*) was strongly inhibited by an increased level of ozone, but largely unaffected by elevated CO_2 (von Tiedemann and Firsching, 2000).

It is expected that changed climate would largely result in a poleward shift of the agro-climatic zones, and corresponding crops that are cultivated in these zones along with the related phytopathogens. Carter *et al.* (1996) projected that under changed climate, maize would be able to grow unfailingly by the year 2050 in southern Finland and outspread further north. Similarly, the potato late blight threat would rise in all potato growing regions while potato diseases caused by nematodes might turn into a problematic issue with an increased number of disease cycles per crop season. Parallel estimates have been made for oak decline disease in oak caused by *Phytophthora cinnamomi.* These predictions suggest that oak decline pathogen would move further north and the disease severity would increase across all the regions including the native environment of the pathogen (Brasier and Scott, 1994). The migration path of these pathogens would follow the distribution pattern of their hosts, while pathogens establishment, survival, and dispersal would largely depend on the physiology of their host and involvement of different biotic and abiotic factors in the new ecosystem/environment (Fig 1).

Most of the aggressive plant pathogens, infecting a range of crop plants, including *Fusarium* spp., *Rhizoctonia* spp., *Sclerotium* spp., *Phytophthora* spp., *Sclerotinia* spp., and many other necrotrophs may move from cultivated crops to wild plant communities (Kodati *et al.,* 2021). Necrotrophs and other plant pathogens with a wide host range might destroy the migrating crops by introducing new diseases. Likewise, less aggressive pathogens might destroy crops grown as monocultures in the nearby vicinity of natural plant communities. There are several well-documented cases where indigenous pathogens have been reported to cause new diseases in introduced crop plants, growing in the vicinity of other indigenous hosts (Keesing *et al.,* 2010; Jones and Baker, 2007). Two best examples that prove this statement include fire blight disease of apple and pear caused by *Erwinia amylovora* in USA and coffee rust caused by *Hemileia vastatrix* in Asia. *E. amylovora* was known to be a minor disease on native members of family Rosaceae in the USA. But the introduction of pears and apples by European migrants in some regions of USA resulted in severe devastation of these plants due to fire blight disease caused by *E. amylovora.* Likewise, the introduction of highly susceptible coffee species (*Coffea arabica*) in Asia during the late 1800s resulted in a severe coffee rust epidemic in the region, before that the coffee rust pathogen was surviving on some alternate hosts in the forests near to coffee estates (Carefoot and Sprott, 1967).

2.2 Plant disease management under changing climate

Because of poor understanding of the impacts of climate change on crop plants or the microbes causing diseases in them, forecasting the possible consequences of disease management practices with any assurance is not feasible till date, though, it is likely that the changed climate would essentially impact the degree of host resistance, pathogenicity in the pathogen and/or efficacy of pesticides and other bio-agents. Intensive investigations are therefore, desired to find out the circumstances where the efficiency of disease management strategies might get hampered due to changed climate. The efficiency of host resistance to diseases might increase considering improved static and dynamic defence mechanisms subsequent to changes in host's morphology, physiology, including nutrient content and availability of water in the soil. However, there are exceptions for instance a number of rust resistance genes may become less effective at warmer temperatures prevailing under the changed climate. However, most of the disease prediction models studied so far are influenced by several biotic and abiotic elements affecting host, pathogen, and their interaction, and the type of disease prediction models used (Fenu and Malloci, 2021). Hence, drawing conclusions solely based on few simulation studies would be inconclusive for breeders to breed for resistance to specific plant disease. Altered genetic resistance of host plants to their pathogens is one of the most significant concerns of climate change effects on different host-pathogen interactions. Changed climate altered host morphology, and physiology has a direct connection with the expression of disease resistance, which could be modified for the benefit of crop plants by applying both traditional and genetic engineering breeding tools. There are indications of structural as well as physiological alterations in plants under changed climate conditions. A substantial rise in the degree of photosynthesis, production of papillae, formation of epidermal layers, accumulation of wax, a higher concentration of silicon at the site of infection, increased fiber content, rise in carbohydrate amount in leaves, a decline in nutrient content, and shift in the synthesis of enzymes responsible for controlling resistance mechanisms, etc. resulting from climate change effects are reported in different host-pathogen interactions (Chakraborty *et al.*, 2000).

The effect of rising CO_2 levels on the magnitude of genetic resistance in host plants has been proved in several studies (Zhou *et al.,* 2017; Paoletti and Lonardo, 2001; Chakraborty *et al.*, 2000). The effect of elevated CO_2 concentration on resistance of a cypress (*Cupressus sempervirens*) clone remained ineffective to canker disease caused by *Seiridium cardinal.* Additionally, the effect of rising temperature and other abiotic factors on the magnitude of genetic resistance by host plants has also been explored. Such effects vary depending on the type of resistance. Several modifications in host morphology, and physiology could possibly amplify the degree of host resistance. However, the biggest risk to the expression of genetic resistance is the modifications in pathogens habits, such as elevated CO_2 concentration may help pathogens to migrate, survive, and cause disease more efficiently and thus, overcome host resistance. The enhanced pathogenicity together with prolificacy of a particular pathogen and proliferating plant canopies fasten the rate of occurrence of plant disease epidemics under beneficial microclimate environment (Chakraborty *et al.*, 2000).

Despite all the above-discussed effects, the changed climate may also have an impact on the efficacy of chemical pesticides used for the management of different pests including plant pathogens, insects, and weeds. Such effects on the efficacy of pesticides could be the results of the following two possibilities: (i) variation in temperature and relative humidity might change the duration and availability of chemical pesticide residues present on plant canopy or in soil and (ii) the infiltration, transport and mode of action of systemic pesticides on and in the plant system are expected to be directly influenced by morphological or physiological characteristics of host plants under elevated temperature or CO_2 concentration. For instance, denser epicuticular wax and epidermal layers on plant stem or leaves (Wolfe, 1995) could slow down the uptake of the chemicals by the crop plants, and flourishing plant canopy might adversely disturb the spray coverage, thereby reducing the concentration of active ingredient in spray suspension on host tissues. Accordingly, disease management strategies including changes in the fungicide application calendar will need to be restructured to minimize crop losses.

Similarly, the pesticide market could face certain drastic changes under changed climate. The pesticides with higher efficacy, a novel mode of action, and higher adaptability to climate change events would be more in demand than their counterparts. The defectiveness and adaptability of some pesticides under fluctuating abiotic factors including precipitation and temperature were analyzed in several US locations using a regression model (Chen and McCarl, 2001). The study concluded that per acre average cost of pesticide increased in wheat, soybeans, cotton, potatoes, and corn with an increase in precipitation. Similar was the case in corn, cotton, soybean, and potatoes when the ambient temperature was increased, however, per acre average cost of pesticide decreased in wheat with increase in temperature. Another aspect that would affect the pesticide market could be the rising awareness among people towards the value of anthropogenic actions in the course of resource exploitation by use of hazardous chemicals. People will unquestionably stress on the adoption of biologically safe non-chemical strategies of plant disease management.

There is no conclusive evidence to prove the influences of changing climate on the effectiveness of biological control of crop diseases. Few reports reveal the possible effect of climate change on the dynamics and structure of the microbes' populations in the rhizosphere and/or phyllosphere. Some crucial soil features such as water content, temperature, nutrient status etc. are expected to face certain alterations under changing climate, which will further influence the activity of microbes inhabiting the rhizosphere (Nosengo, 2003). The rising soil CO_2 concentration up to the tune of 600 ppm could not alter the microbial community; however, climate change effects on plant diversity could indirectly change population structure and dynamics of the microbial community in the soil (Gruter *et al.*, 2006). The elevated CO_2 concentration had a strong association with the population and efficiency of *Metarrhizium anisopliae*, a commonly known entomopathogenic fungus, and *Chlonostachys rosea*, biological control agent of a number of plant pathogens including *Botrytis* (Rezacova *et al.*, 2005). Such studies would be critical for sustaining the usefulness of biological control under changing climate. However, such forecasts are comparatively complicated and largely rely on the effect of several other abiotic and biotic factors that influence the efficiency of biological control. There are speculations that the efficiency of biological control would worsen considering the higher sensitivity of biological control agents towards the extreme weather conditions in the future (Garrett *et al.*, 2006). Conversely, there are arguments that biological control would be more in demand in the future, as increasing awareness in the society of harmful effects of chemical pesticides thereby adopting a more eco-friendly and sustainable ways of managing crop pests including plant pathogens (Lu *et al.,* 2015; Ghini *et al.*, 2008). For meeting such challenges, experts working in different areas of agriculture would have to work through a systematic approach and prioritize the effects of climate change in a broader context, comprising the entire agro-ecosystem.

2.3 Likely effect of climate change on wheat diseases

Wheat is prone to a number of fungal diseases including rusts {black/stem rust (*P. graminis tritici*), yellow/stripe rust (*P. striiformis tritici*) and brown/leaf rust (*P. triticina*)}, powdery mildew (*Blumeria graminis tritici*), tan spot (*Pyrenophora tritici-repentis*), leaf blotch (*Mycosphaerella graminicola*, *Phaeosphaeria nodorum*), spot blotch (*Cochliobolus sativus*), Fusarium head or ear blight (*Fusarium graminearum* and other *Fusarium* species) and more recently wheat blast (*Magnaporthe oryzae* pathotype *triticum*). Globally, a major proportion of wheat yield loss due to biotic stresses is caused by fungal pathogens as against bacterial, viral, or other wheat pests. In general, viral diseases in wheat are predicted to increase with a changing climate (Vassiliadis *et al.,* 2018; Tr bicki *et al.,* 2015). A shift in various climatic conditions including temperature, rainfall, level of CO_2, O_3, and other important gases in the atmosphere, soil factors etc. may influence the occurrence of wheat diseases. Changes in these factors may alter the level of interaction between the host and pathogen by changing or modifying the pathogens population dynamics, geographical distribution, synchronization in their life cycle events, and survival (West *et al.*, 2012). The significant influence of fluctuations in winter temperatures on the occurrence of yellow rust and powdery mildew on wheat has been determined (Jevtić *et al.,* 2020). There was a significant influence of wheat genotypes and climatic elements on the interactions among obligate pathogens and the predominance of one pathogen over another (Jevtić *et al.,* 2020).

There are evidences that suggest the direct positive or negative interaction between climate change and the occurrence of wheat diseases; however such information is not compiled comprehensively. Recently, there was a slow but steady increase in a number of studies published, which speculated the probable occurrence of specific wheat diseases under the influence of changing climatic conditions in the future. Future occurrence of different wheat diseases including rusts (Chakraborty *et al.*, 2011), Karnal bunt (Dumalasova and Bartos. 2009), Septoria tritici leaf blotch (Gouache *et al.*, 2012), Fusarium foot rot (Pettitt and Parry, 1996), Fusarium head blight (Fernandes *et al.*, 2004; Madgwick *et al.*, 2011), and other wheat diseases (Kaur *et al.*, 2008), have been speculated for different agro-climatic zones of the world. A number of reports speculating the effect of climate change on wheat diseases are summarized in Table 1.

Table 1. Likely prediction of occurrence of wheat diseases under climate change scenario

Wheat disease (Pathogen)	**Predicted effect on pathogen/disease**	**Prediction approach**	**References**
Stripe rust *(Puccinia striiformis)*			
Germany	Decrease	Speculation*	von Tiedemann, 1996
North India	Decrease	Speculation	Kaur *et al.* 2008
United Kingdom	Decrease	Speculation	Chancellor and Kubiriba, 2006
Brown/leaf rust *(Puccinia triticina)*			
France	Increase	Simulation**	Caubel *et al.* 2017
Germany	Increase	Speculation	von Tiedemann, 1996
Luxembourg	Increase	Simulation	Junk *et al.* 2016

Canada (Ontario)	Decrease	Speculation	Boland *et al.* 2004
North India	Increase	Speculation	Kaur *et al.* 2008
France	Decrease	Simulation	Gouache *et al.* 2011
Stem rust *(Puccinia graminis)*			
Canada (Ontario)	Decrease	Speculation	Boland *et al.* 2004
North India	Increase	Speculation	Kaur *et al.* 2008
United Kingdom	Sporadic	Speculation	West *et al.* 2012
Spot blotch *(Cochliobolus sativus)*			
South Asia	Increase	Speculation	Sharma *et al.* 2007
North India	Increase	Speculation	Kaur *et al.* 2008
United Kingdom	Increase	Speculation	West *et al.* 2012
Septoria tritici leaf blotch *(Mycosphaerella graminicola)*			
Germany	Increase	Speculation	von Tiedemann, 1996
Canada (Ontario)	Decrease	Speculation	Boland *et al.* 2004
United Kingdom	Decrease	Speculation	Chancellor and Kubiriba, 2006
France	Decrease	Simulation	Gouache *et al.* 2012
Septoria nodorum blotch *(Phaeosphaeria nodorum)*			
United Kingdom	Slight change	Speculation	West *et al.* 2012
Tan spot *(Pyrenophora tritici-repentis)*			
Canada (Ontario)	Decrease	Speculation	Boland *et al.* 2004
United Kingdom	Slight change	Speculation	West *et al.* 2012
Powdery mildew *(Blumeria graminis)*			
Finland	Increase	Speculation	Hakala *et al.* 2011
Sweden	Increase	Speculation	Roos *et al.* 2011
Stinking bunt *(Tilletia controversa)*			
Canada (Ontario)	Increase	Speculation	Boland *et al.* 2004
Tilletia indica *(Karnal bunt)*			
Europe	Increase	Simulation	Baker *et al.* 2000
United Kingdom	Increase	Speculation	West *et al.* 2012
Loose smut *(Ustilago tritici)*			
Canada (Ontario)	Increase	Speculation	Boland *et al.* 2004
Fusarium head/ear blight *(Fusarium species)*			
Sweden	Increase	Speculation	Roos *et al.* 2011
North India	Increase	Speculation	Kaur *et al.* 2008
United Kingdom	Increase	Speculation	West *et al.* 2012
Eyespot *(Oculimacula yallundae)*			
Germany	Decrease	Speculation	von Tiedemann, 1996
Finland	Increase	Speculation	Hakala *et al.* 2011
United Kingdom	Increase	Speculation	West *et al.* 2012
Take all disease *(Gaeumannomyces graminis)*			
Ontario, Canada	Decrease	Speculation	Boland *et al.* 2004

*Expert knowledge based speculations that consider the epidemiology of plant diseases

**Simulation are based on disease forecasting models

The incidence of different wheat diseases has been assumed to shift strongly in Punjab, India (Kaur *et al.*, 2008). Currently, yellow rust is one of the major yield-limiting factors in this region but with increasing temperature, its occurrence is anticipated to reduce in the future. However, the occurrence and prevalence of high-temperature tolerant isolates of *Puccinia striiformis tritici* may become predominant. Conversely, present-day minor diseases in the region including stem rust, foliar blights, brown rust, and Fusarium head blight are expected to upsurge in upcoming decades (Kaur *et al.*, 2008). Such speculations support the fact that changing climatic conditions may change the occurrence of different diseases in a region, and that presently minor diseases may threaten wheat production in the future (Duveiller *et al.*, 2007).

Generally, different abiotic factors like temperature, precipitation, photoperiod, and wind direction and velocity influence the disease causing potential of plant pathogens by affecting almost all the disease cycle events occurring during pathogenesis such as inoculum production and germination, and dispersal. The temperature is one of the most crucial factors that decide the outcome of a particular host-pathogen interaction. Prolonged higher temperature (beyond supra-optimal temperature) conditions will abbreviate incubation and latency period, which will increase the number of disease cycles per crop season and thereby more disease for polycyclic diseases like rusts (Wojtowicz *et al.*, 2017). On the other hand, higher temperature may decline the availability of moisture that in turn will hamper secondary infections by polycyclic pathogens. Conversely, rising temperature may provide congenial conditions for survival of higher temperature-tolerant isolates of a particular pathogen so that the latter could successfully survive, migrate, and establish at warmer geographic locations (Chakraborty, 2013). The survival of temperature tolerant pathogen isolates may also be influenced indirectly through host-plant physiology or climate change driven modifications in cropping patterns such as varying irrigations, changing sowing dates, changing tillage practices (zero tillage etc. in conservation agriculture) etc. Climate change scenarios may also control the expression of host plant resistance to pathogens through the weakening of host or pathogen that may strengthen or weaken the expression of a particular disease resistance gene (Duveiller *et al.*, 2007). This is true for situations where the expression of disease resistance genes is directly influenced by temperature, photoperiod, or precipitation (Chakraborty *et al.*, 2011; Bidzinski *et al.*, 2016).

Certain wheat pathogens, for example, *Septoria tritici*, require frequent rainfall and a consistently extended dew phase during the vegetative growth of wheat for successfully infecting the upper maximum photosynthates contributing leaves of the plant. Other wheat pathogens like *Fusarium* species require just one rainfall ranging between 2–3 mm at the time of flowering for causing severe Fusarium head blight (FHB). *P. triticina* and some other pathogens require only overnight dew to cause successful infection in wheat. Therefore, Septoria blotch could become a predominant disease in the future with warmer winter and frequent rainfall. While, in general wheat rusts and FHB would be more devastating from global warming than STB, since they are poorly dependent on occurrence of repeated and more rainfall, which is anticipated to be irregular in the future (Miedaner and Juroszek, 2021). It is speculated that the global warming would not only promote the above-ground wheat pathogens but also favor the economically significant soil-borne diseases caused by *Phoma* species, *Fusarium*, *Alternaria* etc. (Delgado-Baquerizo *et al.*, 2020).

During the last decade, a drastic change was observed in the pattern of yellow rust throughout the world including the Indian subcontinent and Europe. In India, three new *Yr9* virulent *P. striiformis tritici* pathotypes 238S119, 110S84, and 110S119 having collective virulence to wheat varieties such as Suwon 92 x Omar (*YrSU*), Strubes Dickkopf (*Yr2*, *Yr3a*, *Yr4a*) etc. were reported (Gangwar *et al.*, 2019). Likewise, Warrior and Kranich races of *P. striiformis* were detected on both triticale and wheat in many countries of Europe during 2010-11 (Hovmøller *et al.*, 2016). All these new pathotypes had additional virulence, and were more aggressive than the previous pathotypes on the then cultivated wheat varieties (Hovmøller *et al.*, 2016; Prasad *et al.*, 2020b). Subsequently, though the yellow rust epidemics somehow did not occur in India, two epidemics were reported in European countries during 2013 and 2014 and also less significant epidemics in succeeding years due to high frequency of newly evolved more aggressive and virulent pathotypes. These new pathotypes defeated yellow rust resistance in several previously resistant varieties. These findings suggest that the newly arrived isolates might have a strong propensity to cause severe yellow rust epidemics at warmer temperatures. Similar adaptations for warmer temperature are also reported for yellow rust races evolved in northern and southern France with a certain gain of the southern races in the warmer Mediterranean climate (Vallavielle-Pope *et al.*, 2018). They also observed that since 2000, *PstS1*, *PstS2* have become adapted to higher temperature and have spread worldwide. Likewise, Warrior isolates have spread to both warm and cold parts of Europe. The Warrior isolates have shown a range of infection efficiency and latent period responses to temperature and invasive *PstS2* isolates adapted to warm conditions. We have also observed similar phenomenon with *P. striiformis tritici* pathotype 78S84 which can infect and spread at higher temperature (18^{0}C) than 46S119 which thrives well at lower (15^{0}C) temperature (Bhardwaj *et al.,* 2019). Walter *et al.* (2016) also found that *PstS1* and *PstS2* were more aggressive in warmer areas. Newly emerged (after 2000) isolates of *P. striiformis tritici*, were more aggressive on all the parameters than the old ones at higher temperature. These isolates were able to cause yellow rust diseases in Western Australia and the South Eastern USA, which were otherwise supposed to be too hot for yellow rust epidemics to occur (Milus *et al.*, 2009). Likewise, leaf rust used to be the most common rust disease of wheat in Serbia until 2014; however, due to changed climate conditions, yellow rust predominated over leaf rust and reached up to 90% on the genetic collection tested in the field trials (Jevtić *et al.,* 2017).

The effect of climate change is quite evident from the fact that southern Italy in Europe faced severe stem rust epidemics during 2014-16 as several thousands of hectares area occupying durum wheat was severely infected with stem rust (Berlin, 2017). However, before that this area was thought to be stem rust-free, or if observed, it was in traces. Subsequently, stem rust outbreak occurred in central Sweden during 2017, which was assumed to be caused by a new virulent isolate originating from barberry post sexual recombination (Berlin, 2017). There are predictions that wheat stem rust is going to be a serious threat for North Western Europe (NW Europe) by the year 2050 (Davies *et al.*, 2007). These authors simulated the present-day wheat production conditions of East Africa with that of the European wheat production conditions for 2050. Based on this climate matching system the authors concluded that *P. graminis* will be a possible potential threat for wheat production in most countries of NW Europe including southern England and Ireland (Davies *et al.*, 2007).

The predicted future occurrence of a particular wheat disease for instance wheat leaf rust is not in agreement across the regions/countries (Table 1), which is understandable from the fact that the effect of the climatic conditions is not going to be parallel across different geographic locations. For example, the expected effects of climate change in Northern India might be different from those in Central India or Southern India, due to varying climatic conditions among these regions. Despite influencing the pathogen factors, climate change is also going to affect physiological growth stages in wheat, which would further influence the outcome of the effect of climate change on the wheat-pathogen system (Velásquez *et al.,* 2018). Effect of rising temperature along with changes in other abiotic factors such as, humidity, level of different gases in the atmosphere, soil factors on phenological growth stages of wheat would shift critical wheat development stages (Gouache *et al.*, 2011). For example, about $0.5\text{-}1.0^{0}$C rise in temperature forced early stem elongation and flowering in wheat in Germany (Chmielewski *et al.*, 2004). Conversely, high temperature during winters could endorse and extend the growth and developmental stages in wheat, while increasing temperature and photoperiod effect on vernalization, consequently, could change the heading date (Miglietta *et al.*, 1995). Higher temperature (>34 °C) conditions augment plant senescence and thereby curtail the grain filling period in wheat (Asseng *et al.*, 2011). Despite affecting the grain filling in wheat, accelerated plant senescence also hampers the growth and pathogenesis of wheat pathogens including biotrophic fungi causing powdery mildew and rusts in particular. Though, most plant pathogens suffer under prolonged higher temperature conditions as the latter directly affect pathogen development and pathogenicity. Under such circumstances, it becomes tedious to differentiate if the activities of a particular pathogen have been directly hindered due to higher temperature or indirectly through accelerated senescence in the host plant. A similar situation arises when two or more abiotic factors like temperature, humidity, etc. have either direct or indirect effects on the outcome of a particular host-pathogen interaction. Moreover, the interaction of these factors among themselves and effects on host, pathogens and their interaction is another big challenge to address. One of the strategies to avoid the effects of climate change on the occurrence of wheat diseases is to nullify the effect of climate change in wheat by manipulating heading and flowering timing using genotypes with diverse growth and development patterns or by changing the sowing date. Such strategies would change the growth and development habits and thus, heading and flowering timings in wheat. These alterations would have direct impact on the occurrence of wheat diseases caused by fungi primarily infecting floral parts of the plant such as *Fusarium* species (West *et al*., 2012).

2.4 Future strategies for management of wheat diseases

A shift in distribution and occurrence of wheat diseases under the influence of changing climate has emphasized the need for the development of disease prediction models which can predict the intensity and distribution of important plant diseases in real-field conditions. Moreover, improved disease management strategies including biological control and eco-friendly approaches need to be reoriented in consideration with the changing climate and implemented judiciously for sustainable crop production. Among these strategies, epidemiology-based disease forecasting, monitoring pathogen distribution and dynamics, rapid pathogen detection, and disease diagnosis could significantly contribute in effective wheat disease management (Gautam *et al*., 2013). There is a need to develop and adopt integrated disease management tactics to minimize the dependency on hazardous chemical fungicides. Integration of plant breeding, biotechnology, chemistry, computer science, and other tools could help to counter the risk of wheat diseases in a harmonized manner. Biological control by *Trichoderma* spp., *Bacillus* spp., *Streptomyces* spp., arbuscular mycorrhizal (AM) fungi, and others could provide a sustainable and eco-friendly option of plant diseases management in changing climate scenario. Efforts have been made to manage wheat diseases by application of plant extracts and organic materials directly by their inhibitory effect on pathogens or indirectly by inducing disease resistance in plants. Biological control agents, plant extracts and many other synthetic chemicals of plant origin are reported to induce disease resistance in wheat. Cultural control methods can minimize the intensity of an epidemic or provide long-term partial management of wheat diseases. Use of healthy seeds, time of planting, frequency and amount of irrigation, balanced application of fertilizers (NPK), eradication of alternate hosts, clean cropping, crop rotation, mixed cropping, removal or avoidance of green bridges (volunteer plants, crops grown successively in one area) that may carry inoculum from one season to the other, can significantly reduce the severity and incidence of wheat diseases. All these approaches can be integrated to formulate a specific integrated disease management package for a specific wheat disease in the future under changed climate. Nevertheless, wheat breeding supported by novel molecular tools for disease resistance would remain a critical factor for developing disease-resistant and climate-resilient varieties. Increasing temperature conditions in the future may back the identification and deployment of high-temperature adult-plant (HTAP) disease resistance genes against different wheat diseases including yellow rust. The resistance conferred by HTAP resistance genes is highly durable and mostly non race specific but their expression remains moderate and varies depending upon the temperature and humidity conditions (Line and Chen, 1995). More than 80 QTLs including several yellow rust resistance genes such as *Yr18, 29, 34, 36, 39*, *48, 52*, *Yrns-B1* etc. from 33 wheat varieties have been described to possess HTAP resistance (Huerta-Espino *et al.,* 2020; Chen, 2013). There are recommendations for promoting the strategies for disease resistance breeding including screening of breeding material in a warmer climate (Bryant *et al.,* 2014; Butterworth *et al*., 2010). Multiple disease resistance (MDR) QTLs or genes, such as *Lr67/Yr46/Sr55/Pm46*, *Lr34/Yr18/Sr57/Pm38, Lr46/Yr29/Sr58/Pm39,* and *Sr2/Yr30/Lr27*, conferring resistance to more than one disease, could be promoted to endure the threat of wheat diseases in changed climate (Silva *et al*., 2015). Utilization of such MDR genes or QTLs could be accelerated by marker-assisted or genomic selection tools. Such an approach looks promising especially in situations where the pathogen population keeps on evolving and when there could be a shift in the occurrence of a specific disease in the future. Genomic selection (GS), a promising molecular breeding tool, is an advanced form of MAS which aims to utilize genome-wide genetic markers to envisage the effects of all QTLs and thus figure out a genomic estimated breeding value to deliver more inclusive and consistent selection for various traits including disease resistance (Miedaner *et al*., 2019). GS for plant disease resistance is specifically encouraging since it has already been used extensively for traits like grain yield etc. Furthermore, for diseases like wheat blast, where no reliable resistance sources are available to date except some genomic regions including 2NS Translocation from *Aegilops ventricosa* that confers some resistance to wheat blast pathogen (Cruz *et al.*, 2016), the use of MAS or GS would be limited. Therefore, genome editing and other gene transfer strategies would play a significant role in developing resistant varieties for such diseases in the future (Sánchez-Martin and Keller, 2019).

3. Conclusion

Availability of current understanding about a specific host-pathosystem including the effect of other biotic and abiotic variables, without the interference of changed climate, supports effective management of wheat diseases. But with the inclusion of changing climate issues, disease management could be challenging due to the lack of thorough understanding of disease epidemiology. Addressing such challenges requires detailed information of abiotic and biotic variables, whose role is supposed to be influenced under changed climate. Such information on disease epidemiology and effects of individual climate variables could help develop disease prediction models and thus in predicting disease epidemics at a particular space and time. Despite abiotic climate variables, geographical distribution, host morphology, and physiology along with pathogens, ability to multiply, disseminate and survive will have a direct impact on the occurrence of wheat diseases and the introduction of new disease and/or pathogens. Higher UV-B radiation and elevated CO_2 levels could increase prolificacy in pathogens and thus their evolution. The existing knowledge of climate change effects on wheat diseases occurrence and management is fragmented and therefore, conclusive analysis of possible effects on changed climate on wheat diseases in the future can be based on circumstantial and limited facts. The wheat diseases like damping off, leaf rust, stem rust, Karnal bunt, Fusarium head scab, blight, and blast may increase with the rising temperature. Whereas the stripe rust incidence might decrease. However, the occurrence and prevalence of high-temperature tolerant isolates of *P. striiformis tritici* may become predominant. Considering the significant contribution of different abiotic and biotic factors in the development of plant disease epidemics, modeling tools need to be strengthened for precise and timely prediction of possible changes occurring in the agro-climate scenarios as influenced under changing climate and its effect on different host-pathosystems and impact assessment. Climate change is going to put an extra burden on organizations accountable for the prohibition and quarantine of disease-causing agents, as a plant disease management approach. In certain areas, few economically important diseases may not be favored since the climate at those locations do not let the pathogen establish and proliferate. However, there are chances that these pathogens will migrate to newer areas, establish and cause diseases that were previously absent in those areas. Therefore, exhaustive coordination is required among the researchers from different backgrounds such as plant pathologists, computer scientists, climatologists, epidemiologists, agro-meteorologists, and agronomists to further streamline the future work related to the effect of climate change on fluctuating severity, prevalence, and distribution of wheat diseases and shift in the pathogen population. Future climate change research should primarily focus on minimizing the harmful effects of both biotic and abiotic stresses on plant growth and health; and generating inclusive and pertinent prediction model (s) to predict the effect of changing climate on wheat health and productivity in the future.

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Conflict of Interest

NO

Ethical Compliance Statement

NA

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Review Article

Frontier insect pest management technologies for sustainable rice production

Atanu Seni*

Odisha University of Agriculture and Technology, All India Co-ordinated Rice Improvement Project, RRTTS, Chiplima -768025, Sambalpur, Odisha, India

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***Corresponding author:**
E-mail: atanupau@gmail.com

Abstract

Although effective management of rice insect-pests can be achieved by insecticides but their excessive long term use poses human health and environmental risks in addition to effect on non-targets organisms. Now-a-days, some pest management practices such as agronomic practices like smart fertilizer and nutrient application, real time monitoring and surveillance, ecological engineering through habitat manipulation, biological control by more effective strain, nanotechnology, host plant resistance by RNAi and marker assisted selection, have been introduced and adopted to manage rice insect pests which are eco-friendly in nature and also promote natural pest management. Here, this article intended to discuss the various frontier pest management technologies which will help for sustainable rice production.

Key words: Eco-friendly, ecological engineering, insect resistant, IPM, RNAi, smart agronomic practices

1. Introduction

Rice (*Oryza sativa* L.) is one of the important cereals and staple foods in the world. More than 60% of the global population depends on it for fulfillment of their nutritional requirement (Joshi *et al.,* 2018). In India, it is grown almost one-fourth of the total cropped area and providing food to about more than half of the Indian population (Seni and Naik, 2020). It grows well under different topographic and hydrologic conditions ranging from rain fed upland to lowland as well as in deep water conditions (Seni *et al.,* 2019). The production of rice has been found to be hampered by infestation of various insect pests at different growth stages. Insect pests causing significant yield loss over the years are yellow stem borer [*Scirpophaga incertulas* (Walker)], plant hoppers, both brown plant hopper (BPH), *Nilaparvata lugens* (Stål) and white backed plant hopper (WBPH), *Sogatella furcifera* (Horvath), gall midge [*Orseolia oryzae* (Wood-Mason)], a group of leaf-eating caterpillars like rice leaffolder (*Cnaphalocrocis medinalis* Güenée) and grain sucking bug complex like earhead bug; *Leptocoriza oratorius* (Fabr.) that feed on developing grains (Seni and Naik, 2018; Ali *et al.*, 2019; Jasrotia *et al.*, 2019). Beside insects, other arthropods like rice panicle mite, *Steneotarsonemus spinki* Smiley, was also appeared as a destructive pest of rice (Seni and Mandal, 2021). These pests cause hundreds of millions of dollars of losses every year and threaten food security in regions where rice is grown. For this, rice pest management is crucial to achieve rice production in a sustainable manner (Savary *et al.*, 2006). The yield losses varies from one region to another, however range from 1.2 to 2.2 tons/ha due to the combined attack of diseases, insects, and weeds in Asia (Savary *et al.*, 2012). On the other hand, potential yield gains of at least 10-20% of the current yields may be achieved through effective pest management techniques (Willocquet *et al.*, 2004; Oerke, 2006; Savary *et al.*, 2012).

Since 1970s, Integrated Pest Management (IPM) is in practice and it relies on ecologically based management that aims to suppression of the pests through a combination of techniques such as modification of agronomic practices, mechanical and physical methods, use of resistant varieties, biological control and need based insecticide application. However, IPM was not proved successful as it was thought to be at the beginning due to low adoption and unawareness about its usefulness of management technologies and their application in real farm situation. In addition, inappropriate credit and subsidies, weak public sector and influential agrochemical companies further lead to the failure of IPM on the ground level (Bentley and Andrews, 1996; Savary *et al.*, 2012). As still now, in many rice growing areas insect pest control strategies are solely dependent on various synthetic chemicals which are designed to quickly eradicate insect pests from fields (Savary *et al.*, 2012). However, indiscriminate and excessive use of agrochemicals has led to many negative effects such as development of insecticide resistant in insects, pest resurgence, secondary pest outbreaks besides environmental pollution and human health hazards. With this perspective, focus should be shifted to develop modern pest management technologies that are not solely dependent on insecticides. This will not only increase rice production in sustainable manner but will also improve health and environmental quality.

In this direction, many new technologies and strategies have been developed to tackle the insect pest menace in rice without hampering the environmental quality. In this article will highlight and discuss those frontier technologies, are presently being used for effective pest management in rice aiming towards sustainable rice production.

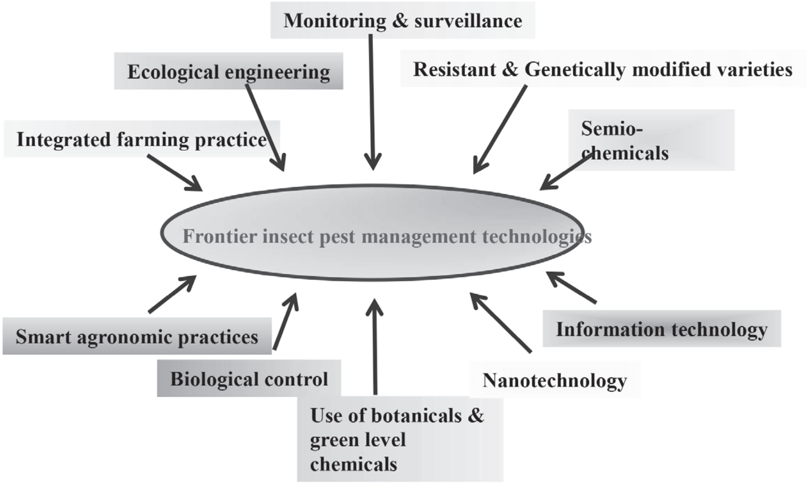

Fig. 1: Different components of frontier insect pest management technologies

2. Frontier insect pest management technologies for rice insect pests

2.1 Pest monitoring and surveillance

Pest monitoring and surveillance is the most important and integral part of Insect pest management programme. It helps to know the occurrence of insect pest, developmental stage and infestation level at certain intervals. In rice, mainly sampling of 25 plants in 5 clusters on a diagonal line of the plot at 7-10 days interval is suitable for determining insect pest's intensity, natural enemy populations and infestation rate (Pasalu *et al.*, 2004). These form the base for taking the management decisions by taking economic thresholds as guidelines. The economic thresholds of common insect pests of rice and yield loss incurred by them are given in table 1 and 2. Installation of light traps is useful for the monitoring and management of certain rice insect pests, mainly planthoppers, stem borers, gall midge and leafhopper. Many light traps are operating throughout north-eastern China and Japan to detect movement of migrating planthoppers (Horgan, 2017). During the spring season in northern hemisphere, brown and white-backed planthoppers migrate many thousand miles from South East Asia to the north east of Asia. In this context, light traps have been very useful for early warning of farmers by ascertain the magnitude, route and environmental factors that favour those movements (Matsumura, 2001; Cheng, 2009). Likewise, rustic light traps have been used in Vietnam as a escape strategy whereby farmers decide rice planting dates on the basis of planthopper catch in light traps (Bentley, 2009). In field installation light trap, the light should be switched on at sunset and switched off, after capturing large population. However, these lights may harm beneficial insects, and thus should not be used continuously throughout the rice growth period (Hong-xing *et al.*, 2017).

Table 1. Economic thresholds of common insect pests of rice

Pest	Economic thresholds	Reference
Stem borer	10% dead hearts or 1 egg mass or 1moth/m^2	Pasalu *et al.*, 2004
BPH and WBPH	10 insects/ hill at veg. whereas 20 insects/hill at later stage	Pasalu *et al.*, 2004
Green leaf hopper	2 insects/ hill in tungro endemic areas. 20-30 insects/hill in other areas	Pasalu *et al.*, 2004
Gall midge	1 gall/m^2 or 10% silver shoot	Pasalu *et al.*, 2004
Leaf folder	2-3 damaged leaves/ hill post active tillering stage	Pasalu *et al.*, 2004
Case worm	1-2 cases/hill	Misra and Jena, 2007
Cutworm	1 damaged tiller/hill or 2 larvae/m^2	Prakash *et al.*, 2014
Earhead bug	1 nymph or adult/hill	Prakash *et al.*, 2014
Rice hispa	2 adults or 2 dead leaf/hill	Prakash *et al.*, 2014
Rice black bug	5 bugs/hill	Prakash *et al.*, 2014
Whorl maggot	25% damage leaves	Misra and Jena, 2007

Table 2. Yield loss caused by major insect pests of rice

Insect	Yield loss	Reference
Yellow stem borer	1-19% in early planted and 38-80% in late transplanted crop	Catinding and Heong, 2003
Plant hopper	10- 90%	Seni and Naik, 2017
Gall midge	0.8% of the total production	Krishnaiah, 2004
Leaf folder	10% flag leaf infestation reduces grain yield by 0.13 g per tiller and the number of fully filled grains by 4.5%.	Murugesan and Chelliah, 1983
Earhead bug	10-40%	Israel and Rao, 1954
Rice hispa	20-28%, Yield reduction from 33.72 g/plant at 5% infestation to 3.50 g/plant at 70% infestation.	Nath and Dutta, 1997
Rice black bug	Ten black bug adults per hill can cause losses of up to 35% in some rice.	http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/insects/item/black-bug
Rice panicle mite	30-90%	Seni and Mandal, 2021

Insect sex pheromones can be used for both monitoring and management purpose by mating disruption or mass killing of insect pest populations. Mass trapping of yellow stem borer can be done by installing of 20 sleeve traps per hectare each with 5 mg pheromone impregnated lures (Pasalu *et al.*, 2004; Misra and Jena, 2007). Whereas, mating disruption can be done by an application of slow release formulation of pheromones @ 40g a.i./ha and it is also found that by adopting this techniques starting from fortnight after planting through multipoint sources could result in season-long control of stem borer and produced grain yields at par with plots received two sprays of conventional insecticides (Pasalu *et al.*, 2004; Misra and Jena, 2007). Verma *et al.,* 2000 tested sex pheromone blend consisting of 2 components viz., (Z)-11-hexadecenal and (Z)-9-hexadecenal @ 3:1 ratio and observed that peak dead-heart and white-ear appeared 2-3 weeks after the highest male moth captures in pheromone traps. They further stated that when trap captures 30 and 19 male moths/week then it caused 10% dead hearts and 5% white ears respectively in field. Beside yellow stem borer, sex pheromone components of striped stem borer; *Chilo suppressalis*, pink stem borer; *Sesamia inferens*, leaf folder; *C. medinalis*, gall midge; *O. oryzae* and rice hispa; *Hispa armigera* have been identified (Misra and Jena, 2007). However, a lot of efforts and refinement studies are necessary in pheromone usages technology which will play an important role in insect pests monitoring and surveillance in near future.

2.2 Smart agronomic practices

Smart agronomic practices for crop protection are those which are helpful to growing crops, and at the same time are useful in pest suppression. Here no large extra cost is necessary for insect pest management. Many times these work very effectively in reducing the multiplication of insect pests. These include:

- Early and synchronous rice planting often less attack by various insect pests like yellow stem borer, gall midge, BPH, WBPH and GLH particularly in wet season and produce more yield. At Chiplima, Sambalpur it was observed that when rice crop was transplanted on 31st July, 2020, produced 3.8 t/ha rice grain whereas when transplanted in 10th September, 2020, rice yield was 1.76 t/ha (var: MTU 7029, 25 days old seedling, without any plant protection measure).
- Application of optimum dosage of nitrogen in 2-3 splits avoids build up of insects such as stem borer, gall midge, leaf folder, BPH and WBPH. Excessive use of nitrogenous fertilizer has positive effects on development, survival, reproduction of rice insect pests by improving their nutritional conditions which ultimately hasten their infestation rate (Balasubramanian *et al.*, 1983; Ma and Lee, 1996; de Kraker *et al.*, 2000; Visarto *et al.*, 2001). Balanced application of N, P, K and other important nutrition elements can improve the plant vigor, and increase the resistant ability against various insect pests (de Kraker *et al.*, 2000). It is found that application of silicon can induce rice resistance or tolerance to heat, drought, lodging, stem borer and plant hoppers (Agarie *et al.*, 1998; Yang *et al.*, 2014; Hong-xing *et al.*, 2017).
- Crop rotation with other non host crop is important to break continuity in insect pest life cycle and population build up (Pasalu *et al.*, 2004; Misra and Jena, 2007).
- Providing alleyways of 30 cm width after every 2-3 metres, is helpful against BPH and WBPH (Misra and Jena, 2007).
- Stubble destruction by ploughing, irrigation or machine after harvesting is helpful to check the carryover of the stem borer and gall midge insects (Pasalu *et al.*, 2004; Misra and Jena, 2007).
- Water management like intermittent draining of water from the fields is helpful when planthopper population become abundant (Pasalu *et al.*, 2004; Behera *et al.*, 2013).

2.3 Host plant resistance

Host plant resistance is the most effective, economical and reliable means for plant protection for centuries ((Pasalu *et al.*, 2004). Before the discoveries of molecular markers, conventional breeding programmes helped to get desired traits for insect pest management. For this, large scale screenings were done to find rare resistant gene from the wild rice species and landraces (Panda and Khush, 1995). In early period, maximum released resistance rice varieties was of the 'vertical', single gene type, and while this had been effective at the releasing time, but evolution of virulent biotypes has become a major setback to that strategy. To overcome this problem, the selection of resistance genes needs to be done with a better knowledge of the virulence composition of the insect pest populations in the target area and the genetics of plant resistance (Behera *et al.*, 2013). Asian rice gall midge (ARGM), *O. oryzae* is one of the serious insect pests of rice in South and Southeast Asia. Till now, in India seven biotypes has been characterized based on their reaction pattern against various groups of rice varieties. It is observed that none of the resistant gene conferred resistance against all the biotypes of gall midge. So, the varietal resistance can be enhanced by combining several resistance genes through gene pyramiding (Fujita *et al.*, 2013; Bentur et al., 2021). Another serious insect pest of rice in Asian rice growing areas is brown plant hopper (BPH), *N. lugens*. There are four biotypes of brown plant hopper have been reported from all over the world and in India biotype 4 is present (Khush and Brar, 1991; Mohanty *et al.,* 2017). Regarding BPH resistance, so far 38 major resistance genes were identified and three genes mainly *bph-5, Bph-6* and *bph-7* showed resistance against biotype 4 only (Behera *et al.*, 2013; Bentur *et al.,* 2021). But, rapid gene flow among migratory insects like plant and leafhoppers may reason for high degree of genetic diversity (Behera *et al.*, 2013) and causes difficulty to manage them. To overcome this problem, uses of molecular techniques are helpful. For this, scientists first identify the effective resistance genes/QTL (quantitative trait loci) from various sources, characterize them genetically and make reliable tightly linked molecular markers for their introgression through marker-assisted backcross breeding (MABB) into popular rice varieties (Chen *et al.*, 2012; Fujita *et al.*, 2013; Horgan, 2017; Mohanty *et al.,* 2017). Till date many QTLs associated BPH resistance has been identified from various land races and wild rice and mapped in different chromosomes like 1, 2, 3, 4, 6, 7, 8, 10 and 12 (Mohanty *et al.,* 2017). Soundararajan *et al.* (2004) reported the presence of BPH resistance QTLs in chromosomes 1, 2, 6, and 7 in the population derived from the cross between IR64 and Azucena and among them QTLs on chromosome 7 were associated with seedling resistance, QTLs on chromosome 2 were associated with antibiosis and QTLs on chromosomes 1, 6, and 7 were associated with tolerance. Likewise, Sun *et al.* (2005) identified three resistance loci on chromosome 4 for BPH resistance in Rathu Heenati. Likewise, Mohanty *et al.,* 2017 identified two QTLs on chromosome 4 for BPH resistance in Salkathi and successfully transferred to two elite rice cultivars namely Pusa 44 and Samba Mahsuri. Similarly, Yao *et al.,* 2016 identified five QTLs associated with African rice gall midge resistance on chromosome 4 in ITA306 x TOS14519 population. So, these QTLs can be integrated into elite rice varieties to make resistant varieties through marker assisted selection.

Currently, research into RNAi technology has gained some attention for controlling of various insect pests in rice (Yu *et al.,* 2014; Horgan, 2017). RNA interference (RNAi) act through gene silencing mechanism by affecting mRNA synthesis at the cellular level triggered by double-stranded RNA (dsRNA). It is observed that by successful delivery of dsRNA molecules into insects by ingestion causes the target gene silencing (Price and Gatehouse, 2008; Bentur *et al.,* 2021), resulting the detrimental effect on physiology and ultimately causes the mortality of the target insect. Pan *et al.* (2018) used RNAi by injecting specific dsRNAs to knock down 135 CP (chitin and cuticular protein) genes in BPH and found that 32 CPs are important for their development and egg production. In further development, Li *et al.* (2015) stated that dsRNAs are stable under diverse environmental conditions and can be absorbed by roots of crop plants. Likewise, Kola *et al.* (2016) observed that by feeding YSB larvae with dsRNA of cytochrome P450 derivative (*CYP6*) and amino peptidase N (*APN*) treated cut stems resulted in increased mortality of the insect.

Another molecular approach, CRISPR (Clustered regularly interspaced short palindromic repeats) based genome editing can be promising in near future to develop resistant varieties against various insect pests. Genome editing can be done by targeting either the host genes or genes in insect population by replacement of nucleotides/domains/motifs or editing of specific bases (Bentur *et al.,* 2021). However, more research is necessary to precise replacement of bases and making them as a viable strategy.

It is found that, transfer of genes in rice expressing snowdrop lectin gene, *Galanthus nivalis agglutinin* (*GNA*), protease inhibitors and *Bt* genes such as *cry1A(b)*, c*ry1A(c)* showed resistance against various insect pests particularly stem borers and both plant and leaf hoppers (Murdock and Shade, 2002; Chen *et al.*, 2012). Transfer of soybean trypsin inhibitor gene and *Allium sativum* leaf agglutinin (*ASLA*) in transgenic rice increase the resistance against the *N. lugens* and *Nephotettix cincticeps* (Lee *et al.*, 1999; Saha *et al.*, 2006). *ASLA* conferred its action in transgenic rice lines by affecting NADH quinone oxidoreductase (NQO) action which is an important component in the electron transport chain (Bala *et al.,* 2013). Pradhan *et al.,* 2016 inserted a vegetative insecticidal protein (*vip*) in MTU 7029 rice variety and found that the transgenic rice showed resistance against various lepidopteran pests like yellow stem borer, leaf folder and rice horny caterpillar. Boddupally *et al.,* 2018 inserted both *Cry 1Ac* and *ASLA* in rice plant and reported that the transgenic rice showed resistance against multiple insect pests including stem borer, leaf folder and BPH.

2.4 Biological control

Use of biological control agents to manage crop insect pests is an important tool for integrated pest management. The successful use of several parasitoids and predators has made biological control as a promising alternative to the chemical control. However, they showed their effectiveness only one or a few insect pests mainly yellow stem borer and leaf folder but not effective against other sporadic pests like gundhi bug, rice hispa, and cutworm

(Pasalu *et al.*, 2004). In comparison to other crops, use of biocontrol agents through inundative or inoculative releases in rice ecosystem has provided sporadic success (Pathak *et al.,* 1996).

In India in rice ecosystem, inundative releases of natural enemies have been restricted to mainly egg parasitoids, particularly *Trichogramma japonicum* and *T. chilonis*, because they are easily multiplied in laboratories. In rice, selection and release of appropriate *Trichogramma* spp. is important for their effectiveness as all *Trichogramma* spp. found in rice ecosystem are not effective in all environmental condition. Among various *Trichogramma* spp., mainly four species *T. japonicum*, *T. chilonis*, *T. ostriniae* and *T. dendrolimi*, are commonly observed in rice fields in China (Guo *et al.*, 2012; Hong-xing *et al.*, 2017). *T. dendrolimi* performs well on parasitizing stem borer eggs at 18 to 26°C while *T. japonicum* performs well at 30 to 34°C (Yuan *et al.*, 2012). In India, it is reported that the inundative release of exotic parasitoid, *T. japonicum* @ 20,000 per acre was effective in reducing stem borer infestation (Pasalu *et al.*, 2004). Likewise, 4 to 9 times releases of *T. japonicum* @ 1,00,000 adults/ha starting from 20 to 38 days after transplanting with an gap of 7-10 days resulted in 4 to 59% reduction in leaf damage due to leaf folder (Pasalu *et al.*, 2004). But in a field test conducted in China reported that parasitism of yellow stem borer eggs, by *T. japonicum* was 9% whereas parasitism by *T. chilonis* was 15% (Tang *et al.*, 2017). It was also evident that *Trichogramma* species parasitized more new eggs of stem borer (<24 h old) compared to older eggs (>24 h old) (Babendreier *et al.*, 2020). Similarly, they parasitized 1–3 day old leaf folder eggs efficiently, but the parasitism of 4-day-old eggs was significantly low (Hong-xing *et al.*, 2017).

Use of microbial pesticides like *Bt (Bacillus thuringiensis),* virus, fungi are another useful approach for rice insect pest management as they are harmless to the humans, natural enemies and environment. Nayak *et al.*, 1978 studied the effect of *Bacillus thuringiensis* var. *Kurstaki* (Thuricide), against different stages of rice yellow stem borers, *S. incertulas* and found that *Bt* had no toxicity effect on egg, pupae and adult stages of stem borer whereas spraying *Bt* @ 1% concentration, at the time of hatching of the larvae, reduced the incidence of dead hearts and white heads by 76.36% and 67.45% respectively under green house conditions. Likewise, the efficacy of *Mamestra brassicae* nuclear polyhedrosis virus on leaf folder at 14 days after spraying was more than 83% (Hong-xing *et al.*, 2017). *Cnaphalocrocis medinalis* granulovirus (CnmeGV), showed synergism action with *Bt* against rice leaf folder (Liu *et al.*, 2013). The initial mortality of leaf folder treated by the agents consisted of CnmeGV and Bt was 3 day shorter than that solely treated with CnmeGV, the mortality was increased by 20.23%, and the persistent time was more than 30 days (Hong-xing *et al.*, 2017). In India, fungal pathogens mainly *Beauveria bassiana* was found promising against rice hispa (Hazarika and Puzari, 1997), whereas *Pandora delphacis* was found promising against BPH (Narayanasamy, 1995).

2.5 Integrated farming system

Despite the traditional rice cultivation, integrated rice farming with animal husbandry such as rice-duck, and rice-fish is an effective mutual benefitted combination because of their healthy co-development (Hong-xing *et al.*, 2017). In a rice-duck system, ducks are introduced into the rice fields to change the microclimate in field, reduce ineffective tillers, promote to enter more sunlight, gas exchange, improve soil health and reduce the insect pests (Long *et al*, 2013; Hong-xing *et al.*, 2017). It is found that, the rice planthoppers in the fourth and fifth generations are reduced by more than 70% in middle rice season and more than 50% in late rice season, respectively (Yang *et al.*, 2004). Similarly, the rice damage caused by stem borer was decreased by 13–47% in middle-season rice and by 62% in late-season rice (Hong-xing *et al.*, 2017). The rice-duck system also helped to increase the number of bio control agents which ultimately reduce the rice insect pests. Yang *et al.*, 2004 observed that the spider population in the rice-duck fields was 1.66–2.61 folds higher than that of the conventional rice fields. Likewise, the parasitization rates of leaf folder larvae were 53–61% in early-season rice field with duck and 29–38% in late-season rice field with duck (Hong-xing *et al.*, 2017). Similarly, rice fish farming also help sustainable rice production by decreasing input costs in terms of fertilizer and insecticide application as fish decreasing insect population by feeding them whereas enhance soil organic matter by their excreta (Ahmed and Garnett, 2011; Rahman, 2016). Although rice duck and rice fish integrated rice farming system is followed in different low lying areas of West Bengal and Assam but that should be popularize in other places in India for sustainable rice production.

2.6 Semio-chemicals

It is established fact that when plants are attack by arthropod herbivores they emanate volatiles chemicals which attract natural enemies (Bruce and Pickett, 2007). Some of those herbivore-induced plant volatiles (HIPV) have been identified, synthesized, used in slow-release dispensers or as sprays. It is evident that under field condition, methyl salicylate, cis-3-hexen-1-ol, (Z)-3-hexenyl acetate and benzaldehyde has resulted in more number of catches of natural enemies (James, 2005; Gurr, 2009). Plants attacked by *N. lugens* produced ethylene 2 to 24 hours after infestation along with HIPV as well as activates salicylate signaling pathways which ultimately affect the more parasitization by attracting *Anagrus nilaparvatae*, a major parasitoid of *N. lugens* (Gurr, 2009). So, the application of such exogenous products on rice plants can lead to more attraction of natural enemies which ultimately help in management of insect pests.

2.7 Ecological engineering techniques

The population size and outbreak frequency of insect pests can be effectively managed by habitat diversification through ecological engineering method (Lu *et al.*, 2015; Gurr *et al.*, 2016; Hong-xing *et al.*, 2017). It was observed that when rice fields were surrounded with nectar-rich flowering plants, more yields were obtained as well as higher natural enemies population were recorded in fields (Lu *et al.*, 2015). Actually, like other plants, rice lacks floral nectar resources that can be used by natural enemies. So, right selection and planting of nectar-rich flowering plants or vegetable patches in rice landscapes can provide year-round resource for natural enemies which not only improve their longevity and reproduction, but also increasing their biological control efficiency (Hong-xing *et al.*, 2017). For effective results, flowering plants should be planted on the bunds of rice fields before rice transplanting and new plantings should be done one month after rice transplanting so as to ensure flowering plants should be available at all rice growing stages (Lu *et al.*, 2015). In China, growing the flowering plant such as *Sesamum indicum*, *Impatiens balsamen*, *Emilia sonchifolia, Trida procumbens, Tagetes erecta* on rice field bund improved the biological management of planthoppers (Lu *et al.*, 2015). Y-tube olfactometer assays indicated that the egg parasitoids *Anagrus optabilis* and *A. nilaparvatae* were significantly attracted by the volatiles from sesame. Similarly, both of these two parasitoids significantly parasitized more BPH eggs in the presence of sesame flowers (Zhu *et al.*, 2015; Hong-xing *et al.*, 2017). Similarly, it was observed that with presence of sesame flowers, adult longevity of predatory bug *Cyrtorhinus lividipennis* was extended, which ultimately helped increased egg consumption and predation rate (Zhu *et al.*, 2015). Likewise, the fecundity of *Trichogramma chilonis*, a common egg parasitoid of many *Lepidopteran* insects, was significantly increased by sesame flowers (Hong-xing *et al.*, 2017). In India, when flowering plants like marigold, balsam and crops like sesame, sunflower were cultivated in rice bund then more numbers of spiders, mirid bug and parasitoids of planthoppers were found in rice fields (Anonymous, 2021). In Bangladesh, growing flowering plants such as sesame, marigold, sunflower and cosmos to rice bunds helped in higher abundance of natural enemies in rice fields and were responsible for more parasitism of planthopper, yellow stem borer, and rice hispa eggs than the broad-spectrum insecticide treated rice plots (Ali *et al.*, 2019). It was observed that yields in ecological engineering strategies adopted rice plots surrounded by sesame and nectar-rich flowering plants with no insecticides applied were at par with rice plots without ecological engineering and sprayed three times (Heong, 2011).

2.8 Botanicals

Use of botanicals is a novel approach as these are consider as harmless to the humans and environment. Unlike synthetic pesticides, botanicals do not kill the insect pests in field condition but reduce their activity by repellency, feeding deterrency, reproductive inhibition and oviposition deterrence (Pasalu *et al.*, 2004). Various greenhouse and field studies have reported that neem formulations are moderately effective against stem borer, leaf folder, plant and leafhoppers (Pasalu *et al.*, 2004; Seni and Naik, 2019). Neem seed kernel extract @ 0.001-0.4% were found effective to repellent the planthoppers (Misra and Jena, 2007). It was also observed that eucalyptus oil @ 1000 ml/ha was found promising against yellow stem borer and plant hoppers whereas Cedar wood oil @ 1000 ml/ha was against gall midge (Seni, 2019).

2.9 Chemical insecticides

Chemical control is one of the quickest and reliable tools of decreasing insect pest populations in rice, particularly in emergency situations where there is no suitable alternative.

Various studies also reported that insect pest outbreaks occurred due to the misuse of insecticides (IRRI, 2011; Ali *et al.*, 2019) which ultimately threatening the whole rice growing areas. Efficacy of chemical control technique depends on the right selection of active ingredient, suitable formulation and application methods on the knowledge of pest life cycle and crop phenology (Pasalu *et al.*, 2004). Beside this, information regarding the most vulnerable stage of the pest, pest intensity and their effect on yield as well as on natural enemies are also important for economic and successful pest management. Further, knowledge of the negative effects of pesticides to the users, consumers and environment is necessary. Among the insecticide formulations, granular formulations of chlorantraniliprole 0.4 GR @ 10 kg/ha, and fipronil 0.3 GR @ 12 kg/ha are effective against stem borer and leaf folder. Among spray chemicals, in situations where leaf folder and stem borer cause problem then cartap hydrochloride 50 SP @ 750 g/ha, fipronil 5 SC @ 1500 ml/ha and rynaxypyr 20 SC@ 150 ml/ha are useful (Seni and Naik, 2020). For plant and leaf hoppers flonicamid 50 WG @ 150 g/ha, pymetrozine 50 WG @ 300 g/ha and triflumezopyrim 10 SC @ 240 ml/ha are very effective (Seni and Naik, 2017; Seni et al., 2019; Seni and Naik, 2020). Farmers should use insecticides for management of rice insect pests only as last resort to avoid economic damage.

2.10 Nanotechnology

Nanotechnology opens up a wide range of opportunities in agriculture like plant protection through the formulations of nano-particle-based pesticides, increase of agricultural productivity by using bio-conjugated nanoparticles (encapsulation), nano based biomarkers which can detect damaging stage of the pest, nanoparticle-mediated gene or DNA transfer in plants for the development of insect resistant varieties. Beside this, nanoparticles can be used for preparation of various types of biosensor, which would be useful in remote sensing devices required for precision farming (Rai and Ingle, 2012). Using nanoparticles and nanocapsules of pesticides can decrease the environmental pollution by reduce the pesticide dose whereas enhance the efficacy of the pesticides. Goswami *et al.* (2010) studied the effects of various types of nanoparticles viz. silver nanoparticles (SNP), aluminium oxide (ANP), zinc oxide and titanium dioxide for the management of rice weevil, *Sitophilus oryzae* and after 7 days they found that 86% and 95% mortality with hydrophobic and hydrophilic SNP and 70% mortality of the insects was noticed when the rice was treated with lipophilic SNP whereas, 100 % mortality was observed in case of ANP. Similarly, Vani and Brindhaa (2013) reported 100% mortality of rice moth, *Corcyra cephalonica* when silica nanoparticle was tested against them.

2.11 Information technology

Proper use of information technology helps rice insect pest management more effectively and economically. Rice knowledge banks, which were developed and made available through specialist websites, could convey the knowledge in various aspects of rice production systems to farmers as well as other government extension workers. Knowledge banks are mainly digital information repositories with simplified retrieval interfaces which help users understand rice crop management, pest problems and, natural enemies as well as other beneficial insects (Horgan, 2017). Several national and international institutes maintain such websites. Several rice knowledge 'apps' have been made available to farmers through smartphones (i.e., IRRI Rice Knowledge Bank), while in others, farmers can get various diagnostic support from remote specialists after answering some questions or uploading photographs of potential pests encountered in their fields (i.e., IRRI Crop manager and IRRI Rice doctor). In India, National Centre for Integrated Pest Management (NCIPM), New Delhi has developed "e-National Pest reporting and alert system" based on the information collected directly from the farmer's fields and then data has been processed carefully so that the system can deliver the outcome immediately to the farming community through short messaging service (SMS) in their own language. Likewise, Kisan Call Centers, formed by Indian Government deliver extension services to the farming community by providing solution to their queries. Although such systems are incepted to help sustainable rice production through effective pest management but, main drawback is unavailability of trained staff always and financial support, and if not regulated properly, they could encourage unnecessary insecticide applications (Horgan, 2017). Government and other private support and proper monitoring for such remote extension activities are necessary to deliver effective results.

3. Conclusion

Excessive use of synthetic chemicals causes environmental pollution, detrimental to natural control agents and insecticidal residue in food grains. For this, some alternative techniques should be promoted to reduce the over reliance on chemical pesticides. For this, a series of eco-friendly techniques such as conservation and utilization of indigenous natural enemies through ecological engineering, integrated farming like rice duck system, use of pheromones and semio-chemicals, smart agronomic practices, resistant varieties are helpful to grow rice in sustainable manner. Regarding new techniques in host plant resistance, QTLs mapping and marker assisted selection has great role in development of resistant varieties but still many efforts are necessary to harness that technology effectively. While other new techniques like RNAi and genome editing have promising role in insect pests management strategy but, more research still needed to make them as a viable strategy.

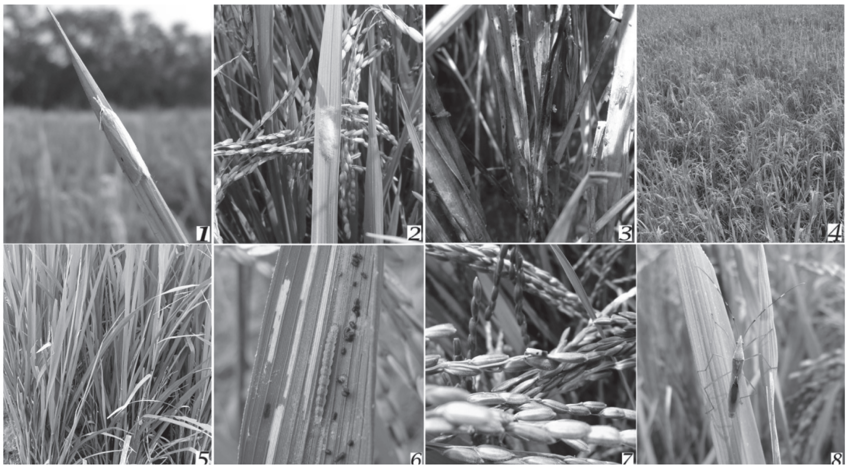

Fig. 2: *1;* Yellow stem borer adult, *2;* Egg mass of YSB, *3;* Plant hopper infested rice plant, *4;* Hopper burn symptom, *5;* Silver shoot produced by gall midge, *6;* Leaf folder larvae *7;* Green leaf hopper, *8;* Rice earhead bug

Conflict of Interest

NO

Ethical Compliance Statement

NA

Author contribution statement

Conceptualization of research and designing of experiments, Data collection and analysis, Preparation of manuscript (AS).

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Research Article

Detection of *Lr19/Sr25* in segregating populations of wheat (*Triticum aestivum* L.) using robust molecular markers

Dharam Pal[1*], Subhash Chander Bhardwaj[2], Hanif Khan[3], Madhu Patial[1], Ram Niwas Yadav[4], Pratima Sharma[1] and Shilpi Kumari[1]

[1]*ICAR-Indian Agricultural Research Institute, Regional Station, Tutikandi Facility, Shimla-171004, India*
[2]*ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla-171002, India*
[3]*ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, India*
[4]*ICAR-Indian Agricultural Research Institute, Regional Station, Karnal-132001, India*

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****Corresponding author:***
E-mail: dpwalia@rediffmail.com

Abstract

Marker assisted breeding (MAB) technology has been proved effective to transfer the genes of interest and also increased the accuracy of selection in wheat improvement programmes. The *Agropyron elongatum* derived *7D.7Ag* translocation carrying *Lr19/Sr25* is not only effective against leaf rust pathotypes but also effective against stem rust race *Ug99* and its variants. Therefore, wheat breeding lines carrying *Lr19/Sr25* translocation segment have been developed worldwide. The present study was aimed for developing wheat genotypes with *Lr19/Sr25* using *Xwmc221*, *PSY1-E1* and *Gb* DNA markers in segregating populations of wheat. We could infer that *Xwmc221*, *PSY1-E1* and *Gb* are effective molecular markers to tag *Lr19/Sr25* in segregating generations of wheat. The data also revealed consistency between host pathogen interaction (HPI) test and *Xwmc221*, *PSY1-E1* and *Gb* molecular markers for selecting lines with *Lr19/Sr25* in segregating generations. This work not only confirmed the robustness of the three *Lr19/Sr25* markers but also demonstrated the application of both genotyping and phenotyping in making full-proof selection of superior progenies with gene of interest in wheat.

Key words: rust resistance markers, MAB

1. Introduction

Among the three wheat rusts, leaf rust, caused by the *Puccinia triticina* is most common, widely distributed throughout the world (Kumar *et. al.*, 2021) and usually causes 10-15% yield loss and also decreases grain quality (Slikova *et al.*, 2003). Leaf rust (*Lr*) can inflict 50% loss in epidemic years (Anonymous, 1992). Among the various techniques to manage the wheat rusts, introgression of genes from novel sources of rust resistance is an effective and environment friendly strategy to combat leaf rust pathogen. As many as 80 *Lr* genes have been reported in wheat and its relatives (McIntosh *et al.*, 2020). Among the *Lr* genes, the *Agropyron elongatum* (Syn. *Thinopyrum ponticum or Lophopyrum elongatum*) derived *7D.7Ag* translocation carrying *Lr19/Sr25* provides protection against leaf rust in Northern India. A large number of recombinant lines using *7D.7Ag* translocation carrying *Lr19/Sr25* have been developed world wide. In India, two wheat varieties PBN142 (HD2189/NI917//Agatha) and WH533 (Agatha/Yacora17) have been released, probably carrying *Lr19/Sr25* due to the presence of Agatha in their pedigree lineage (Tomar *et al.*, 2014). Though virulence to *Lr19* designated as 77-8 has been reported from peninsular India (Bhardwaj *et al.*, 2005) but field population of leaf rust lacks virulence for *Lr19* during 2013-2016 surveys (Bhardwaj *et al.*, 2019). In recent surveys, virulence for *Lr19* has not been observed in India but it was identified in 0.27% of the samples from Nepal only (Bhardwaj *et al.*, 2021). The evolution of *Ug99* and its variants, virulent to many of the *Sr* genes including *Sr31* has created a fear of stem rust epidemics worldwide. The *7D.7Ag* translocation carrying *Lr19/Sr25* not only provides leaf rust resistance but also effective against *Ug99* and its variants (Singh *et al.,* 2011). Besides, it has positive effects on grain yield under favourable conditions (Monneveux *et al.,* 2003). However, the use of *Sr25/Lr19* germplasm was limited until development of a mutant line Agatha-28 (Knott, 1980) through mutation in the linked gene *PSY1-E1* causing undesirable yellow flour (Zhang and Dubcovsky, 2008).

In different studies molecular markers viz., *Xwmc221* (Prins *et al.*, 2001; Somers *et al.,* 2004), *PSY1-E1* (Zhang and Dubcovsky, 2008), *Gb* (Liu *et al.,* 2010) and host-pathogen interaction (HPI) tests have been used for selecting plants with *Lr19/Sr25* in segregating generations. As an outcome of our breeding efforts for leaf and stem rust resistance, segregating and fixed populations of wheat with *Lr19/Sr25* have been developed.

2. Materials and Methods

2.1 Plant material

The wheat cultivar HS240 and rust resistant genetic stocks, FLW20 (*Lr19/ Sr25*) and FLW13 (*Yr15*) were used to develop BC$_2$F$_1$s *viz*, HS240*2/FLW20, HS240*2/FLW13, separately. Both the BC$_2$F$_1$s were further inter crossed to generate F$_1$ (HS240*2/FLW20//HS240*2/FLW13) and subsequently selfed for developing F$_2$, F$_3$, F$_4$ generations. Based on rust phenotyping and marker linkage data, F$_4$ resistant progenies were advanced to F$_5$.

2.2 Seedling resistance tests

The material comprising parents, genetic stocks and segregating generations of the cross HS240*2/FLW20// HS240*2/FLW13 along with the sets of differentials were raised in a mixture of loam soil and farm yard manure using aluminium trays. Seven days old seedlings were inoculated with pure culture (5 mg uredospores per ml in light weight, non-phytotoxic isoparaffinic oil-Soltrol®) of virulent pathotypes *viz*, THTTM (121R63-1 or Ptr77-5), PHTTL (21R55 or Ptr104-2) of *P. triticina* and PTTSF (127G29 or Ptg40-3) of *P. graminis* of Indian sub-continent (Table 1). The inoculated seedlings were atomized with fine mist of water and placed in dew chambers for 48 hrs at 20±2^{0}C for initiation of infection. The seedlings were then shifted on to the green house benches, maintaining temperature 22±2°C for leaf rust and 25±2^{o}C for stem rust with 80% RH till recording of infection types (IT's). The seedling reaction for IT's of rusts was recorded after a fortnight following Stakman *et al.,* (1962).

Table 1: SRT to rust pathotypes and validating wheat genotypes for *Lr19/Sr25* using molecular markers.

S.No	Genotype*	Infection Score to Rust pathotypes			Response to mol. Marker		
		Ptr 77-5	***Ptr 104-2***	***Pgt 40-3***	***Xwmc221***	***PSY1-E1***	***Gb***
1	HS240	3+	3+	;2-	-	-	-
2	FLW20	0;	0;	;2-	+	+	+
3	*Tc+Lr19*	0;	0;	;2-	+	+	+
4	PBW343	3+	2+	2-	-	-	-
5	WBM3617	0;	0;	;1	+	+	+
6	WBM3618	0;	0;	;1	+	+	+
7	WBM3619	0;	;	;2-	+	+	+
8	22	0;	;	;1	+	+	+
9	150	0;	0;	;2+	+	-	+
10	155	0;	;	;2+	+	+	+
11	156	0;	;	;2-	+	+	+
12	157	0;	;	;2-	+	+	+
13	158	0;	0;	;2	+	+	+
14	159	0;	;	;2	+	+	+
15	160	0;	;	;2	+	+	+
16	161	0;	;	;2	+	+	+

17	162	0;	;-	;2-	+	+	+
18	163	0;	;-	;2	+	+	+
19	164	0;	NG	;2-	+	+	+
20	165	0;	0;	;2-	+	+	+
21	169	0;	0;	;2-	+	+	+
22	170	0;	0;	;2-	+	+	+
23	171	0;	;-	;2-	+	+	+
24	172	0;	;-	;2-	+	+	+
25	WBM3617	0;	0;	;1	+	+	+
26	WBM3618	0;	0;	;1	+	+	+
27	WBM3619	0;	;	;2-	+	+	+
28	19	0;	;-	;2-	+	+	+
29	20	0;	;-	;2-	+	+	+
30	21	0;	;-	;2-	+	+	+
31	22-1	0;	0;	;2-	+	+	+
32	23	3+, 0;	;	;2, 3P3-	+/-	+	+
33	24	0;	;-	;2-	+	+	+
34	25	0;	;-	;2-	+	+	+
35	26	0;	0;	;2-	+	+	+
36	27	0;	;-	;2-	+	+	+
37	28	0;	;-	;2-	+	+	+
38	29	0;	0;	;2-	+	+	+
39	30	0;	;-	;2-	+	+	+
40	31	0;	0;	;2-	+	+	+
41	32	0;	;	;1	+	+	-
42	33	0;	;-	;1	+	+	+
43	34	0;	;-	;2-	+	+	+
44	35	0;	;-	;2-	+	+	+
45	36	0;	;-	;2-	+	+	+
46	37	0;	;-	;2-	+	+	+
47	38	0;	;-	;2-	+	+	+
48	39	0;	0;	;2-	+	+	+
49	40	0;	;-	;1	+	+	+
50	43	0;	0;	0;	+	+	+
51	44	0;	;-	;1	+	+	+
52	45	0;	;-	;2-	+	+	+
53	46	0;	;-	;2-	+	+	+
54	47	0;	;-	;2-	+	+	+
55	48	3+	3	3+	-	-	-
56	49	3+	;1	3+	-	-	-
57	50	3+	;2+	3	-	-	-
58	51	0;	0;	;2-	+	+	+
59	56	0;	0;	;1	+	+	+
60	57	0;	0;	;1	+	+	+
61	58	0;	0;	;2-	+	+	+
62	71	0;	;	;1	+	+	+

63	134	0;	0;	;2-	+	+	+
64	140	0;	0;	;2-	+	+	+
65	141	0;	;	;2-	+	+	+
66	142	0;	0;	;2-	+	+	+
67	143	0;	;-	;2-	+	+	+
68	146	0;	;-	;2-	+	+	+
69	147	0;	;-	;2-	+	+	+
70	148	0;	;-	;2-	+	+	+

*1-4 Parents & genetic stocks, 5-7 &25-27 F5 lines, 8-24 & 28-70F4 lines, Infection score of rust pathotypes recorded according to Stakman et al. 1962.
Infection score 0; (naught fleck) / ; (fleck) / ;1, ;2-, 2, 2+ =Resistant, 3-, 3, 3+=Susceptible

2.3 Molecular markers used and DNA isolation

Closely linked molecular markers *viz*, *Xwmc221*, *PSY1-E1* and *Gb* were used to tag *Lr19/Sr25* in segregating populations of cross HS240*2/FLW20//HS240*2/FLW13. DNA was extracted by CTAB method (Rogers and Bendich, 1985). PCR amplification with the primers for *Lr19* was performed in a 20 µl reaction mixture containing 10mM Tris-HCL (pH 8.8), 50 mM KCl, 2 mM MgCl$_2$, 0.1 mM of each dNTPs, 0.75 U *Taq* DNA Polymerase, 22 ng of each of SSR primer and 40 ng genomic DNA. PCR for *Xwmc221* was performed in a Thermal Cycler programmed for 10 minutes at 95°C, 35 cycles [94 °C 1 min, 1 min at annealing temperature (Table 2), 72 °C 1 min] followed by final extension for 7 minutes at 72 °C. The amplified products were separated on 2.5 % high resolution agarose gel, stained with ethidium bromide and visualized in Vilber Lourmet gel documentation system. Allele scoring was performed using Gene Mapper v 4.0 software (Applied Biosystems). Negative controls DNAs were included for comparison in the marker analysis and observations were repeated to ascertain the accuracy of the results.

Table 2: Markers closely linked with rust resistance genes *Lr19/Sr25*, their primer sequences, amplicon size and PCR conditions or annealing temp.

Marker	Type	Sequence(5' -3')	Amplicon size (bp)	AT °C	Reference
Xwmc221	SSR	F ACGATAATGCAGCGGGGAAT R GCTGGGATCAAGGGATCAAT	190	61	Gupta *et al.*, 2006
Gb	STS	F CATCCTTGGGGACCTC R CCAGCTCGCATACATCCA	130	60	Liu *et al.*, 2010
PSY1- E1	STS	F CTACGTTGCGGGCACCGTT R AGAGAAAACCATTGCATCTGTA	191	60 TD	Zhang and Dubcovsky, 2008

AT-annealing temperature; TD-touch down.

3. Results and Discussion

The plants of the cross HS240*2/FLW20//HS240*2/FLW13 selected for *Lr19/Sr25* were carried forward through F$_2$, F$_3$, F$_4$ and F$_5$ generations. Poor performing plants were rejected and the selected F$_1$ plants were selfed to obtain F$_2$. Two hundred and ninety-seven F$_2$ plants were analysed for *Lr19* using *Xwmc221* and positive plants (Fig.1) were carried forward to develop F$_3$ generation. Twenty-three F$_3$ lines were selected and validated for presence of *Lr19* using *Xwmc221* and all were recorded positive for *Lr19* (Fig. 2). Seventy genotypes comprising parents, test stocks, ear to row progenies of F$_4$ and selected F$_5$ bulks were evaluated for seedling resistance to leaf rust pathotypes THTTM (Ptr77-5) and PHTTL (Ptr104-2) and stem rust pathotype PTTSF (Pgt40-3) through HPI test and validated for the presence of *Lr19/Sr25* using molecular markers *Xwmc221, PSY1-E1* and *Gb* (Table 1). Among 60 F$_4$ progenies, 56 lines showed resistant reaction (0; naught fleck), one line (F$_4$-23) showed segregating reaction and three lines (F$_4$-48, F$_4$-49, F$_4$-50) were observed as susceptible (3+) in HPI test to the Ptr77-5. F$_5$ bulks (WBM3617, WBM3618, WBM3619) and F$_4$ progenies except genotype F$_4$-48 were found to be resistant in HPI test to pathotype 104-2, whereas, among 60 F$_4$ progenies, 56 showed resistant reaction. Line F$_4$-23 showed segregating reaction and three lines (F_4-48, F_4-49, F_4-50) were observed as susceptible to the Ptg40-3 (Table 1).

On marker analysis, 56 F_4 progenies were positive for *Xwmc221*, *PSY1-E1*, *Gb* and therefore, validated to carry *Lr19/Sr25* gene (Fig 3-5). Three genotypes (F_4-48, F_4-49, F_4-50) were negative for all the three markers and were also found to be susceptible in HPI test to pathotype Ptr104-2 and Pgt 40-3. One genotype F_4-23 at lane #32 showed two polymorphic fragments of size 190 bp and 209 bp, indicating its heterozygous status with *Xwmc221*. Similar results were also reported by Singh *et al.* (2017) for transferring *Lr19/Sr25* in wheat variety HD2733 using *Xwmc221*. Three F_5 bulks *viz*., WBM3617, WBM3618, WBM3619 which were replicated twice, showed a resistant reaction (;0) in HPI test to the pathotype Ptr77-5 and were also positive for microsatellite markers *Xwmc221*, *PSY1-E1* and *Gb,* indicated the presence of carry *Lr19/Sr25* linked genes.

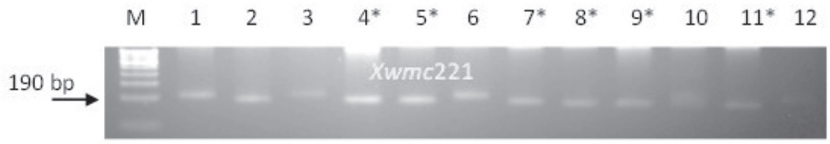

Fig 1. Lane M – 100bp ladder, lanes 1-HS240; 2-FLW20 (*Lr*19); 3-12 representative F_2 population (*Plants positive for *Lr*19)

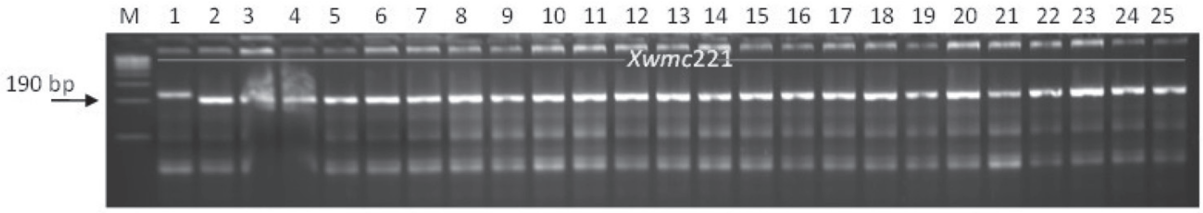

Fig 2. Lane M – 100bp ladder, lanes P1-HS240; P2-FLW20 (*Lr*19); 3-25 representative F3 lines positive for *Lr*19.

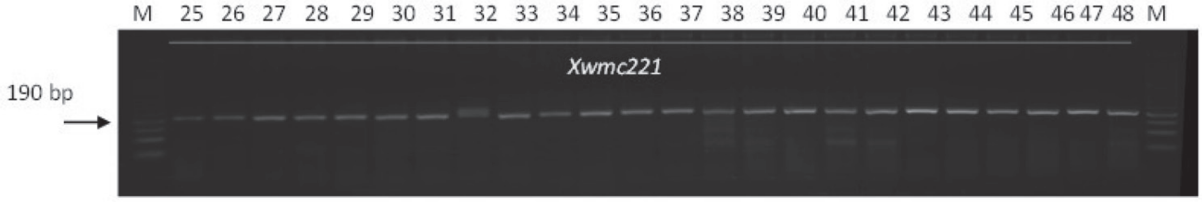

Fig 3. Lane M – 50 Kb ladder, lanes: 25-27 (F5), 28-48 (F4), *Lr*19 positive lines except lane 32 (Line F4-23).

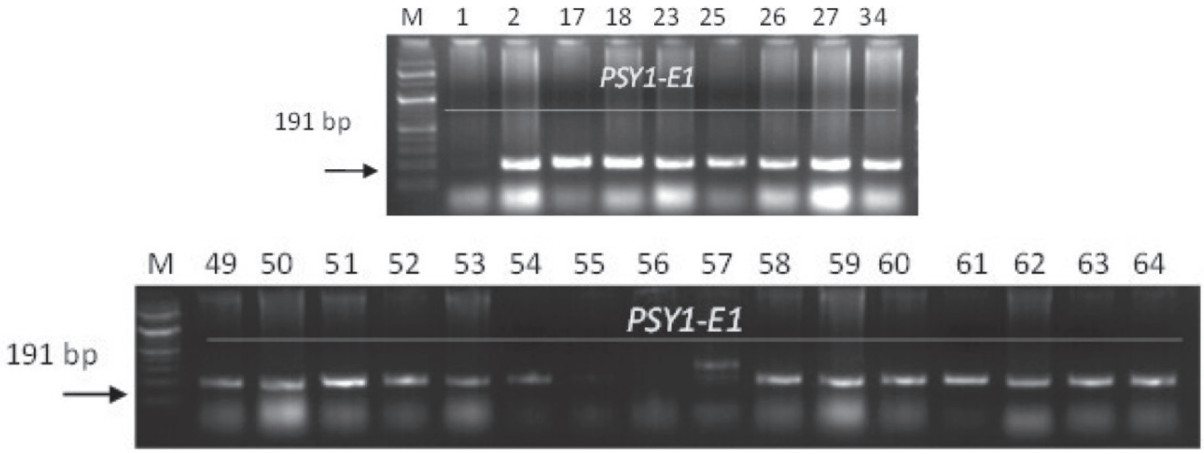

Fig 4a & b- Lane M – 100bp ladder, lanes 1-HS240; 2-FLW20 (Lr19/Sr25); 17, 18, 23, 25, 26, 27, 34, 49-64 representative F4 lines positive for Sr25.

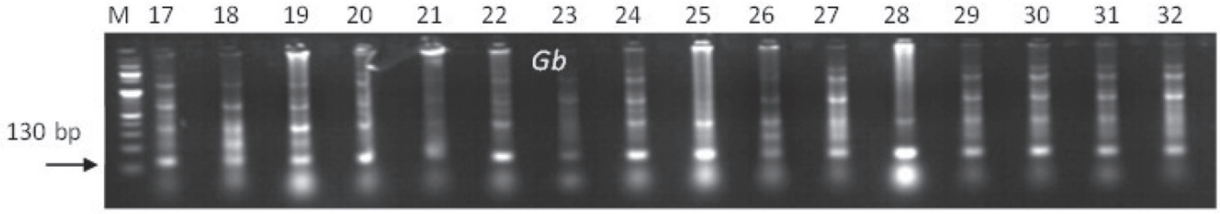

Fig 5- Lane M – 100 bp ladder, lanes: 17-32F4 Sr25 positive lines.

In the present study, we could identify, differentiate and substantiate homozygous and heterozygous resistant plants in different segregating generations for *Lr19/Sr25* using three highly effective microsatellite marker*s*. It has helped in selecting lines with confirmed *Lr19/Sr25* in segregating generations of wheat cross. Microsatellite markers were also used to confirm the presence of *Lr19* in winter wheat cultivars by Tomkowiak *et al.* (2016). In our study, one F_4 line #23 was found to be heterozygous with co-dominant marker *Xwmc221*.

The same line was recorded as positive carrier by two other markers, namely, *PSY1-E1* and *Gb* because of their dominant nature. A dominant SCAR marker $SCS265_{512}$ was used previously for validating *Lr19* (Pal *et al.*, 2015) and microsatellite marker *Sr24*#12 for tagging *Sr24/Lr24* in back cross generations of wheat (Kumaran *et al.*, 2021).

The role of co-dominant microsatellite marker *Xwmc221* was also advocated by Gupta *et al.* (2006) and Singh *et al.* (2017) for identifying heterozygotes and suggested it as an important tool for rapid transfer of *Lr19* into wheat cultivars. *Sr25*, tightly linked to *Lr19*, was counter validated using marker *PSY1-E1* and *Gb* giving an additional support for the validation of *Lr19.* Our results showed consistency between seedling resistance test and molecular marker assisted selection of genotypes for *Lr19/Sr25* using microsatellite markers, *Xwmc221*, *PSY1-E1* and *Gb*. The use of HPI test and molecular markers for validation has resulted in precise and perfect selection of the targeted rust resistance gene in the wheat breeding programme. It is concluded from the present study that *Xwmc221*, *PSY1-E1* and *Gb* are all robust markers to tag *Lr19/Sr25* in rust resistance wheat breeding programme.

A number of earlier workers have advocated the role of *Lr19/Sr25* in wheat improvement programmes. The *7D.7Ag* segment with *Lr19/Sr25* is also known to increase biomass and grain yield by 14% and 20% respectively, in wheat genotypes, Borlaug and Oasis (Singh *et al.,* 1998). A significant increase in yield, biomass and grain number was also reported by Reynolds *et al* (2001) with introgression of *Lr19* in all genetic backgrounds. In another study, *7D.7Ag* translocation increased grain yield potential by 10-15 per cent in a range of genotypes (Singh *et al.,* 2006). Breeding lines with *Lr19/Sr25* from Agatha and Sears' translocations were characterized by high levels of yellow pigment in the endosperm. Mutant lines carrying *Lr19/Sr25* with reduced level of yellow pigmentation in flour were obtained (Knott, 1980). The parent FLW20 involved in hybridization carry *Lr19/ Sr25* from Agatha and therefore, the derived progenies do not possess undesirable trait yellow flour. This work not only confirmed the robustness of three *Lr19/ Sr25* markers for developing leaf and stem rust resistant plants in segregating wheat material but also demonstrated the application of both genotyping and phenotyping in making full-proof selection of superior progenies in wheat.

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Conflict of Interest

Authors declares that they do not have any conflict of interest

Ethical Compliance Statement

NA

Authors Contribution

Conceptualization of research and designing of experiments (DP, SCB), Molecular marker validation (HK, SK, PS), Seedling resistance tests (SCB), Preparation of manuscript (DP, SCB, MP, RN).

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Research Article

Relay cropping of cucurbits in furrows under bed planted wheat for higher profitability

Subhash Chander Tripathi, Raj Pal Meena and Subhash Chander

ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, India

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Corresponding author:
E-mail: subhtripathi@gmail.com

Abstract

A field experiment was condcuted for four consecutive seasons from 2016-17 to 2019-20 at ICAR- Indian Institute of Wheat and Barley Research, Karnal, Haryana with an objective to increase the profitability through relay crops of cucurbits under bed planted wheat crop. Eight relay crops namely musk melon, water melon, bottle guard, ridge guard, bitter guard, cucumber (kheera), cucumber (tarkakari) were planted under bed planting system with wheat. All the cucurbits were relayed in furrows at milk stage of wheat. Combined over four years analysis revealed that wheat + bottle guard produced maximum and signifiantly higher wheat equivalent yield (WEY), gross return, net return and B: C ratio, which was 80.5%, 80.5%, 227.1% and 80.0% higher than sole wheat crop, respectively. Next best profitable treatment was wheat + cucumber (tarkakari), which also produced 39.8%, 112.3% and 40.0% higher WEY, net return and B: C ratio as compared to sole wheat crop, respectively. All the relayed cucurbits were profitable than sole wheat crop and there was no adverse effect on yield attributes and wheat yield . Thus, small and marginal farmers can adopt this relayed cropping sytem to maximise their profit per unit time and space.

Key words: Bed planting, Cucurbits, Profitability, Relay cropping, Wheat

1. Introduction

The small and marginal farmers are incrseaing rapidly and more than 85 per cent farmers belongs to this category in India (Tripathi *et al.*, 2017) . This situation is much more severe in north eastern plain zone (NEPZ). Keeping in mind the slogan of prime minister regarding the doubling the farmers income, land productivity per unit area per unit time requires immediate attention. This will be possible by adopting multiple cropping which increases the profit per unit area and per unit time (Seran and Brintha, 2010; Khan *et al.*, 2014). The small and marginal farmer of NEPZ practice wheat seeding mainly by broadcasting, which is detrimental to wheat yield, may achieve higher spatial and temporal productivity by adopting Furrow Irrigated Raised Bed (FIRB) technology and relay cropping of cucurbits with wheat. In present circumstances, it is important to reduce the cost of production in systematic manner and to make the produce competitive in the global market. In this endeavor, Furrow Irrigated Raised Bed (FIRB) technology can be a stepping stone in encouraging for enhancing the income of farmers. In this planting system, crops are grown on top of beds 40 cm and furrows are utilized as input management zone. This cost will be further reduced if same beds are reshaped for succeeding crops. This technology saves 25 to 50 % seed, 25 % N and 15 to 40 % water in wheat over recommended level (Chauhan *et al.*, 2001). Besides this, it has additional advantages like intercultural operations in standing crop, N placement between the rows by suitable machinery instead of top dressing, manual weeding, lesser *Phalaris minor* germination on the top of beds and reduction in lodging

(Tripathi *et al.*, 2001). Adoption of bed planting for wheat cultivation provides an opportunity to utilize the furrow for intercropping or relay cropping of suitable crops in order to achieve higher profitability. Many intercropping crops like sugarcane, vegetables, seed spices, mentha etc can be grown in furrows along with wheat under bed planting (Sayre and Moreno Ramos, 1997). Diversifying crop rotation improves system robustness through increasing crop resistance and resilience from biotic-induced disturbances and the most diversified cropping systems had a 14% advantage in system robustness (Li *et al,* 2019).

The aim of researcher should be to maximise the profitabilitry of small and marginal farmers without much increase in cost of cultivation. Even small increase in profit of poor farmers will have an impact on their livelihoods. To utilise furrows for relay cropping of cucurbits in wheat under bed planting is one of the options to explore profit maximisation strategy for poor farmers. Keeping this as preamble, number of cucurbits were tried in furrows during milk stage of wheat to maximise the system profitability.

2. Materials and Methods

A field experiment was conducted during *Rabi s*easons of 2016-17 to 2019-20 at ICAR-Indian Institute of Wheat & Barley Research, Karnal (Latitude 29^0 43' N, longitude 76^0 58' E and altitude 245 m) in randomized block design with three replications. The soil was moderately well drained coarse textured sandy loam (62.1% sand, 26.6% silt and 11.3% clay) with low to moderate fertility. Baseline soil samples were collected (0–15 cm depth) from each test site (three) at the start of experiment and analyzed for pH (using a soil water solution of 1:2.5 wt/v), soil organic carbon (Walkley and Black, 1934), available N (Jackson, 1958), available P (Olsen, 1954) and available K (Merwin and Peech, 1951). The soil was having 0.3 % organic carbon, 113.9 kg/ha available N, 12.4 kg/ha available P and 154.9 kg/ha available K with an alkaline pH of 8.3 and EC of 0.13 dsm^{-1}. Meteorogical data recorded during experimental period is represented in figure 1. No major weather extremities were observed and all weather parameters were congenial during crop growth period except rainfall during 2019-20. Wheat variety HD 2967 was sown under bed planting method using recommended rate of seed. At milk stage of wheat, seedlings of musk melon (*Cucumis melo*), water melon (*Citrullus lanatus*), bottle guard (*Lagenaria siceraria*), ridge guard (*Luffa acutangula*), bitter guard (*Momordica charantia*), cucumber (*Cucumis sativus*) and armenian cucumber (*Cucumis melo* var. *flexuosus*) were transplanted. Besides, sole wheat was raised for their comparison. These cucrbits were planted at spacing of 1.0 m between the plant to plant and 1.4 m between the rows consiting of four beds. These were picked up to June in each year. Before transplanting, seedlings of all these relayed crops were raised separately in control environment. All the plants of relay crops survived and started spreading over soil surface. However, spreading speed was increased after wheat harvest. After harvesting of preceding rice crop, the field was prepared using cultivator and disk and seeding was done as per the treatments. Recommended dose of fertilizer (150 kg N, 60 kg P_2O_5 and 40 kg K_2O/ha) was applied to the wheat crop. Full dose of phosphorous in the form of diammonium phosphate and potash in the form of muriate of potash and one third dose of nitrogen in the form of urea was applied as basal i.e before sowing. Remaining two third dose of nitrogen was top dressed in two splits at 1st node stage (DC 31) (Zadoks *et al.*, 1974) and at boot stage (DC 41). Other standard agronomic practices were done as per package of practices for the area. Observations were recorded on above ground biomass, yield and yield attributing characters. Grain yield was calculated from net plot area and converted into q/ha. Bearings of musk melon, water melon, bottle guard, ridge guard bitter guard, and cucumber were taken as yield of these crops. In case of wheat, harvest index (HI) was calculated by dividing grain yield with biomass. Number of earhead per meter row length was counted at two places in each plot and converted to per m^2. Thousand grains weight (TGW) was calculated by taking random grain samples and counted by using Contador electronic seed counter (Pfeuffer, Germany) and weighed. Cost of cultivation was calculated by taking into account the prevailing price of inputs like fertilizer, seed, herbicides, irrigations, tillage operations, transportation charges, management charges, rental value of land and depreciation cost of implements. Economic returns were calculated by taking minimum support price of wheat grain and market price of wheat straw and cucurbits. System productivity in terms of wheat equivalent yield (WEY) was calculated by multiplying yield with minimum support price/market price of each crop in a cropping sequence and subsequently adding and thereafter divided by price of one quintal wheat. The experimental data on wheat yield and yield attributing parameters, WEY, cucurbits yield and economics were subjected to standard statistical analysis as outlined by Gomez and Gomez (1984).

3. Results and discussion

3.1 Year effect

In 2018-19, biomass, grain yield, HI, thousand grain weight, grains/earhead, cucurbits yield, WEY, gross return, net return and B:C ratio were maximum whereas number of earhead/m^2 was lowest as compared to other years. However, system productivity in terms of wheat equivalent yield, gross return, net return and B:C ratio of 2018-19 found non significant with 2016-17. Contrarily, year 2018 recorded significantly lower values with respect to all above mentioned parameters when compared to all other experimental years. This yearly variation in yield and yield parameters was might be due to variation in weather parameters like rainfall and minimum and maximum temperatures during crop growth period (Figure 1). More number of rainy events, higher amount of rainfall and lowering of maximum temperatures during March and April of 2018-19 might attributed the higher yield and yield parameters.

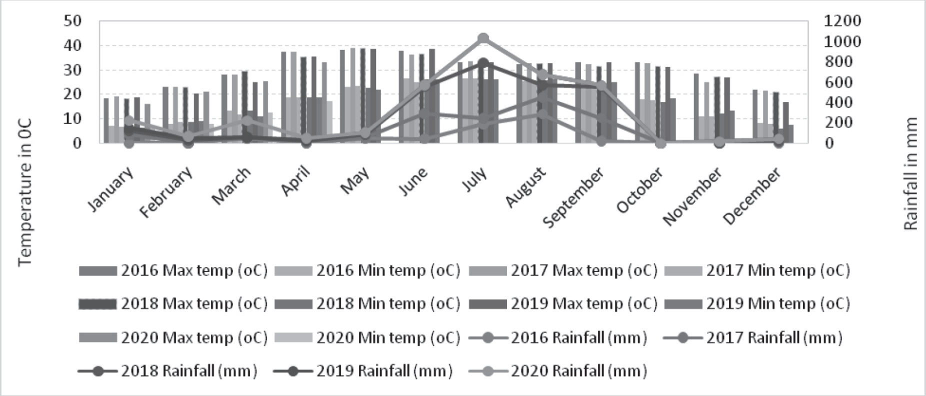

Figure 1. Maximum and minimum temperature and rainfall during the cropping years

3.2 Wheat yield, cucurbits yield and system productivity

Pooled analysis revealed that, relay cropping of cucurbits didn't show any significant effect on wheat yield and yield attributes (Table 1). Among the all cucurbits studied, superior cucurbit yield was obtained under wheat + bottle guard (100.22 q/ha) as compared to other relayed cucurbits. This higher cucurbit yield of bottle guard was mainly attributed to its high yielding potential over other cucurbits. However, wheat + Armenian cucumber was next best combination. On the contrary, the lowest yield was recorded under wheat + cucumber (10.37 q/ha).

Table1. Wheat yield and yield attributes of relayed crops in pooled analysis of four years

Treatments	Biomass (q/ha)	Yield (q/ha)	HI	Earhead/ m^2	1000 grain weight, g	Grains/ earhead
2016-17	146.18	59.55	0.408	450.7	39.19	33.74
2017-18	121.03	50.33	0.416	368.5	41.04	33.37
2018-19	146.15	62.44	0.427	374.0	46.63	36.17
2019-20	130.75	52.56	0.403	479.9	38.83	28.38
CD ($P \leq 0.05$)	3.26	1.57	0.006	16.3	0.61	1.74
Relay cropping						
1. Wheat+musk melon	136.17	55.55	0.407	415.0	41.37	32.6
2. Wheat+-water melon	136.14	56.53	0.417	415.4	41.45	33.2
3. Wheat+bottle guard	133.37	54.98	0.413	418.2	41.08	32.4
4. Wheat+ridge guard	135.83	56.33	0.414	421.3	41.19	32.8
5. Wheat+bitter guard	133.17	55.47	0.417	416.3	41.72	32.6
6.Wheat+cucumber	138.34	54.41	0.408	419.4	41.48	32.7

7. Wheat+ Armenian cucumber	136.20	56.82	0.418	423.2	41.62	32.9
8. Wheat sole	138.99	57.65	0.414	417.7	41.47	34.0
CD (P≤0.05)	NS	NS	NS	NS	NS	NS

All the relayed cucurbits produced higher system productivity in terms of wheat equivalent yield (WEY) than wheat sole crop. Maximum and significantly higher WEY was obtained by wheat + bottle guard (131.1 q/ha), which was 80.5% higher than sole wheat crop. Next highest treatment was wheat + Armenian cucumber which also produced 39.8% higher than sole wheat crop. Significantly the lowest yield was obtained with wheat + cucumber, which was at par with sole wheat crop (Table 2). This was mainly because of higher cucurbit yield and its higher market price. In all the four years, this was the trend where wheat + bottle guard produced maximum WEY followed by wheat + Armenian cucumber, both were significantly higher than all other treatments. These findings are in harmony with the results of Dua *et al.* (2007) and Talukder *et al.* (2016). Tripathi *et al.* (2017) also reported 36.37% higher wheat equivalent yield (92.46 q/ha) under intercropping of wheat on bed + radish in furrow over sole wheat crop. Similarly, Sharma *et al.* (2017) also recorded significantly higher rice equivalent yield with relay cropping of pea at 15 days after flowering of *kharif* rice.

Table 2. WEY, cucurbits yield and economics of relayed crops in pooled analysis of four years

Treatments	Wheat Equivalent Yield (q/ha)				Pooled	Cucurbits yield (q/ha)	Return (Rs/ha)	Net return (Rs/ha)	B:C
	2016-17	2017-18	2018-19	2019-20					
2016-17					100.30		162990	86739	2.14
2017-18					77.47		125883	49633	1.65
2018-19					102.65		166815	90565	2.19
2019-20					88.85		144384	68134	1.89
CD (P≤0.05)					2.46		3997	3997	0.29
Relay cropping									
1. Wheat+musk melon	86.4	73.0	87.0	70.7	79.27	14.36	128813	52563	1.69
2. Wheat+-water melon	93.2	76.5	97.7	105.6	93.25	35.78	151532	75282	1.98
3. Wheat+bottle guard	144.3	98.1	145.7	136.4	131.14	100.22	213094	136844	2.79
4. Wheat+ridge guard	106.4	73.2	110.4	85.1	93.78	37.01	152403	76153	2.00
5. Wheat+bitter guard	102.1	75.1	105.9	72.6	88.93	31.05	144512	68262	1.89
6.Wheat+cucumber	82.7	67.7	86.5	74.7	77.92	10.37	126617	50367	1.66
7. Wheat+ Armenian cucumber	110.3	87.4	110.9	97.7	101.59	48.93	16508.	88833	2.17
8. Wheat sole	76.9	68.8	77.0	68.0	72.67	0.0	118090	41840	1.55
CD (P≤0.05)	6.56	8.14	6.06	6.82	3.24	-	5264	5264	0.07

3.3 Economics

Wheat+bottle guard relay cropping recorded significantly the highest gross return (Rs. 213094/ha, net return (Rs. 136844/ha) and B:C ratio (2.79) as compared to other relay systems (Table 2). Increase in gross return, net return and B:C ratio of wheat + bottle guard relay system over sole wheat crop was to the extent of 80.5% 227.1% and 80.0%, respectively. Next best treatment was wheat + Armenian cucumber which recorded 112.3 % of higher net return and 40.0 % of higher B: C ratio over sole wheat crop. In contrast, the lowest economic return among the relayed crops was obtained by wheat + cucumber (kheera) and it was found non significant with sole wheat crop. These results are in accordance with the findings of Ali *et al.* (2015) and Habimana *et al.* (2019). In similar kind of study, Tripathi *et al.* (2017) also reported that growing of radish in furrows and wheat on to top bed was more profitable than sole wheat crop.

In nut shell, this technology is a boon to small and marginal farmers to maximize their profits through relayed cropping of cucurbits under bed planted wheat crop.

Conflict of Interest

Authors declares that they do not have any conflict of interest

Ethical Compliance Statement

NA

Authors Contribution

Conceptualization of research and designing of experiments (SCT), Data collection and analysis (SCT, RPM), Preparation of manuscript (SCT, RPM, SC).

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Research Article

Stability analysis of quantitative and qualitative traits in heritage rice landrace Zag (red rice) of Kashmir Himalayas

Gazala Hassan Khan[1], Najeebul Rehman Sofi[1], Asif Bashir Shikari[1], Shabir Hussain Wani*[1], Raheel Khan[2], Ashaq Hussain[1], Fayaz Mohiddin[1], Mehdi Rahimi[3] and Nazir Ahmed Bhat[1]

*[*1]Mountain Research Centre for Field Crops, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Khudwani, 192101, Srinagar, J&K, India*
[2]Division of Genetics and Plant Breeding, FoA Wadura, SKUAST Kashmir
[3]Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran.

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Corresponding author:
E-mail: shabirhussainwani@gmail.com

Abstract

Eighteen red rice collections from different sites of the Kashmir Himalayas valley were evaluated during *kharif*- 2017, 2018 and 2019 for stability performance for yield along with its related traits and cooking quality traits. The genotype effect had a significant mean square for all traits except panicle length based on analysis of variance. The genotype×environment interaction showed a significant difference for some of the studied traits, which included kernel elongation ratio, grain yield, number of panicles, 1000 seed weight, kernel length before cooking and kernel length after cooking. When the environment + (G × E) interaction is divided into environment (linear), G x E (linear) and congruent deviation, the mean square by environment (linear) shows that all attributes are taken into consideration. These results showed a significant difference between the environments that can have a great impact on the expression of the studied traits. Similarly, G x E (linear) was observed to be significant for all traits except 50% flowering days, plant height and maturity days, which means that the genotype behaviour of these traits is environmentally predictable and indicates that this is the function of the components of the linear environment. Mean squares due to pooled deviation (nonlinearity) were significant for all traits except 1000 grain weight and kernel breadth before cooking, suggesting that nonlinear composition is important for characteristics that contribute to the total G×E interaction. Thus, the genotypes differ significantly in terms of stability to the environment for such traits. C-5 has a low average for flowering days up to 50% and ripening days relative to the population mean, the row also has a uniform regression coefficient and the least deviation from the regression of yield showing consistent behaviour of the stream over the years. Likewise, C14 has a uniform regression coefficient and the least deviation from the regression for yield as well as a low mean for days up to 50% flowering and days until ripening, showed suitability of the line for consistent yield and early maturity.

Key words: Stability; Eberhart and Russel model; Zag; Red Rice; Adaptability

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for most people and the most important cereal crop and is widely cultivated in many parts of the world and is associated with economic, social and cultural heritage of the region (Amir et al 2019). Rice is grown under various agro climatic conditions thus having greater variability in local germplasm which needs to evaluated (Divya *et al.,* 2020). Rice cultivars with red pericarp are prevalent in the Southern, Eastern, and the hilly tracts of the North-East and North-Western parts of India. The bran layer is rich in polyphenols and anthocyanins which confer antioxidant properties besides characteristic red hue to rice kernel (Chaudhary, 2003). Kashmir valley inhabits number of rice landraces such as *Zag, Nunbeoul, Noor Miri, Qadir Beigh, Kawkadur, Kamad, Mushk Budji* and several others. Of these a red rice, *Zag* is an important landrace of Kashmir known for its red colour and for its high iron and zinc content compared to the white rice varieties (Khan *et al.*, 2020). The Karnah Tehsil of district Kupwara of Kashmir valley is recognized as geographical niche where *'Zag',* a rare red rice landrace, was used to be grown in more than 5000 ha area at an altitude of 1900 m msl (34°02 N latitude and 74°32 E longitude) few decades back. Apart from this, the *Zag* rice landrace is cultivated sporadically elsewhere also in the valley.

Since yield is a quantitative trait, and is highly dependent on the environment, it is rewarding to select a superior genotype based on yield itself when conducted in different environments or over several years (Shrestha *et al.,* 2012) and a stable genotype has potential to perform equally under different environments and years (Suresh et al 2020). Stability assessments in determining genotype performance in different environments can help recommend varieties for general or specific cultivation. Consistency of harvest potential and performance of varieties in different environments are highly desirable (Manjunatha et al, 2018). A number of ecotypes of red rice collected from different identified niches of Kashmir Himalayas were collected, evaluated and conserved at Khudwani centre of Shere Kashmir University. The ear to row method was adopted for their purification followed by multiplication. The varietal trial was constituted and conducted in 2017 and repeated in 2018 and 2019 to identify promising lines with respect to consistency in yield and other associated parameters. In this connection, red rice samples from different corners of the valley were collected and tested for their stability. The objective was to test the collections for their consistency in yield, early maturity *vis. a vis.* for general and specific adaptability.

2. Material and Methods

The experimental materials comprised 18 red rice collections from different ecological niches of the valley (designated as C1 to C18). Access sessions were grown in random block designs replicated 3 times on the experimental farm of MRCFC Khudwani at SKUASTK in spring season 2017, 2018 and 2019. The Khudwani centre is located between elevation 33° 70`N latitude and 75°10`E longitude with average of 1590 meters (amsl) above sea level. Five rows were used for planting each cultivar, the length of each row was 3 meters and the distance between the rows was 20 cm. A standard practice package has been employed to grow healthy crops.

Morphological agronomic traits studied in this study included 13 traits which are: length: breadth ratio (LBR), plant height (PH), number of effective tillers (NT), panicle length (PL), spikelets per panicle (SP), grain yield per plant (GY), spikelet fertility (SF), kernel length before cooking (KLBC), kernel elongation ratio (KER), kernel breadth before cooking (KBBC), kernel length after cooking (KLAC), kernel breadth after cooking (KBAC) and aroma. Twenty grains were randomly sampled from each replication and were dehusked by mini-huller and polished by mini-rice polisher (Kett, Japan). Brown rice was used for kernel length before cooking (KLBC) and kernel breadth before cooking (KBBC) and these traits were measured on them. Also, by dividing the average length by the average width of rice kernel, the L/B ratio was calculated. The method of Juliano *et al.* (1966) was also used to measure the KLAC trait. The method of Murthy (1965) was used to measure the Kernel elongation ratio (ER) trait and this ratio was obtained by dividing the average length of cooked kernel by the average length of the raw rice.

The stability parameters were calculated based on the proposed linear model of Eberhart and Russell (1966) and observations on the phenotypic performance of the 18 genotypes studied in the years 2017, 2018 and 2019 were used for this analysis and was performed using WINDOSAT 9.1.

3. Results and Discussion

In this study, the results of analysis of variance showed that the studied traits (days to 50% flowering, plant height (cm), number of panicles, days to maturity, grain yield, 1000 seed weight, KLBC, KLAC, KBBC, KBAC, KER except panicle length) have very significant mean squares for genotypes. It was found that the mean square of the environment is very important for all characteristics that reveal the various performance of the whole environment. For some traits such as number of panicles, grain yield and 1000 seed weight, KLBC, KLAC and KER, the genotype × environment interaction was significant. When the environment + (G × E) interaction is divided into environment (linear), G x E (linear) and congruent deviation, the mean square by environment (linear) shows that all attributes are taken into consideration. This confirmed that there was a significant difference between the environments and had a great influence on the expression of studied traits. Similarly, G x E (linear) was observed to be significant for all traits except 50% flowering days, plant height and maturity days, which means that the genotype behaviour of these traits is environmentally predictable and indicates that this is the function of the components of the linear environment. For all studied traits, the mean squares due to the pooled deviation (nonlinearity) was significant except 1000 grain weight and KLBC, suggesting that nonlinear composition is important for characteristics that contribute to the total G×E interaction. Thus, the genotypes differ significantly in terms of stability to the environment for such traits.

A pool of ANOVAs showed very significant mean-squared sums of genotypes and environments of all characters investigated. This shows that there is a substantial variation between genotypes in the global environment, as reported by Rashmi *et al* (2017). Substantial (linear) environmental variability indicates a linear contribution of environmental effects and additive environmental variances in these characteristics and is striking with the others researchers' findings such as Saidaiah *et al.* (2010) and Manjunatha *et al.* (2018). In addition, the linear component of the GE interaction was found to be significant for six characters by Babu *et al.* (2005) and Ramya and Senthil Kumar (2008). The stability of 18 sets of red rice based on 10 traits was evaluated by three stability parameters, namely regression coefficient (bi), mean (x), and deviation from the regression (S^2di) using the proposed model of Eberhart and Russell (1966). Based on the model, a reliable genotype for the entire environment is to have high mean yield (xi), unit regression (bi) and minimum deviation (δ^2di) around the slope of the regression. Since the variance of δ^2di is a function of environmental water, we argue that multiple environments with at least a replica for each environment are needed to get a reliable estimate of δ^2di. Therefore, we tested the cultivar response using linear regression taking into account the stability of the mean and deviation in the regression of each genotype. A genotype of $bi = 1$ with a high mean and not significant δ^2di is suitable for general adaptation.

On the other hand, genotypes with insignificant δ^2di, high mean and $bi > 1$ are considered to be lower than the mean in terms of stability. These genotypes are suitable for a favourable environment because they respond positively in better environments but less efficiently in unfavourable environments. Also, a low mean, $bi < 1$ with intentioned δ^2di does not respond favourably to improved environmental conditions and can therefore be considered adapted to particularly harsh environments. Finally, genotypes with any bi value with significant δ^2di were unstable. Estimation of the stability parameters showed insignificant estimates of deviation from the regression (δ^2di) for all traits. However, some genotypes showed significant mean square deviation from regression (S^2di) for some traits that can be referred to days to 50% flowering for genotype C2,C10,C14,C15 and C18; plant height for genotypes C1,C 2, C4, C7,C9,C10,C11,C14 and C15; number of panicles for genotypes C1 and C10; panicle length for genotypes C1, C2, C3, C5, C9,C11, C12 and C16; days to maturity for genotypes C3, C6,C7,C10,C13,C14 and C18; grain yield for genotypes C3, C4,C7 C10,C12, C15, C16 and C18; KLBC for genotype C10, and KER for genotypes C4, C10 and C14. The regression showed that the genotype nonlinear component (the non-uniformity of the regression) exhibiting insignificant mean square deviation (δ^2di) was equal to zero. Thus, it is possible to predict the performance of these genotypes in a specific environment. Therefore, genotypes that could predict performance (i.e., $\delta^2di = 0$) were classified as stable.

Table 1: Stability variance analysis for yield, yield contributing traits in 'zag' genotypes across random environments in the Kashmir valley

Source of variation	**Mean Sum of Suquares**							
	d.f.	**DF (50%)**	**PH**	**NP**	**PL**	**DM**	**GY (t/ha)**	**1000 SW(g)**
Rep within Env.	6	2.9	4.4	0.6	1.0	0.6	0.2	2.2
Varieties	17	48.5**	439.3**	1.7**	3.2	58.9**	2.1**	13.8**
Env.+ (Var.* Env.)	36	36.90**	45.0	3.4**	5.5*	41.1**	3.2**	4.9**
Environments	2	619.5**	333.9**	31.9**	10.9*	562.8**	39.3**	21.4**
Var.* Env.	34	2.6	28.0	1.7**	5.2	10.4	1.1**	3.9**
Environments (Lin.)	1	1238.9**	667.8**	63.8**	21.9**	1125.6**	78.7**	42.7**
Var.* Env. (Lin.)	17	2.2	29.9	2.9**	7.8*	12.6	1.7**	6.8**
Pooled Deviation	18	2.8**	24.7**	0.4**	2.5**	7.7**	0.3**	0.9
Pooled Error	102	1.1	1.4	0.1	0.4	0.7	0.0	0.9
Total	53	40.6	171.5	2.8	4.8	46.8	2.8	7.7

Source of variation	**Mean Sum of Suquares**					
	d.f.	**KLBC**	**KBBC**	**KLAC**	**KBAC**	**KER**
Rep within Env.	6	0. 4**	0.1**	0.02	0.03	0.02**
Varieties	17	0.1**	0.04**	0.3**	0.1*	0.01*
Env.+ (Var.* Env.)	36	0.2**	0.04**	0.3**	0.1**	0.02**
Environments	2	2.9**	0.5**	0.6**	0.8**	0.1**
Var.* Env.	34	0.1**	0.02	0.3**	0.04	0.01*
Environments (Lin.)	1	5.7**	1.01**	1.2**	1.5**	0.3**
Var.* Env.(Lin.)	17	0.1**	0.03*	0.5**	0.1*	0.02**
Pooled Deviation	18	0.02**	0.01	0.1**	0.03**	0.01**
Pooled Error	102	0.01	0.01	0.01	0.01	0.001
Total	53	0.2	0.05	0.3	0.1	0.02

**Significant at 0.01, *significant at 0.05.

DF: days taken to 50% Flowering, NP: Number of Panicles, PH: Plant height (cm), PL: Panicle length (cm), GY: Grain yield t/ha, DM: Days to maturity, 1000 SW: 1000 Seed Weight (g).

KLBC: Kernel Length Before Cooking(mm), KBBC: Kernel Breadth Before Cooking (mm), KLAC: Kernel Length After Cooking(mm), KBAC: Kernel Breadth After Cooking(mm), KER: Kernel Elongation Ratio

Table 2: Mean performance and stability parameters in Zag genotypes for yield and its attributing traits and quality traits across random environments in the Kashmir valley

Genotype	DF(50%)			PH			DM			NP			PL			Yield (T/HA)			1000 SW		
	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i
C1	81.0	1.0	0.2	118.2	0.3	22.9**	12.2a	1.0	1.5**	20.3	0.0	2.5**	116.2	0.8	-0.2	3.0	0.6	0.0	33.033b	2.1	2.1
C2	90.8	1.1	7.85**	127.1	2.0	150.4**	9.2	2.8	-0.1	19.2	4.8	6.1**	124.7	1.4	-0.2	4.0	2.2	0.0	29.9	3.4	2.5
C3	87.6	1.2	-0.2	132.5	1.8	-1.2	10.3	3.0	0.3	19.0	6.9	9.2**	128.3	1.7	46.1**	3.8	2.2	0.3**	29.6	4.2	0.4
C4	85.1	1.1	0.0	122.5	-1.4	7.8*	10.5	0.3	-0.1	21.6b	-0.4	0.7	123.2	1.4	-0.6	5.589a	0.7	0.74**	36.0a	-1.0	0.3
C5	81.3	1.0	-1.0	113.7	0.5	0.1	10.4	0.8	-0.2	20.8	-0.8	2.3*	116.7	0.7	-0.4	3.8	1.2	0.1	31.3c	1.9	0.0
C6	81.1	1.0	0.7	101.1	-0.27*	-1.6	10.1	0.3	-0.1	20.0	0.3	-0.2	117.2	0.4	6.1**	3.8	1.5	0.0	28.8	-0.85*	-1.0
C7	87.8	1.0	-1.1	118.4	1.4	20.6**	11.383b	1.0	0.7*	20.933c	0.0	-0.4	127.5	0.6	52.7**	4.104c	0.2	0.19*	30.2	0.5	-0.9
C8	84.4	1.1	-0.4	123.3	0.8	0.2	10.1	0.9	0.9*	20.9	-0.4	0.6	122.5	1.2	-0.2	4.0	0.31*	0.0	28.8	-1.4**	-1.0
C9	82.8	0.9	-0.7	114.6	0.9	67.3**	10.3	0.6	-0.2	19.7	0.7	2.1*	116.7	0.7	-0.2	3.7	1.3	0.0	28.3	-0.8	-0.8
C10	77.8	0.7	4.23*	110.1	0.3	59.3**	10.8c	1.1	1.6**	20.4	-1.0	-0.2	121.1	1.5	8.7**	2.2	0.2	1.12**	28.7	1.4	-0.4
C11	87.3	1.4	1.6	128.8	1.8	67.35**	8.8	2.9	0.1	18.7	7.1	8.9**	125.6	2.1	0.7	3.7	2.07**	0.0	26.8	1.8**	-1.0
C12	86.2	1.0	-1.1	132.3	1.5	1.9	9.7	0.8	0.1	21.08a	0.2	5.20**	121.3	0.7	1.3	4.146b	0.6	0.28**	30.6	0.9	0.1
C13	81.8	0.8	1.8	114.3	1.5	1.0	10.5	0.6	0.0	19.7	0.4	-0.1	115.9	0.7	7.3**	2.9	1.0	0.0	30.5	1.5	-0.4
C14	80.8	0.7	6.52*	120.0	1.7	12.62**	9.8	0.5	-0.1	20.9	-0.1	0.0	116.2	0.6	2.9*	2.8	0.9	0.0	27.4	-2.1	-0.5
C15	79.4	1.2	5.50*	118.7	1.4	5.79*	9.8	0.2	0.4	19.5	1.2	-0.4	116.1	0.8	-0.4	3.0	0.7	0.6**	32.9	2.8	0.5
C16	77.6	0.8	-0.3	88.0	1.8	0.3	10.7	0.5	0.0	17.4	0.5	2.21*	122.4	1.0	0.2	3.1	1.0	1.5**	32.7	1.7	0.7
C17	78.0	1.0	0.2	94.1	0.4	0.1	10.1	0.2	-0.1	19.1	-0.8	-0.3	114.8	0.7	-0.2	2.0	0.8	0.0	26.8	1.3	-0.8
C18	78.0	1.0	4.77*	113.7	1.6	0.5	10.7	0.8	-0.2	19.8	-0.5	-0.2	116.3	0.9	3.8*	2.9	0.7	0.94**	31.6	0.7	-1.0
Population mean	82.7			116.2			10.3			19.9			120.2			3.5			29.7		

*, **, significant at <0.05 and <0.01, respectively.

DF: days taken to 50% Flowring, GY: Grain yield t/ha, PH: Plant height (cm), NP: Number of Panicles, PL: Panicle length (cm), DM: Days to maturity, 1000 SW: 1000 Seed Weight(g).

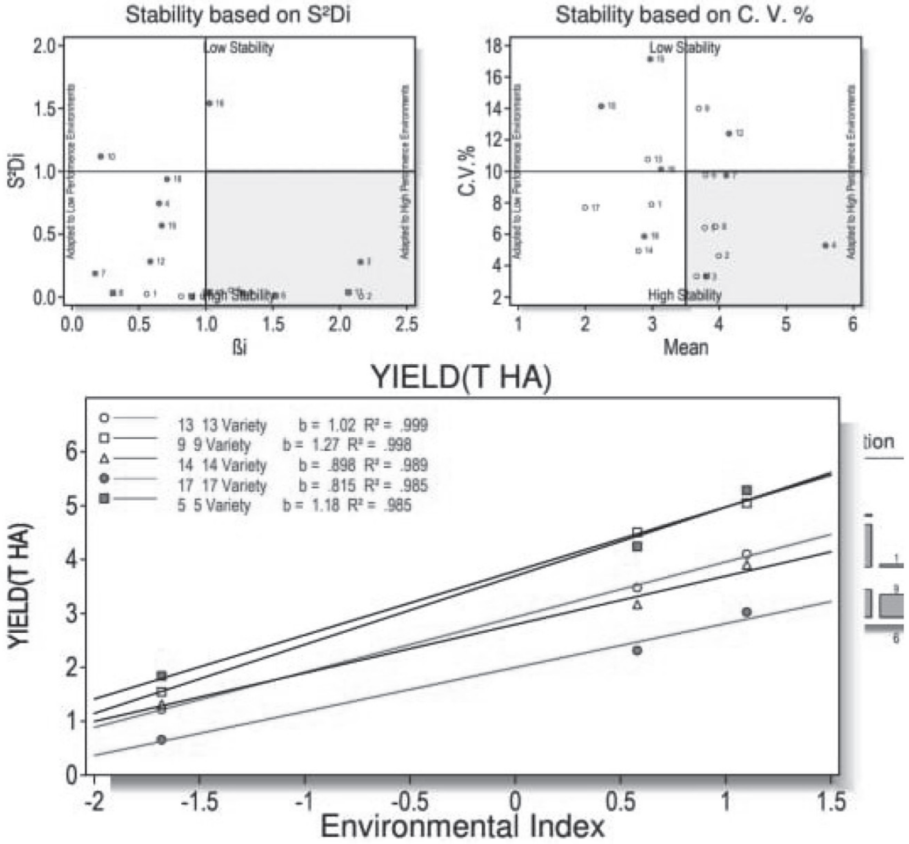
Genotype	KLBC			KBBC			KER		
	Mean	**bi**	**S2di**	**Mean**	**bi**	**S2di**	**Mean**	**bi**	**S2di**
C1	5.7	1.5	0.0	3.1	0.7	0.0	1.5	2.7*	0.0
C2	5.7	0.6	0.0	2.8	0.9	0.0	1.5	0.9	0.0
C3	5.9	1.2	0.0	2.8	0.9	0.0	1.4	1.5	0.0
C4	5.4	0.8	0.0	2.7	1.5	0.0	1.5	-0.7	0.01*
C5	5.6	1.7*	0.0	3.0	0.00**	0.0	1.5	2.4	0.0
C6	5.7	0.7	0.0	2.9	3.1	0.0	1.5	0.7	0.0
C7	5.5	1.1	0.0	2.8	1.2	0.0	1.6	1.8	0.0
C8	5.6	0.9	0.0	2.7	1.5	0.0	1.4	-1.1	0.0
C9	5.4	1.4*	0.0	2.8	0.9	0.0	1.6	1.0	0.0
C10	5.6	0.9	0.12*	3.0	0.00**	0.0	1.6	2.5	0.01*
C11	5.8	1.2	0.0	2.9	1.1	0.0	1.4	2.0	0.0
C12	5.6	1.7	0.0	2.7	1.5	0.0	1.5	0.9	0.0
C13	5.5	1.5	0.0	2.8	0.9	0.0	1.6	0.9	0.0
C14	5.3	1.5	0.0	3.1	0.7	0.0	1.5	0.8	0.0
C15	5.4	0.4	0.0	2.9	0.4	0.0	1.6	1.7	0.02*
C16	5.4	1.4	0.0	2.9	0.6	0.0	1.5	0.3	0.0
C17	5.4	-0.1	0.0	2.8	1.2	0.0	1.5	-0.8	0.0
C18	5.7	-0.2	0.0	3.0	1.1	0.0	1.6	0.7	0.0
Population mean	5.6			2.9			1.5		

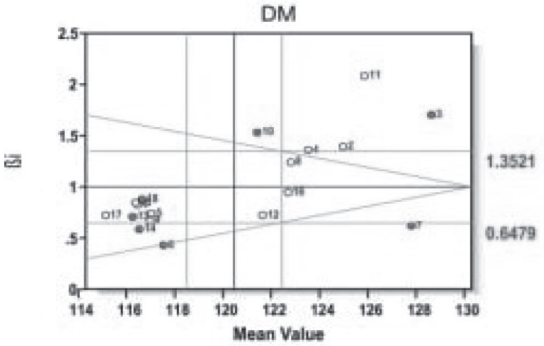
*, **, significant at <0.05 and <0.01, respectively.

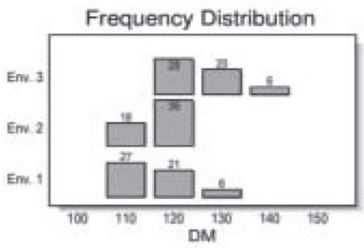
KLBC: Kernel Length Before Cooking(mm), KBBC : Kernel Breadth Before Cooking (mm), KER:Kernel Elongation Ratio

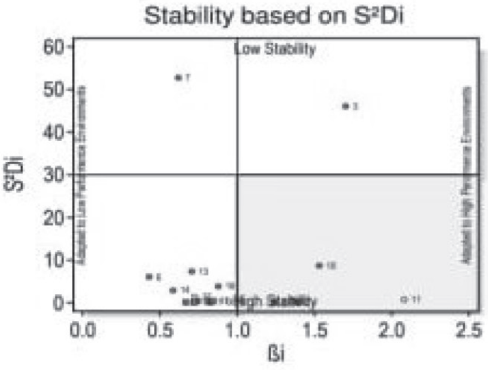
Significant changes in linear regression uniformity for various traits have been reported in relation to genotypes such as C8 and C11 (grain yield); C8 and C11 (1000-seed weight); and C5 and C9 (KLBC); C5 and C10 (KBBC), C1 (KER). Significant and greater than 1 was found in C11 for grain yield and 1000 seed weight and for C8 for 1000- seed weight, C5 and C9 for KLBC, and C1 for KER. Rest of the genotypes showing non-significant regression coefficient (bi) value and deviation from the regression (δ^2di) were average in stability and were either favourably or poorly adapted to the environments. Accordingly, the promising genotypes having maximum number of panicles/plants were C1, C7 and C-10; while those with longer panicle were C12, C4 and C7. For 1000 seed weight, C4, C1 and C5 showed higher mean than that of the population mean. For grain yield, genotypes C4, C12 and C7 were found to be the promising. The line C5 had less mean value for days to 50% flowering and days to maturity than the population mean with unity regression coefficient and least deviation from regression for yield, indicating that this line could show consistent performance across the environments. Furthermore, C-14 had regression coefficient unity and least deviation from regression for yield (Fig. 1) and with low mean value for days to 50% flowering and days to maturity than the population mean, indicating that the consistent performance of the line across the environments (Fig. 2 and 3). The results are in consistency with Manjunathan *et al.* (2018).

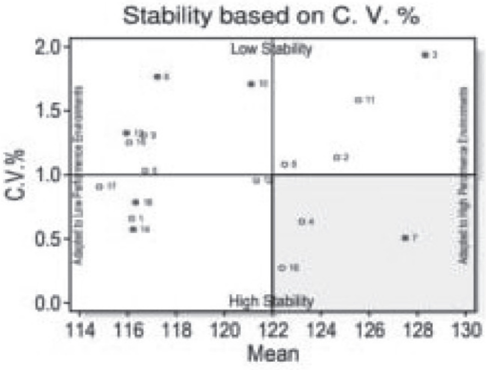
The maximum temperature ranged between 21.7 (°C) to 32.7 (°C), 21.7 (°C) to 32.1 (°C) and 23.2 (°C) to 33.6, during 2017, 2018 and 2019 respectively. The corresponding figures for minimum temperature were 7.3 (°C) to 19.1 (°C), 8.0 (°C) to 18.6 (°C), 8.3 (°C) to 18.6 (°C), respectively. The total rainfall during the crop growing season was 360 mm, 500 mm and 398 mm during 2017, 2018 and 2019, respectively (Fig.3). Lowest yield was recorded in 2019 as compared to 2017 and 2018, This may be the higher average max temp during 2019 (Fig. 3) which would have concurrently resulted into the less

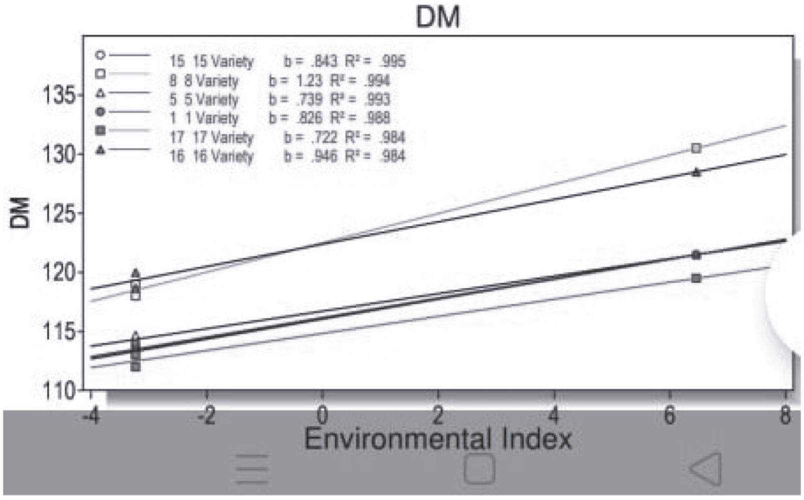












number of days to reach different phenological stages and finally to the reduction in the yield. Despite of the variation in weather parameters across the three years, C 14 and C5 performed in a consistent way throughout the three years.

4. Conclusion

Farmers are cultivating diverse population including local landraces not only to be penalised by various biotic and abiotic stresses but to get more farm income on the sale of local rice varieties. Growing local landraces in addition to high yielding varieties ensure the in-situ conservation of genetic resources for posterity. Since lot of admixtures is often been found in farmers varieties which discourage their cultivation by farmers and grain acceptance by consumers, therefore, pureline method is the option to derive out the better and uniform lines for general cultivation. From present study, it is suggested that the most stable lines identified from three years of continuous study need to be further tested over a greater number of locations to determine their utility for cultivation across broad range of ecologies in the temperate agro-ecosystems.

Compliance with ethical standards

NA

Conflict of Interest

Authors declare that they have no conflict of interest

Author contributions

GHK**,** conduct of trial, analysis of data, preparation of manuscript NRS exploration and collection of different zag lines, ABS, FAM, SHW, RSK, manuscript preparation data recording and data analysis, AH, analysis and interpretation of meteorological data MR, NAB Critical review and finalization

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Research Article

Additive Main effect and Multiplicative Interaction (AMMI) model analysis for yield performance and G×E interaction in a multi-environmental trial of aromatic fine rice in Bangladesh

Shams Shaila Islam[1*], Ahmed Khairul Hasan[2], Abul Bashar Mohammad Khaldun[3] and Novizar Nazir[4]

[1]*Department of Agronomy, Faculty of Agriculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh*
[2]*Department of Agronomy, Faculty of Agriculture, Bangladesh Agricultural University, Mymensigh-2202, Bangladesh*
[3]*Oil Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
[4]*Department of Quality control and Agro Industrial Management, Faculty of Agricultural Technology, Andalas University-25163, Indonesia*

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****Corresponding author:***
E-mail: shaila.hmdstu@gmail.com & shaila@hstu.ac.bd

Abstract

The problem of genotype-environment interaction (G×E) in interpreting multilocus trial analyses and predicting genotype performance can be mitigated by applying Additive Main effect and Multiplicative Interaction (AMMI) model analysis. The model AMMI was used in the present study to determine the effect of genotype, environment, and their interaction, to determine the extent of G×E interaction and to identify the factors contributing to G×E interaction for the best yielding aromatic fine rice genotype grown in four different districts in Bangladesh. Analysis of variance showed that the effect of genotypes, environments, G×E interactions were highly significant for plant height, days to maturity, panicles length, grain yield. Result showed grain yield had highly significant differences for environmental traits like (soil properties, phenological, genotypes) with their interactions which indicated that environments were different and changeability with the genotypes. Large variations in total P, Fe and rainfall identified as the main cause of the observed interaction. Here, Genotype BRRI Dhan34 had the highest mean grain yield values over four locations, respectively. In AMMI model, among four locations Dinajpur with Nilphamari were the majority responsive environments and Dinajpur was the most adjacent responsive location. Therefore, location Dinajpur with BRRI Dhan34 genotype could be considered as a better combination for higher grain yield among the ten aromatic fine rice genotypes.

Key Words: AMMI model; G × E interaction; grain yield; PCA; aromatic fine rice

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods for more than half of the world's population with a global production of more than 700 million tons per year area of 165 million hectares. (Nayak *et al.,* 2019). Local genotypes, including aromatic fine rice, occupied about 12.16% of the total rice area in Bangladesh (Akter *et al.,* 2020). All rice genotypes have different specialty while some are very prevalent for their aroma and scent. Many countries such as India, Thailand, Vietnam, USA, China, etc., are involved in developing special aromatic rice cultivars (Verma *et al.,* 2018). Although the productivity of aromatic fine rice is comparatively low, its demand for internal consumption for export is increasing gradually (Haque *et al.*, 2012).

In agricultural research testing a numerous genotype in several environments is called multi-environment trials (MET). MET is usually conducted to find superior genotypes for better cultivation in the future (Diyah and Hadi, 2016). A variety or genotype is more adaptable if it gives high average yield but has low variation in yielding ability when grown in different environments (Karim *et al.*, 2012). The use of genotype main effect (G) plus genotype-by-environment (GE) interaction (G+GE) AMMI analysis by plant breeders and other agricultural researchers has increased dramatically during the past 5 years for analyzing multi-environment trial (MET) data reported by (Yan *et al.*, 2007).

AMMI model has been expansively applied in the statistical analysis since large portion of the G×E interaction sum of squares visibly separates main and interaction effects which support a breeding program over the check locations (Ebdon and Gauch, 2013 and Rodrigues *et al.,* 2014). Therefore, estimation GEI by AMMI model is the best method stated by (Kindeya *et al.,* 2015).

AMMI model combines ANOVA for the G×E effects with the additive parameters of principal component analysis (PCA) reported by (Gauch and Zobel, 2006). Thillainathan and Femandez (2001) designated that the biplot display of PCA scores plotted against each other provides visual inspection and interaction components. Application of the AMMI model has performed normally throughout the previous two aeras namely (Eberhart and Russell, 1966), variance component methods (Shukla 1972; Gauch *et al.*, 2008; Yang *et al.*, 2009; Rodrigues *et al.,* 2014). With this background, the main objective of the current study was to identify the aromatic rice; perform G x E interaction and find out the influence of environmental components related to G x E interaction to better control the yield of aromatic rice.

2. Materials and Methods

2.1 Experimental plant materials

The experimental plant materials *i.e.*, ten aromatic fine rice genotypes (Table 1) collected from different district of Bangladesh. Four locations differing in latitude, longitude and elevation from the sea level were in Dinajpur (25°37'38" N, 88°38'16" E and 42 m); in Thakurgaon (26°41'83" N, 88°42'16"E and 60 m); Panchagarh (26°20'00" N, 88°33'27" E and 79 m) and Nilphamari (25°48'27" N, 88°41'27" E and 40 m).Top of FormTop of Form The genotypes were evaluated in a RCBD with three replicates in a plot size of 4m×5m with a spacing of 30 cm between rows. Experiments related soil components were described in (Table 2). For the final setting of the experiment, 30-day-old seedlings were used, and one seedling was transplanted per hill. Adequate soil fertility was ensured by applying urea, Triple Super Phosphate (TSP), muriate of potash (MOP), gypsum, ZnSO4 @ 250:130:120:50:10 kg/ha, respectively. Different agronomic actions namely, weed control overall completed by manually, insect and pests by the solicitation of 20 ml per 1 L Cypermethrin 10% w/v EC and 50 ml per 1 L Benfuracarb 20% w/v EC. At 30 days after planting urea fertilizer (46-0-0) was applied.

Table 1: Ten popular aromatic fine rice genotypes with place of collection in Bangladesh

Sl No	Genotypes	Place of collection	Kernel size and shape	Yield (T/ha)
G1	Kataribhog	Dinajpur	Short, medium Scented	2.00
G2	Kalijira (medium grain)	BRRI	Short, medium Scented	1.96
G3	Kalijira (long grain)	Khulna	Short, medium Scented	2.11
G4	BRRI dhan34	BRRI	Short, medium Scented	2.66
G5	BRRI dhan37	BRRI	Short, medium Scented	1.92
G6	Chinigura	Sherpur	Medium, slender Scented	1.21
G7	Basmati	Barguna	Short, bold Scented	2.43
G8	Tulsimala	Sherpur	Short, bold Lightly scented	1.95
G9	Badshabhog	Dhaka	Short, bold Scented	1.35
G10	Gobindhabhog	Jessore	Short, medium Lightly scented	2.38

2.2 Data collection

Yield contributing characters like plant height, days to maturity, panicles length, grain yield data was recorded using ten randomly selected plants in each replication and yield data were finally converted in to (t/ha).

2.3 Statistical analysis

Grain yield which was collected at 12% moisture level. Observations were recorded and the data were statistically analyzed. Here, the contribution of each genotype and environment to GEI is assessed by using the biplot plot in which mean yield values are plotted against scores of the first principal component interaction (PCA1). Correlation coefficient, analysis of variance (ANOVA) with Post Hoc also PCA (Principal Component Analysis) are using SPSS (ver 20) and XLSTAT (ver16).

2.4 Additive main effect and multiplicative interaction (AMMI) method for RCBD analysis

The AMMI method was applied with additive effects to 10 genotypes in three environments, and multiplicative was used for G×E interaction. It affords a symbolic view of the transformed G×E interaction for any interpretation (Kempton, 1984) based on the following AMMI equation:

Where, = Yield for genotype g, environment e and replication r; μ = Grand mean value for trait; = Mean deviations for genotype (genotype means minus grand mean); = Mean deviation for environment; n = PCA axis number reserved in the model; = Singular value for PCA axis n; = Genotype eigenvector values for PCA axis n; = Eigenvector for environment; = Residuals and = Error is used

Table 2: Physico-chemical characteristics of initial soils in the different experimental fields

Soil characteristics	**Locations**			
	Dinajpur	**Thakurgaon**	**Panchagarh**	**Nilphamari**
Soil texture	Sandy clay loam	Loomy	Sandy clay loam	Sandy clay loam
pH	5.25	5.1	5.7	5.21
Organic carbon (%)	0.81	0.55	0.79	0.79
Total N (%)	0.08	0.07	0.08	0.08
Available P (mg/kg soil)	120.34	98.99	121.44	120.00
Exchangeable K (cmol/kg)	0.151	0.055	0.141	0.171
Available S (mg/kg soil)	14	24	14	13

3. Results and discussions

3.1 Analysis of variance result including the partitioning of the G×E interaction of aromatic fine rice

The analysis of result showed significant differences for plant height, days to maturity, panicle length and grain yield for genotype, environment, G x E interaction. The highly significant effect on environment indicates high differentiation of genotypic responses in different environments and existence of wide range of diversity among genotypes (Kulsum *et al.*, 2012). Analysis of variance based on the AMMI model for grain yield is shown in Table 3 indicating that genotype performance was more influenced than environmental factors. The genotype × environment effect interaction could be divided into two components, namely IPCA1 and IPCA2. All significant differences were found for grain yield, indicating that the components of G×E interaction affected the yield of genotypes in different environments and the environments were different. Kumar *et al* (2012) reported the result for hybrid rice at different locations in Bangladesh.

Table 3: Analysis of variance including the partitioning of the G×E interaction of aromatic fine rice

Source of variation	df	Mean sum of squares			
		Plant height	**Days to maturity**	**Panicle length**	**Yield (t/ha)**
Genotype (G)	9	45.22**	69.88**	1.756**	2.22**
Environment (E)	3	263.33**	533.77*	11.26**	1.80**
Replication (R)	2	22.01**	4.82**	1.00*	0.065*
Interaction (G×E) GEI	36	5.033*	2.11**	0.56*	0.142**
AMMI component 1	12	11.65**	3.44**	0.89*	0.32**
AMMI component 2	11	5.67**	2.45**	0.76*	0.14**
Error	18	3.66	0.567	0.578	0.89

Here * $p< 0.05$,**$p<0.01$

3.2 Analysis of variance result for grain yield with soil, climatic and phenological properties

Based on ANOVA result, the average highest grain yield (2.66 t/ha) was found from G4 (BRRI dhan 34) genotype and comparatively low (1.95 t/ha) for G8 (Tulsimala) genotype. High variations occurring in this result were caused by several factors such as soil properties like Fe, total phosphorus, Ca as well as rainfall (Eberhart and Russell, 1966). Changeable environmental features such as rainfall through a single situation can underscore dissimilarity of genotypes in relation to environment across locations. For the different location trials, the location in which the field trials were undertaken showed geographical and environmental dissimilarities (Islam *et al.*, 2020) .The soil properties showed that the highest variation occurred phenological traits like plant height, days to maturity, panicles length showed highest variation. All the findings shown in (Table 4).

Table 4: Summary statistics of grain yield, soil, climatic and phenological properties of ten aromatic fine rice genotypes in 4 locations of Bangladesh

Variables	Sum	Average	Variance	Variables	Sum	Average	Variance
G1	8.012	0.200	0.530	**Fe**	**1068.77**	**26.71**	**7138.41**
G2	3.800	0.096	0.115	CEC	16.05	0.40	1.521
G3	8.420	0.211	0.578	pH	19.95	0.49	2.297
G4	10.63	0.266	0.972	EC	93.76	2.34	52.197
G5	7.68	0.192	0.504	T_max	142	3.55	116.36
G6	4.84	0.121	0.187	T_min	99	2.48	56.61
G7	9.70	0.243	0.681	**Rainfall**	**465.8**	**11.65**	**1254.83**
G8	3.81	0.095	0.121	Humidity	339	8.48	663.54
G9	5.41	0.135	0.211	PH	523.2	13.08	1622.24
G10	9.51	0.238	0.703	DM	2743.2	68.58	5300.17
N	3.78	0.332	0.675	PL	103.6	2.59	63.15
OC	4.44	0.111	0.115	-	-	-	-
Total_P	**726.84**	**18.17**	**3226.57**				
Available_P	29.36	0.73	5.892	-	-	-	-
K	125.02	3.12	91.79	-	-	-	-
Ca	335.15	8.37	664.58	-	-	-	-

Here, N=Total Nitrogen, OM=Organic Matter, OC=Organic Carbon, Total P=Total phosphorus, FG=Filled grains (no), SW=Seed weight (gm), Available P = Available Phosphorus, K=Potassium, Ca=Calcium, Fe=Iron, CEC= Cation Exchange Capacity, pH=pH level, EC=Exchangeable Cation, Tmax=Temperature Maximum, Tmin=Temperature Minimum, PH=Plant Height (cm), PL=Panicles Length (cm) bold letters indicate correlation is significant at the 0.01% level

3.3 Principal Component Analysis (PCA)

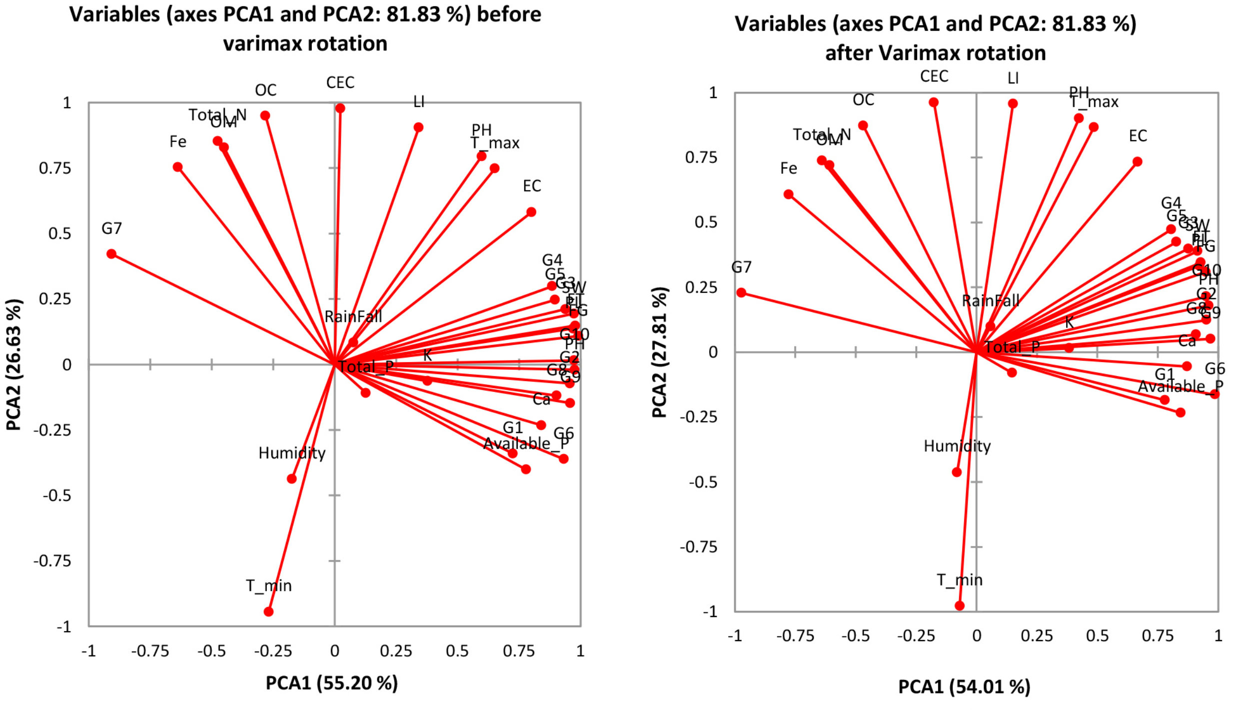
The AMMI biplot provides a visual expression of the relationships between the IPCA1 and IPCA2 with the mean of the genotypes and environments. Principal Component Analysis is a multivariate technique that detects figure arrangements with correspondences and differences between variables set up and arranged in a systematic multivariate system (Islam *et al.*, 2020). Tables 5 and 6 showed that the output of PCA analysis exposed the relation between retained factors and the variables before and after rotation. Figure 1 is the map titled correlation circle (below on axes PCA1 as well as PCA2) showing a projection of the primary variables into the factors planetary. If two variables were away from the center (Xlstat, 2017) but variables were close to each other then they were significantly positively correlated (r near 1). Besides, remained orthogonal, then they do not exist correlated (r near 0); if they were on the opposite side of the center, then they remained significantly negatively correlated (r near -1). From the plot of the component loadings gave a visual representation schemed in planetary that showed exactly how closely related the items to each other as well as with the components.

Table 5: Outputs of PCA analysis between variables and factors before Varimax rotation

Variables	PCA1	PCA2	Variables	PCA1	PCA2
G1	0.725	-0.340	Fe	-0.637	0.753
G2	0.957	-0.073	CEC	0.024	0.977
G3	0.839	0.211	PH	0.598	0.795
G4	**0.885**	**0.299**	EC	0.802	0.582
G5	**0.876**	**0.247**	T_max	0.652	0.748
G6	0.932	-0.361	T_min	-0.267	-0.944
G7	-0.906	0.421	Rainfall	0.077	0.084
G8	0.903	-0.119	Humidity	-0.174	-0.437
G9	0.958	-0.147	PH	0.977	-0.020
G10	0.974	-0.016	PL	0.971	0.137
Total_N	-0.475	0.853	MD	0.343	0.905
OM	-0.449	0.828	-	-	-
OC	-0.281	0.949	-	-	-
Total_P	0.127	-0.108	-	-	-
Available_P	0.779	-0.401	-	-	-
K	0.378	-0.063	-	-	-
Ca	0.841	-0.232	-	-	-

In the current study Fig 1(a) the first principal component axis (PCA1) illustrated 55.20% of entire variation while PCA2 explain 26.63%. Therefore, the two axes together explained 81.83% of the G×E interaction for grain yield with other traits and Fig 1(b) also support the same G×E interaction. Figure 1(a) showed that all the variables have strong relationships with some of the environmental parameters before rotation. Therefore, genotypes were closed with their related environmental traits. Table 4 indicated that most of the genotypes with very strong PCA1 values G1 (0.725), G2 (0.957), G3 (0.839), G4 (0.885), G5 (0.876), G6 (0.932), G8 (0.903), G9 (0.958) and G10 (0.974) appeared with Available_P (0.779), Ca (0.841), EC (0.802), PH (0.977), PL (0.971) in PCA1. On the other hand, in PCA2 only OM (0.828) and OC (0.949) closed to each other. This finding very much similar with (Poramate and Anchalee, 2015).

Although some of the traits shown strong relationship values but they were not closed to each other. Table 6 showed that after rotation the PCA values between factors and the variables changed to some extent. Figure 1(b) also supported the relationships. The correlation round also useful in understanding the significance of the axes. Following this research, the parallel axis link by means of Total_P, Available_P, K, Ca, EC, PH, PL, FT and the vertical axis with Total_N, OM, OC, Fe, CEC, PH, Temp_max, Temp_min, Rainfall, Humidity and LI. These trends revealed that a variable is well linked with an axis.



1a

1 b

Fig 1: AMMI model based on environments focused scaling for comparison the genotypes with the ideal genotype on grain yield. a) before rotation b) after rotation

Table 6: Outputs of PCA analysis between variables after Varimax rotation

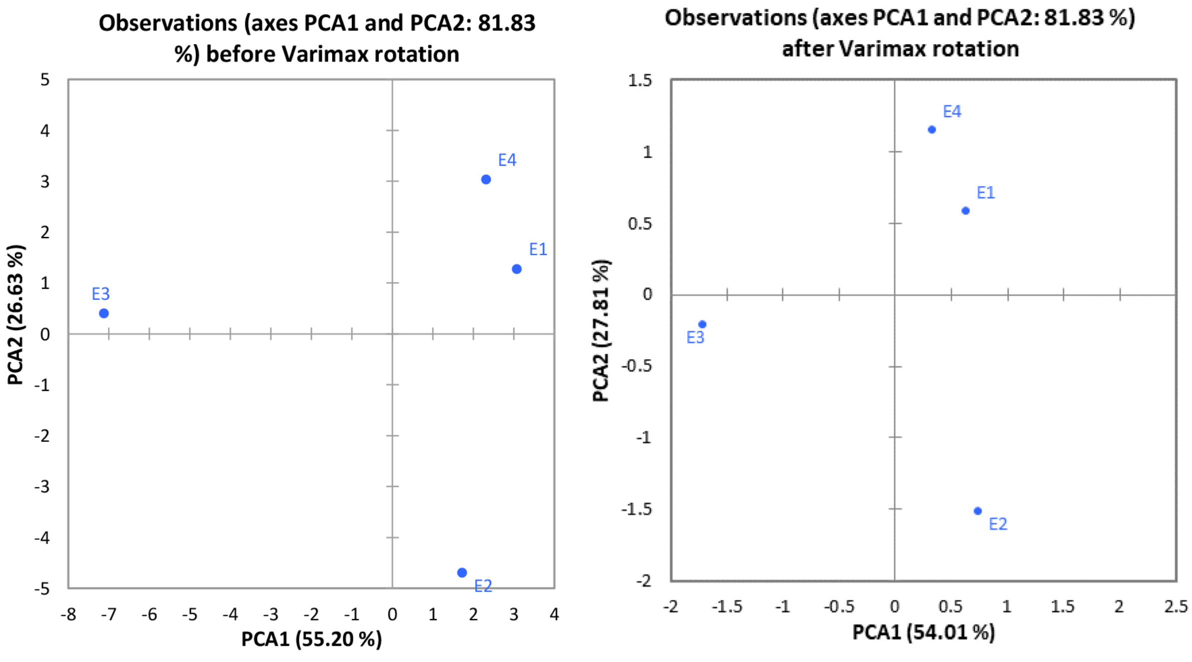
Variables	PCA1	PCA2	Variables	PCA1	PCA2
G1	0.779	-0.185	Fe	-0.777	0.607
G2	0.652	-0.124	CEC	-0.176	0.961
G3	**0.800**	**0.398**	PH	0.424	0.900
G4	**0.806**	**0.473**	EC	0.666	0.733
G5	**0.807**	**0.424**	T_max	0.486	0.865
G6	0.986	-0.164	T_min	-0.069	-0.979
G7	-0.973	0.228	Rainfall	0.058	0.098
G8	0.909	0.068	Humidity	-0.081	-0.464
G9	0.968	0.051	PH	0.961	0.179
G10	0.751	-0.214	PL	0.922	0.332
Total_N	-0.639	0.738	MD	0.151	0.956
OC	-0.469	0.872	GY	0.949	0.309
Available_P	0.845	-0.234	-	-	-
K	0.383	0.015	-	-	-
Ca	0.871	-0.056	-	-	-

3.4 Analysis of variance result for grain yield in four locations

There had a significant effect of environmental parameters for grain yield with four locations. As shown (Table 7), the Tukey's HSD (Honestly Significantly Different) test smeared to wholly pairwise variances among mean values. The risk of 5% chosen values used to define the critical value F, which compared to the standardized difference between the means. Only three pairs appeared significantly different (E1, E3), (E4, E3) and (E2, E3). The means and the groups then categorized founded on this analysis. In conclusion, four location's different environmental parameters showed significantly effects on the yield of genotypes. Based on the result of Table 7, Fig 2 showed the eventual objective of the Principal Component Analysis. Where E1 and E4 environment nearer to the central point was nearer with similar yield and E1 devoured higher yield than E4. After that E3 and E4 took very low yield comparing to other environments. In this case the best environment was E1 (Dinajpur). It was enabled the observations on a two-dimensional map and to identify links that exists grain yield Dinajpur, Thakurgaon, Panchagarh and Nilphamari locations were unique. It showed that every geographical location had its own environmental characteristics and those characteristics had different impact upon the genotypes.

Table 7: Environment /Tukey (HSD)/ ANOVA Analysis of the differences between grain yield with four locations in Bangladesh

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
E1 vs E3	2.061	4.335	2.693	0.001	Yes
E1 vs E2	0.670	1.409	2.693	0.502	No
E1 vs E4	0.403	0.848	2.693	0.831	No
E4 vs E3	1.658	3.487	2.693	0.007	Yes
E4 vs E2	0.267	0.562	2.693	0.943	No
E2 vs E3	1.391	2.925	2.693	0.029	Yes
	Tukey's d critical value:		2.868		



2a **2b**

Fig 2: AMMI observation study for comparison of the locations a) before rotation b) after rotation

4. Conclusion

In summary, the statistical model AMMI was used to determine the G×E interaction pattern of grain yield of ten promising aromatic fine rice cultivars. ANOVA showed that significant differences among genotypes, soil properties and phenological traits indicated the presence of large variability among genotypes and locations for yield, with G4 (BRRI Dhan34) and G7 (Tulshimala) being the first and second high yielding genotypes corresponding to environments E1, E2, E3 and E4. The maximum yielding genotypes G4, G7, G10, G3, G1, G5 and G6, G2, G8 were the low yielding genotypes with wide adaptation. The analysis of the four environments showed that there were significant differences between E1 vs. E3, E3 vs. E4, and E2 vs. E3. PCA analysis also showed that location E1 (Dinajpur) was found to be optimum selection site for identification of broad and adaptive genotype of aromatic rice and for other improvement work on aromatic fine rice.

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Compliance with ethical standards

NA

Conflict of Interest

Authors declare that they have no conflict of interest

Author contributions

Conceptualization of research and designing of experiments (SSI,), Conduction of experiment (SSI, AKH, ABMK), Preparation of manuscript (SSI, AKH, ABMK,NN).

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Research Article

Multivariate analysis and character association studies for yield and nutritional characters in swarna and type 3 RIL population of rice (*Oryza sativa* L.)

Darmagaru Shivani[1,2*], Cheruvuri Naga Neeraja[1], Chandragiri Cheralu[2] and Vuppu Gouri Shankar[2]

[1]*ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, India*
[2]*Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, India*

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Corresponding author:
E-mail: shivani.42agrico@gmail.com

Abstract

The investigation was carried out in one hundred Swarna X Type 3 RIL population of rice to understand the association among yield, yield attributing and nutritional traits. Their direct and indirect effects on the grain yield using correlation and path analysis and the principal component analysis was assessed using multivariate analysis. Significant differences were observed among the RIL population for the traits studied. High values of heritability and genetic advance were observed for plant height, panicle weight, number of filled grains per panicle, panicle weight, 1000-seed weight, grain yield per plant, grain iron and zinc concentration. Character association for the yield attributing traits at both genotypic and phenotypic level revealed significant positive association of grain yield per plant with test weight and plant height. Path coefficient analysis revealed that test weight had highest direct positive effect on grain yield per plant followed by plant height and filled grains per panicle. PCA showed that a cumulative variance of 32.5% from PC1 attributed by grain iron, grain zinc, plant height and test weight would be beneficial in contributing to the total morphological diversity. RILs P45 and P57 have shown higher grain yield per plant with high iron and zinc concentrations. Thus, the trait test weight that showed positive and direct association with grain yield can be focused in selection and can be utilized for improvement in future breeding programmes.

Key words: Correlation, Path, PCA analysis, Variability, Yield and Nutritional traits

1. Introduction

Rice (*Oryza sativa* L.) is the staple food crop for more than half of the world's population. More than 90% of the world's rice is grown and consumed in Asia, whereas 50% of the population depends on rice for food (Tenorio *et al.* 2013). In India, rice accounts for more than 43% of food grain production. It has been estimated that there is a need for 60% more rice production for the expected 9.7 billion global human population by 2050 (Wani *et al.* 2020). Micronutrient deficiency such as Zn and Fe is rampant among Indian population particularly, in the children and pregnant woman (Ritchie *et al.* 2018), therefore, identification of suitable material rich in Zn and Fe content along with yield needs utmost attention for enhancing productivity and production of rice.

The knowledge on genetic variability study is of great significance for the success of any plant breeding programme. Heritability and genetic advance are the most important selection parameters as heritability estimates along with genetic advance helps in envisaging the gain under selection. Heritability along with genetic advance will help in predicting the ultimate effect for selecting superior varieties (Ali *et al.* 2002). Correlation and path analysis determines the association between yield and its components and also brings out the relative importance of their direct and indirect effects with grain yield. Essentially, this kind of analysis could benefit the breeder to choose appropriate selection strategies to improve grain yield.

Multivariate analytical tools have found widespread use in describing the inherent variation among crop genotypes. These tools includes cluster and principal component analysis. Principal component analysis has been helpful in identifying the contribution and the importance of each component to the total variance (Noirot *et al.* 1996) and it has been successfully used in the evaluation of crop germplasm for understanding the correlation and relationship among the variables studied (Zafar *et al.* 2008). Based on these points, the present investigation was carried out with the objective to quantify the genetic variability present in the Swarna x Type 3 RIL population which may be exploited in genetic improvement of rice for grain zinc and iron in addition to yield.

2. Materials and Methods

The material for the present study consisted of one hundred RIL population developed from the cross Swarna x Type 3 using Single Seed Decent method. Field experiment was conducted at ICAR-Indian Institute of Rice Research, Hyderabad. The experiment was laid out in Augmented Block Design including four checks (Swarna, Type 3, BPT 5204 and Chittimuthyalu). Checks were replicated in each block. All the cultural practices are followed as per the package of practices adopted for rice.

Observations were recorded on five randomly selected plants for days to 50 per cent flowering, plant height, panicle length number of productive tillers per plant, panicle weight, number of filled grains per panicle, test weight, and grain yield per plant. Grain iron and zinc concentration were determined by X-Ray fluorescence Spectrometry (XRF) (EDXRF, model-X-supreme 8000) (Paltridge *et al.*, 2012)

Data for the above traits were subjected to statistical analysis *viz.,* Analysis of variance (ANOVA), genetic variability components such as phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability and genetic advance. Correlation coefficients, principal component analysis (PCA) and principal component score was derived using the software SAS v.9.3 to reveal the best relationships among traits.

3. Results and Discussion

In any crop genetic variability is pre-requisite for selection of superior genotypes over the existing cultivars. Variance analysis for all the characters revealed significant differences among the genotypes studied. For all the characters under the study, phenotypic coefficient of variation (PCV) in general was higher than genotypic coefficient of variation (GCV) indicating the influence of environment on the expression of these characters (Table 1). However, the difference between PCV and GCV was less for the characters *viz*, plant height, panicle weight, number of filled grains per panicle and single plant yield indicating low environmental influence and predominance of genetic factors controlling variability of these traits. Similar results were reported by Lakshmi *et al.* (2017) for plant height, by Nandeshwar *et al.* (2015) for panicle weight and by Sameera *et al.* (2015) for single plant yield.

The information of genetic variability alone is of limited use to the breeder unless it is supplemented with the evidence on heritability, which gives a measure of the heritable portion of the total variation. Genetic advance is reliant on phenotypic variability and heritability in addition to selection intensity, so the heritability estimates in addition with genetic advance will be more effective and reliable in predicting the response to selection (Johnson *et al.* 1955). Heritability in the broad sense includes both additive and non-additive gene effects (Hanson *et al.* 1956). Whereas, narrow sense heritability includes only additive components (Johnson *et al.* 1955).

Table 1. Estimates of range, mean and genetic parameters for yield and nutritional traits in rice

Characters	Mean	Range		Coefficient of variability		Heritability (%) broad sense	Gen.Adv as per cent of Mean (at 5%)
		Min.	Max.	PCV %)	GCV (%)		
Days to 50% Flowering	114	101.0	125.0	3.36	2.93	76.03	5.26
Plant Height (cm)	134.6	77.0	167.0	12.64	12.57	98.74	25.72
Panicle Length(cm)	23.9	16.8	28.2	7.67	7.11	85.84	13.56
No. of productive tillers/ plant	10.3	8.2	13.4	10.39	9.17	77.93	16.67
Panicle weight (g)	4.0	2.2	6.0	22.19	22.10	99.23	45.35
Number of filled grains/ panicle	105.4	32.3	171.0	33.58	33.21	97.79	67.65
1000 grain weight(g)	18.8	9.0	24.5	15.51	14.30	90.14	27.95
Grain Iron conc (ppm)	9.7	5.2	16.1	16.44	14.00	72.56	24.57
Grain Zinc conc (ppm)	21.3	14	28.5	13.47	13.02	93.47	25.93
Grain yield/ plant (g)	21.7	11.7	33.2	20.54	20.22	96.98	41.03

In the present study, heritability in broad sense was estimated. High broad sense heritability was recorded for all the characters under study. High heritability coupled with genetic advance was found to be highest for plant height (98.74 and 25.72%), panicle weight (99.23 and 45.35%), number of filled grains per panicle (97.79 and 67.65%), 1000 seed weight (90.14 and 27.95%), grain zinc (93.47 and 25.93%), iron (72.56 and 24.57%) and single plant yield (96.98 and 41.03%). The study clearly showed that there is ample scope to improve all these characters through selection. These results are in conformity with Devi *et al.* (2016), Lakshmi *et al.* (2017) for plant height, number of filled grains per panicle and 1000 seed weight, Satish *et al.* (2017) for panicle weight, Karande *et al.* (2015), Lakshmi *et al.* (2017) for single plant yield. Gangashetty *et al.* (2013) reported similar results for grain zinc and iron concentration.

The complex character such as grain yield is based on the total net effect produced by various yield components relating with one another. The present investigation revealed that there is adequate genetic variability present in the material studied. Among all the characters, plant height, number of filled grains per panicle, panicle weight, 1000-seed weight, grain yield per plant, grain iron and zinc concentration recorded high heritability as well as high genetic advance, indicating the presence of considerable variation and additive gene effects. Hence, response to selection would be quite possible.

3.1 Correlation between characters

Selection based on the magnitude and direction of association between yield and yield attributes is very important in identifying the key characters, as this information can be exploited for crop improvement by designing suitable breeding programmes. Phenotypic and genotypic correlations between yield, yield attributes and nutritional components *viz*., days to 50 per cent flowering, plant height, panicle length, number of tillers per plant, panicle weight, number of filled grains per panicle, test weight, single plant yield, grain zinc and grain iron content were computed separately for the RIL population considering in this study. The results are presented under Table 2. Grain yield per plant was significantly positively correlated with plant height (0.21500) and test weight (0.39373). Test weight showed significant and positive correlation with panicle weight. Similar findings were reported by Nandeshwar *et al.* (2015) and Satish *et al.* (2017). Significant positive association is also observed for grain iron with grain zinc content. Other yield components *viz*., Days to 50% flowering, panicle length, number of tillers per plant, panicle weight and filled grains per panicle showed non-significant positive association with grain yield. Similar findings were earlier reported by Rahman *et al.* (2014) Seyoum *et al.* (2012) and Rao *et al.* (2014) for panicle length, number of tillers per plant and number of filled grains per panicle. While a negative association was found with grain iron and grain zinc content. Similar results were reported by Nagesh *et al.* (2013) for grain zinc and iron content.

Table 2. Genotypic and phenotypic correlation coefficients for yield and nutritional traits in rice

	DFF	PH	PL	NT	PW	FGP	TW	Fe	Zn	SPY
DFF	1.0000	-0.25237**	-0.01350	-0.06742	0.12325	0.06909	0.14333	-0.05973	-0.13330	0.08072
PH		1.0000	0.11451	0.09012	0.10340	-0.02478	0.02336	-0.22693*	-0.18858*	0.21500*
PL			1.0000	-0.00590	-0.08740	0.01530	-0.03999	-0.00078	-0.11041	0.03064
NT				1.0000	-0.02825	-0.05510	-0.04068	-0.05784	-0.07053	0.03782
PW					1.0000	-0.02298	0.28880**	-0.09093	-0.01831	0.10992
FGP						1.0000	0.02035	-0.19668*	-0.08036	0.10278
TW							1.0000	-0.12862	-0.04148	0.39373**
Fe								1.0000	0.66919**	-0.40593**
Zn									1.0000	-0.42433**
SPY										1.0000

* and ** Indicate significance at 5% and 1% levels respectively; DFF: Days to 50% flowering; PH: Plant height; PL: Panicle Length; NT: Total number of tillers per plant; PW: Panicle weight; FGP: Number of filled grains per panicle; TW: Test weight; Fe: Grain iron; Zn: Grain zinc; SPY: Single plant yield.

3.2 Path coefficient analysis

Correlation alone does not provide the true contribution of the characters towards the yield, the genotypic correlations were partitioned into direct and indirect effects through path coefficient analysis, which allows separating the direct effect and indirect effects through additional attributes by apportioning the correlations (Wright, 1923) for better interpretation of cause and effect relationship. The estimates of path coefficient analysis for yield, yield related and nutritional traits given in Table 3. Among the characters studied at genotypic level, Test weight (0.3679) had highest direct positive effect on grain yield per plant followed by plant height (0.1298), number of filled grains per panicle (0.0502), number of tillers per plant (0.016) and days to fifty percent flowering (0.0147). On the other hand, direct negative effect to grain yield was recorded by panicle length, panicle weight, grain iron and grain zinc content.

Table 3. Path analysis of direct and indirect effects for yield and nutritional traits in rice

	DFF	PH	PL	NT	PW	FGP	TW	Fe	Zn
DFF	**0.0147**	-0.0037	-0.0002	-0.0010	0.0018	0.0010	0.0021	-0.0009	-0.0020
PH	-0.0327	**0.1298**	0.0149	0.0117	0.0134	-0.0032	0.0030	-0.0294	-0.0245
PL	0.0001	-0.0006	**-0.0053**	0.0000	0.0005	-0.0001	0.0002	0.0000	0.0006
NT	-0.0011	0.0014	-0.0001	**0.0161**	-0.0005	-0.0009	-0.0007	-0.0009	-0.0011
PW	-0.0033	-0.0028	0.0023	0.0008	**-0.0268**	0.0006	-0.0077	0.0024	0.0005
FGP	0.0035	-0.0012	0.0008	-0.0028	-0.0012	**0.0502**	0.0010	-0.0099	-0.0040
TW	0.0527	0.0086	-0.0147	-0.0150	0.1063	0.0075	**0.3679**	-0.0473	-0.0153
Fe	0.0072	0.0274	0.0001	0.0070	0.0110	0.0237	0.0155	**-0.1206**	-0.0807
Zn	0.0397	0.0562	0.0329	0.0210	0.0055	0.0239	0.0124	-0.1993	-**0.2978**
SPY	0.0807	0.2150	0.0306	0.0378	0.1099	0.1028	0.3937	-0.4059	-0.4243

Thus, it is understood that test weight can be considered as the major yield contributing character. These results are in accordance with the previous studies conductedby Kalyan *et al.* (2017), Lakshmi et al. 2017 and Priya *et al.* 2017. It is also understood that the increased grain yield through the direct effect of test weight is the indirect effect of number of tillers per plant and panicle weight followed by moderate to low indirect effects of panicle length and number of filled grains per panicle. These findings are in concurrence with earlier reports (Padmaja *et al.* 2011, Kalyan *et al.* 2017). Thus, test weight appears to be important trait on which emphasis can be laid as a selection criterion for yield.

3.3 Principal component analysis

The PCA was performed for all the ten traits among the RIL population of rice as indicated in Table 4. On the basis of scree plot (Figure 1), five principal components having Eigen values more than 1 were chosen which showed about 66.6 % variability among the studied traits. The PC1 had 17.9%, PC2 showed 14.6%, PC3 showed 13.3% PC4 exhibited 10.5% and PC5 exhibited 10.23% variability among the RIL population for the traits under study. Principal component one (PC1), principal component two (PC2), principal component three (PC3), principal component four (PC4) and principal component five (PC5) had Eigen values of 1.79, 1.46, 1.33, 1.04 and 1.023, respectively.

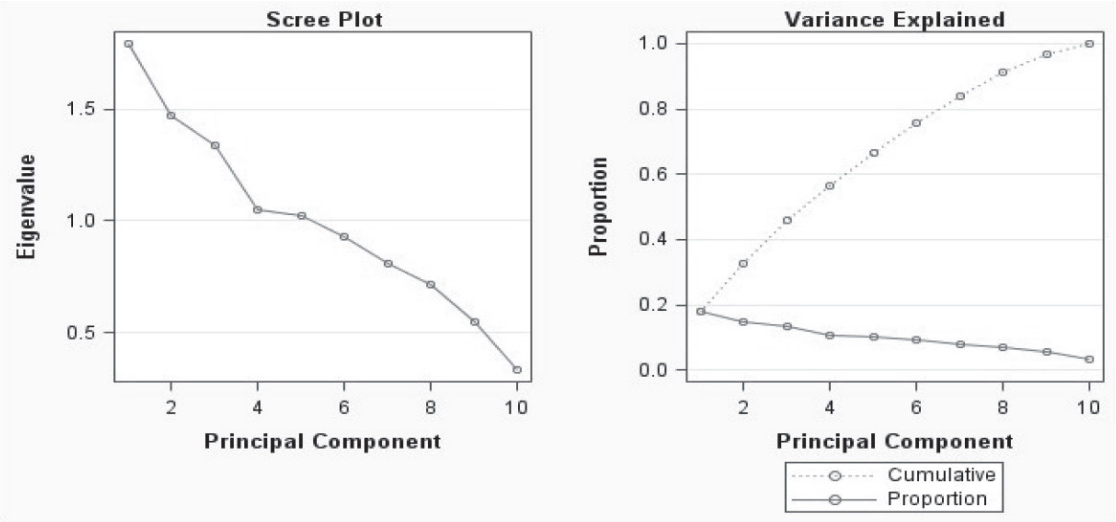

Figure 1. PCA Scree plot and Cumulative variance of RIL population

Table 4. Eigen values, contribution of variability and factor loading for the principal component axis in 100 Swarna x Type 3 RIL population of rice

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	1.79	1.46	1.33	1.04	1.023
Variability (%)	17.9	14.6	13.3	10.5	10.23
Cumulative (%)	17	32.5	45.9	56.4	66.6
Days to fifty percent flowering	-0.10	-0.34	0.32	0.10	-0.35
Plant height	0.11	0.54	-0.25	0.10	-0.16
Panicle length	-0.04	0.25	-0.38	0.55	-0.23
Number of tillers	0.002	0.10	-0.20	-0.45	0.55
Panicle weight	-0.09	0.27	0.41	-0.32	-0.44
Filled grains per panicle	-0.10	-0.27	0.26	0.53	0.37
Single plant yield	-0.37	0.38	0.19	0.20	0.32
Test weight	-0.22	0.41	0.48	0.042	0.12
Grain Iron	0.62	0.18	0.16	0.11	0.028
Grain zinc	0.60	0.05	0.31	0.10	0.16

According to PC 1 grain iron (0.62) and grain zinc (0.60) had relatively higher contributions to the total morphological variability, whereas it is negatively correlated with panicle weight, single plant yield, test weight, number of filled grains and panicle length. The highly positive correlated variables with PC2 were plant height (0.54), test weight (0.41) and single plant yield (0.38), whereas number of filled grains per panicle and days to fifty percent flowering are negatively correlated. The other component PC3 was positively correlated with test weight (0.48) and panicle weight (0.41) and negatively correlated with plant height, panicle length and number of tillers per plant. The component PC4 was positively correlated with panicle length (0.55) and number of unfilled grains per panicle (0.53) and negatively correlated with number of tillers per plant. The variables which are positive and strongly correlated with PC5 were number of tillers (0.55), filled grains per panicle (0.37) and single plant yield (0.32) contributing 10.5% variability and negatively correlated with days to fifty percent flowering, plant height and panicle weight.

On the basis of PCA analysis from Figure 2, the first two principal components (PC1 and PC2)accounted for 45% of the genetic variance. Similar results were reported by Worede *et al.* (2014) for 32.5% of the total variability using the first and second PCs. Selection of traits via grain iron, grain zinc, plant height and test weight lying in these two principal components would be beneficial in contributing to the total morphological diversity.

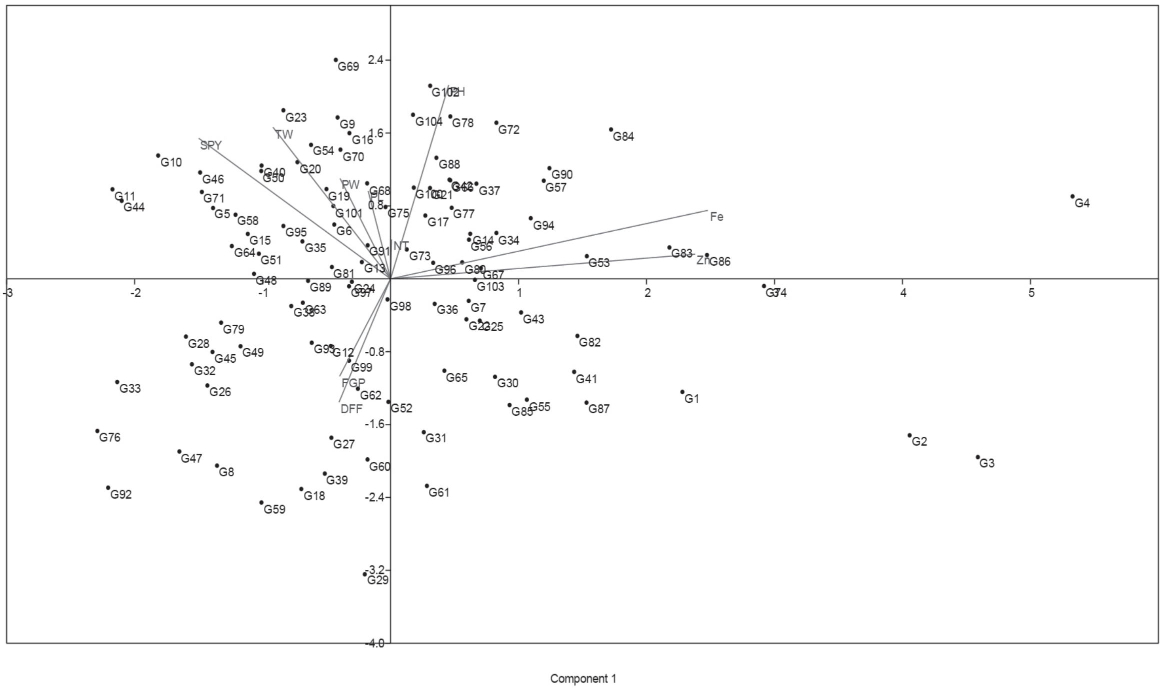

Figure 2. Principal component 1 & 2 of RIL population

4. Conclusion

In the present study critical analysis of character association and path analysis indicated that the test weight possessed both positive association and high positive direct effects. Hence, selection for these trait could bring improvement in yield and yield components. PCA identified grain iron, grain zinc, plant height and test weight in different principal components playing a prominent role in classifying the variation existing in the population. Two RILs *viz.*, P45 & P57 have shown higher grain yield per plant with high zinc and iron concentration presented in the Table 5. These RILs need to be evaluated in multilocation to access further of their yield and nutritional value in different environments. Stable high yielding lines in combination with high Zn and Fe can be a good source of genetic variability for the improvement of rice. These lines also serve as potential donor in further rice biofortification programme especially for enhanced grain zinc and iron content.

Table 5. List of RILs with high grain yield, grain iron and zinc concentration

S. No.	Genotype	Single plant yield (g)	Zinc concentration (ppm)	Iron concentration (ppm)
1	P45	21	28.5	16.1
2	P57	21.7	27.10	16

Compliance with ethical standards

NA

Conflict of Interest

Authors declare that they have no conflict of interest

Authors' contribution

Conceptualization of research (SD, NCN, CC, GSV); Designing of the experiments (SD, NCN, CC); Contribution of experimental material (NCN); Execution of field experiments and data collection (SD, NCN); Analysis of data and interpretation (SD, NCN, GSV); Preparation of the manuscript (SD, NCN, CC, GSV).

Declaration

The authors declare no conflict of interest.

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Research Article

Sensory evaluation and consumer acceptability of zinc biofortified rice by farm women in Telangana, India

Amtul Waris*, Chirravuri Naga Neeraja, Mohammed Mohibbe Azam and Battu Jangaiah#

ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad-500030, TS, India.

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***Corresponding author:**
E-mail: amtul.waris@gmail.com

Abstract

Most of the Indian diets are typically based on cereals and lack micronutrient-rich vegetables, fruits, and flesh foods. The incorporation of zinc biofortified rice in daily diets can help overcome zinc deficiency. The present study aimed to analyze the consumers' acceptance of zinc biofortified rice as the willingness of consumers to accept it is important for the success of biofortification strategy to combat micronutrient malnutrition. The consumers' acceptance of zinc biofortified rice in terms of its hand feel mouth feel texture, taste, and overall acceptability was determined using the Five-point Hedonic scale. Hand-pounded samples of zinc biofortified rice and control were provided to sixty farm women to evaluate by the Home Use Test protocol. The index of acceptability (IA) was worked out for the acceptance of zinc biofortified rice and was found to be greater than 70% for all the parameters. The consumers' socio-economic characteristics did not show a significant relationship with the acceptability of zinc biofortified rice except for hand feel texture. Based on the hedonic categorization suggested by Belmes (2019), the overall acceptability of both the zinc biofortified rice and control are in the acceptable category of hedonic rating. Therefore it can be concluded that the zinc biofortified rice variety DRR Dhan 45 is equally acceptable to the consumers as the control rice. The consumers' socio-economic characteristics did not show a significant relationship with the acceptability of zinc biofortified rice. The zinc biofortified rice can be promoted for use in daily diets to help meet the zinc needs of the family and included in the nutrition intervention programs of the country for overcoming micronutrient malnutrition.

Keywords: Consumer acceptability, sensory evaluation, zinc biofortified rice, malnutrition, farm women

1. Introduction

The micronutrients are very important for various physiological functions and their deficiencies do not lead to physical manifestations as those of macronutrients. Of these micronutrients, deficiencies of zinc and iron are reported to be the most widespread, and their adverse health consequences more severe, , mostly in low and middle-income countries(Gupta *et al.*, 2020). In India, iron and zinc deficiency among children is high (NFHS-4, 2017; and Matthew *et al.*, 2019). Although, zinc and iron both are important micronutrients but this article is focusing on zinc only.

The micronutrient zinc plays an important role in the normal functioning of the body and is integral part of enzyme systems. Mnay important biological functions of zinc include, gene expression, cell division and immunity. (Brown *et al.*, 2004). Adequate dietary intake of zinc helps in normal linear growth of children and has an ameliorating effect on the skin.(Hess and King, 2009). Zinc deficiency in pregnant women may lead to complications during pregnancy. (Kohn *et al.*, 2000; Donangelo and King, 2012).Zinc palys an important role in linear growth and weight gain (Brown *et al.*, 2002). Zinc deficiency in mothers leads to low supply of zinc to the fetus resulting in premature and low birth weight babies (Hess and King, 2009). the most common feature of zinc deficiency may manifest as diarrhea, respiratory infections, impaired immunity and short staure. (WHO, 2002; Ezzati *et al.*, 2002).

A cross-sectional study in India has reported poor cognitive performance of 45% of the adolescent girls due to low plasma concentration of zincsignifying the need to incorporate zinc rich foods in the diets.(Kawade 2012). A high prevalence of zinc deficiency due to low dietary intake has been reported in (64.6%) in pregnant women and 42% in among the nulliparous non-pregnant women in India (Pathak *et al.*, 2008; Pathak *et al.*, 2003). The inadequacy of zinc intake in India is very high and devising an intervention program targeting vulnerable populations is essential (Smith *et al.*, 2019).

Inadequate intake of iron and zinc is one of the most significant determinants for the development of their deficiency (de Benoist *et al.*, 2007). Rice is the staple food but in comparison to other foods, it is poor in iron and zinc content (Hemalatha *et al.*, 2007). Elevated zinc requirement, poor absorption and utilization by the body and increased losses, are some of the common factors resulting in zinc deficiency. Dietary factors play an important role in the development of zinc deficiency in developing countries (Gibson and Anderson, 2009). The high phytic acid content in the cereals and cereal-based diets affects their absorption due the formation of zinc-phytic acid complexes in the intestine (Lonnerdal 2000, Davidsson *et al.*, 2004 and Egli *et al.*, 2004). The bioavailability of zinc is greatly influenced by the presence of several other inhibitors (Davidsson *et al.*, 2004), including calcium and polyphenols (Kim *et al.*, 2011). Unlike iron deficiency, due to the non-specific clinical features the diagnosis of zinc deficiency is difficult and the low level of circulating zinc may be used as an indicator.

The recommended dietary allowances for zinc (mg/d) computed by ICMR are: adult men 12 mg/d, adult women10 mg/d, pregnant women 12 mg/d, lactating women 12 mg/d, boys aged 13-15 yr 11 mg/d, girls 13-15 yr 11 mg/d, and children 7-9 yr 8 mg/d (NIN, 2009).The zinc requirements are high during pregnancy and rapid physiological growth as in children and the inadequate intake leads to higher deficiency.

The pharmaceutical approach of supplementation, the industrial approach of food fortification, and agricultural approaches of dietary diversification and bio-fortification have been advocated as some of the strategies to address micronutrient deficiencies. Crop bio-fortification is increasingly being recognized as a cost-effective and sustainable approach.

Rice is a major staple food consumed widely by the poor population and serves as an ideal crop for fortification. Rice consumption in India was estimated to be 102 million tonnes in 2019-20 and it is expected to increase to 108 million tonnes in 2020-21 (Reidy 2020). Therefore, in the bio-fortification program (Nestel *et al.*, 2006; Pfeiffer & McClafferty, 2007), a major focus is to breed rice containing more Zn. In this direction, the Indian Institute of Rice Research, Hyderabad, has made considerable efforts and developed three bio-fortified high zinc rice varieties, namely, DRR Dhan 45, DRR Dhan 48 and DRR Dhan 49 with a zinc concentration of 22.6 ppm, 24 ppm, and 25.2 ppm respectively in polished grain and all are of medium duration (125-130 days) with an average grain yield of 50q/ha (Yadava et al., 2020). Some more varieties like Zinco Rice, CR Dhan 311, and CR Dhan 315 have been developed and released by other research institutes (Yadava *et al.,* 2020).

Including biofortified varieties in daily diets may help to overcome zinc deficiencies in vulnerable populations *viz*, women and children (Woods *et al.*, 2020). The nutritional intervention program of the Indian Government, Poshan Abhiyan (India.gov.in) can benefit from the biofortified crops in its efforts to reduce undernutrition and stunting as demonstrated in other regions of the world as a simple and cost-effective strategy (Reddy, 2020).

The willingness of consumers and producers to accept new crop varieties will determine whether biofortification can be successfully implemented. The acceptance of biofortified varieties by consumers is an important aspect of the biofortification program (Saltzman *et al.*, 2013). Consumer acceptance of new products is evaluated primarily by three methods, viz. laboratory tests, central location tests (CLT) and home use tests (HUT) (Meilgaard *et al.*, 2007). In HUT, the consumer prepares the food in his/her own way and consumes the food in its own environment. In-home use test the consumers can assess the product as per their expectations (Lawless and Heymann, 2010).

The objective of the present study was to determine consumers' acceptance of zinc biofortified rice variety DRR Dhan 45 through sensory evaluation in home-use testing. The rice variety DRR Dhan 45 is developed by the Indian Institute of Rice Research (IIRR) and released in 2016. It is the first among the high zinc rice varieties notified at the national level and has an overall mean zinc content of 22.6 ppm (Yadav *et al.*, 2020). This is a semi-dwarf, medium duration (125 days) variety with long slender grain and non-lodging type. It is moderately resistant to blast, sheath rot, and rice tungro virus. It is released for the states of Tamil Nadu, Andhra Pradesh, and Karnataka and has good cooking quality with desirable amylose content (20.7%).

2. Materials & Methods

The study was conducted in Nalgonda district of Telangana State, India, which has a high prevalence of stunting (28.3%), underweight (31.3%), wasting (21.2%) and anemia (69.2%) among the child population (Kim *et al.*, 2019) and high levels of anemia among women, 56.2% (NFHS-4, 2017). Convenience sampling was used in the study and ten farm women beneficiaries of the outreach programs of the Indian Institute of Rice Research (IIRR) were selected randomly from each of the six tribal hamlets of Deverkonda Mandal of Nalgonda district of Telangana. Thus the total sample size was 60 farm women. An information session was conducted in the local language with the farm women to obtain their verbal consent. The farm women were provided 500 gm each of hand-pounded rice of the high zinc variety DRR DHAN 45 (Zinc, 22.6 ppm) and check variety (Zinc, 16.7 ppm) (Yadav *et al.*, 2020).

The cooking protocol for both biofortified and control variety was typical of how the rice is cooked by the farm women. The farm women rated the zinc biofortified rice and control with respect to hand feel texture, mouthfeel texture, taste, and overall acceptability. A hedonic test was used on a 5-point hedonic scale (1-very poor, 2-poor, 3- neither poor nor good, 4-good, and 5-very good). The intervals between each score are not the same and also a product that is rated 4 is not necessarily two times as much liked more than a product rated 2. The consumers' scores are measured on an ordered categorical scale and need to be analysed accordingly (Coe, 2002).

2.1 Sensory evaluation testing using modified home use testing (HUT)

Sensory characteristics of zinc biofortified rice (DRR Dhan 45) and control (BPT-5204) were determined using a home use test. At the time of the present study, only small quantity of paddy grain of zinc biofortified rice was available and it could not be commercially milled for consumer acceptability study. Therefore, for home use test, hand-pounded samples of both zinc biofortified and control rice were provided to 60 farm women which is the minimum number of consumers required for a consumer acceptability study (Hough *et al.*, 2006; ISO 8587:2006; and Stone and Sidel, 2004) and hedonic scaling test (Gacula and Rutenbeck, 2006).

The hedonic rating as suggested by Belmes (2019) on a five-point scale was used for the categorization of sensory attributes of both zinc biofortified rice and control. The associated ranges of scores with the level of acceptability were rated as follows: 4.50-5.00 as Highly Acceptable (HA); 3.50-4.49 as Acceptable (A); 2.50-3.49 as Moderately Acceptable (MA); 1.50-2.49 as Slightly Acceptable (SA) and 1.00-1.49 as Not Acceptable (NA). The independent Student's *t* test was used to test the difference in mean scores between the two types of rice**.**

2.2 Index of acceptability (IA)

The index of acceptability (IA) was calculated using the following equation (Fernandes and Salas-Mellado (2017):

IA (%) = (Score x100) / 5

Where, the score represented acceptability reported by the farm women based on the 5-point hedonic scale.

3. Results and Discussion

3.1 Socio-demographic characteristics of the consumers

The socio-demographic characteristics of the respondents indicated that , majority of the respondents (66.7%) belonged to 31-50 age group followed by the 21-30 years age (20%) and about 13% were in the 51-60 years age group. A very high percentage (75%) of the respondents were illiterate followed by 15 percent educated to the primary level schooling followed by nearly seven percent educated up to the secondary level and only three percent belonged to the higher secondary education category. Most of the farm women (36.7%) were having 11-20 years of farming experience followed by 35% having 1-10 years, 23.3% of the respondents had 21-30 years experience and only a very small percentage (5%) had a farming experience in the range of 31- 40 years. It was recorded that 57 percent of the farm women were members of some organizations and 43 percent of them were not having membership in any organization.

3.2 Consumer acceptability of sensory characteristics of zinc biofortified rice and control

The mean sensory scores of zinc biofortified rice and control have been presented in Table 1. None of the farm women rated the hand feel texture as very poor or very good. An equal percentage (37%) of respondents rated it in the ‘poor’ and ‘neither poor nor good’ category. Only 25% of the consumers rated it as ‘good’. The mouthfeel texture was rated as ‘good’ by 56.7 % of the farm women followed by ‘neither poor nor good’ by 30%. An equal percent of farm women (6.7%) rated it as ‘poor’ and ‘very good’. The taste of biofortified cooked rice was rated as ‘good’ by 50% of the farm women and 30% rated it as ‘very good’ followed by ‘poor’(20%) and 11.7% rated it as ‘neither poor nor good’ in taste. The overall acceptability of zinc biofortified rice was rated as ‘good’ by 56.6% of the consumers followed by 25% showing a neutral attitude of ‘neither poor nor good’, 13.3% rated it as ‘very good’ and 5% rated it as ‘poor’.

Similarly, for control, none of the farm women rated the hand feel texture as ‘very poor’, but 5% rated it as ‘poor’. About half of the respondents (51.6%) rated it as ‘good’ followed by ‘neither poor nor good’ (26.7%). It was rated as ‘very good’ by 16.7% of the farm women. The mouth feel texture was rated as ‘good’ and ‘very good’ by 53.3% and 25% of the farm women, respectively. None of the farm women rated it as ‘very poor’ or ‘poor’. Taste was rated as ‘very good’ by 61.7%, ‘good’ by 28.3%, and ‘neither poor nor good’ by 10% of the farm women. The overall acceptability of control was rated as ‘very good’ by 41.7%, ‘good’ by 38.3%, and 20 % of farm women were showing a neutral attitude of ‘neither poor nor good’.

A study from Cuba indicated an overall liking for zinc and iron enhanced rice variety (Padron *et al.*, 2011). While another study in Nicaragua indicated an overall liking for the control in comparison to nutritionally enriched rice (Montecinos *et al.*, 2011). Two biofortified rice varieties and control were equally accepted by consumers in a study in Bolivia (Woods *et al.*, 2020). In a Colombian study, the biofortified variety had a higher overall acceptance compared to the locally consumed variety (Woods *et al.*, 2020). A study among rice consumers in Bangladesh reported the acceptability of smell, colour, and taste of fortified rice by the majority of the participants (Chakravorty and Akhter, 2014). Moretti *et al.* (2005), and Beinner (2010), reported that fortified rice was acceptable to the panelists. Biofortified rice as a good source of bioavailable zinc as compared to rice postharvest fortified has been reported by Marica Brnić *et al.* (2016). Recommending the appropriate cooking method in retaining micronutrient content in cooked rice and educating the homemakers too plays a vital role in the acceptance of fortified rice (Azam *et al.*, 2021).

The results (Table 1) based on the categorization by Belmes (2019) indicate that the hand feel texture of zinc biofortified rice was moderately acceptable while that of control is acceptable. As for mouth feel texture both the zinc biofortified rice and control is acceptable. The taste of both zinc biofortified rice and control are in the acceptable category. Similarly, the overall acceptability of both the zinc biofortified rice and control were in the acceptable category of hedonic rating. However, both zinc biofortified rice and control were not rated in the highly acceptable category. The plausible reason could be that samples of both zinc biofortified rice and control were hand-pound and most of the consumers are accustomed to eat highly uniform and polished white rice. Therefore it can be concluded that the zinc biofortified rice variety DRR Dhan 45 is equally acceptable to the consumers as the control rice. Rai *et al.* (2019) reported no differences in hedonic scores for nonfortified rice and rice blended with fortified rice kernels and concluded that the acceptability of fortified rice primarily depends on the palatability of the fortified rice. Similarly, no difference in mean hedonic scores for rice fortified with ferric pyrophosphate and non-fortified rice was reported by Radhika *et al.* (2011). No significant difference in overall acceptability between the normal and iron-fortified rice products was reported by Sarkar *et al.* (2015).

Table 1: Mean sensory scores of zinc biofortified rice and control (n-60)

Parameters	**Biofortified rice (DRR Dhan 45)**		**Control (BPT-5204)**	
	Sensory Score	Acceptability level	Sensory Score	Acceptability level
Hand feel texture	3.45±0.565^{a}	Moderately Acceptable	3.98±0.724^{b}	Acceptable
Mouth feel texture	3.63±0.713^{b}	Acceptable	4.08±0.590^{b}	Acceptable
Taste	4.02±0.873^{b}	Acceptable	4.38±0.613^{b}	Acceptable
Overall acceptability	3.75±0.750^{b}	Acceptable	4.2±0.567^{b}	Acceptable

All values are means ± SDs. Values in the same row having same alphabet were not significantly different by independent Student's *t* test ($p<.050$).

The calculated Index of Acceptability (IA) of zinc biofortified rice (DRR Dhan 45) and control has been shown in Table 2 and Figure 1. It shows that the IA was more than 70% for all the parameters for both zinc biofortified rice and control except for the hand feel texture of zinc biofortified rice that obtained a slightly lower score on acceptability (69%). According to Spehar and Santos (2002) for a product to be considered acceptable in terms of its sensory properties, it must obtain a minimum score of 70%. Thus it can be concluded that the sensory attributes of zinc biofortified rice are acceptable to the consumers and they may be motivated to include it in their daily diets. The supply-side issues may be addressed to ensure the availability of zinc biofortified rice in the local markets. Consequently, it may be provided under the various nutritional intervention programs especially for children and women from the vulnerable population.

Table 2: Index of Acceptability for zinc biofortified rice and control

Parameters	**Zinc biofortified rice (DRR Dhan 45)**	**Control (BPT-5204)**
	Index of Acceptability	Index of Acceptability
Hand feel texture	69.0	79.6
Mouthfeel texture	72.6	81.6
Taste	80.2	87.6
Overall acceptability	76.5	84.0

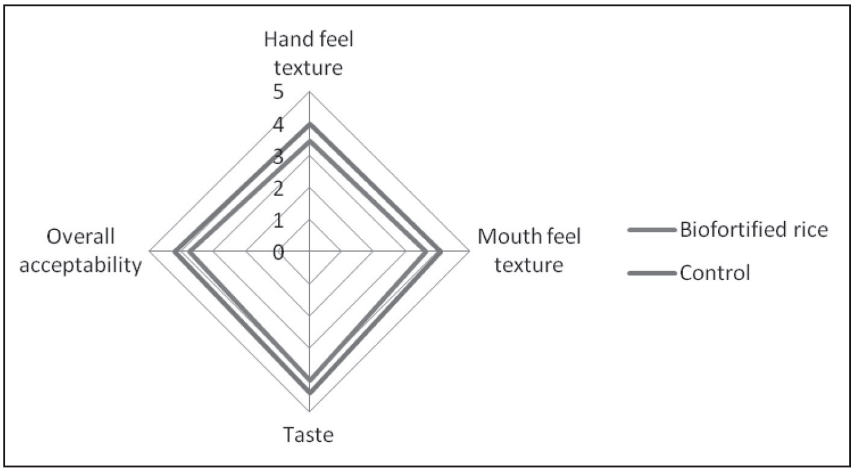

Figure 1: Consumer acceptability scores on a 5 - point hedonic scale
(Scale: 1 very poor; 2- poor; 3- neither poor nor good; 4- good; 5- very good)

3.3 Relationship between socio-economic characteristics and consumer acceptability of zinc biofortified rice

Attempts were made to find the relationship between the personal characteristics of farm women and the acceptability of zinc biofortified rice through the determination of Pearson's correlation coefficient (Table 3). It was found that none of the personal attributes of the farm women indicated a statistically significant relationship. The interaction coefficient for education, membership in organizations and farming experience though positive is insignificant. Whereas, age, family members and farm size have shown negative and insignificant relationship. Thus, it can be concluded that zinc biofortified rice would be accepted irrespective of the age, educational status, family size, farm size, membership status and farming experience of farm women. In a similar study on acceptability of biofortified products, Etumnu (2016) found that consumers' socio-economic characteristics did not have a significant effect on acceptance of biofortified orange flesh sweet potato in Sub-Saharan Africa.

Table 3: Relationships between socio economic characteristics and zinc biofortified rice acceptability

	Acceptability of Zinc biofortified rice	
	Pearson Correlation	-0.079
Age	Sig. (2-tailed)	0.548
	N	60
	Pearson Correlation	0.042
Education	Sig. (2-tailed)	0.747
	N	60
	Pearson Correlation	-0.138
Family Members	Sig. (2-tailed)	0.292
	N	60
	Pearson Correlation	0.132
Membership	Sig. (2-tailed)	0.315
	N	60
	Pearson Correlation	-0.066
Farm Size	Sig. (2-tailed)	0.619
	N	60
	Pearson Correlation	0.064
Experience	Sig. (2-tailed)	0.627
	N	60

4. Conclusion

Consumer acceptance of biofortified rice is an important criterion for its incorporation in the daily diets of families. Zinc biofortified rice, DRR Dhan 45 was acceptable to the consumers and the index of acceptability was greater than 70%. Moreover, based on hedonic scoring both the zinc biofortified rice and control are in the acceptable category. The socio-demographic characteristics of the consumers did not show a statistically significant relationship with the acceptability of zinc biofortified rice and it can be concluded that its acceptance is independent of the personal and social attributes of the consumers. Further studies on consumer acceptability may be undertaken with polished rice with a larger sample both in urban and rural areas and also with different age groups of children as consumers. Based on the acceptability of zinc biofortified rice it is recommended that it may be included in the supplementary feeding programs for children and nutritional intervention programs for women to overcome micronutrient malnutrition.

Compliance with ethical standards

NA

Conflict of Interest

Authors declare that they have no conflict of interest

Authors' contribution

Conceptualization of research and designing of experiments (AW, CNN), Conduction of experiment (AW, MMA, BJ), Preparation of manuscript (AW, CNN).

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Research Article

Identification of heat tolerant barley genotypes based on heat susceptibility index

Suman Devi, Yogender Kumar* and Sachin Shehrawat

Wheat and Barley Section, Department of Genetics and Plant Breeding,
CCS Haryana Agricultural University, Hisar-125004 (Haryana), India

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***Corresponding author:**
E-mail: yogenderkgulia@gmail.com

Abstract

The present experiment was planned to understand the impact of high temperature on grain yield and its component traits in order to select heat tolerant genotypes for future breeding programmes. Fifty barley genotypes were evaluated under two environments created by different dates of sowing *i.e.* timely sown and late sown, during *Rabi* 2016-17. The mean sum of squares due to genotypes based on heat susceptibility index (HSI) revealed the presence of significant variation for all the traits except plant height, spike length and grains per spike. Correlation among HSI of different traits indicated significant positive association of grain yield per plot with harvest index, biological yield, 1000-grain weight, plant height and days to maturity. Out of 50 genotypes studied, HUB 242 exhibited lowest HSI and percent reduction in grain yield under heat stress conditions. The genotypes namely HUB 242, DWRUB 52, RD 2904, BH 902 and IBYT-HI-13 were found most promising based on heat susceptibility index (HSI) of grain yield. However, based on overall rank of HSI of all the traits, IBON-HI-13, IBYT-HI-13, RD 2904, HUB 242 and IBON-HI-3 were identified heat tolerant genotypes. These genotypes could be utilized as promising breeding material for the development of new heat tolerant barley varieties.

Key words: Barley, heat stress, HSI, tolerance

1. Introduction

Barley (*Hordeum vulgare* L.) is an important *rabi* cereal crop grown throughout the temperate and sub-tropical regions of the world. This crop occupied fourth position in total cereal production in the world after wheat, rice and maize (USDA, 2020). Nationally, it is cultivated on an area of 0.62 million hectare producing 1.59 million tonnes grain with productivity of 25.73 q/ha during the crop season 2019-20 (ICAR-IIWBR, 2020). In Haryana state, 44000 tonnes barley was produced from 12,200 hectare area with average productivity of 36.07 q/ha which ranks second after Punjab (37.67 q/ha). Barley can be cultivated in diverse landforms for its tolerance against heat, drought, frost and alkaline soils (Mishra and Shivakumar, 2000). Its cultivation in India is now becoming oriented towards industrial utilization. There is challenge for the breeders to develop genotypes for high yield potential with high malt content and greater stability. Such genotypes with short maturity and good tillering can further overpass the yield gap and can be helpful to meet the demand of quality grain for malting purpose. Generally long duration genotypes are high yielding. But under late sown or terminal heat condition, early maturing/ short duration genotypes may perform better with minimum reduction in grain yield.

Temperature is an important environmental factor influencing the growth and development, and finally the yield of crop plants. Increased ambient temperature as a result of global warming and climate changes is emerging as a great threat to the growth and development of most crop plants. When temperatures are elevated from anthesis to grain maturity, grain yield is reduced because of the reduced time for sink (grain) development. The drastic reduction in morphological and yield contributing traits *i.e.,* plant height, number of tillers/plant, spike length, 1000-grain weight and plant yield under heat stress conditions could be due to the inhibition of photosynthesis which is reflected by the loss of chlorophyll content of the leaves. Vaezi *et al.* (2010) reported reduction in number of spikes per square meter, grain number per spike and 1000-grain weight in barley due to delayed sowing and also observed reduction in grain yield by 39.59% and 31.39%, respectively in two- and six- row genotypes. Modhej *et al.* (2015) also reported an average grain yield reduction in barley and bread wheat genotypes by 17% and 23%, respectively, when these crops were exposed to heat stress after anthesis.

Recent climate change gained the attention of plant breeders due to its adverse effect on crop production. The increased temperature at the far ahead phases of crop period starting from pre-heading to post-anthesis must be understood as chief yield limiting feature (Farooq *et al.,* 2011). The most favorable temperature for barley at grain filling stage is 20°C in sub-tropical regions as depicted by various researchers (Dwivedi *et al.,* 2017). Effect of high temperature on barley growth and development becomes complex after anthesis, as with high temperature (>32°C), period of assimilate accumulation becomes short which results in lower yields (Funaba *et al.,* 2006). It was also observed that even slight increase of 1° C from the optimum ranges of temperature during grain filling had adverse effects on grain yield (Narayanan, 2018). Terminal heat stress, in particular, at post-heading stage causes considerable yield reduction due to stress at critical stages, *i.e.,* anthesis and grain filling (Rehman *et al.*, 2009). At flowering, it causes negative effect on pollen fertility and seed setting which lead to low grain number per spike (Ferris *et al.*, 1998). Furthermore, it shortens the period of grain filling and reduces individual grain weight (Dias and Lidon, 2009; Kaur and Behl, 2010).

The development of barley cultivars with stable performance and higher economic yield under different environments is a primary prerequisite of any breeding program to cope with adverse (abiotic and biotic stress) conditions. Incorporation of heat tolerance in the variety development process is an essential task that breeders would like to achieve by exploring new sources of genetic variability and their utilization (Verma *et al.*, 2021). The adverse effects of heat stress can be mitigated by developing crop plants with improved thermo tolerance using various genetic approaches. The genotypes may display different ability to produce acceptable yield under heat stress. In order to exploit heat tolerance in breeding programmes, a thorough understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermo tolerance is imperative. The heat susceptibility index (HSI) may be used as an indicator of yield stability and a proxy for heat tolerance (Kavita *et al.*, 2016). Hence, the fifty genotypes of barley including six and two-rowed were evaluated for heat tolerance using.

2. Materials and Methods

The experimental material consisted of 50 diverse genotypes of barley including BH 946 and DWRB 101 as check varieties. The material was grown under two different conditions *i.e.* timely (15th November) and late sown (14th December) at Barley Research Area, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar during *rabi* 2016-17 under irrigated condition. The experimental location is situated at latitude of 29° 10’ N, longitude of 75° 46’ E and at an altitude of 215.2 m above mean sea level. The experimental material represented both two (17) and six (33) row types and evaluated in RBD with three replications. Each genotype was grown in a plot size of 3.0 x 0.69 m^2 per replication and the recommended cultural practices were adopted to raise the crop. Observations were recorded on 10 quantitative characters *viz*, days to heading, days to maturity, plant height (cm), spike length (cm), tillers per meter row, grains per spike, 1000-grain weight (g), biological yield (g/plot), grain yield (g/plot) and harvest index (%). Five randomly selected competitive plants in each replication were recorded for all the traits under study except days to heading, days to maturity, biological yield and grain yield which were recorded on plot basis. Further, the value of harvest index was calculated as per the formula given by Donald and Humblin (1976). The weather parameters during the crop season are presented in Fig. 1. Weekly mean maximum temperature varied between 16.9 to 42.9 ^{0}C, whereas, the weekly mean minimum temperature was between 3.2 to 24.6 ^{0}C. Morning RH varied from 46 to 100% while evening RH was highly variable with a range from 16 to 81%. Total amount of rainfall received during the season at Hisar was 59.2 mm.

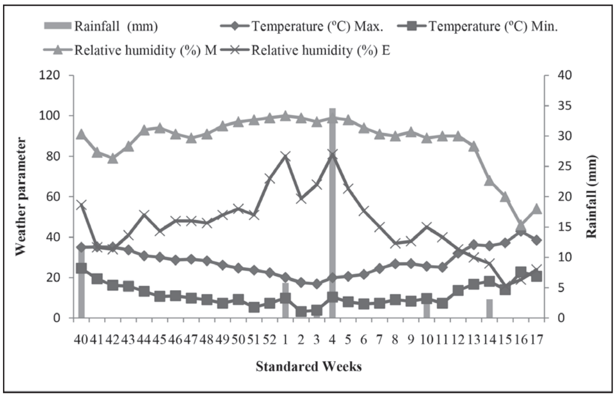

Fig. 1 Weather parameters during the crop season (2016-17)

The recorded data was subjected to analysis using OPSTAT Software (Sheoran *et al.*, 1998). HSI was calculated for grain yield and all other traits by using the formula as suggested by Fischer and Maurer (1978). HSI = [1-YD/YP]/D Where, YP = Mean of genotypes under timely sown, YD = Mean of genotypes under late sown and D = 1- Mean YD of all genotypes/Mean YP of all genotypes.

3. Results and Discussion

The reduction in the performance of barley genotypes under terminal heat condition was expressed in terms of HSI. The HSI of ten quantitative traits were subjected to analysis of variance and mean sum of squares has been presented in Table 1. This table describes the significance of genotypes for HSI of different traits which is pre-requisite for further analysis. The results revealed the presence of significant variance among genotypes for the HSI of all the traits except plant height, spike length and grains per spike. This implies that the magnitude of differences in genotypes was enough to provide scope for selection with improved heat stress tolerance. These results corroborate with the findings of Shehrawat *et al.* (2020) for plant height and spike length.

Table 1. Mean sum of squares for HSI of different traits in barley genotypes

Source of Variation	d.f.	Mean Sum of Squares									
		DH	DM	PH	SL	T/M	G/S	TGW	BY	GY	HI
Replication	2	1.090	0.050	1177.8735	3011224.6615	6.541	20.8315	0.008	5.209	0.324	1.971
Treatment	49	12.138**	0.259**	8.014	17778.833	1.972**	1.983	1.229**	2.216**	0.7099**	3.471**
Error	98	0.426	0.015	6.551	19778.941	0.748	1.658	0.109	0.467	0.167	0.753

DH: Days to heading, DM: Days to maturity, PH: Plant height, SL: Spike length, T/M: Tillers per meter, G/S: Grains per spike, TGW: 1000-grain weight, BY: Biological yield per plot, GY: Grain yield per plot, HI: Harvest index, **: Significant at 1%

The of different traits for 50 barley genotypes have been depicted in Table 2. The genotypes with high positive HSI values are susceptible to high temperature and *vice versa* (Fischer and Maurer, 1978). The estimates of HSI for the important traits under study can be utilized for selection of tolerant genotypes. The HSI for grain yield revealed that the genotype HUB 242 (-0.37) followed by DWRUB 52 (-0.01), RD 2904 (0.11), BH 902 (0.13), IBYT-HI-13(0.18) and IBON-HI-13 (0.18), exhibited minimum HSI, therefore, these entries possessed low heat susceptibility and high yield stability under heat stress condition. In contrast, BH 14-42 (1.79) followed by MGL 105 (1.64) recorded with maximum HSI for grain yield and were identified as highly susceptible to heat. Bahrami *et al.* (2020) also assessed tolerance to terminal heat stress in cultivated (*Hordeum vulgare ssp. vulgare* L.) and wild (*H. vulgare ssp. spontaneum* L.) barley genotypes using phenological and agronomic traits as well as selection indices based on grain yield. Four genotypes namely, 2nd GSBSN-15-35 (-5.81, 0.13), IBON-HI-13 (-3.40, 0.45), IBON-HI-37 (-3.40, 0.52) and 2nd GSBSN-15-8 (-2.16, 0.60) exhibited superiority for days to heading and maturity based on lowest HSI. These genotypes showed low reduction for days to maturity as well as grain yield under late sown as compared to timely sown. Under stress condition, no reduction in plant height was recorded in IBON-HI 37 (-4.08), UPB 1059 (-3.80), IBON-HI-13 (-3.71), 2nd GSBSN-15-8 (-2.06) and IBON-HI-3 (-2.00). The estimates of HSI for spike length highly varied from -188.87 to 215.88. Out of fifty genotypes studied, fourteen showed negative HSI and BH 15-30 (-188.87), MGL-64 (-157.74), MGL-117 (-96.07), BH 15-17 (-80.18) and DWRB 101 (-78.63) genotypes were found highly heat tolerant for this trait. Various morpho-physiological traits were also used by Sallam *et al.* (2018) in order to identifying the tolerant genotypes for heat tolerance improvement in barley through breeding.

Table 2. HSI of different traits in barley genotypes

Sr. No.	Genotypes	RT	DH	DM	PH	SL	T/M	G/S	TGW	BY	GY	HI	Rank based on GY	Overall Rank
1	IBYT-HI-19	6	1.20	1.39	2.97	-64.88	-1.30	0.57	1.29	0.35	1.23	2.11	32	29
2	IBYT-HI-13	6	1.77	0.95	1.58	-4.37	-0.05	0.74	0.32	-0.42	0.18	0.70	5	2
3	IBYT-HI-17	2	1.46	0.94	-0.45	22.46	-0.27	1.97	1.10	-0.63	0.56	1.54	13	11
4	IBYT-HI-16	6	0.12	1.21	0.26	121.43	0.82	0.99	1.50	0.05	1.03	1.83	25	31
5	IBYT-HI-18	6	0.36	1.27	0.40	61.80	1.90	1.08	1.27	1.00	1.10	1.15	30	42
6	IBYT-HI-23	2	2.15	1.25	1.23	-10.18	0.28	1.13	0.50	0.96	1.24	1.52	33	28
7	IBYT-HI-15	6	2.33	1.00	0.73	43.78	1.38	0.63	0.98	0.58	0.46	0.25	10	20
8	IBYT-HI-20	6	0.88	0.82	-0.34	77.58	1.07	1.18	1.77	0.78	1.26	1.81	35	37
9	BH 959	6	2.94	1.25	-0.64	105.20	0.88	1.94	0.92	0.74	1.30	1.93	36	47
10	DWRB 123	2	2.56	1.34	0.07	-16.89	1.37	1.28	1.07	1.07	0.91	0.71	21	33
11	DWRB 137	6	2.43	0.75	1.08	-3.36	0.26	0.35	0.34	0.58	1.06	1.53	27	10
12	MBGSN 145	2	2.32	0.66	0.46	10.18	0.63	2.06	-0.34	1.19	0.75	0.14	17	13
13	RD 2904	2	2.61	0.94	-0.09	19.05	-0.19	1.12	0.37	-0.38	0.11	0.57	3	3
14	RD 2909	6	2.20	1.04	0.41	49.63	3.00	0.29	0.05	1.00	0.42	-0.33	8	17
15	UPB 1059	6	1.53	0.73	-3.80	28.58	1.87	2.04	0.49	0.04	0.63	1.19	14	13
16	HUB 242	6	2.49	1.06	-1.33	68.40	0.62	0.02	-0.38	1.05	-0.37	-2.41	1	4
17	2nd GSBSN-28 (2015)	6	2.33	1.16	-0.11	58.72	1.88	0.52	0.48	0.61	0.44	0.16	9	19
18	2nd GSBYT-23 (2015)	6	1.18	0.80	-0.22	97.06	1.09	2.43	0.79	0.57	1.00	1.38	24	25
19	K 560	6	2.45	0.97	1.76	70.19	1.75	-0.12	0.37	1.22	0.42	-0.92	7	23
20	JB 481	6	0.63	0.66	2.28	21.05	1.78	-0.09	0.90	1.35	1.48	1.70	45	35
21	2nd GSBSN-60 (2015)	6	1.03	0.96	1.50	34.30	0.47	1.24	0.64	0.88	0.72	0.55	15	15
22	2nd GSBYT-02 (2015)	2	0.88	0.85	0.95	69.16	1.46	0.83	1.54	1.22	0.53	-0.45	11	24
23	MGL-58	6	0.63	0.96	1.63	62.95	1.66	0.78	0.63	1.49	1.45	1.63	44	44
24	MGL-62	2	-0.79	1.33	1.67	-33.24	1.62	2.15	0.38	0.77	1.44	2.15	43	39
25	MGL-64	6	1.11	1.02	0.33	-157.74	1.48	0.22	0.07	1.89	0.74	-1.20	16	8
26	DWRB 101	2	2.01	0.63	0.32	-78.63	1.25	1.47	1.04	1.23	0.81	0.08	18	12

27	MGL 105	6	-1.54	0.94	1.43	46.56	0.73	1.18	1.34	0.56	1.64	2.67	49	35
28	MGL-117	6	2.69	0.89	-0.79	-96.07	-0.14	0.55	0.70	-0.46	0.98	2.13	23	6
29	IBON-HI-1 (2015-16)	6	6.06	1.51	-0.72	215.88	0.15	0.16	1.17	1.00	1.49	2.08	47	46
30	IBON-HI-3 (2015-16)	2	-0.13	0.87	-2.00	6.15	-0.09	0.39	1.65	0.09	0.92	1.68	22	5
31	IBON-HI-13 (2015-16)	6	-3.40	0.45	-3.71	192.24	-0.01	4.03	0.62	-0.85	0.18	0.95	6	1
32	IBON-HI-37 (2015-16)	6	-3.40	0.52	-4.08	60.21	0.95	1.66	1.49	-0.68	1.07	2.42	28	16
33	IBON-HI-67 (2015-16)	6	2.09	1.37	-1.00	199.43	0.72	2.24	0.70	0.55	1.21	1.82	31	41
34	BH 902	6	0.13	1.04	0.43	86.39	0.08	1.09	0.93	0.82	0.13	-0.80	4	9
35	2nd GSBSN-15-8	6	-2.16	0.60	-2.06	50.11	0.73	1.02	1.31	-0.99	0.83	2.24	20	7
36	INBON-15-16	6	5.34	1.49	0.55	68.58	1.66	0.81	-0.98	1.08	1.07	1.09	29	45
37	INBON-15-22	6	0.12	0.85	0.17	4.42	2.01	-0.17	1.26	1.41	1.25	1.18	34	22
38	2nd GSBSN-15-35	2	-5.81	0.13	2.17	59.24	0.95	1.86	1.95	0.28	0.54	0.75	12	21
39	AZAD	6	-0.65	1.22	3.40	-22.92	2.18	1.48	1.11	2.06	1.49	0.82	46	48
40	DWRB 143	2	0.66	1.03	-0.22	54.76	1.66	1.99	1.15	2.42	1.39	0.02	40	43
41	BH 13-20	2	2.06	0.56	2.28	1.39	1.12	0.59	1.49	1.08	1.04	0.89	26	25
42	BH 13-22	6	2.89	1.40	1.69	25.03	0.66	1.77	1.14	2.09	1.41	0.52	41	49
43	BH 13-26	2	0.51	1.03	2.09	-78.55	1.56	0.08	1.85	2.34	1.42	0.11	42	38
44	BH 14-25	2	-0.27	0.86	1.41	58.42	0.11	1.55	1.65	1.73	1.51	1.44	48	40
45	BH 14-42	6	-1.13	1.08	1.86	47.72	1.46	1.28	2.04	2.26	1.79	1.67	50	50
46	BH 15-17	2	1.05	0.92	2.28	-80.18	0.11	1.55	1.63	2.37	1.32	-0.18	37	32
47	BH 15-30	6	0.48	1.29	-0.03	-188.87	1.17	1.31	1.70	1.73	1.32	0.69	38	34
48	BH 946	6	0.76	1.52	1.75	14.72	1.34	-0.06	1.08	1.29	0.82	0.19	19	25
49	BH 885	2	0.12	1.03	-0.38	9.86	1.36	1.07	1.63	1.87	1.34	0.76	39	30
50	DWRUB 52	2	1.60	1.19	2.21	-28.45	-0.04	1.57	1.48	0.97	-0.01	-1.33	2	18

RT: Row type, DH: Days to heading, DM: Days to maturity, PH: Plant height, SL: Spike length, T/M: Tillers per meter, G/S: Grains per spike, TGW: 1000-grain weight, BY: Biological yield per plot, GY: Grain yield per plot, HI: Harvest index

The lowest HSI for tillers per meter was found in IBYT-HI-19 (-1.30) followed by IBYT-HI-17 (-0.27), RD 2904 (-0.19), MGL-117 (-0.14) and IBON-HI-3 (-0.09), whereas, for grains per spike the genotypes *i.e.* INBON-15-22 (-0.17), K 560 (-0.12), JB 481 (-0.09), BH 946 (-0.06) and HUB 242 (0.02) showed minimum HSI. The HSI for 1000-grain weight ranged from -0.98 (INBON-15-16) to 2.04 (BH 14-42). The genotypes showed negative values of HSI for a particular trait signifies the better performance of genotype under heat stress than the non-stress condition for that trait, is suitable for climate resilience (Thakur *et al.*, 2020).

The heat tolerant genotypes identified for biological yield per plot were 2nd GSBSN-15-8 (-0.99), IBON-HI-13 (-0.85), IBON-HI-37 (-0.68), IBYT-HI-17 (-0.63) and MGL-117 (-0.46) while for harvest index, HUB 242 (-2.41) followed by DWRUB 52 (-1.33), MGL-64 (-1.20), K 560 (-0.92) and BH 902 (-0.80) exhibited superiority. All the genotypes with low HSI mentioned above showed their superiority for tolerance to high temperature than other genotypes. Ram and Shekhawat (2017) also calculated HSI for various traits in barley in order to select heat tolerant genotypes for future breeding programme. Top ranked genotypes based on HSI of grain yield were HUB

242, DWRUB 52, RD 2904, BH 902 and IBYT-HI-13, whereas, the genotypes *viz.*, IBON-HI-13, IBYT-HI-13, RD 2904, HUB 242 and IBON-HI-3 were identified heat tolerant based on overall rank of HSI of all the traits studied. HSI was also used by Parashar *et al.* (2019) to study the impact of high temperature on yield and its attributing traits for selection of heat tolerant parents and cross combinations in barley. The study by Suresh *et al.* (2017) corroborates our results for identification and /or selection of genotypes based on HSI values.

Correlation coefficients were worked out based on HSI of different traits, to estimate the degree of association among various characters for heat tolerance (Table 3). Grain yield per plot exhibited significant positive association with HI, biological yield, 1000-grain weight, plant height and days to maturity, showing the importance and effectiveness of these traits for detection and screening of high yielding thermo-tolerant genotypes under stress condition. Significant positive correlation was also observed for days to heading with days to maturity and biological yield; days to maturity with biological yield; plant height with spike length and biological yield; tillers per meter with biological yield; and 1000-grain weight with harvest index. Similarly, significant negative correlation was recorded for days to heading with grains per spike, 1000-grain weight and harvest index; plant height with grains per spike; spike length with tillers per meter; grains per spike with biological yield; and HI with biological yield. Correlation among HSI of different characters were also worked out by Shehrawat *et al.* (2020) to estimate the degree of association for heat tolerance.

Table 3. Correlation among HSI of different traits in barley genotypes

Traits	DH	DM	PH	SL	T/M	G/S	TGW	BY	GY	HI
DH	1.000	0.514**	0.024	-0.024	-0.024	-0.234**	-0.441**	0.166*	-0.036	-0.166*
DM	0.514**	1.000	0.131	0.053	-0.032	-0.122	-0.140	0.257**	**0.165***	-0.039
PH	0.024	0.131	1.000	0.622**	-0.125	-0.376**	0.042	0.334**	**0.221****	-0.060
SL	-0.024	0.053	0.622**	1.000	-0.207*	-0.091	0.017	-0.086	-0.013	0.062
T/M	-0.024	-0.032	-0.125	-0.207*	1.000	-0.052	-0.071	0.297**	0.150	-0.084
G/S	-0.234**	-0.122	-0.376**	-0.091	-0.052	1.000	0.021	-0.211**	-0.111	0.071
TGW	-0.441**	-0.140	0.042	0.017	-0.071	0.021	1.000	0.129	**0.309****	0.193*
BY	0.166*	0.257**	0.334**	-0.086	0.297**	-0.211**	0.129	1.000	**0.478****	-0.366**
GY	-0.036	0.165*	0.221**	-0.013	0.150	-0.111	0.309**	0.478**	1.000	0.619**
HI	-0.166*	-0.039	-0.060	0.062	-0.084	0.071	0.193*	-0.366**	**0.619****	1.000

DH: Days to heading, DM: Days to maturity, PH: Plant height, SL: Spike length, T/M: Tillers per meter, G/S: Grains per spike, TGW: 1000- grain weight, BY: Biological yield per plot, GY: Grain yield per plot, HI: Harvest index, *, **: Significant at 5 and 1%, respectively

The high temperature during the reproductive phase of barley poses detrimental effect to the growth and development. But, the genotypes performed differently under heat stress conditions. Some of the genotypes were adversely affected while some could combat with the stress. *Fig.* 2 depicts a radar graph representing the genotypes with per cent reduction in grain yield. One of the promising genotype showing no reduction in grain yield was HUB 242 (-12%). Other tolerant genotypes having minimum reduction in grain yield were RD 2904 (4%), DWRUB 52 (5%), IBON-HI-13 (2015-16) (6%), BH-902 (8%) and IBYT-HI-13 (9%). Pathak *et al.* (2017) also reported reduction in grain yield, spike length, grains per spike and 1000-grain weight in barley under stress condition.

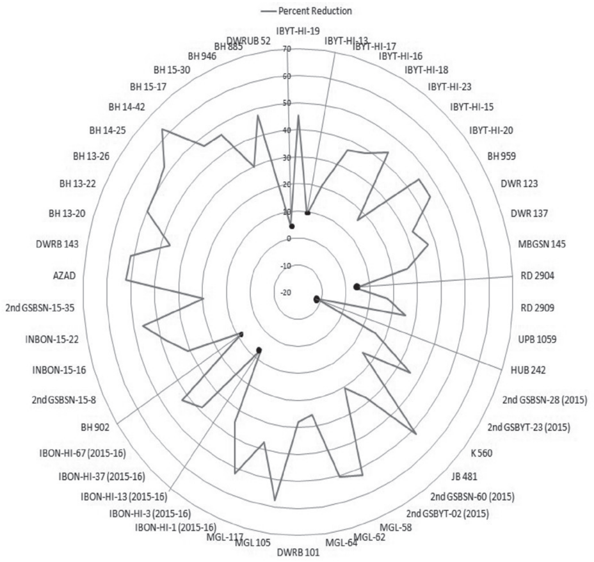

Fig. 2 Per cent reduction in grain yield in barley genotypes

4. Conclusion

From this study, it could be concluded that HSI used under study was recognized as paramount for identifying cultivars with high tolerance to heat stress. The genotypes HUB 242, DWRUB 52, RD 2904, BH 902 and IBYT-HI-13 were found promising based on HSI of grain yield. Likewise, based on overall rank of HSI of all the traits studied, the genotypes *viz*., IBON-HI-13, IBYT-HI-13, RD 2904, HUB 242 and IBON-HI-3 were identified heat tolerant. Hence, study gives an ample opportunity for selection of material to be incorporated in elite cultivars of barley to tolerate the terminal heat stress under North Indian conditions.

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Conflict of Interest

Authors declare that they have no conflict of interest.

Ethical Compliance Statement

NA

Author's Contribution

Conceptualization of research (YK); Designing of the experiments (YK, SD); Contribution of experimental materials (YK); Execution of experiments and data collection (SD, YK); Analysis of data and interpretation (YK, SS); Preparation of the manuscript (YK, SD)

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Short Communication

Genetic diversity among bread wheat (*Triticum aestivum* L.) genotypes as assessed by SSRs

Renuka Sharma[1], Satish Kumar[2], Sanjay Kumar Singh[2], Pradeep Sharma[2] and Gyanendra Pratap Singh[2]

[1]*Assistant Professor, Chandigarh Group of Colleges, Mohali*
[2]*ICAR-Indian Institute of Wheat and Barley Research, Karnal 132001 (Haryana) India*

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****Corresponding author:*** *neprads@gmail.com*

Drought is one among the several climatic factors impeding crop productivity and poses a challenge to global food security. The intensity and frequency of droughts are predicted to increase by 50% to 200% during the 21st century in various geographical regions (Trenberth *et al.*, 2014; Zhao *et al.*, 2017). Raising drought tolerant wheat genotypes is the eventual means of safeguarding the crop against water stress. However, drought tolerance is a complex trait governed by various genes, each with minor effects (Bernardo 2008). Knowledge about germplasm diversity significantly impacts the crop improvement programs by supplying novel sources of gene combinations (Ayana and Beleke, 1998). Prior knowledge of genetic diversity and relationships between the elite lines and cultivars are useful for development of new cultivars. It is highly desirable to characterize genetic diversity among wheat germplasm collections to broaden genetic diversity in future wheat breeding programmes (Haung *et al.*, 2002). Molecular markers have proven their role in crop improvement programs by providing selection precision and accelerating the efforts. Assessing genetic diversity within a narrow genetic pool of novel breeding germplasm could make crop improvement more efficient by the directed accumulation of desired alleles. This is likely to speed up the breeding process and decrease the amount of plant material that needs to be screened in such experiments. Genetic variation in common wheat have been studied using different molecular markers such as RAPDs RFLP, AFLPs, SSR, STS, ISSRs, gene based and MIR based SSRs (Siedler *et al.*, 1994, Gupta and Varshney, 2000, Sharma *et al.*, 2021, Mehta *et al.*, 2021). Since SSRs are multi-allelic nature, co-dominant inheritance, reproducibility, abundance and high polymorphic information content (PIC). A small number of SSR markers are adequate to differentiate the closely related wheat and barley species (Plaschke *et al.*, 1995; Russel *et al.*, 1997; Singroha *et al.*, 2020).

In present investigation, we determined genetic diversity and relationships at the molecular level among the fourteen wheat genotypes using microsatellite markers. The phylogenetic relationships and genetic diversity thus analyzed will assist in parental selection in wheat breeding programmes.

We procured fourteen wheat genotypes from the Germplasm Unit, Indian Institute of Wheat and Barley Research, Karnal and were used for cluster analysis at molecular lene. Fresh and young leaves were used to extract genomic DNA according to the method devised by Saghai-Maroof *et al.* (1984). A total number of 44 GWM were selected, representing each wheat chromosome for genotyping (Table 1). Polymerase chain reaction (PCR) was carried out as described earlier (Sharma *et al.*, 2016). The amplification products were resolved in 2% agarose in 1× Tris-borate EDTA buffer (45 mM Tris-borate and 1 mM EDTA) and were visualized under UV light using Gel Documentation System (Alpha Innotech, USA).

Table 1. SSR primers and their chromosome location used for determining the genetic diversity of wheat genotypes.

S.N.	SSR primers	Chromosome location
1.	Xgwm357,Xgwm666,Xgwm497	1A
2.	Xgwm011,Xgwm131,Xgwm140	1B
3.	Xgwm033,Xgwm106	1D
4.	Xgwm296,Xgwm312	2A
5.	Xgwm120,Xgwm148	2B
6.	Xgwm102,Xgwm349	2D
7.	Xgwm030,Xgwm369,Xgwm155	3A
8.	Xgwm77,Xgwm340	3B
9.	Xgwm71,Xgwm161	3D
10.	Xgwm165,Xgwm397	4A
11.	Xgwm107,Xgwm251	4B
12.	Xgwm624	4D
13.	Xgwm205,Xgwm304	5A
14.	Xgwm67,Xgwm68	5B
15.	Xgwm119,Xgwm292	5D
16.	Xgwm459,Xgwm494	6A
17.	Xgwm219,Xgwm132	6B
18.	Xgwm55,Xgwm469	6D
19.	Xgwm260,Xgwm282	7A
20.	Xgwm146,Xgwm344	7B
21	Xgwm44,Xgwm37	7D

The presence of band was scored as 1 and absence of band was scored as 0 in the binary data matrix. Using the *SAHN* module of the *NTSYS-pc* Jaccard coefficients were used to construct unweighted pair-group method of arithmetic average (UPGMA) dendogram.

In PCR amplification, ninety alleles were identified with different size fragments. The average number of alleles per SSR marker was 3.2, ranging from two alleles for *Xwgm*292 to five for *Xwgm*264. A wide range of alleles of expected fragment sizes was obtained by different primer pairs with strong amplifications. The primesr *Xwgm292* and *Xwgm264* yielded five alleles as shown in Fig. 1a and 1b. However, the higher number of alleles per locus has been reported in wheat (4.6 to 18.1), barley (8.6), and several other crops like tomato (3.1), sorghum (2.3), cucumber (2.6), melon (2.9), and watermelons (2.0) (Fahima *et al.,* 1998; Prasad *et al.,* 2000; Huang *et al.,* 2002; Salem *et al.,* 2008; Mohammadi *et al.,* 2009). The average number of alleles per locus (3.2) in this study was lower in comparison to those reported earlier. We identified a total of 23 alleles at 7 loci with an average of 2.67 alleles per locus in A genome, while 15 loci with an average of 3.0 alleles per locus were detected in B genome. In the D genome 30 alleles were detected with 10 microsatellite loci with an average of 2.87 alleles per locus, suggesting diversity at various levels in three genomes.

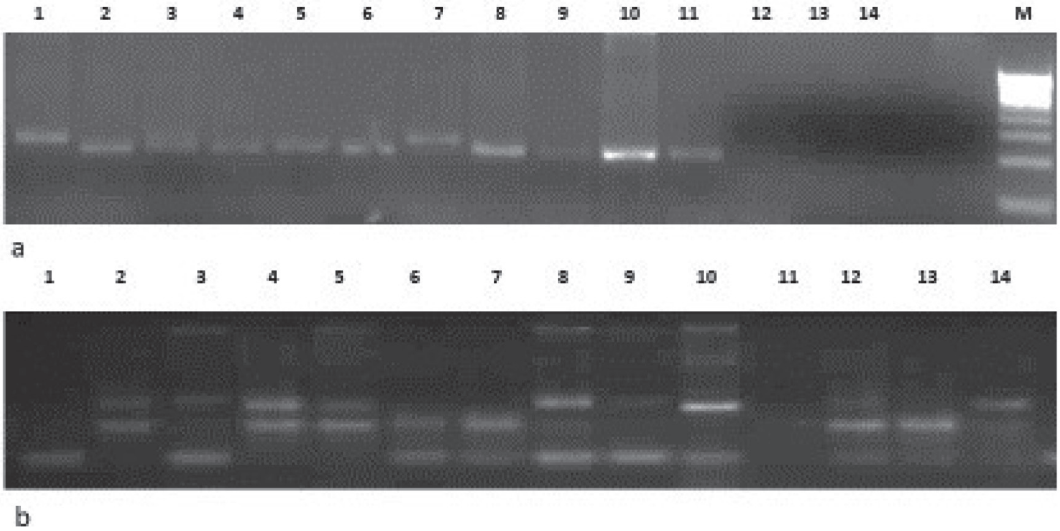

Fig. 1 Gel electrophoresisof amplification productsobtained with microsatelliteprimer pairs Xgwm 292 (a) and Xgwm 264 (b) in 14 wheatgenotypes. M=100 bp standardDNA marker. Lane 1) NI5439, 2) C 306, 3) WH 147, 4) HD 2781, 5) PBW 175, 6) WR 544, 7) HUW468, 8) PBW 343, 9) HD 2733, 10) GW 322, 11) MACS 2496; 12) HD 2932, 13) HUW 234 and 14) Raj 4037.

The lowest allele per locus among the seven homoeologous chromosome groups was obtained in group 6 identifying only one allele for *Xgwm* 148. The group 2 chromosomes were identified to have the highest no. of alleles as presented in Table 2. In a genome-wise comparison, the B genome was found to be highly diverse (0.65) followed by D (0.50) and A (0.44) genomes. For homoeologous chromosome groups, the highest PIC value of 0.53 was observed for group 2 chromosomes markers and the lowest value of 0.27 for group 7 markers. The observations made in this study are contrary to those made by Iqbal *et al.* (2009), where they reported highest PIC value for A genome followed by D and B genomes. Similarly, we report highest value of PIC for homologous group 2 chromosomes in contrast to homoeologous group 7 chromosomes as reported by Roder *et al.,* (2002;) and Haung *et al.,* (2002). This difference can be attributed to the genotypes belonging to different geographical regions and the set of different primer pairs used in this study.

Table 2. Genetic diversity according to genomes and chromosomes across 32 microsatellite loci

Genome	Number of alleles	Gene diversity	Mean no. of alleles/locus
All genomes	90	0.48	3.20
A	23	0.44	2.67
B	36	0.65	3.00
D	31	0.50	2.87
Chromosome			
Group 1	16	0.39	2.50
Group 2	22	0.53	3.20
Group 3	10	0.41	2.67
Group 4	14	0.52	2.95
Group 5	10	0.44	2.87
Group 6	06	0.27	2.00
Group 7	12	0.43	3.00

This analysis therefore identifies the divergence of alleles specific for a particular geographical region. The maximum value of PIC (0.53) in this study is in accordance to the earlier studies where PIC values ranged between 0.23-0.90 (Plaschke *et al.*, 1995, Prasad *et al.*, 2000, Mohammadi *et al.*, 2009). However, the mean PIC value in our results corroborates those reported by Bohn *et al.* (1999). We observed highest genetic diversity confirming that there is highest polymorphism in B genome and A genome is based on polymorphism studies least genetic diversity. The highest polymorphism among B genome is also reported by Eujayl *et al.* (2002) and Wang *et al.* (2007) in wheat as well as by Cho *et al.* (2000) in rice.

It might be correlated to evolution of each of the three wheat genomes. The B genome has originated from species closely related to the *A. speltoides*, a cross-pollinated species, whereas A and D genomes are traced to have originated from *T. urartu* and *Ae. tauschii*, respectively and are self-pollinating species. In general, a cross pollinating species exhibit higher genetic diversity in comparison to a self-pollinating species. This might be the reason why, B genome is highly diverse in primitive hexaploid wheat as comparison to genomes A and D. During the course of evolution of the hexaploid wheat, tetraploid wheat crossed with *Aegilops tauschii*, and produced the hexaploid wheat. Consequently, the opportunity of the gene exchange of the D genome with A or B genome was lower than that between B and A genome. Evidences also suggest that B genome chromosomes are rich in repetitive DNA sequences and the length of B genome is longer than A and D genomes.

For all possible pairs of varieties, the genetic similarity (GS) coefficient ranged from 0.50 to 0.92. The similarity coefficient generated a tree for cluster analysis using UPGMA as shown in Fig. 2. The varieties C306/NI5439 (drought tolerant) had highest similarity of~ 0.92. Apart from this more pairs *viz.* HD 2781/C306, HD2733/HD 2781 and HD2932/MACS 2496 also showed high degree of commonness. The dendrogram based on UPGMA algorithm grouped the fourteen wheat varieties into two major clusters, I (10 varieties), and II (04 varieties). However, two varieties in cluster I (PBW 175) and II (GW 322) showed considerable diversity with other varieties in their respective clusters. The clusters I, and II were further divided into two sub-clusters (Ia: five varieties, Ib: three varieties; and IIa: four varieties) as shown in Fig. 2. Similar investigations have been carried out by Ram *et al.,* (2007) using SSR markers.

It was postulated that biased selection of material in the previous breeding program might have resulted into high level of similarity and narrowed the genetic base of wheat germplasm. It is further suggested that more polymorphic

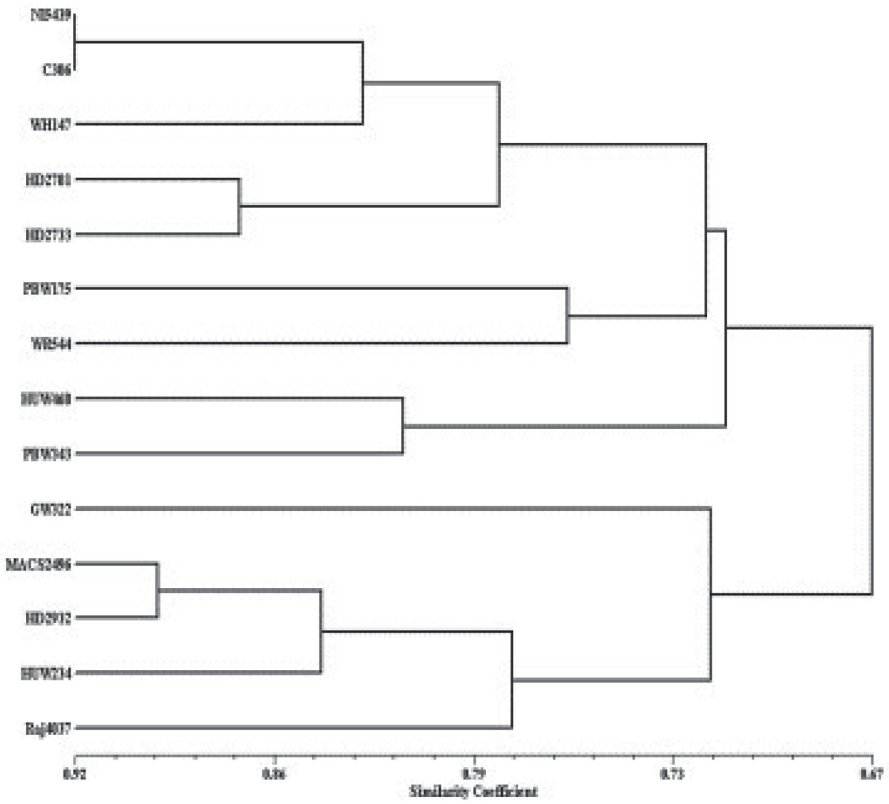

Fig. 2 UPGMA dendrogram of 14 wheat genotypes based on 32 SSR markers.

microsatellite markers could be used for efficient screening of the wheat germplasm by saturating more regions of the wheat genome and these microsatellite marker data will be useful in identifying diverse parents and for maintaining genetic variation in germplasm for trait improvement.

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Compliance with ethical standards

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Author contributions:

RS, PS worked for the wet lab experiments. PS, SK, SKS edited the manuscript. All Authors read the manuscript.

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Short Communication

Comparative evaluation of different fungicides and bio-agents for management of spot blotch of barley caused by *Bipolaris sorokiniana* (Sace. Ex Sorok)

Javed Bahar Khan, Sugam Katiyar, Charul Kanchan, Jitendra Kumar and Pravir Kumar Gupta*

Section of Rabi Cereals, C.S.Azad. Univ. of Agri. & Tech. Kanpur (UP)-208002

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****Corresponding author:*** *pkguptacsau@rediffmail.com*

Keywords: Difenconazole, *Hordeum vulgare*, Per cent growth inhibition, *Psudomonas fluorescens*, Systemic fungicide, *Trichoderm harzianum.*

Barley (*Hordeum vulgare* L) is one of the first domesticated cereal crop after maize, rice and wheat, which contributes 5.5-6.0% of the global cereals and 11.5-12 per cent of the coarse cereals production (Pal *et al.* 2012, Kumar *et al.* 2013b, Kumar *et al.* 2014). Barley is used as food crop in many countries of Africa, Middle East, South America and Asia. During 2019-20 in India, the area under the crop was 0.62 million hectare, productivity 25.73 q/ha with a production of 1.59 million metric tonns (Anonnymous 2019-20). In India, barley is an important coarse cereal crop being grown in *Rabi* (winter) season in Northern plains. Barley occupies area in 10 states viz. Himanchal Pradesh, Jammu & Kashmir, Bihar, Maharashtra, West Bengal, Punjab, Haryana, Madhya Pradesh, Uttar Pradesh and Rajasthan (Randhir Singh et al. 2014). Malt is the second largest use of barley and among cereals, barley is preferred for Kernel and its enzymatic activity (α and β amylase mainly) (Shaveta *et al.* 2019). The utilization of barley for malting and brewing industry has increased recently with the increase in consumption of beer, health drinks and other malt based products in India (Randhir Singh *et al.* 2014). Currently it has received attention of research workers to boost its production through the adoption of new technology and high yielding varieties. Like other cereals, barley is also exposed to various diseases which are ultimately responsible for significant yield reduction and poor grain quality. Barley is exposed to several biotic stresses, however, some of these are rust, spot blotch, net blotch, powdery mildew, loose smut and covered smut (Gangwar *et al.* 2018). Among these, spot blotch caused by *Bipolaris sorokiniana* is an important fungal disease causing severe losses up to 25-30 per cent reduction in grain yield and also reduces the grain quality (Randhir Singh *et al.* 2014). Keeping in view, the importance of disease, especially with reference to the state of Uttar Pradesh, it is better to have management well in advance. The management of disease can be done through cultural practices and use of resistant genotype, but there is need for efficient management of disease with eco-friendly means.

In vitro, six systemic fungicides against *Bipolaris sorokininia* viz; Propiconazole, Tebuconazole, Azoxystrobin, Difenconazole, Azoxystrobin + Difenconazole and Mancozeb and five bio-agents viz. *T.viride-1, T. viride-2, T. harzianum-1, T. harzianum-2 and Pseudomonas fluorescens* were tested against the pathogen to find out their relative efficacy in inhibiting the growth of fungus in culture in four dilutions (25 ppm, 50 ppm, 100 ppm and 200 ppm) using poisoned food technique proposed by Nene and Thapliyal, (1993). The desired concentrations were obtained by adding appropriate amount of stock solution of fungicides to potato dextrose agar (PDA) medium in petri dishes. PDA without fungicides served as control. Each plate was inoculated with a 5mm mycelial disc of the pathogen i.e. fungus taken from 7-days old culture. The inoculated plates were incubated at 25 ± 1^0C till the fungus covered the whole plate in control. For antagonistic activities of five bio-control agents, dual culture technique was used (Rahman 2009). Five mm diameter disc of actively growing pathogen (*B. sorokiniana*) taken from the margin of 10-days old culture was placed at one end of petri dish containing solidified PDA medium. The inoculation of mycelia disc (5mm) of antagonist at opposite end of petri dish was done 3-days after the placement of pathogen in order to adjust the slow growth rate of pathogen. The disc of pathogen and antagonist were placed at equal distance from the periphery of petri dish. In case of bacterial antagonist, culture disc (5mm diameter) of the pathogen was placed at one end of the petri dishes containing PDA medium and after three days the bacterial antagonist was streaked on opposite side of the growing pathogen colony. The plate containing PDA medium inoculated with pathogen only, served as control. The both experiments were conducted under Complete Randomized Design with four replications for each treatment. The inoculated plates were incubated at 25 ± 1^0C.

The redial growth of colony was recorded and per cent inhibition of each treatment was calculated by using formula given by Vincent (1927).

$$\text{Growth inhibition(I)} = \frac{\text{Radial growth in control (C)– radial growth in treatment (T)}}{\text{Radial growth in control (C)}} \text{X } 100$$

Where,

I = Per cent growth inhibition

C = Growth of pathogen (mm) in control

T = Growth of pathogen (mm) in treatment

All the six fungicides significantly inhibited the mycelial growth of *B. sorokiniana* at all doses (25, 50, 100 and 200 ppm). There was a significant decrease in the respective mycelial growth and accordingly more inhibition was observed at higher concentration (200 ppm) than lower concentrations. A significant interaction between pathogen and concentration of fungicides was observed (Table-1). Among the fungicides viz. Propiconazole, Azoxystrobin, Azoxystrobin + Difenconazole and Tebuconazole were most effective. Propiconazole showed 91.51, 97.67, 100.00 and 100.00 per cent inhibition, while Tebuconazole exhibited 87.20, 95.11, 100.00 and 100.00 per cent mycelial inhibition at 25, 50, 100 and 200 ppm concentration respectively and followed by Azoxystrobin exhibiting 87.20, 94.18, 100 and 100 per cent mycelial inhibition at 25, 50, 100 and 200 ppm, respectively. Next to these fungicidal treatment against the *B. sorokininia* was Azoxystrobin + Difenconzole which showed inhibition of 87.20, 94.18, 95.34 and 100 per cent followed by Difenconazole recording mycelial growth inhibition of 85.34, 87.20, 90.69 and 96.27 per cent. Mancozeb was least effective fungicide exhibiting 27.90, 31.39, 48.83 and 89.18 per cent inhibition respectively. Fungicides Propiconazole, Tebuconazole, Azoxystrobin and Azoxystrobin +Difenconazole were found most effective exhibiting 100 per cent inhibition at 200ppm followed by Difenconazole (96.27) and Mancozeb (89.18) per cent. The similar findings were reported by Mahapatra and Das, 2013. Padha (2008) reported that Propiconazole and Tebuconazole completely inhibited mycelial growth of *B. sorokiniania* at 25 ppm and higher concentration. No growth of *Drechslera sorokiniana* had been reported when fungicide Mancozeb was used (Chattannavar *et al.* 1985). Hasan *et.al.* (2012) reported that systemic fungicides completely inhibited the growth of pathogen at lower concentration under laboratory condition. Bio-agents viz; *T. viridi-1, T. viridi-2*, *T. harzianum-2, T. harzianum*-1 and *Pseudomonas fluorescens* were evaluated against *B. sorokiniana* and showed highly significant inhibiting effect on mycelial growth. Data presented in Table-2 showed that *T. harzianum*-1 was most effective exhibiting 77.90 per cent mycelial inhibition of the pathogen followed by *T. harzanium-2* (74.80 per cent) and *T. viride-1* (74.41 per cent). These three were at par with each other. Bacterial bio-agent *P. fluorescens* was least effective reducing 47.22 per cent mycelia growth of pathogen. Hasan *et. al.* (2008) found that species of *Trichoderma* inhibited the mycelia growth of *F. monilifarmae* in the range of 32.50 to 45.00 per cent. Hasan (2013) reported that *T.harzanium* isolates RVT-103 effectively reduced radial growth of *B. sorokiniana* (45.45 %). According to Harman (2006), *Trichoderma spp.* sensed the presence of target fungi and appeared to grow towards them.

Table 1: *In vitro* evaluation of different fungicides against *B. sorokiniana.*

Treatment	**Mean radial growth (mm) at different concentration (ppm)**							
	25		**50**		**100**		**200**	
	Growth (mm)	**Inhibition (%)**	**Growth (mm)**	**Inhibition (%)**	**Growth (mm)**	**Inhibition (%)**	**Growth (mm)**	**Inhibition (%)**
Propiconazole	7.30**	91.51	2.00**	97.67	0.00**	100.00	0.00**	100.00
Tebuconazole	11.00**	87.20	4.20**	95.11	0.00**	100.00	0.00**	100.00
Azoxystrobin	51.60**	40.00	12.00**	86.04	4.80**	94.41	0.00**	100.00
Difenconazole	12.60**	85.34	11.00**	87.20	8.00**	90.69	3.20**	96.27
Azoxystrobin + Difenconazole	11.00**	87.20	5.00**	94.18	4.00**	95.34	0.00**	100.00
Mancozeb	62.00**	27.90	59.00**	31.39	44.00**	48.83	9.30**	89.18
Control	86.00	-	86.00	-	86.00	-	86.00	-
SE (d)	1.32	-	1.31	-	0.70	-	0.57	-
CV (%)	4.68	-	6.30	-	4.33	-	4.96	-
S.E.m±	0.93	-	0.93	-	0.50	-	0.40	-
CD (1%)	2.83	-	2.82	-	1.51	-	1.22	-

** Significant at 1 % level

Table 2: Effect of antagonists on the growth of *B. sorokiniana* by dual culture method:

Antagonist	Mycelial growth (mm)	Growth inhibition (%)
Trichoderma viride-1	22.00**	74.41
T. viride-2	26.33**	69.37
T. harzianum-1	19.00**	77.90
T. harzianum-2	21.66**	74.80
Pseudomonas flourescens	45.33**	47.22
Control	86.00	-
SE (d)	1.53	-
CV (%)	5.13	-
S.E.m+	1.08	-
CD (1%)	3.33	-

** Significant at 1 % level

Conclusion

Among the fungicides Propiconazole, Tebuconazole, Azoxystrobin and Azxoxystrobin + Difenconazole completely inhibited the mycelia growth of pathogen at 200 ppm concentration under *in-vitro* condition. Among bio control agents, *T. harzianum-1* (77.90 per cent) was found most effective antagonist against pathogen followed by *T. harzianum-2* (74.80per cent) and *T. viride*-1 (74.41per cent).

Compliance with ethical standards

NA

Conflict of Interest

Authors declare that they have no conflict of interest

Authors' contribution

The compliance of referee comments has been made. The manuscript Comparative Evaluation of Different Fungicides and Bio-agents for Management of Spot Blotch of Barley Caused by Bipolaris sorokiniana (Sace. Ex Sorok) is a part of M. Sc. Ag. Thesis of Mr. Sugam Katiyar submitted in C S A Univ. of Agri. and Tech., Kanpur.

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Short Communication

Identification of promising sources of hulless barley (*Hordeum vulgare* L.) for important quality traits

Dinesh Kumar[1], Ramesh Pal Singh Verma[1], Sneh Narwal[2], Jogendra Singh, Rekha Malik[1],
Ajit Singh Kharub[1] and Gyanendra Pratap Singh[1]

[1]*ICAR-Indian Institute of Wheat and Barley Research, Karnal – 132001, India*
[2]*ICAR-Indian Agricultural Research Institute, New Delhi – 110 012, India*

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****Corresponding author:*** *dinesh.kumar3@icar.gov.in*

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Barley is one of the oldest domesticated crops by human being and used as staple food for quite a long time (Haas *et al.,* 2018). Over the period of time wheat and rice replaced barley from regular diets and this led to significant decrease in its area and production. However, in last two decades barley has made its place among the nutraceutical grains especially because of relatively higher content of soluble dietary fibres as compared to other cereal grains except oats (Derakhshani *et al.,* 2020). Barley contains significantly higher levels of a soluble fibre called mixed linkage β-1-3;1-4 glucans (popularly known as beta glucans) *vis-à-vis* wheat and rice. Beta glucans have been shown to reduce low density lipoprotein cholesterol (LDL Cholesterol) and thus providing protective role against cardiovascular diseases (Ames *et al.,* 2008). Regular consumption of beta glucan is also reported to reduce the blood sugar and thus helpful in prevention and management of type-II diabetes (Ames *et al.,* 2008). Consumption of soluble fibre rich diet has been shown to protect against certain kinds of colon cancer (Madhujith *et al.,* 2005). The health benefits of barley were probably known to ancient civilizations and some studies suggest the use of barley in management of type-II diabetes in Indo-Vedic Civilization (Sarkar *et al.,* 2015 there in). Barley has very low glycemic index among the cereal grains. The lower glycemic index foods are considered healthy option especially in case of type-II diabetes management. Soluble dietary fibres, by increasing the viscosity of stomach and intestinal contents, is believed to reduce the overall intestinal enzymatic activity, and to decrease post-prandial plasma glucose levels. Besides the beta glucans, the amylose percentage is also important parameter as higher amylose content starches are degraded slowly in the human gut (Aldughpassi *et al.,* 2012).

At present majority of the barley production is consumed as animal feed (65-70%), next major use is for malting (25-30%) and very lesser amount is used directly as food (2-5%). Barley as a food is mainly consumed in some African countries and higher Himalayas' especially Tibet region (Ullrich, 2011). With the changing food habits and life styles, the importance of nutraceuticals or healthy foods is on rise and expected to be a part of the regular diet in urban population of developed and developing countries (Narwal *et al.,* 2015). Barley, Oats and Millets are the Grains of Future and hence need to have high yielding varieties with good quality and tolerance to biotic and abiotic stresses. In case of barley most of the varieties developed in India are hulled ones and cater to the need of feed and malt barley segment. The hulless varieties are preferred over the hulled ones for direct consumption as barley-based foods, since the adhered hull led to poor texture, mouth feel and undesirable colour to the processed products (Narwal *et al.,* 2017). The removal of hull needs extra efforts and may also lead to loss of nutrients from upper layers of the grain. The hulless varieties are available, but have lower yields as compared to the hulled varieties. The hulless breeding programme is focussed on increasing the yield with better tolerance to biotic and abiotic stresses. However, the quality component is equally important as for higher flour recovery and better health promoting activities the grains must possess certain quality parameters. In the present study, four exotic germplasm introductions, 19 hulless indigenous landraces, and six released hulless barley cultivars were screened for grain physical and biochemical quality parameters to identify sources of better quality for their potential use in hull less barley improvement programme of the country.

A set of 29 genotypes were grown in three replications at ICAR-IIWBR, Karnal during 2017-18 in rabi season following the recommended cultural practices of feed barley for North Western Plains Zone. The cleaned grains were analysed for thousand kernel weight, plump grain percentage, grain protein, beta glucans content, amylose percentage and test weight using standard EBC procedures. Thousand kernel weight was estimated by counting thousand grains on Pfeuffer make grain counting machine and then weighing the grains on electronic weighing machine. Grain plumpness was determined using Pfeuffer make Sortimat machine where 100 g grains were separated over 2.8 mm, 2.5 mm and 2.2 mm screens; the grains retained over 2.8 and 2.5 mm were considered plump ones. Protein content was measured using Foss make NIR machine. Beta glucans and Amylose content were quantified using Megazyme make enzymatic kits. Test weight was estimated using ICAR-IIWBR developed hectolitre apparatus.

The hullless barley genotypes have normally lesser yields and one of the reasons for this is relatively lower values of thousand grain/kernel weight as compared to hulled ones. Therefore, it is of paramount importance to identify the hulless genotypes with higher thousand grain weight. Three genotypes, BCU 8038, BCU 7998 and BCU 8023 had significantly higher thousand grain weight as compared to the best Indian check Geetanjali and comparable to exotic check Atahualpa (Table 1). The rate of grain filling and grain filling duration are important determinants of thousand kernel weight and genetic variation is available for these features. However, both rate and duration of grain filling are greatly affected by genotypic and environmental interaction (Sakuma and Schnurbusch, 2019). In this study, three sources have been identified under the similar growing conditions indicating the genetic variation in the thousand kernel weight.

Another important grain physical trait related with flour recovery is percentage of bold or plump grains. In this study, the grains retained on 2.5 mm screen were considered as percentage of total plump grains. In case of malt barley, the minimum desirable percentage of plump grains is 90 %, though no such standard is available for food barley, however higher the value more will be the flour recovery. Three genotypes, BCU 8041, DWR 62 and DWR 80 had bold grain percentage of more than 70 %. The major contributor to the grain dry matter are polysaccharides especially starch and normally plump grains result from higher starch deposition in the endosperm. For increasing the grain plumpness source-sink dynamics are very important as more deliverance of photosynthates to grain and its conversion to storage molecules decides the grain size/weight (Dreccer *et al.,* 1997). However, besides several other quality parameters genotype x environment interaction and cultural practices are also very important (Mckenzie *et al.,* 2005). Though the present study has been conducted only for one year at one location, however it has provided important preliminary insight into relative performance of promising sources for further detailed study.

Grain protein content varied from 9.2 to 14.3 per cent, though higher protein content is desirable in food barley provided it is not because of reduced starch or lesser plump grains. In this study, no such genotype could be identified having higher protein content coupled with higher plump grain percentage. In barley, the major storage proteins are hordeins (prolamines), therefore identification of better sources of hordein content and better nutritional composition is required for hulless barley. There are four major types of hordeins based upon the amino acid composition and molecular weight and at molecular level the hordein protein families are coded by *Hor-1, Hor-2, Hor-3, and Hor-4* located on chromosome 1H (Tanner *et al.,* 2019). Though genotype is the major determinant of grain protein content (Kumar *et al.,* 2012), the content is also significantly affected by the cultural practices especially nitrogen fertilization and growing environment.

The hulless barley grain is considered a good source of mixed-linkage (1 → 3), (1 → 4)-β-D-glucans (β-glucans) which contribute to the major portion of soluble fibres. The beta glucan content varied from 4.6 to 7.3 per cent in the genotypes tested, with the highest content in BCU 8028 (7.3 % dwb). Three other genotypes had beta glucan content of more than 6 percent besides the checks. The genotype BCU 8028 was found to contain highest grain beta glucan content in preliminary screening done during 2014-15 (Fig. 1). The genotypes were also screened for beta glucan polymorphism molicular level using CAPS marker HvCslF6, however no significant differences were discernible in this study (Fig. 2). Barley contains approximately 2–11% of β-glucans and content is affected by genetic and environmental factors (Al-Ansi *et al.,* 2020). The soluble dietary fibre content is relatively higher in hulless barley as hull causes dilution effect on most of the nutrients except the insoluble fibres in hulled barley. The higher content of grain beta glucans in barley is the major reason for labelling barley as health promoting grain. β-glucans lower plasma cholesterol (mainly LDL cholesterol), bring down post-prandial blood glucose, lower glycemic index of barley and reduce the risk of colon cancer. Health benefitting effects of β-glucans are mainly due to their property of making viscous mass in the gut (Peckz *et al.,* 2017).

Amylose content also contributes in increasing the resistant starch content and the percentage is mainly genotypically determined however, in this one-year study no significant differences could be inferred from the data.

There is a positive correlation (0.48) between protein and beta glucan content; and between thousand grain weight and plump/bold grains. This correlation may help in better understanding of the food quality traits in future.

Table 1: Grain physical and biochemical trait values in hulless barley grains

Genotype	**Origin**	**TGW (g)**	**Bold grain (%)**	**Thin grain (%)**	**Protein (% dwb)**	**Beta glucan (% dwb)**	**Amylose (%)**	**Test wt (kg/hl)**
BCU 8023	I	45.1	53.0	14.7	12.2	6.0	26.3	76.9
BCU 8024	I	37.9	31.9	21.7	11.1	5.3	25.7	75.8
BCU 8025	I	42.7	36.8	17.1	9.4	5.3	24.3	76.2
BCU 8026	I	38.4	55.9	12.0	10.0	5.5	26.3	76.2
BCU 8027	I	38.3	52.2	11.1	11.5	6.3	21.5	77.3
BCU 8028	I	36.3	48.6	13.7	11.1	7.3	27.6	78.4
BCU 8029	I	36.7	27.5	22.9	9.5	4.6	23.5	78.2
BCU 8030	I	39.9	40.0	14.6	10.7	5.5	24.5	77.4
BCU 8031	I	40.9	44.6	14.6	11.9	5.5	26.8	77.4
BCU 8032	I	39.9	27.0	20.6	10.7	5.8	30.7	77.2
BCU 8033	I	38.4	24.1	25.0	11.0	5.5	28.1	76.6
BCU 8034	I	40.3	62.3	14.7	11.0	5.9	28.1	75.9
BCU 8035	I	39.1	50.2	17.1	10.6	5.0	28.3	74.5
BCU 8036	I	39.5	22.9	25.9	10.1	5.6	22.3	77.6
BCU 8037	I	44.1	51.4	11.4	10.8	5.6	22.9	77.5
BCU 8038	I	49.1	61.3	7.6	10.4	5.9	24.2	78.8
BCU 8039	I	42.9	40.4	16.4	10.6	5.7	26.9	77.1
BCU 8040	I	40.1	32.8	21.1	10.5	5.3	27.2	76.9
BCU 8041	I	44.7	73.6	5.6	9.2	4.9	23.6	65.6
BCU 7998	E	47.1	65.5	6.1	10.1	6.1	23.5	78.4
DWR 62	E	37.5	72.3	3.7	10.2	4.9	24.8	78.7
DWR80	E	41.5	71.9	4.1	9.8	5.5	26.5	75.9
DOLMA	C	36.0	9.9	45.3	10.2	6.5	26.2	75.6

NDB943	C	39.3	46.5	9.3	10.9	5.8	22.2	79.1
KARAN16	C	37.9	33.3	21.8	10.0	5.3	29.8	75.2
BHS352	C	37.6	20.6	34.0	10.3	6.6	29.7	76.4
GEETANJALI	C	40.8	64.2	4.6	9.7	5.3	32.8	79.6
HBL 276	C	32.5	12.7	43.1	11.1	6.0	26.1	75.4
ATAHULAPA	E	46.7	56.5	7.4	14.3	6.5	30.8	66.5
LSD (5%)		4.2	10.7	6.1	0.8	0.9	NS	1.4

I= Indigenous landrace, E= Exotic, C= Released Cultivar, LSD= Least Significant Differences

In this preliminary study genetic differences among different genotypes were noticed and BCU 8028 for higher grain beta glucan content; BCU 8038, BCU 7998 and BCU 8023 for higher thousand grain weight and BCU 8041, DWR 62 and DWR 80 for higher bold grain percentage were identified. These genotypes may provide important clues at biochemical and molecular level to assist breeders in development of improved hulless barley genotypes for food purposes.

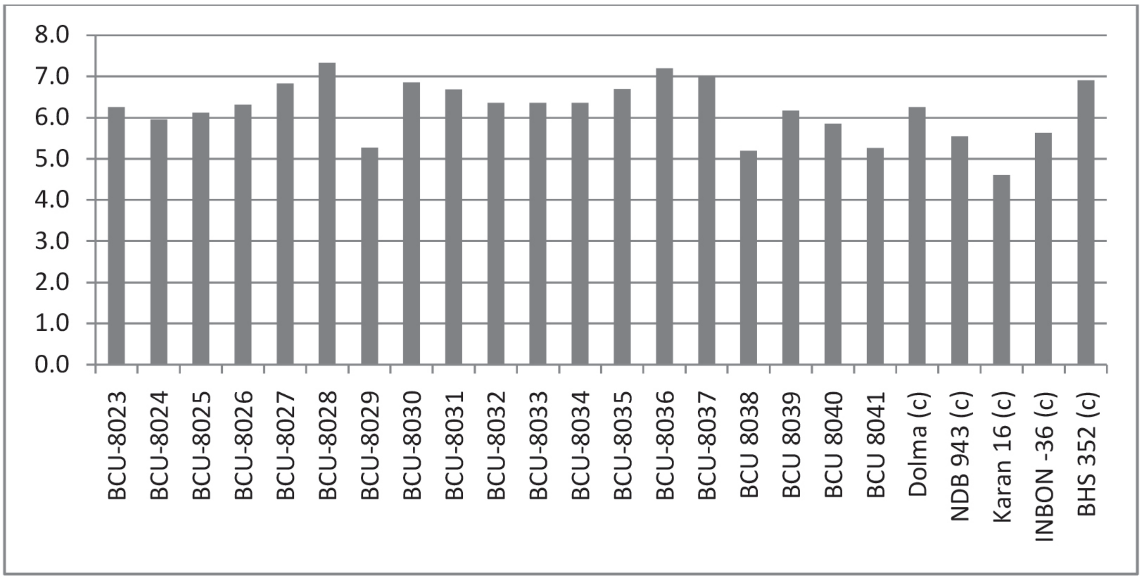
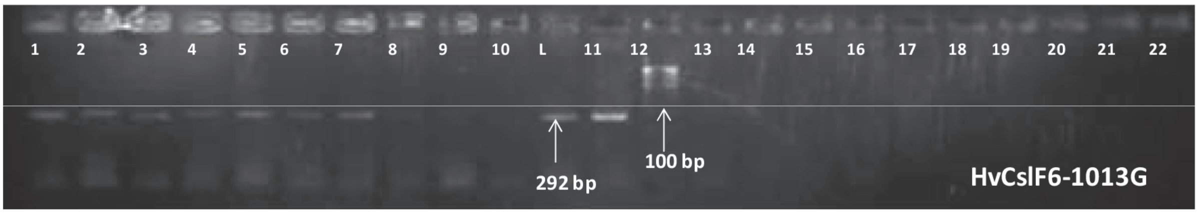


Fig. 1: Beta glucan content (% dry weight basis) in barley genotypes (2014-15)



Well 1-22: BCU8023,8024,8025,8026,8027,8028,8031,8032,8033,8034,8035,8036, 8037,8038, 8039,8040,8041, INBON 36,KARAN 16, BHS 352, DOLMA, NDB943

Fig. 2: Agarose gel showing the polymorphism for the marker HvCslF6 with respect to beta glucan in Hulless landraces and checks

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Conclusion

Barley is one of the unique cereals having health promoting properties in its grains. There is a renewed interest in food barley in past few years and need is being felt for high yielding hulless genotypes with superior food quality parameters. In this study 19 land races collected from Leh and Ladakh region, four exotic germplasm introductions, breeding lines and six released hulless cultivars were evaluated in 2017-18 for seven grain quality parameters. Promising genotypes for grain beta glucan content and thousand kernel weight have been identified. A positive correlation has been observed between grain beta glucan content and protein content.

Compliance with ethical standards: NA

Conflict of Interest: Authors declare that they have no conflict of interest

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Varietal Release

HI 1633 (Pusa Vani): A bio-fortified bread wheat variety for late sown, irrigated conditions of Peninsular Zone of India

Sakuru Venkata Sai Prasad*[1], Jang Bahadur Singh[1], Divya Ambati[1], Rahul Madho Rao Phuke[1], Thorehalli Linganna Prakasha[1], Akilesh Nandan Mishra[1], Kailash Chandra Sharma[1], Anil Kumar Singh[1], Gyanendra Pratap Singh[2], Jai Bhagwan Sharma[3], Pradeep Kumar Singh[3], Harikrishna Yadav[3], Ram Kumar Sharma[3], Naresh Kumar[3], Rajbir Yadav[3], Kiran Bapusaheb Gaikwad[3], Sanjay Kumar[3], Prasanth Babu Hanumanthappa[3], Raghunandan Katlutkor[3], Vaibhav Kumar Singh[3], Murugasamy Sivasamy[4], Tapas Ranjan Das[3], Dharam Pal[5], Vinod[3], Anju Mahendru Singh[3], Neelu Jain[3], Manjeet Kumar[3], Paramasivan Jayaprakash[4], Venu Kumaran Vikas[4], Madhu Patial[5], Ram Niwas Yadav[6] and Kumble Vinod Prabhu[7]

[1] *ICAR-IARI, Regional Station, Indore, M.P*
[2] *ICAR-Indian Wheat and Barley Research Institute, Karnal*
[3] *ICAR-Indian Agricultural Research Institute, New Delhi*
[4] *ICAR-IARI, Regional Station, Wellington, Tamil Nadu*
[5] *ICAR-IARI, Regional Station, Shimla*
[6] *ICAR-IARI, Regional Station, Karnal*
[7] *PPVFRA, Government of India, New Delhi*

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Corresponding author: *sprasad98@gmail.com*

Abstract

A new bread wheat variety HI 1633 (Pusa Vani) has been released and notified by the Central Sub-Committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops, Government of India for commercial cultivation under irrigated and late sown conditions of Peninsular Zone of India. HI 1633 has average yield of 41.7 q ha^{-1} and showed superiority over checks. The potential yield of HI 1633 is 65.8 q ha^{-1} and found resistant to black and brown rusts. HI 1633 found to have excellent chapati quality (7.63), biscuit quality (7.08), high grain hardness (>80.0), test weight (80.3 kg hl^{-1}) and sedimentation value (45.0 ml). It has high protein content (12.4 %) and presence of 5+10 subunit of *Glu-D1* reflecting higher gluten strength. It has good amount of micronutrients *viz.*, iron (41.6 ppm) and zinc (41.1 ppm) content making it rich in nutritional qualities. This variety has been recommended for irrigated late sown conditions and would contribute to increasing wheat production and alleviate the socio-economic status of farmers of Peninsular zone in India.

Key Words: Biofortified wheat, disease resistance and quality

Introduction

Wheat is the most important staple food crop of the world. It provides food to 36 % of the global population and 20% of the food calories. Wheat cultivated area in India is more than 30 million hectares with the production of nearly 108 million tonnes (IIWBR Director's Report, 2020). The late sown area of wheat in peninsular zone is also in an increasing trend due to diverse crop cultivation practices and in this area needs wheat varieties resistant to black and brown rusts along with tolerance to terminal heat stress. However, improvement of quality traits and micro- nutrient content along with yield in wheat are the new objectives of the breeding programmes to attain food and nutrition security in India.

Development and Notification of HI 1633: The variety HI 1633 was developed from the cross **GW 322/ PBW 498** through modified pedigree method**.** HI 1633 developed by the ICAR-Indian Agricultural Research Institute, Regional Station, Indore was released by the Central Sub-Committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops and notified vide S.O. 500 E, dated 29.1.2021 for commercial cultivation under irrigated, late sown conditions of the Peninsular Zone, which is the potential area for wheat crop and comprises states like Maharashtra, Karnataka and Plains of Tamil Nadu.

Yield superiority and adaptability: HI 1633 was advanced to national coordinated trials of late sown trials (NIVT 3B) during 2017-18 (ICAR-IIWBR, 2018). Under Co-ordinated trials of AICW&BIP (NIVT 3B, AVT I & AVT II), HI 1633 was evaluated at 30 locations during 2017-18 to 2019-20 out of which it appeared 21 times in the first non-significant group indicating its wider adaptability and stable yield (Table 1, ICAR-IIWBR 2020). Evaluation of HI 1633 under late sown conditions along with the checks showed that HI 1633 had an average yield of 41.7 q/ha and performed superior over checks. The potential yield of HI 1633 is 65.8 q/ha in Pune during 2018-19 (ICAR-IIWBR, 2019) of Peninsular zone. It showed significant yield advantage of 6.4%, 6.4% and 4.3% over the checks HD 2932, Raj 4083 and HD 3090, respectively. HI 1633 was early to flower (55-60 days), maturity (100-105 days), and possessed bold grains (TGW 42.0g).

Distinguishing morphological characteristics: Wheat variety HI 1633 has semi-erect growth habit and green foliage colour and anthocyanin pigmentation was absent on coleoptile at boot stage. It has semi-erect, medium sized having green flag leaf, very strong waxiness on sheath and blade. Peduncle of HI 1633 is medium, with strong waxiness, white coloured, weak waxy tapering spike which bears white awn. The lower glume has narrow sloping shoulder with long straight beaks. It possesses amber coloured, oblong, medium sized, hard grain with medium germ width.

Table 1: Performance of HI 1633 and other checks in Peninsular Zone

Items	Year of testing	No. of trials/ location	Proposed variety	Check Varieties			CD
			HI 1633	**HD 2932**	**Raj 4083**	**HD 3090**	
Mean yield (q/ha)	NIVT 3B (2017-18)	5	42.3	38.7			2.9
	AVT I (2018-19)	12	45.9	42.5	44.0	42.2	1.4
	AVT II (2019-20)	13	37.6	36.4	34.8	38.0	1.4
	Weighted Mean		41.7	39.2	39.2	40.0	-
% increase / decrease over the checks	NIVT 3B (2017-18)			9.3*	-	-	-
	AVT I (2018-19)			8.0*	4.3*	8.8*	-
	AVT II (2019-20)			3.3	8.0*	-1.1	-
	Overall Weighted Mean			**6.4**	**6.4**	**4.3**	-
Frequency in the first top non-significant group	NIVT 3B (2017-18)		4/5	3/5	-	-	-
	AVT I (2018-19)		9/12	6/12	7/12	8/12	-
	AVT II (2019-20)		8/13	6/13	3/13	7/13	-
	Overall performance		**21/30**	**15/30**	**10/25**	**15/25**	-

* Significantly superior

Performance in agronomical evaluation: In the agronomical trials under irrigated and late sown conditions, HI 1633 was high yielding genotype (35.10 q/ ha); and showed yield superiority in the range of 0.2 to 4.6 per cent over check varieties MACS 6478, Raj 4083 and MACS 6222 and was on par with HD 2932. It showed significant increase (0.52 to 4.88% in overall mean) for 1000 grain weight over all the checks; and on par with Raj 4083 (Anonymous, 2020).

Table 2: Performance of HI 1633 and checks under various agronomic conditions

Experiment / Item	Sowing time	Proposed Variety	Check Varieties			
		HI 1633	**HD 2932**	**Raj 4083**	**MACS 6222**	**MACS 6478**
Yield (q/ha)	Normal	37.61	38.81	37.42	35.46	40.66
	Late	35.85	36.86	34.86	34.32	36.59
	Very late	31.85	33.23	31.35	30.31	27.84
	Mean	35.10	36.30	34.54	33.36	35.03
% loss in comparison with Normal Sowing	Normal : Late	4.68	5.02	6.84	3.21	10.01
	Normal : Very late	15.32	14.38	16.22	14.52	31.53
	Late : Very late	11.16	9.85	10.07	11.68	23.91
% superiority over checks & qualifying variety	Normal		-3.09	0.51	6.06*	-7.50
	Late		-2.74	2.84	4.46	-2.02
	Very late		-4.15	1.59	5.08	14.40*
	Over mean		-3.31	1.62	5.22*	0.20

CD (P=0.05) :*Sowing(A)=1.19; Genotypes(B)= 1.67; B within A= 2.88, A within B = 2.95*

* Significantly superior

Table 3: Quality performance of HI 1633

Quality Trait	HI 1633	Checks		
		HD 2932	**Raj 4083**	**HD 3090**
Protein %	12.4	12.4	12.2	12.4
Fe (ppm)	41.6	37.4	41.5	39.8
Zn (ppm)	41.1	37.2	38.4	40.2
Grain Hardness index	78.0	71.0	75.8	77.8
Sedimentation value (ml)	45.4	50.2	58.7	50.8
Phenol test (max score 10)	7.1	4.5	7.1	7.6
Wet Gluten (%)	34.4	32.7	34.4	34.2
Dry Gluten (%)	10.5	10.4	10.9	10.7
Gluten Index	58	81	76	61
Chapati quality	7.63	7.83	7.62	7.58
Bread Loaf Volume (ml)	548	543	563	573
Bread Quality	6.60	6.40	7.25	7.47
Biscuit Quality- Spread Factor	7.08	7.29	7.19	7.28
HMW subunits				
Glu-D1	5+10	2+12	5+10	5+10
Glu-A1	2*	2*	1	1
Glu-B1	7	17.18	7+8	7
Glu-1 Score	8	8	10	8

Resistance to major disease and pests: HI 1633 has high levels of field resistance to stem (ACI: Max.-2.0; Mean-1.9) and leaf (ACI: Max.-10.0; Mean-4.2) rusts under artificial inoculations. It showed seedling resistance (all stage resistance) to 27 virulent pathotypes each of stem and leaf rusts. The postulated stem rust resistance gene *Sr31* has been reported to be resistant to all Indian pathotypes of stem rust. It also showed high levels of adult plant resistance to prevalent and virulent stem rust pathotypes 40A and 117-6; and leaf rust pathotypes 77-5, 77-9 and 104-2 (ICAR-IIWBR, 2020b). It showed good levels of resistance to leaf blight, Karnal bunt, *Fusarium* head blight, loose smut, foot rot and flag smut. It was not affected by major insect pests.

Grain quality: HI 1633 is a bread wheat genotype with good quality *viz.,* good chapati quality (7.63), biscuit quality (7.08), high grain hardness (>80.0), test weight (80.3 kg/hl) and sedimentation value (45.4 ml). It has high protein content (12.4%) and protein quality (*Glu* score of 8/10) for high molecular weight subunits and presence of 5+10 subunit of Glu-D1 reflecting higher gluten strength (ICAR-IIWBR,2020c). It has good amounts of essential micronutrients like iron (41.6 ppm) and zinc content (41.1 ppm) making it rich in nutritional qualities; and termed as **biofortified wheat.** It meets all desirable components for better biscuit, and chapati making qualities that makes it also favorable for industrial purpose.

In nutshell, the biofortified and high yield potential variety HI 1633 couples with stress tolerance to terminal heat and plasticity for sowing time with resistance to major insect pests, stem and leaf rusts makes this variety a suitable choice for the farmers of Peninsular Zone of the country.

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Varietal Release

VL *Gehun* 967: A high yielding, rust-resistant wheat (*Triticum aestivum* L.) variety, suitable for rainfed organic conditions of Uttarakhand hills of India

Lakshmi Kant[1], Sanjay Kumar Jain[2], Borehalli Rangaswamy Raghu[3] and Dibakar Mahanta[4]

ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora- 263601, Uttarakhand
[1]ICAR-VPKAS, Almora, Uttarakhand
[2] ICAR-NIBSM, Raipur, Chhattisgarh
[3] ICAR-IIHR, Bengaluru, Karnataka
[4] ICAR-IARI, New Delhi

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****Corresponding author:*** *lkant_vpkas@yahoo.com, lakshmi.kant@icar.gov.in*

Abstract

VL *Gehun* 967 is a rust resistant high yielding variety, released by Uttarakhand seed Sub-Committee and further notified by Central Sub-Committee on Crop Standards, Notification and Release of Variety. It has been recommended for cultivation under rainfed organic timely sown production conditions of Uttarakhand hills. VL *Gehun* 967 recorded an average grain yield of is 1.97 t ha^{-1}, which is 12.71% higher than the best check VL *Gehun* 907. This variety has recorded maximum average coefficient of infection (ACI) of 17.4 for stripe rust and 4.0 for leaf rust under artificial inoculation conditions indicating that it is resistant to both stripe and brown rust. It possesses 9.78 to 10.07% average protein, 76.37 to 79.73 kg/hl^{-1} hectoliter weight and 7.63 to 7.56 very good chapatti quality score, therefore, possessing very good quality for chapatti making and good flour recovery. The large scale cultivation of this variety in Uttarakhand hills under organic conditions would enhance wheat productivity and also help in reducing the inoculum load of rusts in hills of Uttarakhand due to its better resistance.

Key Words: Stripe and leaf rust, chapatti quality, winter x spring wheat derivative.

Introduction

Wheat is the most important winter cereal crop of Northern hills zone of India comprising the states of Uttarakhand hills, Himachal Pradesh, UTs of Jammu & Kashmir and Laddakh, hilly regions of North Eastern States and West Bengal. Wheat is cultivated in this region in around 1.39 m ha area (Gupta and Kant, 2012; Kant *et al.,* 2020), approximately 3.7% of the Country's wheat acreage. Although area and production wise, it is very small but cultivation of rust resistant varieties in this zone is one of the strategy to manage the rust inoculum load in the north-western plains, the wheat bowl of India. Uttarakhand (3.421 lakh ha) has largest area under Northern hills zone (NHZ). Around 50.2% (1.717 lakh ha) area is under hills, whereas 49.8 % (1.703 lakh ha) is under plains. The wheat productivity in plains (3.5 t ha^{-1}) is higher than the national average (3.0 t ha^{-1}) whereas, the productivity of hills (1.02 t ha^{-1}) is far below the national average (Anonymous, 2016; Chanda *et al.,* 2017). This may be attributed mainly to small and fragmented land holdings, unavailability of inputs (seed and fertilizer, etc.) at appropriate time and place, and poor extension of latest technologies. In addition to this, the prevalent varieties, *viz.*, 'VL *Gehun* 907' and 'HS 562', under rainfed as well as irrigated timely sown conditions of NHZ including Uttarakhand have started showing susceptible reactions to the new virulent pathotypes of stripe and leaf rust pathogens under changed climatic conditions. Therefore, farmers are left with limited alternatives to cultivate the above varieties. Further, the wheat cultivation in hilly areas is constrained due to predominant rainfed cultivation and still be considered as organic by default as farmers rarely apply fertilizers and chemicals.

The most effective strategy to manage the menace of rust diseases is deploying rust resistant high yielding wheat varieties. However, for deployment, we require specific set of varieties, with wider adaptability as well as capability to yield higher under rainfed organic conditions of hills. Therefore, a breeding programme was undertaken at ICAR- *Vivekananda Parvatiya Krishi Anusandhan Sansthan* (VPKAS), experimental Farm, Hawalbagh, India (29° 36'N and 79°40' E and 1250 m above msl) in 2007-08 in order to meet demand for an appropriate high yielding and rust resistant variety for rainfed organic conditions of Uttarakhand hills.

Entry number 6065 was selected from 2nd STEMRRSN during the Wheat field day during 2007-08 at ICAR-IIWBR, Karnal. The same was evaluated at evaluation nursery at Hawalbagh and finally 5 plants were selected. During 2008-09, 3 plants were selected from progeny 6065-2. During 2009-10 progeny of plant no 4 selected and harvested as bulk. During 2010-11 it was assigned VW 20138 number and evaluated in station trials under timely sown rainfed and irrigated conditions at experimental farm, Hawalbagh, Almora, Uttarakhand following Alpha lattice design with 2 replications. Under both the conditions, it yielded better than the best check VL *Gehun* 907, therefore, further evaluated as VL 967 under IVT, AVT-I and AVT-II-timely sown rainfed and irrigated trials of All India Coordinated Project (AICW&BIP) at 28 and 15 different locations, respectively, following randomized complete block design having 4 replicates in IVT and 6 in AVT, during 2011-12, 2012-13 and 2013-14 in the states of Himachal Pradesh, the then Jammu & Kashmir and Uttarakhand of northern hills. During 2012-13, 2013-14 and 2014-15 crop season, VL 967 was also tested at 14 locations following randomized complete block design with 3 replications in Uttarakhand State Varietal Trials (SVT) under organic conditions. During the final year of testing in 2014-15, it was also tested in farmers' field. The crop was sown within the recommended sowing time of second fortnight of October under rainfed conditions. The uniform crop geometry of 6 row plot of 3 m length with 23 cm row to row distance was followed over all the locations. The crop received 60 Kg ha^{-1} N, 60 Kg ha^{-1} P and 40 Kg ha^{-1} K as a basal dose and 30 Kg ha^{-1} N as a top dressing each after first irrigation and at the jointing stage under irrigated conditions whereas under rainfed conditions 60 Kg ha^{-1} N, 30 Kg ha^{-1} P and 20 Kg ha^{-1} K was applied as basal dose. Under rainfed organic trials only 20 t ha^{-1} FYM was provided as basal dose. Data on ancillary, yield, and disease susceptibility were recorded at all the locations and compiled at IIWBR, Karnal coordinated trials. However, the SVT data was compiled by assistant director, regional agriculture testing and demonstration station (RATDS), Haldwani, Uttarakhand. The individual location as well as for pooled data was subjected to the standard analysis of variance.

Artificial epiphytotic conditions were created for disease screening in a multi-location plant pathological screening Nursery. The disease recording was done as per Nayar *et al.* (1997) at all the locations and then compiled at ICAR-IIWBR, Karnal.

It was identified for release in the SVT meeting of Uttarakhand held on 30.09.2015 at Directorate of Agriculture, Dehradun, Uttarakhand. Subsequently, it was released by Uttarakhand State Seed Sub-Committee meeting held at Dehradun on 05.02.2018.

Grain yield ability and adaptability: VL *Gehun* 967 recorded grain yield of 1.99 t ha^{-1} (14 locations' weighted mean) with 12.71% advantage of grain yield over VL *Gehun* 907, the best check, under rainfed organic conditions of Uttarakhand hills. VL *Gehun* 967 has shown stable performance over the locations, occupied top ranking position in the first non-significant group of entries including checks under organic conditions of Uttarakhand hills. Under NHZ, it recorded average grain yield of 4.39 t ha^{-1}, which was at par with the best check HS 507 (Table 1). It has shown its flexible adaptation with higher grain yield (20.4%) under late sowing (27th November) in comparison to all checks under organic irrigated timely sown condition (Table 2). It showed grain yield superiority over all the checks under late sown condition. In the farmers' field trials conducted by department of agriculture, Uttarakhand, VL *Gehun* 967 yielded 2.6 t ha^{-1} under organic hills trials.

Table1: Grain yield performance of VL *Gehun* 967 and checks under organic rainfed conditions in Uttarakhand hills.

Testing years (no of locations)	Grain yield (t ha^{-1})		
	VL 967	VL 907 (C)	UP 2572 (C)
RF-2012-13 to 2014-15 (17) Hills organic	1.99	1.76 (12.71)	1.67 (19.07)
Frequency in the top non-significant group	4/14	1/14	0/14

	VL 967	HS 507	VL 804	VL 907	HPW 349
Northern Hills Zone (Rainfed)	3.03	3.08	2.84 (6.69)	3.09	3.21
Frequency in the top non-significant group	4/28	6/28	2/28	2/23	2/23
Northern Hills Zone (Irrigated)	4.39	4.43	4.33 (1.39)	4.39	4.14 (6.04)
Frequency in the top non-significant group	2/15	2/15	0/15	0/9	0/9

Number of locations and % increase of weighted mean over checks are given in parentheses.

Table 2. Adaptability to Agronomic Variables

Name of proposed variety/Hybrid:- VL *Gehun* 967			Adaptability Zone - Uttarakhand hills				
			Production condition- Timely sown organic irrigated				
Nature of Expt.	Item		VL 967 (P)	VL 804 (C1)	VL 907 (C2)	HS 507 (C3)	HPW 349 (C4)
Sowing date experiments	Yield (t ha^{-1}) under recommended N level	i) Normal (07.11.13)	6.37	7.16	7.55	7.22	7.31
	Percentage gain or loss when sown	ii) Late (27.11.13)	7.67 (20.4 %)	7.11 (-0.7 %)	7.35 (-2.7 %)	7.38 (2.2 %)	6.82 (-6.7 %)

Response to diseases: VL *Gehun* 967 showed better resistance to stripe rust in comparison to check UP 2572 under SVT and Check VL 804, VL *Gehun* 907 and HPW 349 under AICRP trials under field conditions. VL *Gehun* 967 also has the better stripe rust resistance and recorded ACI ranging 0.09 – 17.4 under artificial conditions. Similarly, the ACI of 2.0 to 4.0 for leaf rust was recorded under artificial inoculation conditions (Table 3).

Quality traits: VL *Gehun* 967 possesses 9.78 to 10.07 % protein, 76.37 to 79.73 Kg hl^{-1} hectoliter weight and 37.33 to 37.67 ml sedimentation value. In addition, it showed chapatti making score of 7.63 to 7.56, therefore, it has very good chapatti quality and good flour recovery. It possesses 32.7 to 34 ppm iron and 35.4 ppm zinc content. The zinc content is 11.07 to 12.85 % higher than the best check (Table 4).

Table 3: Response of VL *Gehun* 967 and checks against leaf and stripe rusts under natural and artificial epiphytotic conditions in NHZ.

Rust/Condition			Reaction against stripe and leaf rusts*				
	Item		Proposed variety VL 967 (P)	HS 507 (C1)	VL 804 (C2)	VL 907 (C3)	HPW 349 (C4)
Leaf Rust							
Natural	1st year (11-12)	IVT	5S	0	0	-	-
	2nd year (12-13)	AVT	NR	NR	NR	NR	NR
	3rd year (13-14)	AVT	0	0	tR	tR	0

Artificial	1st year (11-12)	IVT	10S (2.0)	5S (1.0)	60S (16.8)	20S (9.0)	60S* (12.2)
	2nd year (12-13)	AVT	20S (4.0)	60S*(12.8)	40S (16.0)	5MS (1.6)	10S (3.6)
	3rd year (13-14)	AVT	10S (3.4)	20S (4.0)	80S (28.8)	40S (10.4)	5S(1.6)
Skipe Rust							
Natural	1st year (11-12)	IVT	10S (2.0)	5S(1.0)	60S (16.8)	-	-
	2nd year (12-13)	AVT	0	0	10S	10MS	0
	3rd year (13-14)	AVT	10S (5.7)	10S (4.0)	60S (23.7)	30S (12.7)	20S (8.3)
Artificial	1st year (11-12)	IVT	10MS(0.9)	20S (3.9)	20S (6.5)	20S (5.4)	5S (0.8)
	2nd year (12-13)	AVT	20S (7.8)	20S (4.6)	60S (15.7)	40S (10.9)	20S (7.0)
	3rd year (13-14)	AVT	60S(17.4)	20S (8.4)	80S (34.9)	80S (20.5)	40S (11.0)

*Highest score, average coefficient of infection (ACI) is given in parentheses.

Table 4. Data on Quality Characteristics (Rainfed)

Quality Characte-ristics	Years	Proposed variety VL 967 (P)	HS 507 (C1)	VL 804 (C2)	VL 907 (C3)	HPW 349 (C4)
Zonal mean						
Hectolitre weight (kg/hl)	**Rainfed**	76.37	78.27	79.87	77.55	79.55
	Irrigated	79.73	80.97	81.67	78.75	80.95
Protein content (%)	**Rainfed**	9.78	10.54	9.96	10.04	9.81
	Irrigated	10.07	10.34	10.44	11.31	10.17
Sedimentation value (ml)	**Rainfed**	37.33	41.67	36.33	40	51.5
	Irrigated	37.67	39.67	37	39	52.5
Chapatti quality	**Rainfed**	7.63 (Very Good)	7.56	7.62	7.44	7.61
	Irrigated	7.56 (Very Good)	7.5	7.65	7.42	7.62
Phenol test (Max. Score 10)	**Rainfed**	6.5	5.7	5.23	6.3	4.97
	Irrigated	6.5	5.53	4.97	6.33	5.07
Iron Content (ppm)	**Rainfed**	32.7	33.17	32.27	32.27	33.73
	Irrigated	34	37.67	32.3	35.13	33.07
Zinc Content (ppm)	**Rainfed**	35.4	30.73	31.87	30.27	31.3
	Irrigated	35.4	31.37	28.1	31.37	30.17

Varietal description: VL *Gehun* 967 has semi-erect growth habit, green foliage, with medium wax on whole plant body, ear shape is tapering with medium compact ear density, average height of 75-80 cm, and maturity is 165-170 days in rainfed organic hills conditions of Uttarakhand. It has amber bold grains with 45-48 g thousand grain weight.

Demonstration at farmers' field: Farmers' field trials were conducted during rabi 2014-15 crop season. These were conducted at 6 different locations of district Almora. The average grain yield of 2.57 t ha^{-1} was recorded in these farmers' field trials. Farmers' response to this variety has been positive and they are very enthusiastic to grow this variety.

Conclusion

VL *Gehun* 967 is a high yielding disease resistant wheat variety which has performed well under inorganic rainfed and irrigated timely sown conditions under NHZ as well as rainfed organic timely sown conditions of Uttarakhand hills. It has shown stability in terms of wider adaptability, high yield, very good chapatti quality and high resistance to stripe as well as leaf rusts. The farmers' field trials conducted in Uttarakhand have shown its potential and acceptability among the farmers. It will provide an alternative to wheat variety VL *Gehun* 907 and replacement of UP 2572 in hills. The release of VL *Gehun* 967 would increase the wheat productivity of Uttarakhand as well as provide much needed diversity for rust diseases.

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