

POTENTIAL EFFECTS OF GLIBENCLAMIDE ON PROTEIN EXPRESSION IN ALCL₃-INDUCED NEUROTOXICITY: IMPLICATIONS IN NEURODEGENERATIVE DISORDERS

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Abstract

Alzheimer's disease (AD), the most common form of dementia, affects 46.8 million people worldwide while Type 2 diabetes mellitus (T2DM), a metabolic disorder, affects 382 million people globally. Both these devastating diseases share some common pathological features including insulin resistance. Based on this common pathological similarity the present study aimed to investigate the effects of an antidiabetic drug on hippocampal protein expression in a mouse model of aluminum chloride (AlCl₃) induced neurotoxicity that displays brain atrophy and neuronal damage as in neurodegenerative disorders (NDDs). Age matched male Balb/c mice were divided into 4 groups and administered with AlCl₃, Glibenclamide (GLI) (10mg /body weight), AlCl₃ followed by GLI and control. Significant expression alterations were observed for seven proteins while substantial restoration of protein expression was also detected, as an effect of GLI administration. However; it is worth mentioning that GLI exhibit negative regulation of expression for few of the expressed proteins. In conclusion, GLI may have the potential to restore altered protein expression during neurotoxicity with few exceptions, which is speculated to be dependent on the nature of the protein. Further characterization of the expressed proteins will be helpful to validate the observed significant effects of GLI that may provide a novel approach to combat cellular and metabolic alterations in neurotoxicity and neurodegenerative disorders.

Keywords: Alzheimer's disease, Type 2 Diabetes Mellitus, Neurotoxicity, Neurodegeneration, Antidiabetics

1. INTRODUCTION

Type 2 diabetes Mellitus (T2DM) and Alzheimer's disease (AD) constitute major burden of affected population and are estimated to increase in upcoming years

with approximately 46.8 million people living with dementia in 2015 (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016).

According to International Diabetes Federation (IDF) almost 382 million people are affected with T2DM worldwide, this number is expected to increase to 592 million by 2035 (IDF, 2013). It is estimated that the T2DM patients have twice the risk of developing dementia and AD while those taking insulin have four times the risk (Gudala et al., 2013). Insulin plays a significant role in memory formation by stimulating the synthesis of choline acetyltransferase (ChAT), which involves in the biosynthesis of acetylcholine, a potent neurotransmitter in memory formation and learning. (Hasselmo, 2006). Insulin also mediates beta precursor protein and beta derived proteins (Watson and Craft, 2003). Hence low levels of insulin are linked with low production of acetylcholine, a possible link between the two pathologies i.e. AD and T2DM (Kroner, 2009; Rivera et al., 2005). Moreover, the amyloid beta-derived diffusible ligands (ADDLs) disturb this normal process of memory formation by binding to insulin receptors and altering the conformational anomaly that leave cells resistant to insulin. ADDLs also have the potential to reduce plasticity of synapse and cause oxidative damage (Viola et al., 2008). Advanced glycation end products (AGEs), end products of Maillard reactions, formed as a result of linkages between carbonyl group of sugars and amino group of proteins, lipids etc., form

unstable complex compounds (Yamagishi et al., 2007; De Felice et al., 2008). Activation of receptors for AGEs (RAGE), stimulate the oxidative stress by triggering reactive oxygen species (ROS) and nuclear factor- κ B (NF- κ B) which further stimulate various inflammatory mechanisms (Sugimoto et al., 2008). Diabetic neuropathy is reported in case of AGE/RAGE pathway activation, which increase the oxidative load and cause neurological dysfunction. AGEs have been reported in peripheral nerves, kidney, retinal vessels and central nervous system (CNS) of diabetes patients. Oxidative stress also induces the formation of AGEs, making a vicious cycle (Sato et al., 2006). Diabetic individuals are more vulnerable to develop AD by AGEs production (Valente et al., 2010). AGEs are also implicated in neurofibrillary tangles and plaques (Zhu et al., 2007).

Tau protein tangles and beta-amyloid protein plaques are shown to have the AGEs. Hence, T2DM and AD, both cause the production of AGEs, which aggravates the oxidative burden on the neural cells, thus presenting another justifying link between the two pathological conditions (Takeuchi and Yamagishi, 2008). Sulfonylureas have been studied for various neurological pathologies. Potential effects of glimepiride on cell proliferation and neuroblast differentiation on dentate

gyrus hippocampal region was reported showing reduced proliferation and differentiation which have been alleviated by glimepiride (Yoo et al., 2011). Another study highlighted the positive impact of glyburide (glibenclamide) in restoring the activities of superoxide dismutase and catalase in the brain sections of streptozotocin-induced diabetic rat (Nazaroglu et al., 2009). Glibenclamide (GLI) also mediates the decrease in contusion expansion rate in patients with moderate and severe traumatic brain injuries sustaining cerebral contusions (Khalili et al., 2017). In addition, GLI significantly reduces cerebral edema and decreases the rate of hemorrhagic conversion following ischemic stroke (Khanna et al., 2014). In this study, aluminium chloride (AlCl₃) induced neurotoxicity mouse model of AD was established. AlCl₃ has been used in various research studies to induce AD in mice (Sun et al., 2009; Shati et al., 2011).

The AlCl₃ induced mouse model was an imminent choice as AlCl₃ can cross blood brain barrier and has the ability to produce oxidative stress after accumulating in glial and neural cells (Yuan et al., 2012). Thus oxidative stress, a major stigma of AD occurs in mouse model. Moreover; the model imitates the pathological events taking place in AD brain like neuronal degeneration, which is not observed in

many transgenic AD mouse models. The aim of this study is to investigate the potential effects of antidiabetic drug on protein expression during neurotoxicity. T2DM is a metabolic disorder and affects different organs including brain leading to cognitive functioning impairment. Previous studies concluded that T2DM and AD share common mechanisms, so antidiabetic drugs might be effective for alleviating the complexities of neurodegenerative disorders.

2. MATERIALS AND METHODS

Animals

All experiments were conducted in agreement with the decrees of the Institute of Laboratory Animal Research, Division on Earth and Life Sciences, National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals: Eighth Edition, 2011). Institutional Review Board (IRB) of Atta ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST) approved the procedures and experiments, planned for this project. Three months old male Balb/c mice (n=24) were bred and maintained at animal house, ASAB. Wood shavings served as bedding of each cage (40x20.5x20.5cm) containing 4 mice, with a unique identity of each mouse. Temperature was maintained at 25 °C ± 1, light and dark cycle of 12 hours

was provided matching with the day light hours. A standard diet *ad libitum*, that consists of crude protein (30%), crude fiber (4%), crude fat (%) and moisture (10%), was provided to the animals with equivalent access to water and food during the protocol.

Animal Treatment

Balb/c mice (weight range 35-42g) were randomly divided into six groups containing four mice each. U-100 insulin syringe 30G x 5/16” (0.3mm x 8mm) needle was used for subcutaneous injection for the administration of AlCl₃ and the drug; Glibenclamide (GLI) (Daonil[®], Sanofi Aventis Pakistan Ltd). The drug was also up-titrated to 10 mg/kg and the time span was down-regulated from 4 weeks to 2 weeks. The detailed treatment strategy is stated in Table 1.

S.No	Experimental Groups	Treatment	Duration (days)
1	Control	-Normal water -Distilled water	21
2	AlCl ₃	Subcutaneous injection of 0.2ml of 3% AlCl ₃	8
3	GLI 10mg	-GLI 10mg/kg in distilled water	14
4	AlCl ₃ + GLI 10mg	-Subcutaneous injection of 0.2ml of 3% AlCl ₃ -GLI 10mg/kg in distilled water	14

Animal Dissection

The mice were anesthetized by chloroform and then decapitated/sacrificed. Brain tissues were removed and separated in a petri dish placed on ice. Hippocampus region of brain was detached and added in an eppendorf tube and instantly stored at - 80 °C till further use.

Protein Extraction

The frozen Hippocampus tissues were weighed (approx 50mg) and a100ul of ice cold lysis buffer (7M urea, 2M thiourea and 4% v/v triton X-100/ CHAPS,1% beta mercaptoethanol, 10mM phenyl methane sulfonyl fluoride (PMSF; 200mM stock)) was added. The tissues were then homogenized using sonicator UP400S Hielscher Ultrasound Technology. To increase the solubility, the tissue lysate was kept at room temperature for one hour and vortexed repeatedly. Centrifugation was carried out at 14000 rpm for 10 min at 4 °C and the supernatant was collected and subsequently stored at -20 °C. The pellet was treated as same and the finally the two supernatants were pooled and centrifuged at 14,000 rpm, for 90 mins at 4 °C. The final supernatant constituting the total protein extract was stored at -80 °C till further use.

Protein Quantification by Bradford

Protein Assay

Bradford protein assay (Bradford, 1976) was used to determine the total protein

concentration. Bradford reagent (coomassie blue G250, methanol, 85% orthophosphoric Acid (H₃PO₄), water) was prepared and the precipitates were removed by filtration with Whatmann#1 filter paper and volume was made up to 1 litre. The reagent was stored in a dark bottle at -4 °C.

The dilutions were prepared from 1mg/ml BSA stock solution. Standard concentrations of BSA included 0.0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ug/ul. All the samples were diluted with double distilled water (ddH₂O) in ratio of 1:20. Bradford reagent was added in sample and standardized dilutions of BSA, followed by gentle vortexing. Absorbance was measured at 595nm for each dilution.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE)

Laemmli standard protocol (Laemmli, 1970) was used to perform the sodium dodecyl sulphate poly acrylamide gelelectrophoresis (SDS-PAGE). Mini protean Tetra cell (Bio-Rad) was used to carry out gel electrophoresis according to the manufacturer's protocol. Protein sample (50ug) dissolved in sample diluting buffer (0.125M Tris/HCL, 20% glycerol, 10% betamercaptoethanol, 10% SDS, 0.5% bromophenol blue) was resolved on 10% resolving gel (distilled water, 30% acrylamide solution, Tris-HCl pH 8.8, 10% SDS, 10% APS, TEMED). The electrophoresis was run at 90V for 45 min

for the visualization of protein bands. The gel was stained with 0.025% Coomassie brilliant blue and later detained with 75ml Glacial acetic acid, 25ml 100% ethanol, until background became clear.

Image and Statistical Analysis

Gel image analysis and quantification of protein bands was carried out by using Lab Image[®] gel image analysis software (Bio-Rad). Relative quantity of each protein band set the basis for calculating the differential expression of proteins. The statistical analysis of the data was observed by One Way ANOVA. The value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS

Total Protein Quantification

Protein concentration in all groups was measured by comparing absorbance value of colored reaction product with the plotted standard curve. The intensity of colored product is a directly related to protein concentration (Figure 1).

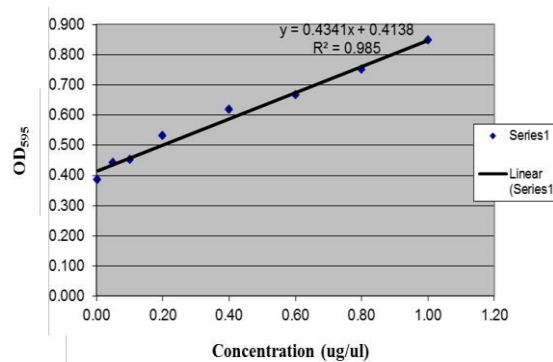


Figure 1: Bradford Standard curve plotted
foreight values: Concentration has been

plotted as independent variable (x-axis). Absorbance was measured at 595nm and plotted as dependent variable (y-axis). The line represents the linear regression for entire set of standard points. The value for linear regression was $R^2=0.985$

Differential Expression Analysis of Hippocampal Proteins

The image quantitation analysis revealed several proteins exhibiting altered expression in treated groups compared with control. Proteins of 115 kDa, 100 kDa, 89 kDa, 64 kDa, 50 kDa, 33 kDa, and 15 kDa, showed altered expression in AlCl₃-treated group. Interestingly, the administration of GLI (10mg), significantly restored the expression of 115, 100 and 89 kDa proteins to normal (Figure 2).

A 100 kDa protein had showed an increased expression in AlCl₃-treated group while the expression level was restored in the AlCl₃ + GLI group. The GLI-treated group also showed normal levels of protein expression. Quantitative analysis of 89 kDa protein showed a remarkable rise in expression as a result of AlCl₃ treatment while this expression was restored when AlCl₃ treatment was followed by GLI. Quantification analysis of 116 kDa protein showed considerably decrease expression in Al group. Proteomic analysis also revealed a remarkable decrease in expression in AlCl₃ + GLI group and GLI group as compared to control. A 64 kDa protein showed

increased levels of expression in GLI group and AlCl₃+ GLI group as compared to control and AlCl₃-treated group, while there was no significant difference in expression level in AlCl₃-treated group as compared to control.

Increase in expression levels was also observed for a 50 kDa protein in GLI group as compared to AlCl₃+ GLI group, AlCl₃-treated group and control. Quantitative analysis of 33 kDa protein also showed interesting results in all groups. Expression was increased in AlCl₃-treated group while a further rise in expression level was observed in presence of GLI in AlCl₃ + GLI group. However, the analysis revealed a decrease in expression in GLI group as compared to AlCl₃ + GLI group and AlCl₃-treated group. Analysis of 15 kDa protein showed a remarkable rise in expression levels in AlCl₃ + GLI group, GLI group as compared to control group (Figure 3).

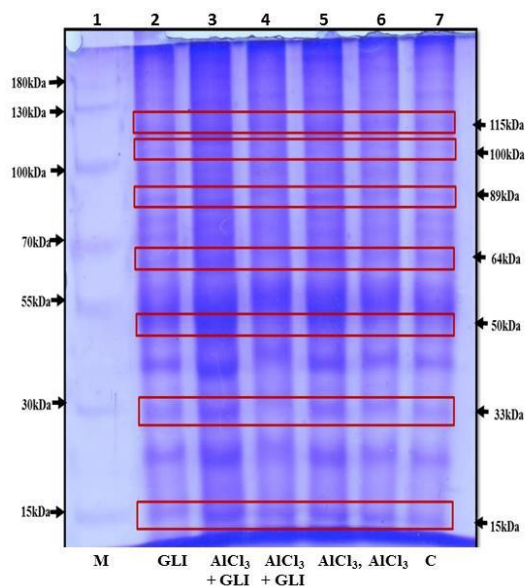


Figure 2: Differential expression pattern of proteins isolated from hippocampus, separated by SDS-PAGE: Extracted proteins were resolved on 12.5% separating gel and stained with coomassie brilliant blue Lane 1: Protein Marker, Lane 2: GLI (10 mg), Lane 3: AlCl₃ + GLI (10 mg), Lane 4: AlCl₃+ GLI (10 mg), Lane 5: AlCl₃, Lane 6: AlCl₃, Lane 7: Control

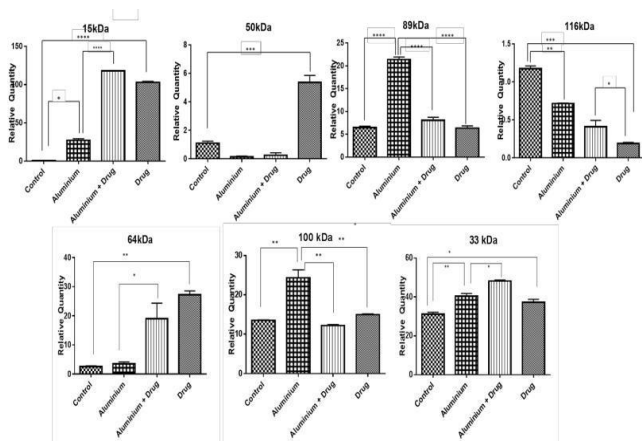


Figure 3: Expression graph of differentially expressed hippocampal proteins. Histogram representing relative quantity of 15 kDa, 50 kDa, 89 kDa, 116 kDa, 64 kDa, 110 kDa and 33 kDa proteins in control, AlCl₃-treated group, AlCl₃ + GLI group and GLI-treated group; showing significant differential expression. The graphs were generated by GraphPad Prism 6 software. Data was analyzed using one-way ANOVA, followed by multiple comparison Bonferroni test. The data is shown as the mean ± SEM, **p* < 0.05; ***p* < 0.01; ****p* < 0.001. To check the consistency samples were run in triplicates.

4. DISCUSSION

The study aimed to investigate the potential effects of an antidiabetic drug; GLI, on neurotoxicity induced in AlCl₃ mouse model for AD. A total of 22 hippocampal proteins were expressed out of which seven proteins showed altered expression.

Proteins of 100 kDa, 89 kDa, 116 kDa, 64 kDa, 50 kDa, 33 kDa and 15 kDa were altered by AlCl₃ administration. GLI showed an affirmative effect on expression levels of two proteins i.e. 100 kDa and 89 kDa. As GLI binds to sulfonylurea binding subunit 1 (SUR1) (Ashcroft, 1996; Sola et al., 2015) and inhibits its activity that further leads to inhibition of necrotic cell death, potency of anti-inflammatory response and stimulation of neurogenesis. Several potential effects of GLI were assessed in rodent models of ischemic stroke (Ortega et al., 2013), traumatic brain injury (Simard et al., 2009a), hemorrhagic stroke (Simard et al., 2012), spinal cord injury, neonatal encephalopathy of premature and metastatic brain tumor (Thompson et al., 2013; Kurland et al., 2013). GLI appeared to be highly effective in decreasing the cerebral edema intensity, infarct volume and mortality in rat models of ischemic stroke (Simard et al., 2009b, Zhou et al., 2009). This might be linked with the increased expression of 100 kDa and 89 kDa proteins. As GLI exhibits therapeutic ability against brain injury so it may have the potential to restore the expression levels of proteins, which are involved in brain functioning and have modified role in neurodegenerative disorders like AD. In cytotoxic edema, which is a result of depolarization of channels due to ATP depletion, GLI binds to SUR1 and blocks it, thus preventing

cytotoxic edema and brain damage after traumatic brain injury (Zweckberger et al., 2014). Actually, the permeation of GLI in BBB is enhanced after traumatic or ischemic injury, which might be due to collapse of BBB and low pH environment that exists in the brain injury. As GLI is a weak acid and is taken up by the CNS easily after injury (Kurland et al., 2013), such environment makes it to cross BBB conveniently. In our case the 100 kDa and 89 kDa proteins which showed normalized expression following the drug administration, it is speculated that they might possess a significant role in functioning of neuronal mechanism of memory formation and cognitive functioning, however further validation studies are required for their structural and functional characterization.

Interestingly, GLI besides its positive effects on protein expression has also demonstrated negative effects on few proteins by altering their normal expression. In case of 116 kDa protein, decrease in expression level was observed in $AlCl_3$ -treated group as compared to control. However; contrary to the expected effect GLI reduced its expression further. GLI is known to have the higher tendency of causing hypoglycemia as compared to other agents of the same group. The reason is the gradual decrease in the ability of beta cells to produce insulin, which is the common drawback of all anti diabetic drugs

(Rendell, 2004). Moreover, an early GLI treatment may help protecting beta cells against the autoimmune attack, which triggers the development of type 1 diabetes (Lamprianou et al., 2016). Further elucidation of this 116 kDa protein might reveal its importance in pathogenesis and involvement in AD.

GLI showed dramatically varied results in rest of the proteins which were differentially expressed in treated groups. In case of 64 kDa and 50 kDa proteins, GLI seemed to have the potential impact in mediating the expression levels of proteins; however, effect of the drug has been diminished in the presence of $AlCl_3$ -induced neurotoxicity. Similarly in case of 33 kDa and 15 kDa proteins, the aberrantly increased expression due to $AlCl_3$ -induced neurotoxicity is further increased in presence of GLI when $AlCl_3$ followed by GLI. Based on these findings it is speculated that the aberrant protein expression levels might be associated or are the consequences of $AlCl_3$ -induced neurotoxicity and the high affinity of proteins for $AlCl_3$ (Cheng et al., 2018) which was also triggered by GLI. This inverse regulation of protein expression can be linked with the nature of the proteins, however it is also noteworthy that the GLI has the potential that restores and normalize the aberrant protein expression but the exact molecular mechanism is still not clear.

Further elucidation of these aberrantly expressed proteins will clear their potential role in the pathogenesis of both diseases, which might be helpful to reveal the various other aspects associated with these metabolic disorders.

5. CONCLUSION

The present study enunciates certain facts that might open a new research gate towards the therapeutic strategies of neurological disorders. Moreover, the study also focuses on the dynamic effects of sulfonylurea on proteome expression of hippocampus. As proteins have a significant role in developmental and pathological changes involved in neurodegenerative disorders like AD, it might also lead to the new link between the two diseases, concluding the reason for development of one disease leading to the other.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author's roles *SZ, substantial contribution to conception, design of the study and finalization of the manuscript; MM, all experimental work, analysis and data interpretation.

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CHARACTERIZATION OF DIFFERENT *AEGILOPSTAUSCHII* ACCESSIONS IN SIMILAR DURUM WHEAT BACKGROUND

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Abstract

In wide hybridization, resistance against pests, diseases and insects have largely been exploited previously. Synthetic hexaploid (SH) wheats carry a wide range of resistances against *C. sativus*, *Tilletia indica* and *S. tritici* due to the presence of D-genome. In addition, SH wheats carry tolerance/resistance against drought, heat, salinity, cold, water logging, leaf rust, mineral toxicities, sprouting, stripe rust, powdery mildew, loose smut and cereal cyst nematode (CCN). From the primary set of 1014 D-genome synthetics prepared by International Center for Wheat and Maize Improvement (CIMMYT), Mexico, an experimental set was categorized by crossing different accessions of *Ae. tauschii* with same durum cultivar (female parent). Two main goals were studied during this experiment; (i) inheritance of different genes, and (ii) effect of cytoplasmic inheritance if any. The experimental sub-set was comprised of 90 entries. Phenological characterization, screening against stripe rust (Yr), Karnal bunt (KB) and molecular characterization with 41 SSRs markers were done in this study. The total loci/alleles scored was 191 out of which 185 were polymorphic loci/alleles thereby giving the percentage of polymorphism as 96.85%. Genotype 89 showed the best KB and Yr resistance and is also the most diverse line based on SSRs.

Key words: *Aegilopstauschii*, *Triticum turgidum*, reciprocal crosses, stripe rust, Karnal bunt

1. INTRODUCTION

Wheat is one of the most domesticated crops. World's population is increasing day by day and it will be doubled by 2050, hence food demand is increasing accordingly. So as to encounter the prospering worldwide populace, sustainable wheat profitability is fundamental. Regardless of considerable advances in plant systems, worldwide wheat production is as yet undermined due to a large number of pathogens and pests (Moffat, 2001). Within the tribe *Triticeae*, approximately 325 species reside; about 75 annual and 250 perennial species (Dewey, 1984). This provides the scope of natural diversity for improvement in the customary wheat germplasm by nearly or remotely related alien species. Regular projects on wheat breeding are around various cross mixes of germplasm dwelling in a similar gene pool that undergo genetic recombination followed by trait segregation, evaluation and ultimate varietal release. So as to increase the genetic diversity in wheat, scientists started to focus on novel genetic resources from nearby wheat progenitors. There are many accessions of wheat progenitors based on A-, B- and D-genomes. Due to close proximity of A & D-genomes, they are more advantageous than B-genome for bread wheat improvement. Cytogenetic tests have

also revealed greater closeness of the 7-chromosomes of the D genome wild diploid than the A-genome chromosomes with their respective D and A genomes. Accessions of these two diversity sources reside in the primary gene pool, can be hybridized with ease, allow for swift gene transfer via homologous recombination and have extensive diversity for global biotic/abiotic stress/constraints that limit wheat production. Higher genetic proximity tilts the ideal decision of exploiting D-genome diploid *Ae. tauschii* ($2n = 2x = 14$). Moreover, just a couple of accessions were employed in the natural amphiploidization event, also known as natural hybridization. Subsequently, plants with low genetic diversity were created. Integral to this are the perceptions of Kihara (1944) and McFadden and Sears (1946) on the role of *Ae. tauschii*. They encouraged current researchers to center their attention towards wheat improvement on this wild diploid by means of different conventions (Mujeeb-Kazi and Hettel, 1995). So also, the wild wheat emmer is a proficient wellspring of target qualities for good attributes, which includes genes for drought and salinity tolerance, protein production like required storage proteins, large spikes, amount of photosynthate, grain size, herbicidal response, large grain, resistance to diseases like powdery mildew, rusts, soil-born

mosaic virus, abundant tillering and other quantitative traits genes (Jaradat, 2011).

Karnal bunt of wheat is caused by *Tilletia indica* (Syn. *Neovossia indica*, a fungus that infects wheat (Mitra, 1931) and *Triticale* (Agarwal, 1977) during flowering (Bedi, 1949). Currently, it is considered a quarantine pest by twenty-one countries. The loss in yield is not serious due to Karnal bunt, but it damages the quality of flour and the tolerable range of its infection is 3%. This fungus produces trimethylamine, which in turn adversely affects odor and taste of the flour. It is not possible to control the production of chemicals by fungus therefore developing Karnal bunt resistant cultivars is the most suitable option. Synthetic hexaploid wheat derived from durum wheat x *Aegilopstauschii* is one of the potential sources to introgress resistance against Karnal bunt in bread wheat (Gorafi et al., 2018). Despite of the gigantic advances in rust control, it is still considered a major biotic stress of wheat around the globe, especially in Pakistan (Latif et al., 2018). Improvement in human progress has greatly been influenced by the events of rust infections in cultivated cereals (Roelf et al., 1992). Stripe rust commonly known as yellow rust is the most important among all rusts. It is present in more than 60 countries around the world with the

exception of Antarctica. Amid the most recent decade, many episodes of stripe rust epidemics have been reported in many countries causing huge losses in wheat production. The severe epidemics throughout the world indicate that virulence is present for most of the known stripe rust resistance genes which are race-specific in nature. Its damage can be controlled by resistance breeding which is inexpensive as well as eco-friendly. Globally, a chain of yellow rust resistance genes *Yr1*, *Yr41* and many conditionally selected genes are being recognized (McIntosh, 2008). In China, many genes are effective like *Yr5*, *Yr10*, *Yr11*, *Yr15*, *Yr12*, *Yr26*, *Yr13*, *Yr14*, *Yr24*, *YrZH84* whereas other genes have lost their efficiency like *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9* (Wan et al., 2007). A large pool of variability is present in wild relatives of wheat which can be utilized for improvement of bread wheat (Mujeeb-Kazi, 2006; Singh et al., 2007). A set of 90 D-genome synthetic hexaploids with subsets of same durum parent but different *Ae. tauschii* parents were screened against stripe rust and Karnal bunt to identify the expression and suppression of resistance provided by the parents.

2. MATERIALS AND METHODS

From the primary synthetics, an experimental set was categorized by

crossing 90 different accessions of *Ae. tauschii* with same durum cultivar which was kept as a female parent (Table 1). This experimental subset was made to study the inheritance pattern of different genes and to investigate the effect of cytoplasmic inheritance if any. Screening of 90 genotypes against stripe rust and Karnal bunt was done along with phenological and molecular characterization using SSRs (Tables 1 & 2).

a. Phenotypic Evaluation

Phenological data of the following traits was taken from each genotype by randomly selecting five plants and the arithmetic mean was computed. Phenotypic data included days to flowering, days to physiological maturity, plant height, awn color, thousand grain weight, number of grains per spike and spike length. Standard protocols were applied to collect phenological data according to Afzal et al. (2017). Planting was done in the fields of National Agricultural Research Center (NARC), Islamabad in 2-meter-long rows with inter-space of 30cm.

b. Stripe Rust Studies

Stripe rust resistance studies at seedling stage and at adult plant stage were conducted in the fields of Crop Disease

Research Program (CDRP), Murree; Punjab and Wheat Wide Crosses, NARC, Islamabad, respectively. In order to identify new genetic stocks with stripe rust resistance at both plant stages while screening was done for two years. The inoculum for disease was collected from different cities of Pakistan such as Khushab, Mirpur, Chitral, Sialkot, Rawalpindi, Nowshera, Kotli, Sheikhopura, Chakwal, Gilgit, Jugglot, Muzaffarabad, Kasur, Peshawar, Rawalakot, Faisalabad, Abbottabad and Skardu. Revival and increase in inoculum was done in a greenhouse on a susceptible cultivar named “Morocco” at CDRP, Murree.

c. Glasshouse Evaluation for Seedling Resistance

Planting of 90 accessions of D-genome synthetics was done in glasshouse in disposable pots at CDRP, Murree. Inoculum of urediospore suspension was prepared with mineral oil and petroleum ether in a ratio of 30:70. In order to evaporate the oil, plants were then placed outdoor for 2 hours. The inoculated plants were then shifted in a preset dew chamber set at 10°C with 16 hours light and 8 hours dark, photoperiod for 48 hours. Afterwards, plants were shifted to preset glasshouse with 6-10°C temperature. Three weeks after inoculation, type of infection was noted on

a 0-9 scale (McNeal et al., 1971). By that time, the check susceptible variety “Morocco” showed the maximum infection. The plants were assigned with infection types; 0-3 infection types were taken as resistant, 4-6 as intermediate resistant and 7-9 were taken as highly susceptible.

d. Evaluation of Adult Plant Resistance

Under field conditions at NARC Islamabad, assessment of adult plant resistance for evaluating stripe rust resistance along with synthetic hexaploid (SH) wheat lines and their parents was done. Screening of germplasm was done by planting them in 1m row with inter-row space of 30cm. After planting, epidemic of stripe rust was established. Inoculum carrying urediospore suspension in mineral oil and ether (30:70) was sprinkled on spreader rows and the lines to be screened. For the prevention of escape from the inoculation, it was repeated three times.

Data were recorded three times with a 10 days interval for evaluating the severity of disease and type of infection. When Morocco (a susceptible check) reached 100% disease severity, first phase for disease data recording was started. According to the modified Cobb scale, the severity of disease on plants was calculated

as the percentage of rust infections (Peterson et al., 1948). Table 3 shows the description of data recording of response with respect to the infection type.

e. Karnal Bunt Screening

To ensure the genetically heterogeneous fungus population, teliospores from different wheat growing lands of Pakistan were utilized. Teliospores were isolated by the following protocol. Water-tween-20 solution was added to infected kernels and was first shook, centrifuged (3,000 rpm) and then sieved using a 60-micron mesh. This process removed all the residues of kernels. After sterilizing their surface with 0.5% HOCl solution, they were centrifuged for 2 minutes and incubated at room temperature after rinsing them in sterile distilled water and placing them on 1.5% water agar. Following 5-8 days, growing teliospores were shifted to potato-dextrose agar (PDA) and sterile water was added to it. Colonies of fungus were scratched from the media following nine days on to extra PDA plates. After 8-10 days, the fungal colonies on PDA plates were cut into small pieces and placed on the top of sterile glass petri-plates. Some sterile water was added to the base of each plate and afterward the allantoidsporidia were counted every 2h using a haemocytometer and the

concentration of spores was adjusted to 10,000 spores per ml.

Inoculation was done at booting stage (stages 48-49 as indicated by Zadoks et al., 1974) on five arbitrary tillers from each entry. Infusion of 1 ml/tiller was done by hypodermic syringe from the sporodial suspension. There were 2-3 dates of planting amid each cycle to be tested. 10 spikes were evaluated for contamination at maturity and the general rate disease was ascertained for every entry. The inoculation and subsequent scoring was done for two consecutive cycles in NARC fields.

f. Molecular Evaluation

For molecular evaluation and determination of molecular diversity of synthetics, SSRs were utilized. Under controlled condition of temperature and light, sowing of four seeds in jiffy pots of each genotype was done in plant growth chambers at Wheat Wide Crosses, Islamabad. DNA extraction was later done from young leaves harvested from all genotypes of synthetic hexaploid wheat. Modified DNA extraction protocol was utilized (Blatter et al., 2004). Unequivocally scorable amplified DNA fragments were transformed into binary character matrices. The data matrix was then used to calculate the GS index of genetic similarity (Nei) cluster analysis of the genetic distance (1-GS) matrix.

$$\pi = \sum_{ij} x_i x_j \pi_{ij} = \sum_{i=1}^n \sum_{j=1}^i x_i x_j \pi_{ij}$$

Where x_i and x_j are the respective frequencies of the i th and j th alleles, π_{ij} is the number of allele differences per locus between the i th and j th alleles, and n is the number of alleles in the sample. The summation is taken over all distinct pairs i, j , without repetition. Similarity values were calculated for each line, and clustering was done by the Un weighted Pair-Group Method with Arithmetic Averages (UPGMA) (Sneath and Sokal, 1973) using the POP gene software package. The dendrogram was generated using the same software.

3. RESULTS AND DISCUSSION

a. Stripe Rust Studies

At seedling stage, 37.7% (34 out of 90) and 95.6% (22 out of 23) of the durum parents showed resistance in this screening experiment (Table 4). Same genotypes at adult plant stage were 41.4% (37 out of 90) resistant and in durum plants, 86.9% (20 out of 23) plants were found to be resistant under field conditions in NARC.

Resistance found at both seedling stage and adult plant stage was 19 out of 90 (21.1%) in SHs (1, 13, 14, 17, 19, 20, 34, 37, 38, 42, 62, 63, 67, 72, 74, 80, 81, 87, 89) and 9 out

of 10 (90%) in durum parents (1, 4, 5, 8, 11, 12, 13, 22, 26). Presence of resistance in the germplasm is a clear indication that they have resistance genes against stripe rust and can be utilized in breeding programs for further exploitation.

There is a clear indication of presence of minor genes in those plants which were susceptible at the seedling stage but resistant at adult plant stage. Therefore, adult plant resistance (APR) is of great importance in acquiring durable resistance against rust diseases. 18 out of 90 (20%) in this experiment (2, 3, 5, 7, 11, 15, 22, 23, 31, 33, 53, 55, 58, 71, 73, 82, 83, 84) were found to be good candidates in terms of providing durable resistance to wheat cultivars, although no durum parent showed adult plant resistance.

b. Karnal Bunt Studies

Examination of grains for evaluation of Karnal bunt was done after artificial inoculation. After hand threshing, grains collected from each entry were rated on the scale from 0 to 5 after thorough examination. Entry with the scale of 0 was taken as resistant, all else from 1 to 5 were considered as susceptible. In our study, 30 out of 90 entries (33.3%) including 4, 9, 10, 11, 13, 14, 16, 17, 29, 31, 32, 39, 40, 42, 45, 47, 49, 53, 59, 66, 67, 68, 81, 82, 83, 84, 86, 87, 89 and 90 were completely

immune (Table 4). All durum parents were also immune to Karnal bunt.

c. Molecular Studies

To evaluate the genetic diversity of D-genome synthetic hexaploids, simple sequence repeats (SSR) primers were utilized. Genetic polymorphism was detected by using 275 SSR primers, at DNA level (Röder et al., 1998). Samples which gave no amplification were discarded for further analysis.

Efficiency of primers to amplify the genotypes ranged from maximum 39 genotypes (gwm129-5A) to 2 (gwm68-5B, gwm284-3B) in this experiment. The scorable bands ranged from 2 (gwm68-5B) to 66 (gwm129-5A) in this experiment. Population genetic analysis showed that 96.5% of alleles (185 out of 191) were polymorphic. Range of scorable bands was from 50-600bp in this experiment.

d. Similarity Matrix

The dendrogram was generated by conducting bivariate analysis. It was used to estimate genetic diversity by utilizing Nei and Li's coefficient (1979). The range of similarity matrix was found to be from 75.5% (minimum) to 100% (maximum). Minimum range was between 2 and 90

while maximum was present in 33 different combinations.

In this experiment, there was only one with two sub-clusters; A and B (Figure 1). Sub-cluster A contained 38 genotypes carrying the most diverse line of the group i.e., 27. The other diverse lines of this sub-cluster were 14, 54 and 61. Sub-cluster B contained 61 genotypes with 1 as the most diverse line and 4, 5 and 41 as other good examples.

There is a good amount of genetic diversity observed in the response of synthetic hexaploid wheats against stripe rust, which is accredited to the A and B-genomes and/or to the D-genome (Ma and Mujeeb-Kazi, 1995; Assefa, and Fehrman, 2000; Rizwan et al., 2007). Lange and Jochemsen (1992) generated 22 Synthetic hexaploid wheats from 11 stripe rust resistant wild emmer and 8 *Ae. tauschii* accessions. However, resistance in one or both parents was frequently suppressed in the synthetic lines, indicating the presence of suppressor genes on the AB and/or D-genomes (Kema et al., 1995; Plamenov and Spetsov, 2011). Bai and Knott (1992) have reported that the transfer of leaf rust and stem rust resistance genes from wild emmer wheat to cultivated bread and durum wheat revealed that the suppression of resistance was more common in the D-genome of bread wheat and there was a specificity of the suppressors. The

dilution of leaf rust resistance was noted in amphiploids with *T. durum* when compared to the diploid resistant parent (*Ae. tauschii*) (Kerber and Dyck, 1969). The same has also been noted by Nelson et al., (1997) in case of powdery mildew, leaf rust, yellow rust and glume blotch. The resistance of durum has also been noted to get suppressed by *Ae. tauschii* in amphiploids, e.g., *Lr23* (Trottet et al., 1982). The complete expression of the resistance of *Ae. tauschii* in common wheat, however, is investigated for green bug resistance (Harvey et al., 1980), Hessian fly (Gill et al., 1987), cereal cyst nematode (Zhang et al., 2016) and wheat curl mite (Dhakal et al., 2017). Lage et al. (2003) screened 58 synthetic hexaploids for green bug resistance. All *T. dicoccum* parents were susceptible but a few *Ae. tauschii* parents exhibited high level of resistance. The occurrence of suppressor genes for green bug resistance in the A and/or B-genomes of *T. dicoccum* is clearly indicated in some synthetics. The resistance from diverse *Ae. tauschii* accessions was expressed in a different way when crossed with the same *T. dicoccum*, demonstrating diversity between the resistance genes, which are present in the tested synthetic hexaploid wheats. Therefore, it cannot be said with clarity if the resistance gene suppressors are present in durum or *Ae. tauschii*, due to limited knowledge of the variability, structure,

function and activity of suppressor genes in Triticeae (Rafique et al., 2012).

Significant roles in the expression or suppression of genes for different traits under study have been influenced by the interactions among genomes (A, B and D). To delineate the genomic effects using same durum cultivars with diverse D-genome accessions formed an appropriate germplasm set to study. There were ten such sub-sets i.e., 10 durums with various *Ae. tauschii* accessions. Variable trends in expression are shown in Table 4. The first sub-set is comprised of durum cultivar which is no. 4; 'Altar 84', combining with eight D-genome accessions. Concentrating on the main traits that are thought to be key players in breeding; plant height at maturity, days to physiological maturity and 1000 kernel weight were found to be 73 to 120 cm, 105 to 172 days and 26.1 to 44.1g respectively. All genotypes were found to be susceptible to Karnal bunt except 83 and 89. Genotype 63 and 89 were found to be resistant against yellow rust at both seedling and adult plant stages. Altar 84 was resistant against both Karnal bunt and yellow rust. These observations clearly indicate that the expressivity of the genomes is affected by the accession. This pattern clarifies why suitable SH accessions ought to be chosen in breeding since trait masking among genomes is typically

present. The observation in patterns of variable expression with other durum cultivars and the D-genome accessions proves that accessional diversity can disentangle yield points of SH wheat providing a choice to choose what should be the correct synthetic for improving wheat.

Information in Table 4 enables selection to be made for synthetics: 27, 34, 44, 67 and 76. Genotype 67 exhibits resistance against both Karnal bunt and yellow rust at seedling and adult plant stages, hence proving to be the best line in this set of germplasm. Since, Altar 84 (a durum parent) was resistant to both Karnal bunt and yellow rust giving variable results when crossed with the accessions of *Ae. Tauschii*, it indicates that the accessions were abrogating the resistance in durum cultivars. Consequently, choosing accessions in which corresponding SH indicated resistance against Karnal bunt and yellow rust would be perfect for use in direct crossing as their effect on the bread wheat A- and B-genomes would ideally not be penalizing. It is a clear demonstration of the fact that *Ae. tauschii* has favorable genes.

The above pattern is also well-expressed in other groups where other durum cultivars and *Ae. tauschii* accessions are used (Table 4). Molecular analysis using SSRs of group

1 of SH combinations comprised of Altar 84 and 8 *Ae. tauschii* accessions demonstrated diversity as follows; entries 1, 63, 78 and 83 were assembled together. Entries 13, 18 and 89 were found to be the most diverse. Consolidating the stress data, resistance at both seedling and adult plant was present in entries 63 and 89. SSR based polymorphism showed that the genotype 89 is the most diverse among all with ideal resistance against Karnal bunt and yellow rust. This empowers integration of different components for adding productivity to the breeding programs.

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Table 1: Synthetic entries numbered from combining same durum cultivars and different *Aegilopstauschii* accessions

Group No.	D-genome Synthetic Hexaploids Entry Numbers	Durum Parent No.	Total No. of SH Entries
1	1, 18, 30, 52, 63, 66, 78, 83, 89	D-4	9
2	2, 3, 14, 19, 26, 43, 49, 51, 65	D-8	9
3	4, 11, 20, 35, 37, 38, 42, 47, 64	D-13	9
4	5, 8, 9, 10, 12, 21, 23, 27, 58	D-12	9
5	6, 15, 22, 31, 45, 50, 56, 74, 75	D-23	9
6	7, 32, 48, 53, 68, 71, 72, 76, 86	D-26	9
7	13, 16, 17, 25, 36, 46, 57, 61, 88	D-5	9
8	24, 39, 40, 41, 60, 62, 73, 85, 90	D-1	9
9	28, 29, 33, 34, 55, 59, 69, 79, 82	D-11	9
10	44, 54, 67, 70, 77, 80, 81, 84, 87	D-22	9

*: SH entry numbers are similar to data base maintained in CIMMYT Wide Crosses program in Mexico with durum cultivar pedigree details given in Table 2.

Table 2: Pedigree/Parentage of the germplasm used in this study

S. No.	PEDIGREE
1	ALTAR 84/AE.SQUARROSA (191)
2	68.111/RGB-U//WARD/3/AE.SQUARROSA (328)
3	68.111/RGB-U//WARD/3/ AE.SQUARROSA (321)
4	CETA/AE.SQUARROSA (540)
5	D67.2/P66.270/AE.SQUARROSA (213)
6	GARZA/BOY//AE.SQUARROSA (286)
7	GAN/AE.SQUARROSA (268)
8	D67.2/P66.270//AE.SQUARROSA (220)
9	D67.2/P66.270//AE.SQUARROSA (222)
10	D67.2/P66.270//AE.SQUARROSA (308)
11	CETA/AE.SQUARROSA (1016)
12	D67.2/P66.270//AE.SQUARROSA (221)
13	DVERD_2/AE.SQUARROSA (1027)
14	68.111/RGB-U//WARD/3/AE.SQUARROSA (329)
15	GARZA/ BOY//AE.SQUARROSA (467)
16	DVERD_2/AE.SQUARROSA (221)
17	DVERD_2/AE.SQUARROSA (214)
18	ALTAR 84/AE.SQUARROSA (220)
19	68.111/RGB-U//WARD/3/AE.SQUARROSA (452)
20	CETA/AE.SQUARROSA (327)
21	D67.2/P66.270//AE.SQUARROSA (633)
22	GARZA/BOY//AE.SQUARROSA (276)
23	D67.2/P66.270//AE.SQUARROSA (218)
24	CROC_1/AE.SQUARROSA (205)
25	DVERD_2/AE.SQUARROSA (295)
26	68.111/RGB-U//WARD/3/AE.SQUARROSA (463)
27	D67.2/P66.270//AE.SQUARROSA (257)
28	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (215)

29	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (223)
30	ALTAR 84/AE.SQUARROSA (333)
31	GARZA/ BOY//AE.SQUARROSA (265)
32	GAN/AE.SQUARROSA (182)
33	CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA (273)
34	CPI/GEDIZ/3/GOO// JO/CRA/4/AE.SQUARROSA (296)
35	CETA/AE.SQUARROSA (661)
36	DVERD_2/AE.SQUARROSA (402)
37	CETA/AE.SQUARROSA (174)
38	CETA/AE.SQUARROSA (1024)
39	CROC_1/AE.SQUARROSA (886)
40	CROC_1/AE.SQUARROSA (444)
41	CROC_1/AE.SQUARROSA (518)
42	CETA/AE.SQUARROSA (256)
43	68.111/RGB-U//WARD/3/AE.SQUARROSA (325)
44	DOY 1/AE.SQUARROSA (188)
45	GARZA/BOY//AE.SQUARROSA (307)
46	DVERD_2/AE.SQUARROSA (1022)
47	CETA/AE.SQUARROSA (796)
48	GAN/AE.SQUARROSA (236)
49	68.111/RGB-U//WARD/3/AE.SQUARROSA (326)
50	GARZA/BOY//AE.SQUARROSA (270)
51	68.111/RGB-U//WARD/3/AE.SQUARROSA (316)
52	ALTAR 84/AE.SQUARROSA (332)
53	GAN/AE.SQUARROSA (180)
54	DOY 1/AE.SQUARROSA (255)
55	CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA (453)
56	GARZA/BOY//AE.SQUARROSA (278)
57	DVERD_2/AE.SQUARROSA (333)
58	D67.2/P66.270//AE.SQUARROSA (217)
59	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (193)
60	CROC_1/AE.SQUARROSA (170)
61	DVERD_2/AE.SQUARROSA (1031)
62	CROC_1/AE.SQUARROSA (213)
63	ALTAR 84/AE.SQUARROSA (304)
64	CETA/AE.SQUARROSA (235)
65	68.111/RGB-U//WARD/3/AE.SQUARROSA (322)
66	ALTAR 84/AE.SQUARROSA (507)
67	DOY 1/AE.SQUARROSA (510)
68	GAN/AE.SQUARROSA (163)
69	CPI/GEDIZ/3/GOO// JO69/CRA/4/AE.SQUARROSA (633)
70	DOY 1/AE.SQUARROSA (349)
71	GAN/AE.SQUARROSA (408)
72	GAN/AE.SQUARROSA (201)
73	CROC_1/AE.SQUARROSA (333)
74	GARZA/BOY//AE.SQUARROSA (439)
75	GARZA/BOY//AE.SQUARROSA (350)
76	GAN/AE.SQUARROSA (285)
77	DOY 1/AE.SQUARROSA (333)
78	ALTAR 84/AE.SQUARROSA (219)
79	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)
80	DOY 1/AE.SQUARROSA (1030)
81	DOY 1/AE.SQUARROSA (515)
82	CPI/GEDIZ/3/GOO// JO69/CRA/4/AE.SQUARROSA (637)
83	ALTAR 84/AE.SQUARROSA (502)
84	DOY 1/AE.SQUARROSA (517)
85	CROC_1/AE.SQUARROSA (224)

86	GAN/AE.SQUARROSA (890)
87	DOY 1/AE.SQUARROSA (458)
88	DVERD_2/AE.SQUARROSA (1029)
89	ALTAR 84/AE.SQUARROSA (211)
90	CROC_1/AE.SQUARROSA (879)
	Durum Parents
D-1	CROC_1
D-4	ALTAR84
D-5	DVERD_2
D-8	68.111/RGB-U//WARD
D-11	CPI/GEDIZ/3/GOO//JO/CRA
D-13	CERCETA
D-22	DECOY1
D-23	GARZA/BOY
D-26	GAN

Table 3: Response and observation of host wheat plant against stripe rust

Reaction/response	Observation
No disease	0
Resistant	R
Resistant-Moderately Resistant	RMR
Moderately resistant	MR
Moderately resistant-Moderately Susceptible	MRMS
Moderately Susceptible	MS
Moderately Susceptible-Susceptible	MSS
Susceptible	S

Table 4: Phenological and disease characterization of D-Genome Synthetic Hexaploids and their Durum parents

Entry	Flow	HT	AWN	P. MA	GWT	G/S	SL	KB	Yr (S)	Yr (A)
1	66	73	LB	105	44.1	13	9.5	+	1	10MRR
2	60	105	LB	99	33.5	19	12	+	89	TR
3	66	77	LB	105	38.2	15	10.5	+	89	TR
4	60	90	LB	99	36.6	12	9	-	8	70S
5	66	113	LB	110	32.0	13	8.5	+	67	30MR
6	70	103	LB	109	29.7	19	12	+	8	70S
7	73	91	LB	112	39.2	18	12	+	78	10R
8	67	118	LB	107	32.2	14	10.5	+	8	90S
9	70	121	LB	109	35.4	14	12	-	8	90S
10	65	98	LB	105	31.0	13	9	-	89	70S
11	65	104	LB	105	27.0	13	9.5	-	89	0

12	70	104	LB	109	31.0	14	10	+	78	70S
13	66	96	LB	106	34.0	14	10.1	-	1	10R
14	70	120	LB	109	52.9	15	10.3	-	1	0
15	149	108	LB	179	28.8	17	9	+	56	10MR
16	71	102	LB	110	33.0	16	11.5	-	67	70S
17	70	110	LB	109	49.9	14	10	-	12	5R
18	70	103	LB	109	27.9	12	8	+	67	70S
19	70	86	LB	109	32.0	16	12	+	1	0
20	70	102	LB	108	49.8	15	11.5	+	1	0
21	70	115	LB	109	24.0	13	9	+	78	90S
22	70	90	LB	110	25.0	13	10	+	9	TR
23	75	90	LB	113	26.2	14	8.5	+	8	10R
24	74	88	LB	112	27.0	15	13	+	67	30MSS
25	69	90	LB	107	23.7	13	10.5	+	78	30MSS
26	73	90	LB	112	28.5	14	10.5	+	78	30MSS
27	141	117	LB	168	33.5	8	11	+	89	70S
28	66	73	LB	105	25.4	21	14	+	78	90S
29	92	103	LB	105	41.0	14	9.5	-	78	30MSS
30	70	82	LB	108	37.5	16	10.5	+	78	70S
31	73	75	LB	112	26.0	13	11	-	8	TR
32	62	100	LB	101	32.5	12	7	-	8	90S
33	59	100	LB	97	34.6	14	13	+	8	0
34	60	110	LB	100	35.5	13	13	+	0	0
35	62	110	LB	101	27.3	12	9.5	+	8	90S
36	65	90	LB	106	35.2	13	10	+	56	30MSS
37	146	90	LB	178	25.0	22	9	+	0	10MR
38	126	135	LB	175	30.0	20	13	+	1	10MRR
39	77	93	LB	113	37.0	13	10	-	8	30MSS
40	77	90	LB	113	35.2	13	10.5	-	7	40MS
41	68	83	LB	106	55.7	14	10	+	67	40MS
42	62	80	LB	101	46.2	12	8.5	-	0	10R
43	72	99	LB	109	49.8	14	8.5	+	78	10MS
44	65	109	LB	105	59.2	15	10	+	56	30MSS
45	64	85	LB	103	30.5	13	9	-	9	30MS
46	70	97	LB	106	44.0	14	9.5	+	9	70S
47	79	69	LB	114	31.0	14	10	-	78	90S
48	72	97	LB	109	34.6	15	11	+	78	90S
49	70	89	LB	109	35.7	12	7.5	-	67	90S
50	72	76	LB	109	39.2	13	11	+	89	30MSS
51	68	102	LB	106	45.0	12	9.5	+	67	70S
53	68	100	LB	106	37.2	14	9.5	-	67	10R
54	70	100	LB	110	37.0	12	8	+	67	90S
55	140	153	DB	182	40.0	13	12	+	78	10R
56	72	80	LB	111	43.2	15	10	+	9	90S
57	141	104	LB	177	40.0	15	12	+	56	90S
58	71	75	LB	110	31.0	15	13	+	78	10R
59	70	113	LB	108	32.5	19	12	-	34	30MRMS
60	65	97	LB	105	53.4	18	10	+	78	30MRMS
61	129	113	LB	177	40.0	19	10	+	89	90S
62	72	96	LB	112	39.8	20	11	+	1	10R
63	79	62	LB	116	37.9	12	7	+	1	10R
64	73	100	LB	109	35.5	14	10	+	89	30MSS
65	77	109	LB	115	32.2	15	12	+	78	90S
66	75	120	LB	113	35.2	16	12	-	78	90S
67	146	120	LB	172	41.0	30	13	-	0	0
68	68	113	LB	106	41.0	15	11	-	89	70S
69	63	104	LB	101	40.0	12	9	+	78	70S

70	149	100	LB	181	40.0	14	12	+	78	90S
71	66	100	LB	106	36.6	15	13	+	78	10R
72	140	95	LB	121	35.0	15	10	+	0	10R
73	142	72	LB	181	35.0	20	10	+	78	10R
74	144	128	LB	176	30.0	2	11	+	0	10R
75	150	97	LB	180	16.0	8	10	+	67	70S
76	141	102	LB	161	33.0	37	13	+	67	70S
77	133	114	LB	178	37.0	26	12	+	78	90S
78	136	99	LB	172	31.0	7	11	+	78	70S
79	74	119	LB	112	28.7	14	10	+	78	70S
80	139	110	LB	182	30.0	15	10	+	0	10MR
81	78	100	LB	116	35.9	15	11	-	0	0
82	72	109	LB	109	36.6	14	10	-	78	0
83	73	103	LB	110	26.1	12	9	-	45	10R
84	143	119	LB	175	40.0	12	13	-	45	0
85	74	112	LB	112	52.3	12	10	+	9	70MSS
86	67	86	LB	105	41.0	13	10	-	89	70MSS
87	76	72	LB	113	31.1	12	10	-	0	0
88	144	184	LB	184	30.0	13	10	+	89	90S
89	75	117	LB	112	27.3	14	10	-	0	10R
90	74	103	LB	112	32.1	16	13	-	78	90S
D-1	87	86	LB	101	45	45	9	-	0	10R
D-4	89	78	LB	108	33.0	26	6	-	0	TR
D-5	87	76	LB	112	37.6	18	6	-	0	10R
D-8	95	103	LB	108	32.2	30	9	-	0	0
D-11	85	102	LB	99	46.0	28	6	-	0	0
D-12	98	96	LB	110	37.0	41	10	-	0	5R
D-13	88	102	LB	100	41.1	28	7	-	0	10R
D-22	89	103	LB	115	34.8	48	9	-	0	0
D-23	100	68	LB	115	12.5	9	8	-	23	20MRMS
D-26	82	104	LB	98	35.5	41	9	-	0	5R

Abbreviations in the first row are as follows: **FLOW:** Days to Flowering; **HT:** Plant Height at Maturity (cm); **AWN:** Awn color (LB = light brown, AW = Amery white, Y = yellow, DB = dark brown); **P. MA:** Days to Physiological Maturity; **GWT:** 1000-grain weight (g); **G/S:** No. of grains/spike; **SL:** Spike length (cm); **KB:** Karnal bunt; - = immune, + = susceptible; **Yr (S):** Yellow rust screening at seedling stage; **Yr (A):** Yellow rust screening at adult plant stage where R = Resistant, TR = Trace resistant, MR = Moderately resistant, MS = Moderately susceptible, M = Overlapping of MR-MS, MSS = Moderately susceptible to susceptible, S = Susceptible, TS = Trace susceptible;