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# NUST JOURNAL OF NATURAL SCIENCES

**Volume 4** **June – Dec, 2018** **Issue: 1**

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NUST Journal of Natural Sciences, Vol. 4, Issue 1, 2018, pp.1-7

# JUNK FOOD ADDICTION AMONG MEDICAL STUDENTS IN RAWALPINDI- A KAP STUDY

**Nosheen Zaidi [1] and Najma Javed [2]**

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## Abstract

Use of high calorie junk foods has increased dramatically in the recent past, leading to many social and health problems. Pakistan is facing the same situation. It is therefore, a strong need to develop public health strategies targeting to reduce intake of junk food. Study was conducted over period of 9 months (from January to August 2013) among students of Foundation University Medical College. It was a cross sectional study. A total of 445 students were selected. Response rate was 85.5%. Male to female ratio was 1: 1.7. The percentage of girls was more because there are more female students as compared to male students in every class. Age ranged from 17-29 years with mean of 21 years. The percentage of Boarders to Non-boarders was 41% (182) and 59% (262) respectively.38% (169) of students take junk food daily while 39% (174) on weekly basis. Majority 169 (38%) students prefer home deliveries; while 111 students (25%) prefer to dine in. However 37% like to take food with them. Knowledge about non communicable diseases was quite good however the term 'junk food' was not accurately defined by all students. Around 200 (23%) of students spend Rs. 1000 for buying junk food weekly, while 142 (32%) spend more than 1000 rupees.191 (43%) of the students have experienced an increase in junk food frequency since they are living in hostel, 174 (39%) have no idea or didn't notice any change whereas 80 (8%) had no change in their dietary habit despite of being living in hostel. On consuming junk food, 209 (47%) of the students have a pleasant feeling while majority 249 (56%) have no elevation of mood following junk food. Most (231, 52%) of the students said that they are not addicted to junk food. Junk food is quickly replacing the traditional home made and healthy foods. Health Education sessions regarding risks and hazards associated with junk food consumption must be done regularly in schools/ colleges to prevent junk food epidemic especially among youth.

**Keywords:** Social norms, Health messages, Junk food

## 1. INTRODUCTION

Eating is the normal physiological process. Junk food is an informal term applied to certain foods that perceived to have little or no nutritional value but there ingredients are considered unhealthy for consumption [1, 2] but taste good.

Junk food is usually ready to eat foods rich in saturated fats, salt or sugar containing little or no fruit, vegetables, or fiber. In addition to this junk food is easy to carry, purchase and consume[3]. Junk food includes sugar, donuts, cookies, chips, candy bars, fried foods, muffins, burgers, bread, milkshakes, coca cola, pizza and canned foods etc.[4].The junk food is psychologically addictive. Initially they are given to the children as "reward" for being good and this early conditioning persists long into adult hood [5]. This leads to indulgence in over eating; causing changes in the brain resulting in tough behavior with compulsion to eat and thus junk food addiction [6].The term addiction means psychological dependence, which is a mental or cognitive problem, not just an illness.

Addiction has three stages. The first stage is Bingeing that is defined as escalation of intake with a high proportion of intake at one time followed by signs of withdrawal, which become apparent when the abused substance is no longer available. The final stage of addiction is craving, resulting from enhanced motivation[7].Junk food alters brain activity similar to drugs like cocaine or heroin whereas high calorie foods may cause compulsive eating and obesity[8].

The foremost side effect of Junk food addiction is obesity. Obesity increases the risk of many metabolic and chronic diseases and is an independent risk factor for death from coronary heart disease [9].

This study was done to know the reasons of using junk food despite its hazardous effects on health including related financial burden and to create health awareness among students.

## 2. OBJECTIVES

- To determine the reasons of using junk food and associated financial burden among students of Foundation University Medical College
- To create awareness among students.

## 3. DURATION AND TYPE OF STUDY

Study was conducted over period of 9 months (from January to August 2013) among students of Foundation University Medical College. It was a cross sectional study.

Inclusion Criteria: Students of Foundation Medical University willing to participate in the study

Exclusion Criteria: House Officers working in attached hospital

## 4. METHODS

**Subjects:** The study cohort of medical students with age of 17-29 years was selected. Participants were students of Foundation University Medical College, Rawalpindi. A total of 470 students were approached and objective of the study was shared. Out of 470, 445 agreed to participate in the study and gave written consent. All enrolled students were briefed about the study. A close ended questionnaire was used to collect data. There was a pretesting of the questionnaire in the field. Some editing was done. Questions were designed under three heads. Section one had information on demography while others three captured data on knowledge, attitude and practices regarding junk food respectively.

Trained students handed over questionnaires to the participants and facilitated them in case of quarry. It took about 30 minutes to fill questionnaire by the participants. Data was entered and after cleaning of the data, analysis was done using SPSS (Statistical Package for Social Scientists) software version 15.

The study had been approved by the Ethical Committee Members of the institution.

## 5. RESULTS

A total of 445 students were selected. Response rate was 85.5%. Male to female ratio was 1: 1.7. The percentage of girls was more because there are more female students as compared to male students in every class. Age ranged from 17-29 years with mean of 21 years. The percentage of Boarders to Non-boarders was 41% (182) and 59% (262) respectively.

There were 105 students from first year, 98 from second year, 97 from third year, 85 from fourth year, and 60 students were from final year. A total of 445 students filled the questionnaire in the entire college.

## 6. KNOWLEDGE

Being medical students, majority of the students had knowledge of diseases like obesity, diabetes, hypertension and food poisoning however knowledge regarding depression and libido was less. (Table-1)

| Hazards | Frequency | Percent | Valued percent | Cumulative percent | T test significance |
|---|---|---|---|---|---|
| Obesity : | | | | | |
| **Yes** | 414 | 93.0 | 93.0 | 93.0 | 1.09 |
| **No** | 31 | 7.0 | 7.0 | 100.0 | |
| **Total** | 445 | 100.0 | 100.0 | | |
| Diabetes Mellitus : | | | | | |
| **Yes** | 313 | 70.3 | 70.3 | 70.3 | 1.34 |
| **No** | 132 | 29.7 | 29.7 | 100.0 | |
| **Total** | 445 | 100.0 | 100.0 | | |
| Hypertension : | | | | | |
| **Yes** | 332 | 74.6 | 74.6 | 74.6 | 1.29 |
| **No** | 113 | 25.4 | 25.4 | 100.0 | |
| **Total** | 445 | 100.0 | 100.0 | | |
| Depression : | | | | | |
| **Yes** | 160 | 36.0 | 36.0 | 36.0 | 1.69 |
| **No** | 285 | 64.0 | 64.0 | 100.0 | |
| **Total** | 445 | 100.0 | 100.0 | | |
| Food poisoning : | | | | | |
| **Yes** | 370 | 83.1 | 83.1 | 83.1 | 1.20 |
| **No** | 75 | 16.9 | 16.9 | 100.0 | |
| **Total** | 445 | 100.0 | 100.0 | | |
| Increased libido: | | | | | |
| **Yes** | 139 | 31.2 | 31.2 | 31.2 | 1.73 |
| **No** | 306 | 68.8 | 68.8 | 100.0 | |
| **Total** | 445 | 100.0 | 100.0 | | |

Table 1: Frequency and percentages of knowledge of different junk food related illness among medical students of Fauji Foundation University Medical College, Rawalpindi.

Further, the term ‘junk food’ was not accurately defined by all students, a product taken as a junk food by one group of students was not a junk food for others sowing lack of knowledge about what exactly junk food is.

All students receive pocket money from their parents on monthly or weekly basis and spend as per their needs (Figure 1).

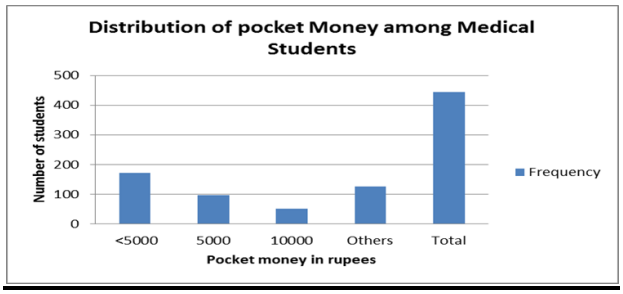


**Figure 1: Frequency of pocket money amongst students**

## 7. PRACTICES

Regarding consumption of junk food, 38% (169) of students take it daily while 39% (174) on weekly basis. This indicates an overall high level of inclination towards junk food intake. When it comes to boarders, majority 169 (38%) students prefer home deliveries; while a percentage of 111 (25%) prefer to dine in. However 37% like to take food with them.

Around 200 (23%) of students spend Rs.1000 for buying junk food weekly, while 142 (32%) spend more than 1000 rupees, 103 had no idea. More than 50% of the students admitted having a craving for the junk food (Figure 2).

When asked about risk factors for indulgence into junk food addiction, 191 (43%) of the students have experienced an increase in junk food frequency since they are living in hostel, 174 (39%) have no idea or didn't notice any change whereas 80 (8%) had no change in their dietary habit despite of being living in hostel.

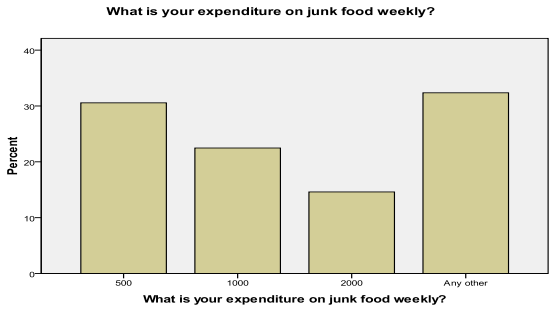


**Figure 2: Distribution of amount spent on junk food weekly**

## 8. ATTITUDE

On consuming junk food, 209 (47%) of the students have a pleasant feeling followed by 11(25%) and 27 (5.8%) who feel satisfied and depressed respectively and 22% had no effect. When students were asked about the role of junk food in elevating mood, majority 249 (56%) responded in No. Majority (231, 52%) of the students said that they are not addicted to junk food.

## 9. DISCUSSION

The results of this study indicated enhanced practices of junk food intake in undergraduate medical students of Foundation University, Rawalpindi. It was found that junk food intake is more in boarders as compared to the day scholars.

The study showed that students have a strong psychological and social inclination towards junk food, well aware of its hazards, but still there is a high prevalence of junk food intake. Thamarai et al in their study revealed the same findings. Based on their study, 93.3% of medical students were aware of the definition and 61.7 % were aware of the composition of the junk foods. 46.3% of students consumed 1-5 cans of soda drinks per week, 29.7% students reported the frequency of eating pizza per week may vary[10].

Current study showed that around 200 (23%) of students spend Rs.1000 for buying junk food weekly, while 142 (32%) spend more than 1000 rupees, 103 had no idea. Same are the findings in study[10] with results that 39.7 % of students spending less than Rs.200 per month. At the same time, 13 % students have mentioned the amount may exceed Rs.1000 and above per month.

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NUST Journal of Natural Sciences, Vol. 4, Issue 1, 2018, pp.8-19

# DIFFRACTION BY CASCADED THICK HALF PLANES COMPOSED OF PEMC METAMATERIAL

**Naeem ul Haq[1], A. B. Mann[2] and Saeed Ahmed[3]**

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## Abstract

An analytic solution of plane wave diffraction by three parallel thick half planes composed of PEMC metamaterial is developed. Duality transformation introduced by Lindell and Sihvola is applied to transform the field produced by three semi-infinite, parallel, thick, PEC half planes to the case of three semi-infinite, parallel, thick half planes in PEMC medium. It is observed that PEC medium is the limiting case of PEMC medium. Numerical results are also produced and discussed for the effects of thickness and admittance parameters on the amplitude of the diffracted field. Numerical results are found to be in good agreement with the available numerical results on PEMC metamaterial.

**Keywords:** Diffraction, Duality transformation, PEMC medium, Wiener-Hopf technique, cascaded thick half planes.

## 1. INTRODUCTION

Diffraction of electromagnetic waves by canonical geometries such as half planes, strips, slits waveguides etc has been the subject of numerous past investigations. Lee [1,2] studied and analyzed in detail the diffraction problems related to open ended staggered plate waveguide, open ended parallel plate waveguide, bifurcated waveguide and by array of parallel plates. Usually in dealing with problems comprising of parallel plates it become cumbersome to decouple the arising Wiener-Hopf (WH) equation and thus one has to work in the domain of matrix WH equations. A comprehensive procedure for tackling the matrix version of WH equations is not yet available because it is not normally easy to split the matrix into factors being regular in appropriate half planes and these factors should have algebraic growth at infinity. The non

commutativity of the factor matrices and the requirement of the radiation conditions also present further problems. Nevertheless the development and improvement of this technique is progressing steadily. For example the Wiener-Hopf Hilbert method introduced by Hurd [3] Rawlins [4] and Rawlins and Williams [5] is a powerful tool in the case when kernel matrix has only branch point singularities, while the Daniele-Kharapkov method proposed by Daniele [6] Kharapkov [7] and Jones [8] is effective for the class of matrices having only pole singularities and branch-cut singularities.

Diffraction problems related to series of parallel plates have a long and rich history. Jones [9] attempted the plane wave diffraction problem by three parallel soft semi-infinite plates which required the factorization of 3x3 matrix arose in the related WH equations. Jones tackled the problem [9] by reducing it to two WH equations which then required the factorization of a 2x2 kernel matrix instead of a 3x3 kernel matrix. Later Ibrahams [10] reconsidered the problem [9] and showed that product decomposition of involved 2x2 kernel matrix would be much simpler if Jones [9] would not have considered the system of WH equations as a fully coupled system. Asghar etal. [11] extended Jones analysis [9] from plane wave diffraction to the cases of line source and point source diffraction in still air as well as when the medium is convective. Alkumru [12] also extended Jones analysis [9] to the case of thick and impedant plates instead of three semi-infinite, parallel, plates being thin and perfectly conducting.

What has not been done is the consideration of PEMC medium for the work reported in [12]. The aim of this paper is to study the plane wave diffraction by three, semi-infinite cascaded thick half planes in a PEMC metamaterial by using the duality transformation introduced by Lindell and Sihvola [13]. Plane wave diffraction from three, semi-infinite cascaded thick half planes will help understanding the diffraction process in PEMC medium and will go a step further to complete the discussion with respect to parallel thick half planes. Since our method of solution also consists of integral transforms, the WH technique and the method of steepest descent so the notation to be used principally is that of reference [12]. In order to avoid repetition we shall omit details of calculations and shall only report necessary computational steps and major calculations essential for understanding the effects of PEMC metamaterial. Some graphs showing the effects of thickness parameter $b$ and admittance parameter $M$ are plotted and discussed. A good agreement with the

existing literature on PEMC metamaterial is observed.

The work presented in this paper is not only mathematically important but may also have engineering applications. As polarization is always an important factor to consider in the field of transmission and reception of signals which may carry audio, video or any type of data for most of the wireless communications. Multipath effects are the common reception problems in dynamic complex electromagnetic environments. In order to reduce signal fading caused by multipath effects, diversity techniques are therefore applied to the antenna system at the receiving site [14]. Different kinds of polarization techniques have been analyzed and different pros and cons have been discussed in [15]. Hence in case of PEMC metamaterial based waveguides we can control the polarization by choosing the suitable value of parameter $M$ and hence we can control the scattering phenomenon.

## 1. FORMULATION OF THE PROBLEM

The geometry of the problem is depicted in Fig. 1.

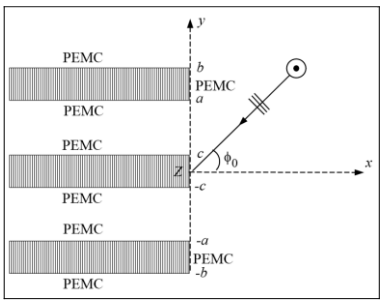


Fig. 1: Geometry of the Problem.

In semi-infinite waveguide region, scattered field is expanded into normal modes and Fourier integral transform is used elsewhere. Alkumru [12] used image bisection principle to split up the problem into even and odd excitation modes which result into a modified WH equation of second kind whose solution contains an infinite many constants satisfying an infinite number of algebraic equations for which [12] has to resort on numerical solution of this system of infinite many algebraic equations. A time factor of the form $e^{-i\omega t}$ is assumed and suppressed throughout the analysis. Let the three thick, semi-infinite, cascaded and impedance plates are located at the positions $S_1 = \{(x,y,z);\ x \in (-\infty,0), y\in(a,b); z\in(-\infty,\infty)\}$, $S_2 = \{(x,y,z);\ x \in (-\infty,0), y\in(-c,c); z\in(-\infty,\infty)\}$, and

$$S_3 = \{(x,y,z); x \in (-\infty,0), y \in (-b,-a); z \in (-\infty,\infty)\}.$$

The horizontal and vertical walls of the geometry depicted in Fig. 1 have same impedance $Z = \eta Z_0$, where $Z_0$ is the characteristic impedance of the free space. Diffraction of an $E_z$-polarized plane wave defined as

$$E_z^i = u_i(x,y) = e^{-ik(x\cos\phi_0 + y\sin\phi_0)}, \quad (1)$$

where $k = \frac{2\pi}{\lambda}$, is the free space wave number and $\phi_0$ is the angle of incidence is considered by three parallel, thick, semi infinite half planes for which the even and odd excitation modes will be dealt separately in sequel. Detailed formulation can also be seen from [12]. The field reflected from the plane $y = b,$ having impedance $\eta$ can be written as follows:

$$u_1^r(x,y) = \frac{\eta\sin\phi_0 - 1}{\eta\sin\phi_0 + 1} e^{-ik[x\cos\phi_0 - (y-2b)\sin\phi_0]}. \quad (2)$$

**Analytic solution of the problem for even excitation mode**

Let us fiest consider the even excitation mode. Since the total field is symmetric at the plane $y = 0,$ the normal derivative of the total electric field must vanish at $x \in (-\infty, \infty),\ y = 0.$ The Helmholtz equation satisfied by the total field $u_T^e(x,y)$ is

$$\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + k^2\right) u_T^e(x,y) = 0, \quad (3)$$

where the superscripts $e$ and $o$ denote the even and odd excitation modes. The supporting boundary conditions on the semi-infinite cascaded half planes located at $-\infty < x < 0,\ y = b,\ y = a$ and $y = c$ are

$$\left[1 + \frac{\eta}{ik}\frac{\partial}{\partial y}\right] u_T^e(x,b) = 0, \quad (4)$$

$$\left[1 - \frac{\eta}{ik}\frac{\partial}{\partial y}\right] u_T^e(x,a) = 0, \quad (5)$$

and

$$\left[1 + \frac{\eta}{ik}\frac{\partial}{\partial y}\right] u_T^e(x,c) = 0. \quad (6)$$

The boundary condition on the plane $0 < x < \infty,\ y = 0$ is

$$\frac{\partial u_T^e(x,0) = 0}{\partial y}. \quad (7)$$

The boundary condition on the wall $x = 0,$ $y \in \{(0,c) \cup (a,b)\}$ is

$$\left[1 + \frac{\eta}{ik}\frac{\partial}{\partial x}\right] u_T^e(0,y) = 0. \quad (8)$$

In addition to the above mentioned boundary conditions the following continuity relations in the regions $0 < x < \infty,\ y = b$ and $x = 0,$ $c < y < a$ should also be considered to complete the solution of the boundary value problem i.e.,

$$u_T^e(x,b^+) = u_T^e(x,b^-) \quad x > 0, \quad (9)$$

$$\frac{u_T^e(x,b^+)}{\partial y} = \frac{u_T^e(x,b^-)}{\partial y} \quad x > 0, \quad (10)$$

and

$$u_T^e(0,y^+) = u_T^e(0,y^-) \quad c < y < a, \quad (11)$$

$$\frac{\partial u_T^e(0,y^+)}{\partial x} = \frac{\partial u_T^e(0,y^+)}{\partial x} \quad c < y < a, \text{ (12)}$$

where the superscripts $+$ and $-$ denote that the corresponding limits are attained from above or below side of the respective half plane. For analysis of even mode excitation problem it is convenient to express the total field $u_T^e(x,y)$ in different regions as

$$u_T^e(x,y) = \begin{cases} u_i^e(x,y) + u_1^r(x,y) + u_{1(diff)}^e(x,y), & \text{if } -\infty < x < \infty,\ y > b; \\ u_2^e(x,y), & \text{if } c < y < a,\ x < 0; \\ u_3^e(x,y), & \text{if } 0 < y < b,\ x > 0, \end{cases}$$

(13)

where $u_j\,(j=1,2,3)$ are the scattered fields in the different regions. By substituting Eqs.(1), (2) and (13) in Eqs. (3-12) we shall arrive at:

$$\left[\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + k^2\right] u_{1(diff)}^e(x,y) = 0 \quad y > b, \text{ (14)}$$

subject to the boundary conditions

$$\left[1 + \frac{\eta}{ik}\frac{\partial}{\partial y}\right] u_{1(diff)}^e(x,b) = 0 \quad x < 0, \quad (15)$$

$$\left[1 - \frac{\eta}{ik}\frac{\partial}{\partial y}\right] u_2^e(x,a) = 0 \quad x < 0, \qquad (16)$$

$$\left[1 + \frac{\eta}{ik}\frac{\partial}{\partial y}\right] u_2^e(x,c) = 0 \quad x < 0, \qquad (17)$$

$$\frac{\partial u_3^e(x,0)}{\partial y} = 0 \quad x > 0, \qquad (18)$$

$$\left[1 + \frac{\eta}{ik}\frac{\partial}{\partial x}\right] u_3^e(0,y) = 0 \quad x > 0,\ y \in \{(0,c) \cup (a,b)\}. \text{ (19)}$$

The continuity conditions shall take the form

$$u_{1(diff)}^e(x,b^+) - u_3^e(x,b^-) = -\frac{2\eta \sin\phi_0}{\eta \sin\phi_0 + 1} e^{-ik[x\cos\phi_0 + b\sin\phi_0]} \quad x > 0,\ y = b,$$

(20)

$$\frac{\partial}{\partial y} u_{1(diff)}^e(x,b^+) - \frac{\partial}{\partial y} u_3^e(x,b^+) = \frac{2ik\sin\phi_0}{\eta \sin\phi_0 + 1} e^{-ik[x\cos\phi_0 + b\sin\phi_0]} \quad x > 0,\ y = b,$$

(21)

$$u_2^e(0,y^+) = u_3^e(0,y^-)\ x = 0,\ c < y < a, \text{ (22)}$$

$$\frac{\partial}{\partial x} u_2^e(0,y^+) = \frac{\partial}{\partial x} u_3^e(0,y^-)\ x = 0,\ c < y < a. \text{ (23)}$$

Since $u_{1(diff)}^e(x,y)$ satisfies the Helmholtz equation in the range $x \in (-\infty, \infty)$, its Fourier transform with respect to $x$ gives

$$\left[\frac{d^2}{dy^2} + (k^2 - \alpha^2)\right] F^e(\alpha,y) = 0, \qquad (24)$$

with

$$F^e(\alpha,y) = F_-^e(\alpha,y) + F_+^e(\alpha,y), \qquad (25)$$

where $F_+(\alpha,y)$ and $F_-(\alpha,y)$ are regular funtion of $\alpha$ in half planes, $\mathrm{Im}(\alpha) > \mathrm{Im}(k\cos\phi_0)$ and $\mathrm{Im}(\alpha) < \mathrm{Im}(k)$ respectively. The general solution of the Eq. (24) satisfying the radiation condition for $y \to \infty$ is read as

$$F_+^e(\alpha,y) + F_-^e(\alpha,y) = A^e(\alpha) e^{iK(\alpha)(y-b)}, \text{ (26)}$$

where $K(\alpha)=\sqrt{k^2-\alpha^2}$ is the square root function defind in the complex $\alpha$ plane cut along $\alpha=k$ to $\alpha=k+i\infty$ and $\alpha=-k$ to $\alpha=-k-i\infty$ such that $K(0)=k$. We observe that the mathematical problem in the transformed complex plane $\alpha$ is same as that of Alkumru [12]. Therefore omitting the details of calculations and following the standard WH method [16] and the procedure used in [17] i.e., letting $\eta=0,$ we can obtain the results for three perfectly conducting (PEC), semi-infinte, parallel thick half planes in case of even excitation case as:

$$ik\frac{\chi_+(\alpha)}{N_+^e(\alpha)}R_+^e(\alpha)=-2k\sin\phi_0\frac{e^{-ikb\sin\phi_0}}{\alpha-\alpha_0}\frac{N_-^e(\alpha_0)}{\chi_-(\alpha_0)}-\sum_{m=1}^{\infty}\frac{K_m^e\sin(K_m^e b)}{\alpha+\alpha_m^e}\frac{N_+^e(\alpha_m^e)}{\chi_+(\alpha_m^e)}\frac{\phi_m^e}{2\alpha_m^e},\quad(27)$$

and in case of odd excitations as

$$ik\frac{\chi_+(\alpha)}{N_+^o(\alpha)}R_+^o(\alpha)=2k\sin\phi_0\frac{e^{-ikb\sin\phi_0}}{\alpha-\alpha_0}\frac{N_-^o(\alpha_0)}{\chi_-(0,\alpha_0)}+\sum_{m=1}^{\infty}\frac{K_m^e\cos(K_m^e b)}{\alpha+\alpha_m^o}\frac{N_+^o(\alpha_m^o)}{\chi_+(0,\alpha_m^o)}\frac{\phi_m^o}{2\alpha_m^o},\quad(28)$$

where $\alpha_0=k\cos\phi_0$, $\alpha=k\cos\phi$, $\alpha_m^e=\frac{2m\pi}{b}$,

$\alpha_m^o=\frac{(2m+1)\pi}{b}$, $K_m^e=\sqrt{k^2-(\alpha_m^e)^2}$,

$K_m^o=\sqrt{k^2-(\alpha_m^o)^2}$,

$\phi_m^e=f_e^m-\alpha_m^e g_m^e=\frac{2K_m^e R_+^e(\alpha_m)}{b\sin(K_m^e b)}$, and

$\phi_m^o=f_m^o-\alpha_m^o g_m^o=\frac{2K_m^o R_+^o(\alpha_m)}{b\cos(K_m^o b)}$.

Further the value of $\chi_+(\alpha),\ \chi_-(\alpha),\ N_+^e(\alpha)$ and $N_-^e(\alpha)$ at $\eta=0$ can be obtained from [12].

## 2. ANALYSIS OF THE FIELDS

The diffracted field in the region $y>b$ for the both even and odd excitations can be obtained by taking the inverse Fourier transform of $F^e(\alpha,y)$ and $F^o(\alpha,y)$ as:

$$u_{1(diff)}^e(x,y)=\frac{1}{4\pi}\int_{?}A^e(\alpha)e^{iK(\alpha)(y-b)-i\alpha x}d\alpha\quad(29)$$

and

$$u_{1(diff)}^o(x,y)=\frac{1}{4\pi}\int_{?}A^o(\alpha)e^{iK(\alpha)(y-b)-i\alpha x}d\alpha.\quad(30)$$

where ? is a straight line parallel to real axis lying in the strip $\mathrm{Im}(k\cos\phi_0)<\mathrm{Im}(\alpha)<\mathrm{Im}(k)$. To determine the far field behavior of the diffracted fields $u_{1(diff)}^{e,o}(x,y)$, we need to compute the expressions of $A^{e,o}(\alpha)$ by using Eqs. (27, 28) by bearing in mind that

$$R_+^{e,o}(\alpha)=\frac{K(\alpha)}{k\chi(\alpha)}A^{e,o}(\alpha).\quad(31)$$

Introduce the substitutions

$$x=\rho\cos\phi,\ y-b=\rho\sin\phi,\quad(32)$$

In Equations (29, 30) omitting the details of calculations, the asymptotic evaluation of the integrals in Eqs. (29, 30) by using the method of steepest decent gives the diffracted field valid for $y>b,$ for three parallel, semi-infinite PEC thick half planes

by taking the surface impedances $\eta$ equals to zero in [12] as

$$u_{1d}(\rho,\phi)=\frac{u^{(e)}_{1(diff)}(\rho,\phi)+u^{(o)}_{1(diff)}(\rho,\phi)}{2}, \quad (33)$$

with

$$u^{(e)}_{1(diff)}(\rho,\phi)\sim\left\{u_0D^e(\phi,\phi_0)+\frac{\sin\phi e^{i\frac{\pi}{4}}}{\sqrt{2\pi}}\frac{N^e_-(\alpha)}{\chi_-(\alpha)}\times\sum_{m=1}^{\infty}\frac{K^e_m\sin(K^e_m b)}{2\alpha^e_m}\frac{N^e_+(\alpha^e_m)}{\chi_+(\alpha^e_m)}\frac{\phi^e_m}{\alpha^e_m-\alpha}\right\}\frac{e^{ik\rho}}{\sqrt{k\rho}} \quad (34)$$

and

$$u^{(o)}_{1(diff)}(\rho,\phi)\sim\left\{u_0D^o(\phi,\phi_0)+\frac{\sin\phi e^{i\frac{3\pi}{4}}}{\sqrt{2\pi}}\frac{N^o_-(\alpha)}{\chi_-(\alpha)}\times\sum_{m=1}^{\infty}\frac{K^o_m\cos(K^o_m b)}{2\alpha^o_m}\frac{N^o_+(\alpha^o_m)}{\chi_+(\alpha^o_m)}\frac{\phi^o_m}{\alpha^o_m-\alpha}\right\}\frac{e^{ik\rho}}{\sqrt{k\rho}}, \quad (35)$$

where $u_0=e^{-ikb\sin\phi_0}$,

$$D^e(\phi,\phi_0)=e^{-i\frac{3\pi}{4}}\sqrt{\frac{2}{\pi}}\frac{\sin\phi\sin\phi_0}{\cos\phi+\cos\phi_0}\frac{N^e_-(\alpha)N^e_-(\alpha_0)}{\chi_-(\alpha)\chi_-(\alpha_0)} \quad (36)$$

and

$$D^o(\phi,\phi_0)=e^{i\frac{3\pi}{4}}\sqrt{\frac{2}{\pi}}\frac{\sin\phi\sin\phi_0}{\cos\phi+\cos\phi_0}\frac{N^o_-(\alpha)N^o_-(\alpha_0)}{\chi_-(\alpha)\chi_-(\alpha_0)}. \quad (37)$$

We have taken $u_{1d}(\rho,\phi)=E^s$ so the amplitude of diffracted field can be written as

$$|E^s|=20\log_{10}(u_{1d}(\rho,\phi)\sqrt{k\rho}). \quad (38)$$

The diffracted field from the three parallel PEC thick half planes can be transformed to three parallel thick PEMC half planes by the concept of PEMC introduced by Lindell and Sihvola [13, 18]. It is a generalization of both PEC and PMC media. PEMC medium is defined by a scalar parameter *M,* known as an admittance of the surface. The PEC canonical problems have been transformed to PEMC structures by many researchers[19,20,21,22,23,24,25,26,27,28,29,30,31] through the duality transformations. Here we present an analytic scattering theory for three parallel PEMC thick half planes which is a generalization of the classical diffraction theory. The diffracted fields by PEMC interface and isotropic medium can be obtained from the known problem of PEC objects. In general, the diffracted (scattered) field has a cross-polarized component which gives nonreciprocal effect. This means that PEC and PMC are the limiting cases with no cross-polarized component. Because the PEMC medium does not allow electromagnetic energy to enter and an interface of such a medium behaves as an ideal boundary to the electromagnetic field. It has also been observed theoretically that a PEMC material acts as a perfect reflector of electromagnetic waves but differs from the PEC and the PMC. Our interest is to transform scattered field from three parallel PEC thick half planes to three parallel PEMC thick half planes of the concerned problem by using the duality

transformations [13].

The transformed fields can be obtained from the field diffracted by three parallel PEC thick half planes with the help of duality transformations as

$$\begin{pmatrix} E_d^s \\ H_d^s \end{pmatrix} = \begin{pmatrix} M\eta_0 & \eta_0 \\ \frac{-1}{\eta_0} & M\eta_0 \end{pmatrix} \begin{pmatrix} E^s \\ H^s \end{pmatrix}, \quad (39)$$

With $E_d^s = M\eta_0 E^s + \eta_0 H^s$ (40)

and

$$H_d^s = -\frac{1}{\eta_0} E^s + M\eta_0 H^s, \quad (41)$$

where $E^s$ and $H^s$ are the diffracted (scatterred) fields while $E_d^s$ and $H_d^s$ are the transformed fields from the three parallel PEC thick half planes and these fields satisfy the condition

$$\eta_o H_d^s = -u_z \times E_d^s. \quad (42)$$

Further the following transformation gives scattered field from the three parallel PEMC thick half planes is

$$\begin{pmatrix} E \\ H \end{pmatrix} = \frac{1}{(M\eta_0)^2 + 1} \begin{pmatrix} M\eta_0 & -\eta_0 \\ \frac{1}{\eta_0} & M\eta_0 \end{pmatrix} \begin{pmatrix} E_d^s \\ H_d^s \end{pmatrix}, \quad (43)$$

where $E$ is the field diffracted (scattered) by the three parallel PEMC thick half planes can be written as

$$E = \frac{1}{(M\eta_0)^2 + 1} \left[ ((M\eta_0)^2 - 1)E^s - 2M\eta_0 E^s \right], \quad (44)$$

with $E^s = u_{1d}(\rho, \phi) = \frac{u_{1d}^{(e)}(\rho, \phi) + u_{1d}^{(o)}(\rho, \phi)}{2}$. (45)

## 3. RESULTS AND DISCUSSION

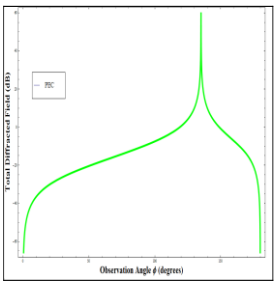


Fig. 2 (a)

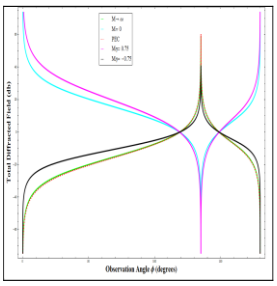


Fig. 2 (b)

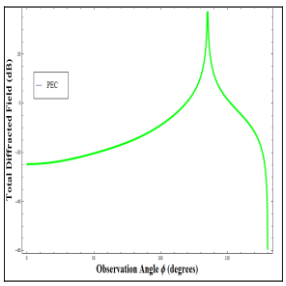


Fig. 2 (c)

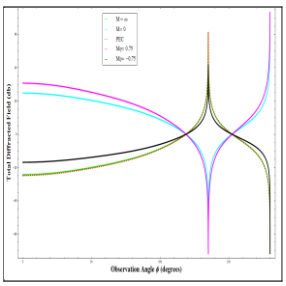


Fig. 2 (d)

Mathematica Software is employed for the numerical and graphical results; we have reproduced the results given by Lindell and Sihvola [13, 18] to get the better understanding of the behavior of cascaded PEMC thick half planes. It can be observed from the article [12] for the PEC case ( $\eta = 0$ ), that amplitude of the diffracted field increases with increasing thickness b. An attempt is made to verify the theoretical

results by the graphs. In all cases, the behavior of the diffracted field by cascaded PEC and PEMC thick half planes for thickness $b = \lambda/4$ and $b = \lambda/2$ against the observation angle $\phi$ is depicted via graphs 2 (a-d). As we can see that in the graphs, the dotted red line is overlapping the behaviour of the diffracted field at $M = \pm\infty$ with green line whereas for the value $M = 0$, the behavior corresponds to PMC case with cyan line. In the same way, the behavior of the crossed-polarized field for the values $M\eta_0 = 0.7$ shown by the magenta line while $M\eta_0 = -0.7$ represented by the black line. The PEMC medium shows maximal behavior as $M\eta_0 \to \pm 1$. The practical application of such a material is not in practice but the validation of the theoretical results available and verified. These numerical results describe the complete PEMC theory.

## 4. CONCLUSION

In this work, plane wave diffraction by cascaded PEMC thick half planes is investigated. The co-polarized and the cross-polarized fields depend on the parameter $M$. It is concluded that the both coupled electric and magnetic fields excitation can be observed analytically at the same time in the PEMC theory which leads to a most general case for the diffraction (scattering) theory. It is also incurred that the parameter $M$ plays an important role in PEMC theory to interlink the PEC and PMC media and further through parameter M polarization in waveguides, antennas etc can be controlled which has significant applications in WLAN [14, 15]. The cross-polarized scattered fields vanish in the PEC and PMC cases and are maximal for $M\eta_0 = \pm 1$. As a check, we can see that $M = \pm\infty$, corresponds to PEC case and $M = 0$, corresponds to a PMC case.

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# ETHICAL ISSUES IN GENOMICS

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## Abstract

Bioethics are usually associated with ethical issues that emerge from advances in medical sciences and practices. These basically include the ethical guidelines that should be followed during any research or medical procedure or practice that is to be conducted. The genetic material present in the form of DNA, which encodes guidelines for cellular life, is termed as genome. Genome of many organisms has been sequenced completely and with this genetic manipulation is possible. To characterize and manipulate the genome many techniques and procedures have been developed like Whole Genome Sequencing (WGS) and genome editing respectively. Some other techniques that come under the umbrella of functional genomics generate huge amount of data that can be characterized and manipulated according to the requirement. These new procedures and approaches have raised many ethical concerns and issues that are being addressed in this review.

**Keywords:** Bioethics, Functional genomics, Genomics, Genetic manipulation, Whole Genome sequencing

## 1. INTRODUCTION

Bioethics is defined as the study of the debatable issues that are promptly ascending due to rapid advancements and progressions in the fields of biotechnology, medicine and other fields of biology (Capron & Michel, 1993). The word bioethics was first used in the 20th century by Fritz Jahr (Lolas, 2008). However, in the 1970s, Van Rensselaer Potter who was an American botanist gave somewhat a more extensive connotation to the term bioethics as he included harmony

towards the biosphere as well thereby creating the concept of what can be called as *global ethics.* Global ethics served as link between different fields such as medicine, biology, ecology and incorporated human values to ensure the well-being and survival of the humans as well as animals and other species (Goldim, 2009; Lolas, 2008). Bioethics is a fundamental part of the research that cannot be neglected, and the importance is ever increasing. Bioethics deals with several issues ranging from organ transplant, abortion, surrogacy, gene therapy, cloning genetic engineering, recombinant DNA research and astroethics and life in space (Callahan, 1970; Gutiérrez-Samperio, 2001; Malcom, 1978; Mautner, 2009; Muzur, 2014). Different bioethicists may have a difference of opinion on how different situations and controversial matters should be handled. Principles of bioethics i.e. autonomy, non-malfeasance, beneficence and justice should be considered while conducting research, clinical studies or any experiments as these principles sets ethical guidelines which should be followed under all conditions for the aim of global benefit (McCormick, 2013).

Field of genomics is meticulously linked to bioethics. Genomics is defined as the complete study of genome of an organism by the application of various techniques (Organization, 2002, 2004). Genomics may be regarded as a discipline in genetics as both the fields are closely interlinked. Techniques in genomics may involve gel electrophoresis, PCR, blotting, microarrays, chromatin immuno precipitation, DNA and genome sequencing and sequence alignment assays (Bickel, Brown, Huang, & Li, 2009; Saraswathy & Ramalingam, 2011). Rapid advancement in genomics has elicited researches that allows us to understand systems as complex as brain (Kadakkuzha & Puthanveettil, 2013). Genomics studies determine the complete sequence of DNA of different organisms along with very fine and precise genetic mapping. The field of genomics may also comprise of studying different phenomenon like epistasis, heterosis, and pleiotropy. It may also cover the interaction and relation between the loci and alleles in the genome of an organism (Pevsner, 2015). Scientific studies and researches focused on studying single genes cannot be included under the umbrella of genomics until and unless the effect of gene on the entire genome is encompassed thus revealing the pathways in which this gene is involved and other analysis related to its function (Robinson, 2002). Genomics has widespread applications in various fields such as social sciences, biotechnology medicines

and anthropology (Barnes & Dupré, 2009). It also finds its pertinence in synthetic biology, bioengineering (Baker, 2011; Church & Regis, 2014). Genomics even has role in the conservation of species as conservationists utilize the genome information to appraise several genetic factors like to determine whether an organism is heterozygous or homozygous for a disorder or anomaly that is recessive in nature (Frankham, 2010). Evolutionary patterns and processes are also studied by bringing genomics into play (Allendorf, Hohenlohe, & Luikart, 2010).

With quick progression in genomics technology and techniques a number of ethical questions are being raised about what is ethically right and what is not as genomics actually involves playing and manipulating the genome. Is sequencing the genome of an individual right? Is it okay to know all the good and bad genes in it? Is it lawful to edit the genome even if it is only for the therapeutic purpose? Is the privacy maintained during survey analysis for genomics study? All these and many other questions are being faced by our society and addressing them is exceedingly important. A brief overview of ethical guidelines for genomic research consent is shown in Figure 1

Following are some of the communal ethical issues that arise with the genomics study

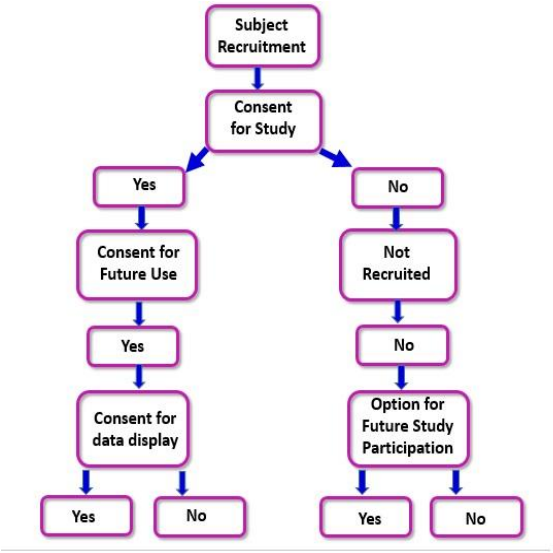


**Figure1: Brief overview of ethical guidelines for genomic research**

## GENOME SEQUENCING

WGS (Whole Genome Sequencing) is the new powerful tool for genetic research and gene discovery in human. The diverse nature of WGS research is increasing the concern about the ethical issues related to it. Ethical considerations raised by WGS are somewhat like those raised by other applied genomic technologies in research. In every genetic research informed consent is an important requirement. The common rule makes it mandatory that researchers obtain informed consent for research that involves "a living individual". So, research permission be informed to the

person to sequence his/her genome. An informed consent must address the requirements of data protection and protection of human subject. WGS approaches should also be described in consent document but it is not necessary to explain the detail of a specific methodology. Goals of the research should be made clear to the participants in the informed consent procedure so that the patients may not expect direct therapeutic benefits from research. (Pinxten & Howard, 2014). Data obtained from genome sequencing of the research subject also possibly reveal information about DNA sequence of his/her close relatives, the consents of whom were not taken for participation in the research. This is a serious issue therefore, it is proposed that researcher conducting whole genome sequencing research should discuss this issue with the participant and encourage him/her to involve his/her close relatives in making decision about research participation. Also, the information generated about the third party, by the research activity, must be protected and kept confidential (McGuire, Caulfield, & Cho, 2008)

Sharing of data and samples for secondary use in other related researches may pose high risk to participant's privacy and autonomy if no mention of secondary uses was made in consent document. So, either the secondary user of data should stay within the primary study consent or re consent the participants for conducting further studies (Manasco, 2005).

WGS research usually reveals many results, which to return and share with participants is an important question as some people may assume these results to be helpful in making clinical decisions. The results obtained from lab research can never be used for this purpose unless results are confirmed in a clinical lab. If confirmed, researcher should notify physician and participant as these results may have an impact on treatment. If the results are of commercial benefit then it should be communicated to the participant and his/her consent should be taken before commercializing the results (Manasco, 2005). Results of unknown or no clinical significance are not returned to the participant (Wolf, 2013)and results of clinical validity but of no clinical utility (results that cannot be acted upon) are returned if the research subject prefer them(Wolf, 2013).

## FUNCTIONAL GENOMICS

The development and application of global experimental approaches to assess gene function and interaction by using information provided by structural genomics is referred as Functional

genomics. It includes large-scale experimental methodologies or high-throughput combined with statistical or computational analysis of the results (Hieter & Boguski, 1997). Functional genomics as a mean of assessing phenotype is different from other approaches primarily with respect to the scale and automation of biological investigations. Modern functional genomics approaches would examine how 1,000 to 10,000 genes are expressed as a function of development in a single experiment. For years animals are used in research with modifications or manipulations for benefit of humans to identify human gene function, it represents a species modified to accommodate humanness, not just an animal enrolled in research. On the other hand, such experimentation imposes suffering to animals. According to epistemological theory gene sequences are same and doesn't matter in which specie they are present but from an ethical perspective gene sequences in different organisms is different (Hoeyer & Koch, 2006). Ethical issues regarding animal welfare can arise in all stages of genetically engineered animals such as invasiveness of procedures or methods, requirement of large number of animals and unpredicted welfare concerns. Other ethical issues include concerns over intellectual property, patents of genetically engineered animals and methods to create them (Caulfield & Gold, 2000).

## CREATION OF BIOBANKS

Biological materials, an important tool in research and its associated databases for sample exchange among different organizations. Basic research on human biomaterials reveals structure, function, composition of cells, and sub cellular components, helps in molecular diagnostic of diseases and development in targeted therapies, pharmacogenetics and pharmacogenomics the way to personalized medicine also influenced by research. Apart from enormous benefits of research on samples taken from biobanks, ethical issues must be considered(Cambon-Thomsen,Ducournau , Gourraud, & Pontille, 2003). In the context of biobank, number of questions arises regarding the inform consent that when consent must be obtained and what information must be included in consent. With respect to biobanks privacy is another ethical issue. Different techniques are used to minimize these issues such as limit access to date, using privacy enhancing approaches. Another debating topic is intellectual property and ownership of participants, data, samples,

entire databases, and downstream products (Haga & Beskow, 2008)

## GENOME EDITING

To amend any inherited genetic disorder gene editing is a potent tool which can either be done by using engineered nucleases like Zinc Finger Nucleases (ZNF), CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), TALEN (Transcription Activator like Effector Nuclease), Homing Endonuclease (HE) and Mega-TAL (Mega -Transcription activator like), induce double stranded breaks (DBS), which is repaired by DNA repair mechanism (Boissel et al., 2014; M Scharenberg, Duchateau, & Smith, 2013) or vectors like adeno-associated virus (AVV) and synthetic nucleotide templates(Khan, Hirata, & Russell, 2011). Genome editing is the modification in the genetic makeup, although it has emerged as a new therapeutic approach to cure disorders at genetic level as in case of hematopoietic diseases (Corrigan-Curay et al., 2015; Porteus, 2015)but there are several ethical concerns associated with germ line and somatic cell therapy. A committee regarding gene editing and ethical considerations related to it has been formed in conjunction with National Academy of Medicine (NAM), Royal Academy of Sciences, National Academy of Sciences (NAS) and Chinese Academy of Sciences (Moreno & Vaillancourt, 2016).

In germ line genome editing, intended or unintended, several ethical concerns arise. The spontaneous germ line alterations and hindrance in the normal functioning of the genes are attributed to the issues related to unintended genome editing. In case of intentional genome editing in which zygote is modified to correct genetic diseases but this may change the human gene pool or might re-create or create what exists or not naturally and usually a mosaic of edited zygote is formed which lead to practical limitations to germ line genome editing apart from ethical concerns(Kohn, Porteus, & Scharenberg, 2016).

There are no unique ethical concerns related to somatic cell modification through genome editing but these are associated with the nature, extent and applications of the gene editing process for example the clinical trials for HIV-1 were conducted using the cells edited using ZNF that disrupted the CCR5 HIV co-receptor (Tebas et al., 2014). There are ethical concerns regarding use of genome editing to enhance a function as in case of over expression of a therapeutic protein in resistance to HIV infection by

knocking out CCR5 or to modify HSC for the treatment of monochromatic leukodystrophy (Biffi et al., 2013) might lead to certain other cosmetic changes(Kohn et al., 2016) .

Gene editing poses serious genotoxic effects that need to be assessed and analyzed before any of the process is used. The integrating vectors might lead to uncontrolled integrations that may suppress the tumor suppressor genes or activate the proto-oncogenes while in case of nuclease mediated genome editing the DSBs may lead to the creation of an insertion or deletion and certain chromosomal aberrations due to wrong fusion of two DSBs. There are four limitations to the genome editing risk assessment;

1. The spontaneous mutagenesis in genome along with chromosomal rearrangements.
2. The current genotoxicity assays through sequencing has limit of detection 1: 10,000 thus there is a possibility that there could be undetected mutations and rearrangements in modified cells.
3. Whole genome sequencing can be used to assess genotoxicity but then it has low sensitivity and it might miss any oncogenic mutation.
4. There are many therapies that cause genotoxicity to cells that are not target to therapy, these should be recognized and assessed for risks. Functional genotoxic assays might be helpful, but no such tests have been established for human stem cells (Kohn et al., 2016). Although genome editing is a promising approach for treatment of various disorders but the raising ethical concerns and issues must be addressed and must be evaluated. At present there is no method efficient enough to evaluate the risks posed by the genome editing but protocols for safety assessment should be formed and issues like genotoxicity should be incorporated in them.

## 2. CONCLUSION

Genomics is promptly developing fields that will advent even more as the time progresses. Further research and insights in genomics holds great potential of saving lives, improving its standard and bringing a cure to diseases which are fatal at the present moment. As Spiderman says and we quote that with great power comes great responsibility so as studies in genomics advances more and more ethical issues will be raised these ethical issues must be addressed according to the need of time and ethical guidelines must be set and followed stringently for the greater good of humanity.

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NUST Journal of Natural Sciences, Vol. 4, Issue 1, 2018, pp.30-50

# A NOVEL MULTI-EPITOPE PEPTIDE VACCINE CONSTRUCT AGAINST INFLUENZA A VIRUS AND *STREPTOCOCCUS PNEUMONIAE* CO-INFECTION: AN *IN SILICO* APPROACH

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## Abstract

Viral and bacterial respiratory tract co-infections in the same host often result in severity and heightened pathology of illness compared to single infections. This has proven to be true for combined infections with Influenza A virus and the bacterium *Streptococcus pneumoniae*. Separate vaccines do exist for each individual infection but they prove to be ineffective and non-specific when the infection has multiplied in case of co-infection. The study utilised in silico approaches and proposed a structural design for multi-epitope peptide vaccine having the ability to target co-infection caused by A/New York/392/2004 (H3N2) and R6 strains of Influenza A virus (NCBI Accession: PRJNA15622) and *Streptococcus pneumoniae* (NCBI Accession: PRJNA278), respectively. Epitope prediction followed by protein prioritization was performed using the reference sequence of each strain to short list the epitopes that can later be used for constructing multi-epitope structure. The multi-epitope constructs having Cholera Toxin Subunit B as adjuvant and $(Gly_4Ser)_3$ as flexible linker were then analyzed for their ability to induce an effective immune response in human body for which Macrophage receptor with collagenous structure, Toll-like receptor 2, 4 and 5 were taken as Pattern Recognition Receptors. The significant immune response generated through each Pattern Recognition Receptor helped to conclude that multi-epitope peptide structures can be used as probable candidates for the design of vaccine. The combination of the epitopes LWSYNAELL and FTGKQLQVG of Influenza A virus and *Streptococcus pneumoniae*, respectively, induced highly significant immune response in case of each Pattern Recognition Receptor when tested through in-silico predictive tools.

**Keywords:** Co-infection, Influenza A virus, Multi-epitope, *Streptococcus pneumoniae*.

## 1. INTRODUCTION

Bacterial-viral co-infections are best described with the case of influenza. However these co-infections are extended to other respiratory viruses, as well, such as Respiratory syncytial virus (RSV), Parainfluenza virus (PIV), Rhinovirus, Adenovirus, and human Metapneumovirus (hMPV) [1]. Several factors including enhanced susceptibility to co-infection with bacterial strains lead to increased virulence [2].Pneumonia and other lower respiratory tract infections are associated with the prevalence of respiratory viruses, particularly Respiratory syncytial virus (RSV) and Influenza [3], [4]. Besides, the bacterial strains in case of positive cultures are almost always *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae*, or a combination of these bacteria[5]–[8]

Post influenza bacterial infection and combined viral-bacterial pneumonia are the two types of bacterial respiratory infection that occur during influenza virus infection. The former, however, can be recognized more easily compared to the latter in terms of clinical diagnosis mainly because it is during the recovery phase from influenza that this infection tends to take place[9]. The combined viral-bacterial infection involves the interaction of the virus with the host response along with the inflammation induced by bacteria, which leads to increased bacterial colonization and outgrowth followed by viral replication. Hence, both the viral replication and bacterial growth will be affected by the host response[10], [11].On the other hand, virus-induced changes to the host are involved in case of post influenza pneumonia. Besides, the absence of virus makes this infection less complicated[12], [13].

The research carried out over the years is suggestive of the fact that several factors of host, virus and bacteria are involved in viral-bacterial pneumonia[10], [12]–[15]. Mechanisms that play critical role in either post-influenza pneumococcal pneumonia or combined viral-bacterial infection have been identified through mouse studies and are summarized in Table 1[16]–[18]. Currently, the focus of these studies is mainly on combined viral-bacterial pneumonia (bacterial challenges up to 7 days after influenza) [14], [15], [19], [20], while other studies aim to investigate post-influenza pneumonia (bacterial challenges ranging from 14 days up to 35 days after influenza infection) [12]–[14].

Once the influenza virus gains entry in to epithelial cells and replicates, the mucociliary clearance of the virus gets

impaired due to the reduction in mucociliary velocity. Hence the events lead to increased burden of *S. pneumonia* 2 hours after bacterial challenge [14]. It is the role of influenza-induced damage to the airway epithelium that influences the severity of both combined viral-bacterial infection and post-influenza pneumococcal pneumonia. The ultimate effect of the influenza-induced damage to the airway epithelium is the increased colonization of bacteria at the basal membrane[21]. This preferred infection and replication of the influenza virus in the airway epithelial cells leads to induction of an antiviral response that aims to remove the virus through transcription and translation based inhibition of the viral replication [22]. Apoptosis also occurs to eradicate the virus in the form of apoptotic bodies that are subsequently removed by alveolar macrophages[23]. However, the viral infection results in TNF-α and IL-12 dependent production of IFN-γ by T cells and endogenous IFN-γ production by APCs. It is then IFN-γ that down regulate the scavenger receptor express by alveolar macrophages, termed as Macrophage receptor with Collagenous structure (MARCO), which has a role in bacterial phagocytosis. However, owing to the viral infection, this phagocytosis also gets inhibited. Prolonged desensitization of alveolar macrophages to bacterial TLR ligands such as lipoteichoic acid (TLR2), lipopolysaccharide (TLR4) and flagellin (TLR5) also occurs due to IAV infection [13] and lasts for several months owing to the longer life span of macrophages that reside in airway epithelium. Hence, the desensitized macrophages produce decreased number of chemokine. Decreased NFκB activation and consequently reduced recruitment of neutrophils to the site of infection also takes place. Eventually, this antiviral mechanism and altered macrophage function leads to an increased risk of bacterial colonization and enhanced bacterial invasion upon secondary infection with *S. pneumoniae* in case of mice [13], [18].

**Table 1. Host factors involved in Combined Viral-Bacterial Pneumonia and in Post-influenza Pneumonia.** MARCO, TLR 2, 4 and 5 are identified as the PRRs that play a critical role in IAV-SP co-infection and further affect the signaling of cytokine and chemokine such as IFN-γ, IFN-α/β and IL-10. Abbreviation: MARCO, Macrophage receptor with Collagenous structure; TLR, Toll-like receptor; PRR, Pattern recognition receptor; IFN, Interferon; IL, Interleukin.

| Host factors | Combined Viral-Bacterial Pneumonia | Post-influenza Pneumonia |
|---|---|---|
| Pattern recognition receptors | MARCO[a] | TLR2[b]<br>TLR4[b]<br>TLR 5 [b] |
| Cytokine/Chemokine | IFN-γ[a]<br>IFN-α/β[c] | IL-10[d] |
| Immune cells | Neutrophil function[e, f, g, h, i]<br>Neutrophil recruitment[j, k, l]<br>Neutrophil apoptosis[m, n]<br>Macrophages[i, o]<br>Monocytes[i] | Neutrophil function[d]<br>Neutrophil recruitment[b] |
| Mechanical factors | Epithelial injury[p]<br>Mucociliary velocity[q] | Unknown |

[a]Sun & Metzger, 2008; [b]Didierlaurent et al., 2008; [c]Shahangian et al., 2009; [d]van der Sluijs et al., 2004; [e]LeVine et al., 2001; [f]McNamee & Harmsen, 2006; [g]Abramson & Hudnor, 1994; [h]Verhoef, Mills, Debets-Ossenkopp, & Verbrugh, 1982; [i]Abramson, Mills, Giebink, & Quie, 1982; [j]Larson, Parry, & Tyrrell, 1980; [k]Shahangian et al., 2009; [l]Ruutu, Vaheri, & Kosunen, 1977; [m]Engelich, White, & Hartshorn, 2001; [n]Colamussi, White, Crouch, & Hartshorn, 1999; [o]Debets-Ossenkopp, Mills, Van Dijk, Verbrugh, & Verhoef, 1982; [p]Plotkowski et al., 1986; [q]Pittet et al., 2010.

The heightened pathology and increased morbidity and mortality associated with IAV-SP co-infection is a serious concern and currently no vaccine exists that can concurrently target the co-infection of IAV and *S. pneumoniae*. The periodic administration of more than one vaccine or even administration of same vaccine to boost immune response to target co-infection is not only time-consuming but cost ineffective as well. It is therefore necessary to propose a vaccine construct having the ability to target co-infection with single administration without compromising on the immune response generated. In this regard multi-epitope peptide vaccine construct can prove to be potential candidates because of their ability to incorporate the epitope of each pathogen, which eventually broadens the scope of immune responses generated in the human body. The study aims to initially analyze the immune response generated by each targeted receptor of human body involved in IAV-SP co-infection. Based on this analysis the study aims to propose the best

combination contained within the construct that can be incorporated in the vaccine formulation. If the multi-epitope peptide based vaccine construct proves to induce an efficient immune response similarly during biological validation, then it will help prevent the detrimental effects of IAV-SP co-infection and will also help to design vaccine with different combinations for other types of co-infection as well.

## 2. MATERIALS AND METHODS

The steps of general methodology applied to the study have been enlisted in Fig 1.

Since the study involved strains of two different pathogens, therefore, the tools for target prediction utilized at each step also varied as shown in Fig 2.

### 2.1 Strain Selection

Influenza A Virus strain A/New York/392/2004 (H3N2) was selected from influenza viruses and *S. pneumoniae* R6 strain was selected from *S. pneumoniae* species.

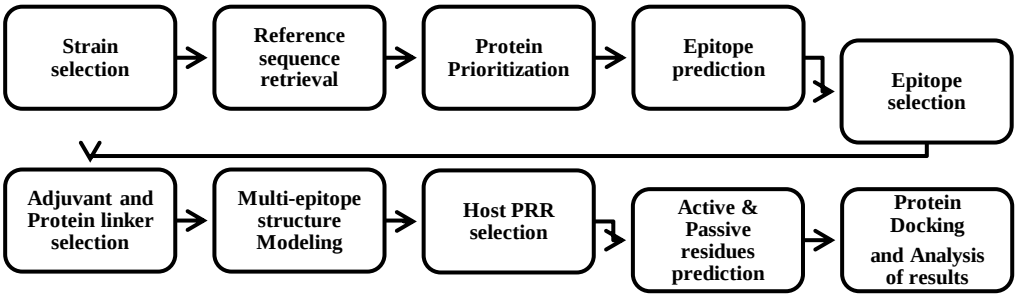


**Figure 1.General Methodology applied to the study.** The study focused on applying in-silico approaches to predict the vaccine targets for multi-epitope peptide construct. Protein prioritization followed by epitope prediction led to finalization of probable vaccine candidates which were then modeled to analyze the protein interactions.

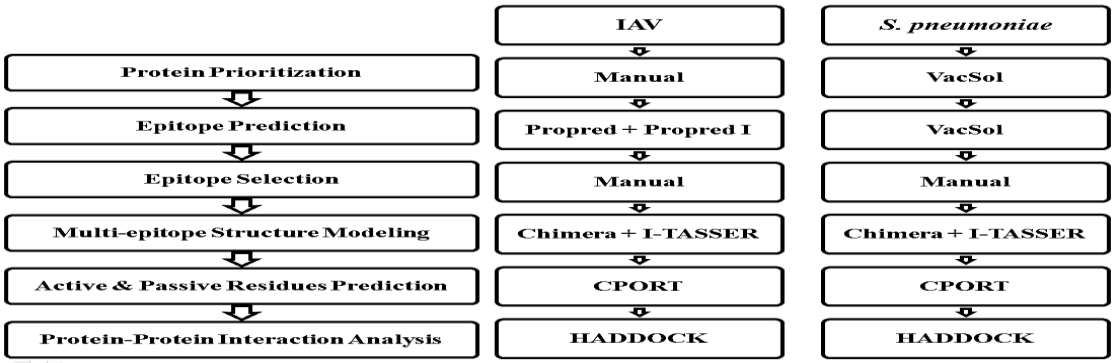


**Figure 2.Tools utilized for target prediction.** The tools were first identified based on the studies reported over the years. Some steps were performed manually to facilitate the performance of sub-steps involved.

### 2.2 Reference Sequence Retrieval

The complete genomes Protein sequences (Reference sequence) of each pathogen was retrieved from National Center for Biotechnology Information (NCBI) with accession numbers PRJNA15622 and PRJNA278 for IAV and *S. pneumoniae* respectively. Reference sequences were selected as they are updated to reflect current knowledge of sequence data and biology. Some other features of Reference Sequence collection include non-redundancy, explicitly linked protein and nucleotide sequences along with format consistency and data validation.

### 2.3 Protein Prioritization and Prediction of T cell epitopes

Protein prioritization was manually performed for IAV, whereas, VacSol, an in house pipeline designed at Integrative Biology Laboratory, ASAB, NUST was used to prioritize proteins in case of *S. pneumoniae*. VacSol, a high throughput *in silico* pipeline uses subtractive reverse vaccinology to predict potential therapeutic targets in prokaryotic pathogens [35]. This pipeline also helped to predict both B and T cell epitopes of the prioritized proteins of *S. pneumoniae*. The determination of the T cell epitopes required both HLA I and HLA II binding peptide sequences. HLA class I binding promiscuous epitopes in the reference sequence of IAV were predicted by the help of ProPred I (www.imtech.res.in/raghava/ProPred1/) [36]. 4% default threshold value was opted with proteasome and immunoproteasome filters enabled at 5% threshold value to maximize the efficiency of determining T cell epitopes. The epitopes determined by ProPred I have the ability to bind to 47 HLA class I alleles. However, ProPred [37] was used at a cut off value of 3% threshold to predict epitopes for HLA class II alleles. ProPred predicts antigenic epitopes that have the ability to bind to 51 HLA class II alleles.

### 2.4 Epitope Selection

#### 2.4.1 Antigenic Prediction

Analysis of the antigenic properties of the predicted epitopes was performed using VaxiJen version 2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [38]. To obtain antigenic sequences a threshold value of 0.5 antigenic score was maintained, which gives 87% accurate results for viruses. Alignment-independent prediction of protective antigens is performed by Vaxijen server on the basis of the physiochemical properties of the antigens [39].

#### 2.4.2 Class I Immunogenicity Prediction

All the HLA-I binding antigenic epitopes of IAV were scanned for MHC-I immunogenicity using Immune Epitope Database (IEDB) Analysis tool

(http://www.iedb.org/) [40]. Default parameters were selected to perform the immunogenicity prediction, which uses amino acid position within the peptide and their properties [39].

#### 2.4.3 Validation of Predicted Epitopes

To comment on the novelty of the predicted epitopes IEDB database was utilized as it contains experimentally confirmed data characterizing antibody and T cell epitopes studied in NHPs, homo sapiens and other animal species [39].

### 2.5 Adjuvant and Protein Linker Selection

Cholera Toxin B (CTB) was investigated as a classical mucosal adjuvant that has the ability to enhance vaccine immunogenicity [41]. Hence, CTB was used as an adjuvant for the multi-epitope construct because of its efficient generation of immune response during the infection of IAV and *S. pneumoniae*.

$(Gly_4Ser)_3$, a flexible protein linker commonly used for protein engineering and design [42], [43], was selected as the protein linker for the multi-epitope vaccine construct against IAV-SP co-infection.

### 2.6 Multi-epitope Structure Designing and Modeling

UCSF Chimera 1.11.2 was used for multi-epitope structures designing that involved linkage between the adjuvant, IAV epitope and *S. pneumoniae* epitope through the flexible protein linker $(Gly_4Ser)_3$[44]. The different combination of constructs were later modeled using I-TASSER (http://zhang.bioinformatics.ku.edu/I-TASSER), which is a server for protein structure and function prediction [45].

### 2.7 Host Pattern Recognition Receptors Selection

Macrophage receptor with collagenous structure (MARCO) and Toll-like receptors (TLRs) were selected as the Pattern Recognition Receptors (PRRs) of the host because of their immune response generated during IAV-SP co-infection. The TLRs included TLR 2, TLR 4 and TLR 5. The Protein Data Bank (PDB) file of each receptor was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) (http://www.rcsb.org/pdb/home/home.do) [46].

### 2.8 Determination of Protein-Protein Interactions

CPORT (Consensus Prediction Of Interface Residues in Transient complexes)(http://haddock.science.uu.nl/services/CPORT/) was used for the prediction of active and passive residues involved in the interaction of each multi-epitope and the selected PRRs [47]. A combination of six methods is utilized by CPORT to accomplish the task [48]–[53].

The structures of each combination of multi-epitope construct against each PRR were fed into guru level interface HADDOCK (High Ambiguity Driven protein-protein DOCKing) (http://milou.science.uu.nl/services/HADDOCK2.2/haddock.php) server using default settings [54], [55]. Guru level interface being an advanced HADDOCK interface allows the identification of flexible regions from the simulation perspective, unlike easy level interface. The top clusters in each case were refined for better orientation, which led to improved HADDOCK scores. To analyze intermolecular and intramolecular interactions PDBsum was utilized [56].

# 3. RESULTS AND DISCUSSION

## 3.1 Prioritized Proteins and Predicted Epitopes

Based on the requirement and methodology of the study, the epitopes were predicted using the prioritized protein of each pathogen. Hemagglutinin (HA) was the prioritized protein of IAV, whereas, Probable Thiol Peroxidase (Tpx) was prioritized in case of *S. pneumoniae*.

### 3.1.1 Influenza A Virus Prioritized Proteins

The proteins of IAV were prioritized on the basis of three factors as summarized in Table 2. Proteins having greatest % identity with humans and annotation were preferred. Besides, Host Apical and Virion membranes were the preferred subcellular locations for the proteins that were needed to be prioritized. Although both Hemaglutinnin and Neuraminidase were obtained as the prioritized proteins but based on the requirements of selecting one best protein, only Hemaglutinnin was considered for further predictions.

**Table 2. Prioritized proteins of IAV.** The proteins of IAV were prioritized on the basis of their homology with humans, subcellular location and annotation. Analysis of each factor utilized respect tool mentioned within the bracket.

| Protein | % Identity with Humans (UniprotKB: Blastp) | Subcellular Location (UniprotKB and Virus-mPLoc ) | Annotation (UniprotKB: 5-point-system) |
|---|---|---|---|
| **PA-X protein** | 22.2 – 30.4 | • Host Cytoplasm | 2/5 |
| **Hemagglutinin** | 20.2 – 34.1 | • Host Apical Cell Membrane<br>• Virion Membrane | 3/5 |
| **Matrix protein 2** | 31.9 | • Host Apical Cell Membrance | 2/5 |
| **Matrix protein 1** | 25.3 – 36.4 | • Host nucleus<br>• Peripheral virion membrane protein | 2/5 |

| | | | |
|---|---|---|---|
| | | (cytoplasmic side)<br>• Host nucleus | |
| **Neuraminidase** | 20.9 – 27.7 | • Host Apical Cell Membrance | 3/5 |
| **Nucleocapsid protein** | 22.9 – 36.1 | • Host Nucleus | 2/5 |
| **Non-structural protein 2** | 21.3 – 39.5 | • Host Nucleus<br>• Virion | 2/5 |
| **Non-structural protein 1** | 34.4 – 26.5 | • Host cytoplasm<br>• Host nucleus | 3/5 |
| **Polymerase acidic protein** | 18.5 – 30.1 | • Host cytoplasm<br>• Host nucleus | 2/5 |
| **Polymerase basic protein 1** | 27.4 – 33.3 | • Host cytoplasm | 2/5 |
| **PB1-F2 protein** | 33.3 – 52.9 | • Host cytosol<br>• Host mitochondrion inner membrane<br>• Host nucleus | 2/5 |
| **Polymerase basic protein 2** | - | • Host nucleus | 2/5 |

### 3.1.2 Hemaglutinnin Predicted Epitopes

The epitope prediction of Hemaglutinnin resulted in two T-cell epitopes demonstrated in Table 3.

### 3.1.3 *S. pneumoniae* Prioritized Proteins and Predicted Epitopes

The use of VacSol helped in prioritizing two proteins of *S. pneumoniae* based on the factors summarized in Table 4. However, based on the requirement of prioritizing one best protein, Probable thiol peroxidase was selected for further predictions. Each prioritized protein of *S. pneumoniae* was further used to predict T-cell epitopes based on the factors enlisted in Table 5.

**Table 3. Predicted epitopes of Hemaglutinnin.** The epitope prediction of Hemaglutinnin, based on MHC-I and II allele count, immunogenicity and antigenicity, gave two T-cell epitopes. VaxiJen was used for antigenicity check, whereas, Class I Immunogenicity IEDB tool was used for immunogenicity. Abbreviation: MHC, Major Histocompatibilty Complex; IEDB, Immune Epitope Database.

| T Cell Epitope | MHC-I Allele Count | MHC-II Allele Count | Location | Immunogenicity (IEDB) | VaxiJen (Threshold = 0.5) | |
|---|---|---|---|---|---|---|
| | | | | | **Score** | **Antigenicity** |
| **IEVTNATEL** | 12 | 2 | 50 | 0.17425 | 0.8869 | Probable Antigen |
| **LWSYNAELL** | 8 | 0 | 437 | 0.05113 | 0.6384 | Probable Antigen |

**Table 4. Prioritized proteins of *S. pneumoniae*.** Six factors were taken in to account by VacSol to prioritize the proteins of *S. pneumoniae*.

| Protein | Non-Homologous | Localization | Essential | Virulent | Helices < 2 | Annotated |
|---|---|---|---|---|---|---|
| **Zinc-binding lipoprotein AdcA** | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | 0 | Periplasmic | 1 | 3 | IN: 1 | 40.42 % |
| **Probable thiol peroxidase** | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | 0 | Periplasmic | 1 | 4 | OUT: 0 | 100 % |

**Table 5. Predicted epitopes of *S. pneumoniae*.** VacSol predicted a total of five T-cell epitopes based on MHC-I and MHC II allele count. VaxiJen score for antigenicity was also taken in to consideration. Abbreviation: MHC, Major Histocompatibilty Complex.

| Protein | T Cell Epitope | Location | MHC-I Allele Count | MHC-II Allele Count | VaxiJen (Threshold = 0.5) | |
|---|---|---|---|---|---|---|
| | | | | | **Score** | **Antigenicity** |
| Zinc-binding lipoprotein AdcA | FLLCLGACG | 21 | 6 | 6 | 0.4059 | Probable Antigen |
| | LESDPQNDK | 286 | 3 | 5 | 0.9905 | Probable Antigen |
| | MVKEVSGDL | 51 | 40 | 6 | 0.806 | Probable Antigen |
| Probable thiol peroxidase | LAGLDNTVV | 70 | 6 | 4 | 1.2005 | Probable Antigen |
| | FTGKQLQVG | 12 | 2 | 2 | 1.4001 | Probable Antigen |

### 3.2 Multi-epitope Peptide Vaccine Construct Design

The study aimed to propose a multi-epitope peptide vaccine construct, hence the best epitope of each pathogen, an adjuvant and a linker were incorporated in to the construct as demonstrated in Fig 3.

### 3.3 Multi-epitope Peptide Vaccine Construct Combinations

A total of four combinations were proposed using one best epitope from each pathogen. The combinations have been represented in Table 6.

**Figure 3. Proposed design of Multi-epitope peptide vaccine constructs.** LWSYNAELL and FTGKQLQVG were incorporated as IAV and *S. pneumoniae* epitopes, respectively.


**TABLE 6. Combinations for multi-epitope peptide vaccine construct.** The table represents the possible combinations involving Hemaglutinnin and Probable Thiol Peroxidase T-Cell Epitopes.

| Multi-epitope | Hemaglutinnin T- Cell Epitope | Probable Thiol Peroxidase T-Cell Epitope |
|---|---|---|
| 1 | IEVTNATEL | LAGLDNTVV |
| 2 | LWSYNAELL | LAGLDNTVV |
| 3 | IEVTNATEL | FTGKQLQVG |
| 4 | LWSYNAELL | FTGKQLQVG |

## 3.4 Protein-Protein Interaction

HADDOCK results summarized in Table 7 helped to identify the best multi-epitope combination for the vaccine construct. The visual representation of the interaction of multi-epitope 4 with MARCO, TLR 2, TLR 4 and TLR 5 has been indicated in Fig 4, 5, 6 and 7, respectively.

**TABLE 7. Protein-Protein interaction results.** Based on the lowest HADDOCK and RMSD score, multi-epitope 4 was proposed as the best combination.

| | IAV EPITOPE | SP EPITOPE | RECEPTOR | HADDOCK SCORE | RMSD SCORE |
|---|---|---|---|---|---|
| MULTIEPITOPE 1 | IEVTNATEL | LAGLDNTVV | MARCO | -143.4 +/- 0.6 | 0.3 +/- 0.2 |
| | | | TLR2 | -119.3 +/- 1.5 | 0.3 +/- 0.2 |
| | | | TLR4 | -138.0 +/- 3.3 | 0.2 +/- 0.1 |

| | | | | | |
|---|---|---|---|---|---|
| | | | TLR5 | -129.8 +/- 7.6 | 0.3 +/- 0.2 |
| MULTIEPITOPE 2 | LWSYNAELL | LAGLDNTVV | MARCO | -138.4 +/- 1.6 | 0.3 +/- 0.2 |
| | | | TLR2 | -132.4 +/- 4.1 | 0.3 +/- 0.2 |
| | | | TLR4 | -124.8 +/- 1.2 | 0.3 +/- 0.1 |
| | | | TLR5 | -212.2 +/- 5.3 | 0.3 +/- 0.2 |
| MULTIEPITOPE 3 | IEVTNATEL | FTGKQLQVG | MARCO | -141.6 +/- 6.5 | 0.3 +/- 0.2 |
| | | | TLR2 | -107.2 +/- 1.0 | 0.3 +/- 0.2 |
| | | | TLR4 | -152.3 +/- 4.2 | 0.3 +/- 0.1 |
| | | | TLR5 | -262.0 +/- 6.4 | 0.3 +/- 0.2 |
| MULTIEPITOPE 4 | LWSYNAELL | FTGKQLQVG | MARCO | -143.9 +/- 3.3 | 0.3 +/- 0.2 |
| | | | TLR2 | -131.8 +/- 6.0 | 0.3 +/- 0.1 |
| | | | TLR4 | -171.4 +/- 4.1 | 0.3 +/- 0.2 |
| | | | TLR5 | -262.0 +/- 6.4 | 0.3 +/- 0.2 |

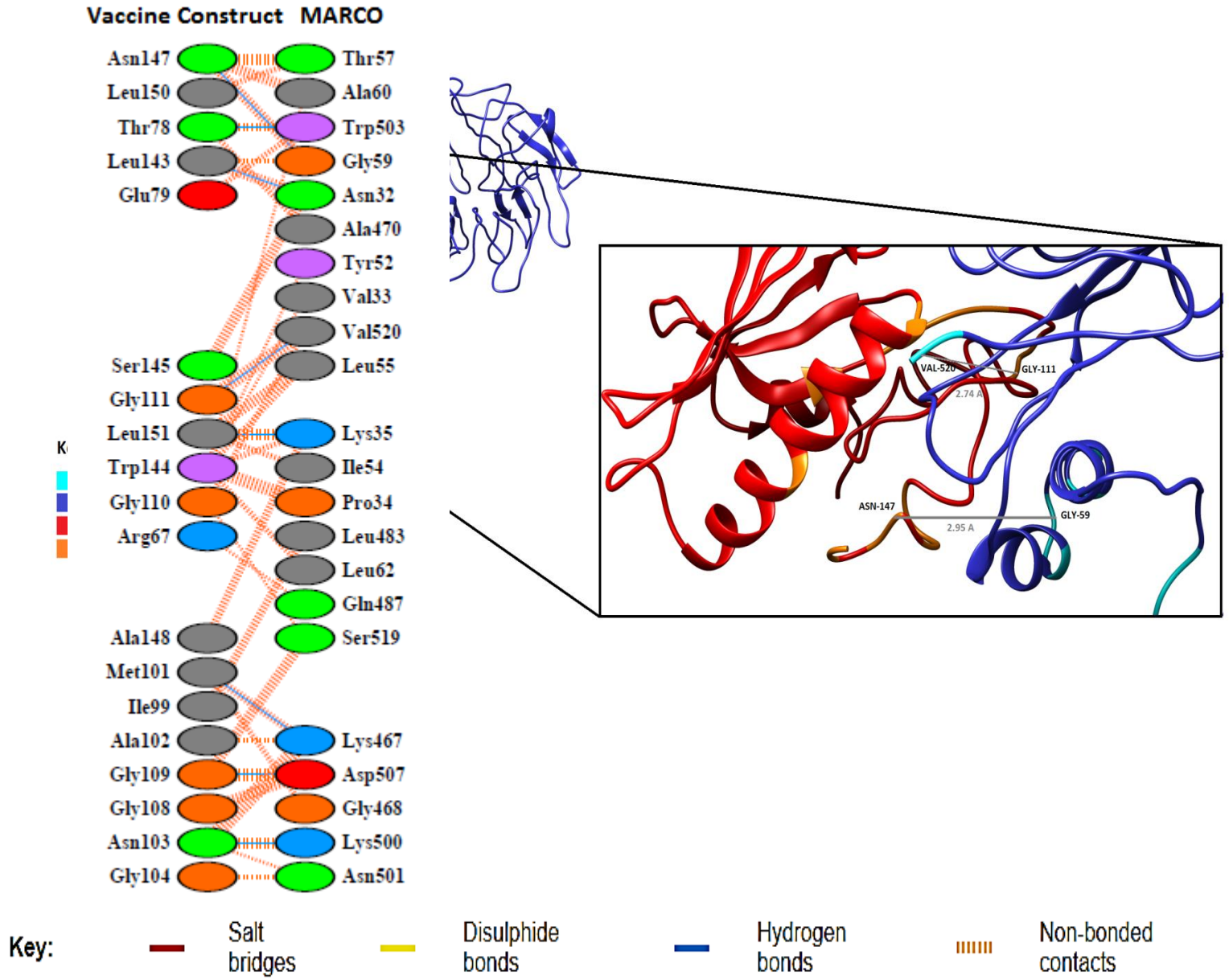


**Figure 4. Interaction between Vaccine Construct (Multi-epitope 4) and MARCO.** The figure indicates the interacting residues an construct and MARCO. Abbreviation: MARCO, Macrophage receptor with collagenous structure.

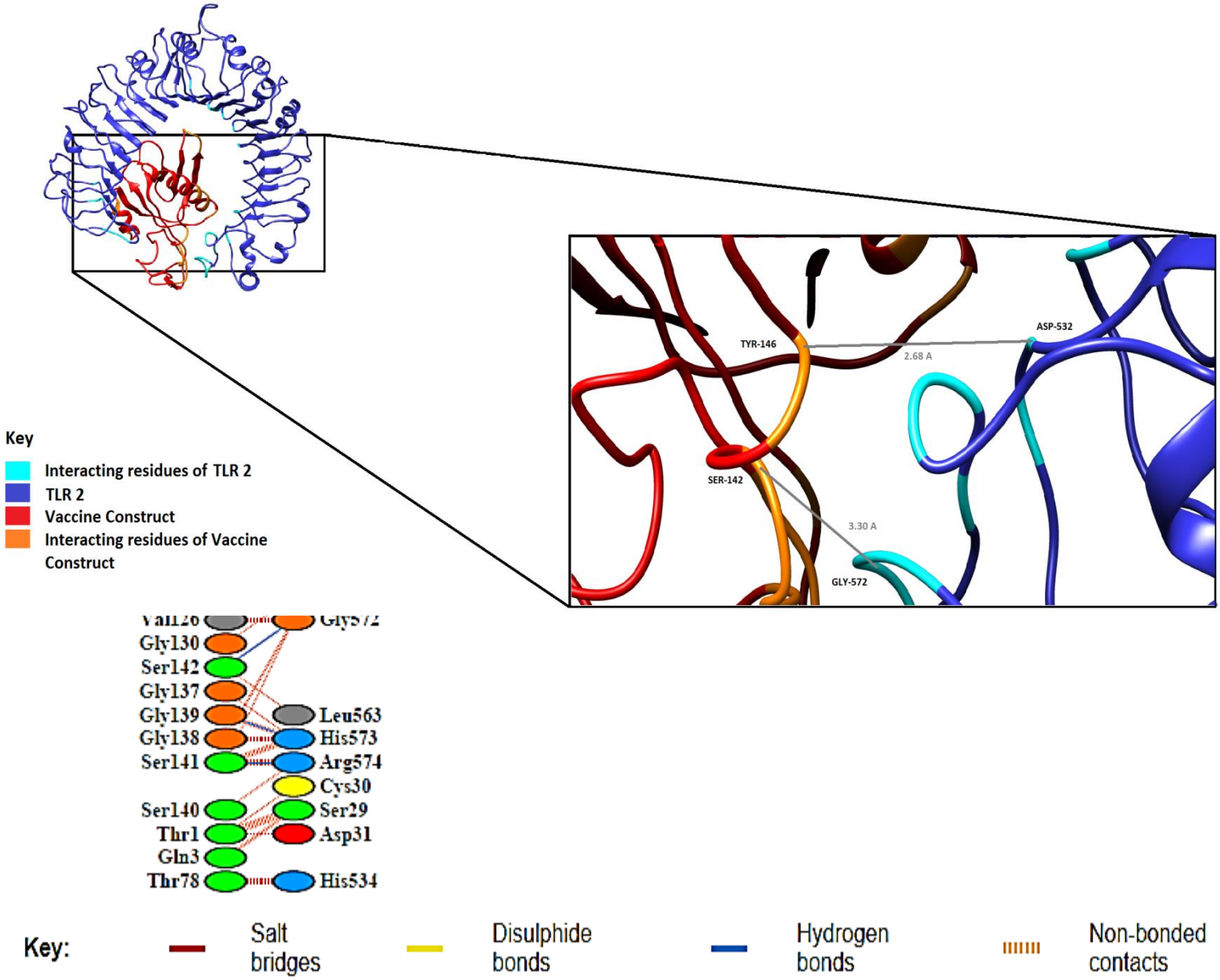


**Figure 5. Interaction between Vaccine Construct (Multi-epitope 4) and TLR 2.** The figure indicates the interacting residues vaccine construct and TLR 2. Abbreviation: TLR, Toll-like receptor.

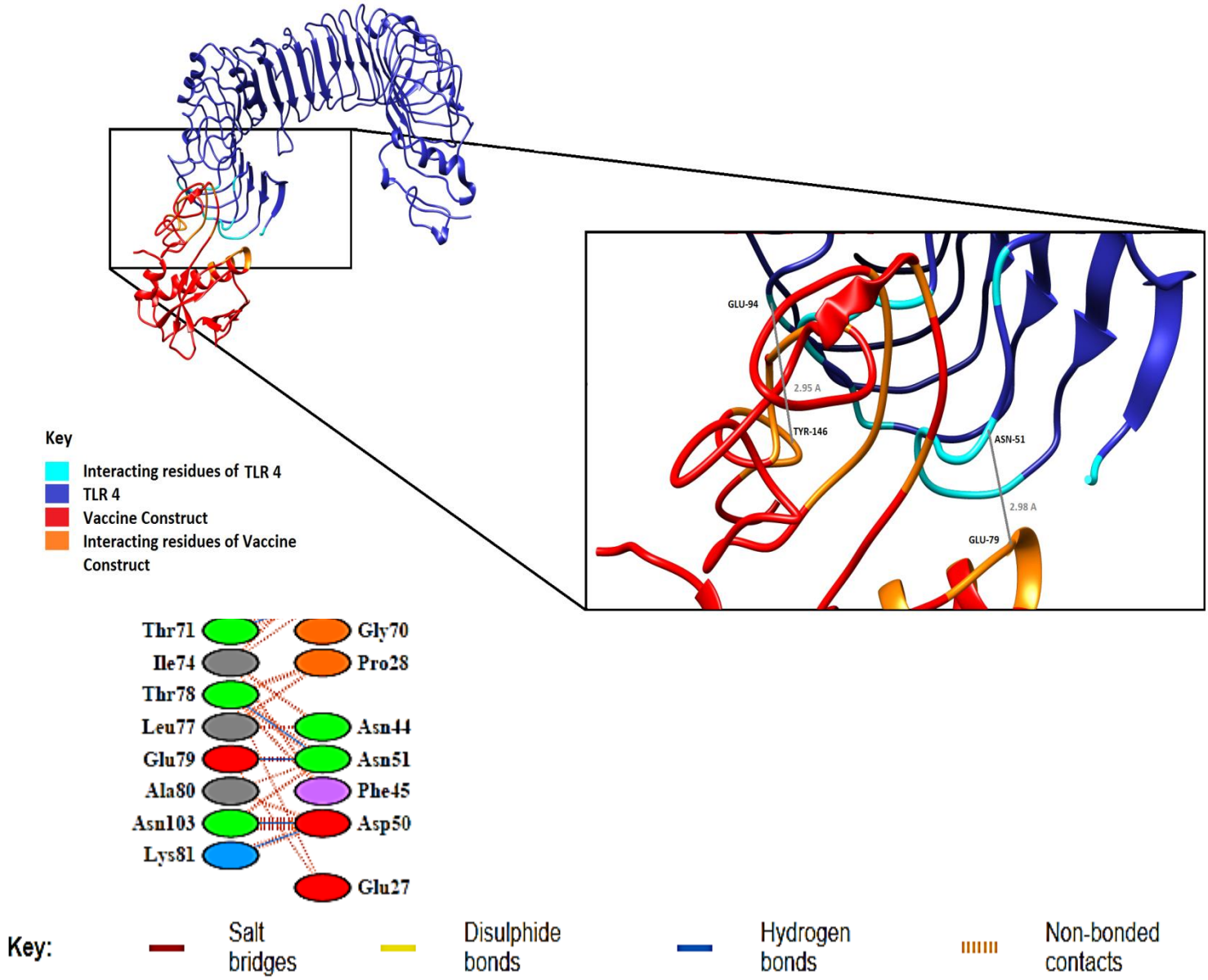


**Figure 6. Interaction between Vaccine Construct (Multi-epitope 4) and TLR 4.**The figure indicates the interacting residues a vaccine construct and TLR 4. Abbreviation: TLR, Toll-like receptor.

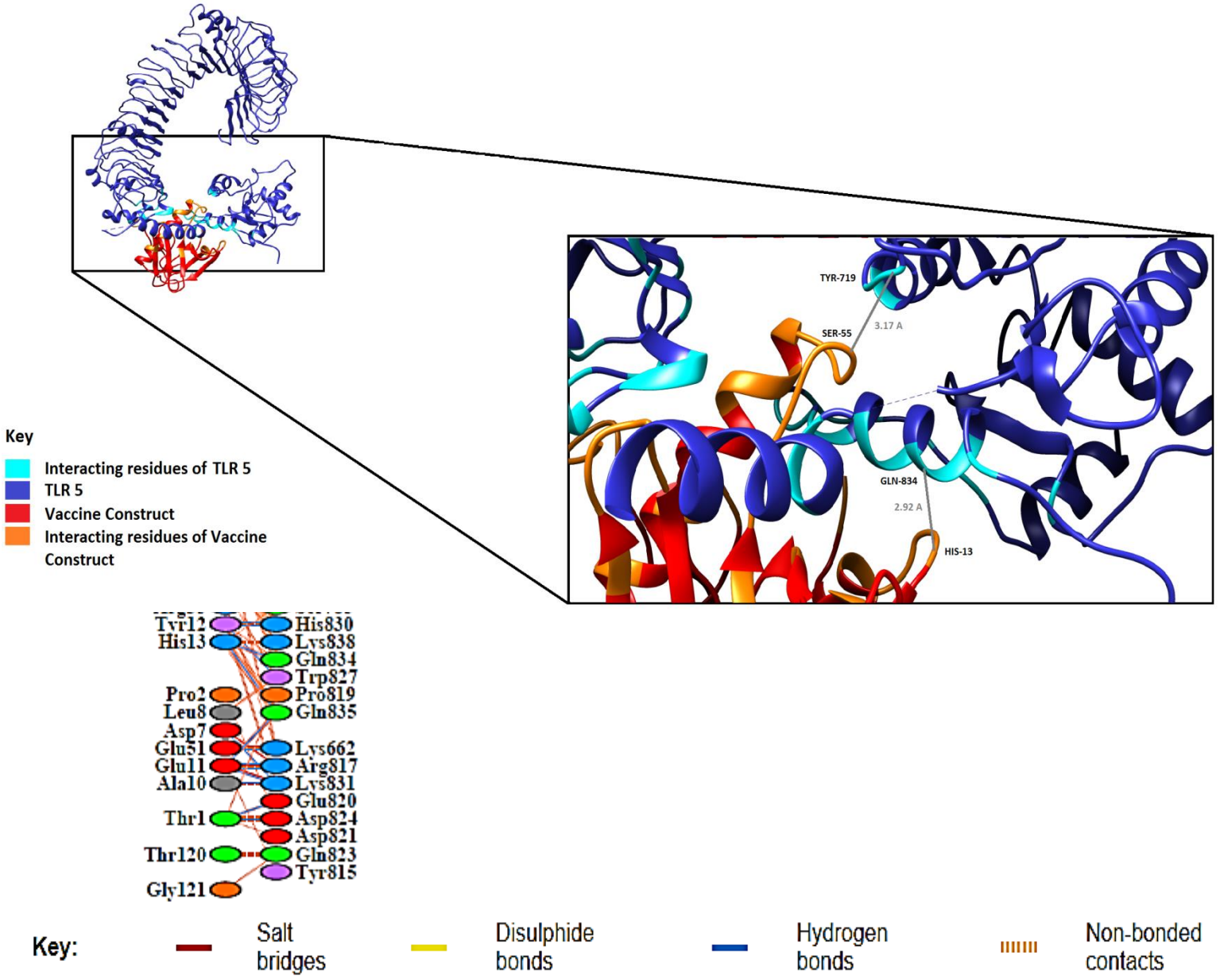


**Figure 7. Interaction between Vaccine Construct (Multi-epitope 4) and TLR 5.** The figure indicates the interacting residues and bo vaccine construct and TLR 5. Abbreviation: TLR, Toll-like receptor.

## 4. CONCLUSION

The results of this in-silico study helped in concluding that multi-epitope peptide based construct can be used as probable candidate against infectious diseases that involve co-infection of multiple pathogens. This hypothesis has been proven true particularly for the co-infection of IAV and *S. pneumoniae*. Significant Immune response was generated by each PRR particularly in case of the combination of multi-epitope 4 construct, which led to the conclusion that the very combination has the greatest potential to induce an effective immune response in human body if and when validated through animal model. The study considered PRRs as the only immune targets of human body; however, this approach can further be extended to Human Leukocyte Antigens (HLAs) in order to broaden the scope of the study in terms of immune response generated by the vaccine construct. Based on the promising results of this in-silico predication, the results can further be validated using animal model or the Lab-on-a-chip approach. Different adjuvants can also be made use of in order to boost the immune response generated. The scope of the study can also be broadened by including other strains of each pathogen. Besides, there exists an interesting link between microorganism infection and carcinogenesis, which has recently gained immense attention from researchers worldwide. Hence, the link between IAV-SP co-infection and Lung cancer can be studied in depth and by identifying the common signaling pathways a multi-epitope peptide vaccine construct or fusion proteins can also be proposed against this fatal combination.

## Conflict of Interest

The author declares that there is no conflict of interest.

## Acknowledgement

The author would like to thank the lab members at Integrative Biology Laboratory of Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology for providing the guidance and assistance with the Bioinformatics tools required to complete the study.

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# POTENTIAL EFFECTS OF GLIBENCLAMIDE ON PROTEIN EXPRESSION IN ALCL$_3$-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 ($AlCl_3$) 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 $AlCl_3$, Glibenclamide (GLI) (10mg /body weight), $AlCl_3$ 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 Maill ard 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-κB (NF-κB)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 ($AlCl_3$) induced neurotoxicity mouse model of AD was established. $AlCl_3$ has been used in various research studies to induce AD in mice (Sun et al., 2009; Shati et al., 2011).

The $AlCl_3$ induced mouse model was an imminent choice as $AlCl_3$ 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 $\pm$ 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 mositure (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 $AlCl_3$ 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<br>-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% $AlCl_3$<br>-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 sufonyl 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% othrophosphoric Acid ($H_3PO_4$), 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 ($ddH_2O$) 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).

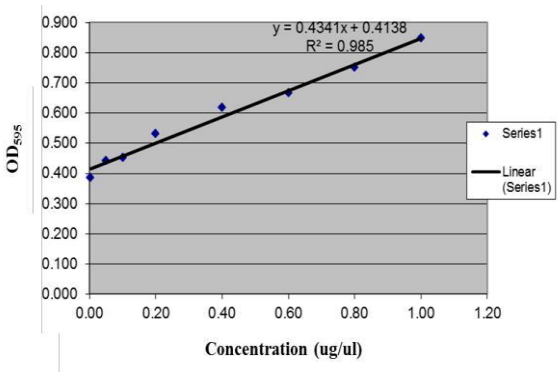


**Figure 1: Bradford Standard curve plotted foreight values:** Concentration has been

plotted as independent variable (x-axis).Absorbance was measured at 595nm and plotted as dependent bariable (y-axis). The line represents the linear regression for entires 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 $AlCl_3$-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 $AlCl_3$-treated group while the expression level was restored in the $AlCl_3$ + 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 $AlCl_3$ treatement while this expression was restored when $AlCl_3$ treatement 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 $AlCl_3$ + GLI group and GLI group as compared to control. A 64 kDa protein showed increased levels of expression in GLI group and $AlCl_3$+ GLI group as compared to control and $AlCl_3$-treated group, while there was no significant difference in expression level in $AlCl_3$-treated group as compared to control.

Increase in expression levels was also observed for a 50 kDa protein in GLI group as compared to $AlCl_3$+ GLI group,$AlCl_3$-treated group and control. Quantitative analysis of 33 kDa protein also showed interesting results in all groups. Expression was increased in $AlCl_3$-treated group while a further rise in expression level was observed in presence of GLI in $AlCl_3$ + GLI group. However, the analysis revealed a decrease in expression in GLI group as compared to $AlCl_3$ + GLI group and $AlCl_3$-treated group. Analysis of 15 kDa protein showed a remarkable rise in expression levels in $AlCl_3$ + GLI group, GLI group as compared to control group (Figure 3).

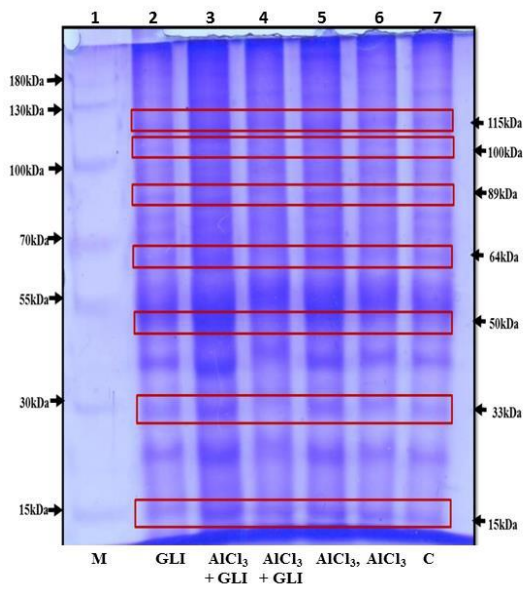

**Figure 2: Differential expression pattern of proteins isolated from hippocampus, seperated by SDS-PAGE:** Extracted proteins were resolved on 12.5% seperating gel and stained with coomassie brilliant blue Lane 1: Protein Marker, Lane 2: GLI (10 mg), Lane 3: $AlCl_3$ + GLI (10 mg), Lane 4: $AlCl_3$+ GLI (10 mg), Lane 5: $AlCl_3$, Lane 6: $AlCl_3$, Lane 7: Control

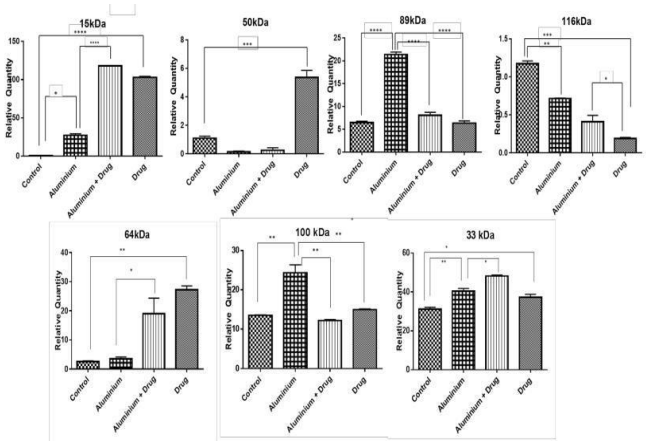


**Figure 3: Expression graph of differentially expressed hippocampal proteins.** Histogramrepresenting relative quantity of 15 kDa, 50 kDa, 89 kDa, 116 kDa, 64 kDa, 110 kDa and 33 kDa proteins in control, AlCl3-treated group, AlCl3 + GLI group and GLI-treated group; showing significant differntial expression. 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 $AlCl_3$ 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 $AlCl_3$ 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 AlCl3-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.

## ACKNOWLEDGEMENTS

This research was supported through BS student's research grant by National University of Sciences and Technology (NUST), Islamabad, Pakistan.

## 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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NUST Journal of Natural Sciences, Vol. 4, Issue 1, 2018, pp.64-82

# 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, Tilletiaindica* 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*, *Triticumturgidum*, 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 *Tilletiaindica* (Syn. *Neovossiaindic*, 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℃ with 16 hours light and 8 hours dark, photoperiod for 48 hours. Afterwards, plants were shifted to preset glasshouse with 6-10℃ 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 f 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 form 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 allantoidsporodia 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 xi and xj are the respective frequencies of the ith and jth alleles, πij is the number of allele differences per locus between the ith and jth 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 Fehrmann, 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 ofD-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;

# KARNAL BUNT RESISTANCE IN SYNTHETIC HEXAPLOID/BREAD WHEAT DERIVATIVES

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## Abstract

Karnal bunt is caused by a smut fungus (*Tilletiaindica*), which results in blackening of seeds in wheat. It is one of the most common fungal diseases of wheat and is subjected to quarantine in many countries of the world. The disease symptoms are usually blackening of seeds and fishy smell. The flour made by infested wheat is unlikely to be purchased and consumed by the public. The disease spores stay potent for a long period of time for upto 5 years or more, thereby reducing the chances of its eradication. Certain methods have been used to increase the resistance of plants against Karnal bunt, which includes use of fungicides as well as exposure to artificial extreme environments to kill the pathogen. Breeding for disease resistance is of huge interest to scientists as it is cheap and also results in better quality of crop for export purposes. We screened a set of synthetic/bread wheat derivatives against Karnal bunt and identified two resistant derivatives for breeders.

**Key words:** *Tilletiaindica*, quarantine, bread wheat, Karnal bunt, fungus

## 1. INTRODUCTION

Wheat is one of the most important leading cereal grains in the world (FAO, 2011). It has been cultivated for more than 10,000 years in South Asia, which is its geographic center of origin. Bread wheat has been grown in Nile valley in 5000 BC. Later, it spread to Mediterranean region for domestication (Leonard and Martin., 1963).

Nowadays, wheat is cultivated most successfully at 25ºC with maximum and minimum growth temperatures of 32ºC and 3ºC respectively. World population is constantly increasing; thereby the demand for food production is also increasing alongside.

Wheat provides 21% of the total food calories to around 94 under-developed countries in the world (Braun *et al.,* 2010). It belongs to Poaceae; the family of long grasses. Poaceae is considered to be the world's largest family of flowering plants. It includes approximately 10,000 species, classified under 600-700 genera, diverged from ancestral progenitor about 50 to 70 million years ago. Most of the grasses in this family are polyploids. Wheat is a polyploid, which belongs to the tribe *Triticaea;* economically most important tribe of the family. *Triticaea* constitutes both annual and perennial forms of 25 genera, which include both wheat and other wild relatives of wheat (Mujeeb-KaziandVahidy, 1994).

Bread wheat is one of the best examples of evolution characterized through allopolyploidy. *Triticumaestivum*is allohexaploid that got evolved after two hybridization steps, integrated by spontaneous chromosome doubling. The first natural cross between B and A diploid donors of wheat genome occurred around 9000 BC resulting in tetraploid emmer wheat (BBAA), currently known as durum wheat. Around 6000 BC, another cross between this tetraploid wheat (BBAA) and another wild D-genome donor occurred, resulting in the production of common bread wheat (BBAADD), which is hexaploid in nature. The A-genome donor of wheat is *Triticumuratu,* D-genome is *Aegilopstauschii* and the donor for B-genome is probably a wild wheat relative of *Triticum*genus (Feldman., 2001).

*Tilletia*is a group of smut fungi, which infects grasses either systemically or locally. *Tilletiaindica;*which infects wheat along with *Tilletiahorrida;*which infects rice, are both locally infecting species of *Tilletia.* These species are known to infect economically important crops thus threatening food security (Carris*et al.,* 2006). Karnal bunt is an important disease of wheat, which deteriorates theflour quality in addition to monetary losses. It is difficult to differentiate between teliospores of Karnal bunt and other related fungi (Singh and Gogoi, 2011). Spread of infection and susceptible period of infection is heading and anthesis of wheat (Wei-ChuanandGui-Ming., 2010). Karnal bunt disease is observed to be at maximum when the inoculum contains 50,000 sporidia per ml (GurbirandSatvinder, 2005).

Karnal bunt is not involved in severe wheat losses but Karnal bunt infested seeds produce a chemical; trimethylamine, which affects the odor and palatability of the whole meal. Resistant cultivars provide the best option to control Karnal bunt as its chemical control is not feasible. Although, Karnal bunt is a rare disease, it needs to be controlled for reduction of economic losses.

## 2. MATERIALS AND METHODS

The germplasm of bread wheat by synthetic derivatives (BW/SH) was provided by Dr. Abdul Mujeeb Kazi, Wheat Wide Crosses, National Agricultural Research Center, Islamabad. The pedigree of germplasm is provided in Table 1.

**Table 1: Pedigree of 209 Synthetic/Bread wheat derivatives used in the study**

| | |
|---|---|
| 1. | MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 2. | MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 3. | 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/5/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 4. | MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/4/ CETA/AE.SQUARROSA(895) |
| 5. | TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA(205)/3/3*BUC/6/FCT/6/DOY1/AE.SQUARROSA(458) |
| 6. | OPATA//CETA/AE.SQUARROSA(895) |
| 7. | 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/6/CETA/AE.SQUARROSA(895) |
| 8. | OPATA//DOY1/AE.SQUARROSA(372) |
| 9. | CHAPIO/INQALAB 91/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878) |
| 10. | D67.2/P66.270//AE.SQUARROSA(223)3/ARLIN_1/T.MONOCOCCUM(95) |
| 11. | TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/CROC_1/AE.SQUARROSA(444) |
| 12. | MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/4/ DOY1/AE.SQUARROSA(372) |
| 13. | MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 14. | D67.2/P66.270//AE.SQUARROSA(223)/3/ARLIN_1/T.MONOCOCCUM(95) |
| 15. | URES/PRL//BAV92/3/YAV_2/TEZ//AE.SQUARROSA(249) |
| 16. | GAN/AE.SQUARROSA (897)//OPATA/3/D67.2/P66.270//AE.SQUARROSA(223) |
| 17. | OPATA//CETA/AE.SQUARROSA(1031) |
| 18. | OPATA//DOY 1/AE.SQUARROSA(255) |
| 19. | OPATA//INQALAB 91/FISCAL |
| 20. | OPATA//CETA/AE.SQUARROSA(1031) |
| 21. | OPATA//ROK/KML// AE.SQUARROSA(214) |
| 22. | OPATA//DOY 1/AE.SQUARROSA(517) |

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| 23. OPATA//DOY 1/AE.SQUARROSA(517) |
| 24. OPATA//ALTAR 84.AE.SQUARROSA(J BANGOR) |
| 25. OPATA//68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 26. OPATA//68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 27. OPATA//AE.SQUARROSA(1026)/DOY 1) |
| 28. OPATA//68.112/WARD//AE.SQUARROSA(369) |
| 29. OPATA//68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 30. GANAE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB91/5/ BKH-94 |
| 31. BKH-93/6/CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OAPTA |
| 32. PBW-343/6/YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/OPATA |
| 33. CHIR3/CBRD//OPATA |
| 34. SAAR/INQALAB 91/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 35. SAAR/INQALAB 91/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 36. SAAR/INQALAB 91/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 37. MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/PASTOR/4/SARSABZ |
| 38. MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD/4/KAMBARA |
| 39. PBW-343/6/YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA(498)/5/OPATA |
| 40. CHIR3/CBRD/3/GAN/AE.SQUARROSA (897)//OPATA |
| 41. MAYOOR//TK SN1081/AE.SQUARROSA (222)/4/SABUF/3/BCN//CETA/AE.SQUARROSA(895)/5/GAN/AE.SQUARROSA (897)//OPATA |
| 42. KAUZ/3/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 43. BKH-93/BORLOUG M95 |
| 44. BKH-93/BORLOUG M95 |
| 45. FUS/BW-595-(ALTAR 84/AE.SQUARROSA (224)//2*YACO/7/OPATA/6/68.111RGB-U//WARD/3/FGO/4/…) x INQ-120-(162 SAAR/INQALAB 91) |
| 46. FUS/BW-586-(ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/MAYOOR//TK SN1081/AE.SQUARROSA (222)/4/KUKUN) x SH/BW.R.KB-1-(ALTAR 84/AE.SQUARROSA (221)//YACO) |
| 47. SARSABZ//CHIR3/CBRD |
| 48. OPATA/PASTOR |
| 49. PBW-343*2/CHAPIO/3/D67.2/P66.270//T.BOEOTICUM(66) |
| 50. PBW-343*2/CHAPIO/3/D67.2/P66.270//T.BOEOTICUM(66) |
| 51. OPATA/5/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(1038) |
| 52. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 53. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 54. ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 55. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 56. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 57. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA)(248) |
| 58. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 59. ALTAR 84/AE.SQUARROSA(224)//2*YACO/3/MAYOOR/TK SN1081/AE.SQUARROSA(222)/4/KUKUN/5/GAN/AE.SQUARROSA(248) |
| 60. MH 97/2/D67.2/P66.270//T.BOEOTICUM(66) |
| 61. TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA(227) |
| 62. TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA(227) |

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| 63. TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA(227) |
| 64. RABE/2*MO88/5/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(1038) |
| 65. RABE/2*MO88/5/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(1038) |
| 66. RABE/2*MO88/5/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(1038) |
| 67. MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 68. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OAPTA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 69. MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 70. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 71. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 72. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 73. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 74. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OAPTA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 75. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 76. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 77. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 78. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 79. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 80. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 81. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 82. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 83. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA(208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 84. CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OPATA/6/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(783) |
| 85. TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA(273) |
| 86. OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(878) |
| 87. INQALAB 91/TSAPKI//SCA/AE.SQUARROSA(518) |
| 88. ALTAR 84/AE.SQUARROSA(221)//YACO/3/INQALAB 91/4/D67.2/P66.270//T.BOEOTICUM(66) |
| 89. CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA(TAUS)/4/WEAVER/5/2*KAUZ/6/DOY1/AE.SQUARROSA) (458) |
| 90. CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA(TAUS)/4/WEAVER/5/2*KAUZ/6/DOY1/AE.SQUARROSA) (458) |
| 91. CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA(TAUS)/4/WEAVER/5/2*KAUZ/6/DOY1/AE.SQUARROSA) (458) |
| 92. CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA(TAUS)/4/WEAVER/5/2*KAUZ/6/DOY1/AE.SQUARROSA) (458) |
| 93. CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA(TAUS)/4/WEAVER/5/2*KAUZ/6/DOY1/AE.SQUARROSA(458) |

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| 94. SERI/5/68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA(392) |
| 95. TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/6/OPATA/7/ SCA/AE.SQUARROSA(518) |
| 96. MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD/4/ARLIN_1/T.MONOCOCCUM(95) |
| 97. MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD/4/ARLIN_1/T.MONOCOCCUM(95) |
| 98. CHAPIO/INQALAB 91/4/68.111/RGB-U//WARD/3/AE.SQUARROSA(452) |
| 99. CHAPIO/INQALAB 91/4/68.111/RGB-U//WARD/3/AE.SQUARROSA(452) |
| 100. CHAPIO/INQALAB 91/4/68.111/RGB-U//WARD/3/AE.SQUARROSA(452) |
| 101. CROC-1/AE.SQUARROSA(224)//KAUZ/3/CETA/AE.SQUARROSA(895) |
| 102. OPATA/PAS//DOY1/AE.SQUARROSA(1024) |
| 103. MAYOOR//TKSN1081/AE.SQUARROSA(222)/3/OPATA/4/ DOY1/AE.SQUARROSA(515) |
| 104. DOYI/AE.SQUARROSA(1018)/6/CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OPATA |
| 105. DOYI/AE.SQUARROSA(1018) x CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OPATA |
| 106. DOYI/AE.SQUARROSA(1018) x CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OPATA |
| 107. MAYOOR/TK SN1081/AE.SQUARROSA (222)/3/PASTOR/4/CROC_1/AE.SQUARROSA(444) |
| 108. MAYOOR/TK SN1081/AE.SQUARROSA(222)/3/PASTOR/4/CROC_1/AE.SQUARROSA(444) |
| 109. PBW-343//DOY1/AE.SQUARROSA(188) |
| 110. OPATA//CETA/AE.SQUARROSA(895) |
| 111. OPATA//INQALAB 91/AC8528 |
| 112. OPATA//INQALAB 91/AC8528 |
| 113. OPATA//GAN/AE.SQUARROSA(408) |
| 114. OPATA//DOY1/AE.SQUARROSA(458) |
| 115. OPATA//DVERD_2/AE.SQUARROSA(333) |
| 116. OPATA//DVERD_2/AE.SQUARROSA(333) |
| 117. OPATA//DVERD_2/AE.SQUARROSA(333) |
| 118. OPATA//DVERD_2/AE.SQUARROSA(333) |
| 119. OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(629) |
| 120. OPATA/6/x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA(629) |
| 121. OPATA//ALTAR 84/AE.SQUARROSA(J BANGOR) |
| 122. OPATA//ALTAR 84/AE.SQUARROSA(J BANGOR) |
| 123. OPATA//ALTAR 84/AE.SQUARROSA(J BANGOR) |
| 124. OPATA//ALTAR 84/AE.SQUARROSA(J BANGOR) |
| 125. OPATA//ALTAR 84/AE.SQUARROSA(J BANGOR) |
| 126. OPATA//CETA/AE.SQUARROSA(1027) |
| 127. OPATA//ALTAR 84/AE.SQUARROSA(205) |
| 128. OPATA//INQALAB 91/TSAPKI |
| 129. OPATA//DOY 1/AE.SQUARROSA(1026) |
| 130. MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/PASTOR/4/MH-97 |
| 131. PAS/4/MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT |
| 132. PAS/4/MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT |
| 133. MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/6/ YAV_3/SCO//JO69/CRA/3/YAV/79/4/AE.SQUARROSA(498)/5/OPATA |
| 134. MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/FCT /6/YAV_3/SCO//JO69/CRA/3/YAV/79/4/AE.SQUARROSA(498)/5/OPATA |

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| 135.KAUZ/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 136.KAUZ/4/MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD |
| 137.MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/PASTOR/4/SARSABZ |
| 138.BKH-93/4/MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT |
| 139.PASTOR/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 140.PASTOR/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 141.PASTOR/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 142.BKH-93/4/MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT |
| 143.BAV/4/MAYOOR//TK SN1081/AE.SQUARROSA(222)/3/CBRD |
| 144.BKH-94/4/D67.2//P66.270//AE.SQUARROSA(257)/3/OPATA |
| 145.BKH-94/4/D67.2//P66.270//AE.SQUARROSA(257)/3/OPATA |
| 146.CROC-1/AE.SQUARROSA(205)//BORL95/3/ALTAR84 |
| 147.KAMBARA/INQALAB |
| 148.INQALAB/7/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA |
| 149.INQILAB 91/RABI//INQALAB |
| 150.INQALAB/7/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 151.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ) |
| 152.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ) |
| 153.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 154.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 155.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 156.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 157.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 158.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 159.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 160.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 161.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 162.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 163.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 164.CHAPIO/INQALAB 91/4/PICUS/3/KAUZ*2/BOW//KAUZ |
| 165.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/BCN/6/ 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 166.TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/ 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 167.TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/ 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 168.TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/ 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 169.TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA(205)/3/3*BUC/6/CNO/7/ 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 170.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |
| 171.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |
| 172.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |
| 173.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |
| 174.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |
| 175.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |
| 176.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/BCN/4//6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/ |
| 177.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/ PBW-343 |
| 178.MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT/4/PASTOR |

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| 179.MAYOOR/PASTOR |
| 180.MAYOOR/PASTOR |
| 181.MAYOOR/PASTOR |
| 182.MAYOOR/PASTOR |
| 183.MAYOOR/PASTOR |
| 184.CHIRYA/PBW-343 |
| 185.CHIRYA/PBW-343 |
| 186.CHIRYA/PASTOR |
| 187.CHIRYA/Weebill-1 |
| 188.CHIRYA/Weebill-1 |
| 189.CHIRYA/BKH-94 |
| 190.DOY1/AE.SQUARROSA(224)//HANS/PRL |
| 191.ALTAR 84/AE.SQUARROSA(193)//PASTOR |
| 192.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/ BKH-94 |
| 193.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/BKH-94 |
| 194.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/BKH-94 |
| 195.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/BKH-94 |
| 196.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/BKH-94 |
| 197.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/ PBW-343 |
| 198.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 199.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/ PBW-343 |
| 200.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 201.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 202.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 203.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 204.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 205.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 206.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 207.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |

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| 208.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |
| 209.GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA(895)/3/MAIZ/4/INQALAB 91/5/PBW-343 |

## 2.1 Karnal Bunt Screening

Teliospores from various wheat growing areas of Pakistan were collected to guarantee the genetically heterogeneous fungal population. To isolate teliospores, infected kernels were shaken in water-tween-20 solution (2-3 drops of tween 20/100ml of water) for 15seconds, centrifuged at 3,000 rpm and sieved through a 60 micron mesh to remove the kernel residue. These were then surface sterilized with 0.5% sodium hypochlorite by centrifuging for about two minutes, rinsed with sterile distilled water and plated on 15% of water agar for incubation at room temperature. After 5-8 days, germinating teliospores were transferred to potato dextrose agar (PDA). Nine days later, the fungal colonies were scrapped and further incubated on PDA plates.

After another 8-10 days, PDA fungal colonies were cut into small pieces and placed on the lid of sterile glass petri-plates. A small amount of sterile water was added at the bottom of each. Then the allantoids sporidia were counted every 24h using a hemocytometer and spore concentration was adjusted to 10,000/ml. three random tillers were taken from each genotype and inoculated at booting stage.

The inoculation was performed by injecting approximately 1ml of the sporodial suspension with hypodermic syringe into each tiller. After inoculation, tillers were tagged to indicate the date of inoculation. At the maturity of plants, the inoculated spikes were collected from each genotype and graded for infection. Overall percentage of infection was also calculated among all the spikes.

## 3. RESULTS

### a. Scoring Of Karnal Bunt Infected Seeds

It is observed that among all 209 lines, only two lines showed less than 3% infection (which is the International trade limit set for wheat movement/trade and human consumption) on artificial inoculation and hence are resistant to Karnal bunt infection. These lines are genotypes number 39 and 143 showing 2.56% and 2.27% infection respectively. In the rest of 207 lines, mean infection rate was 14.75% with an upper limit of infection of 66.67% (Table 2).

**Table 2: Karnal bunt scoring on the basis of infection**

| S. No. | Total Grains | Infected grains | 0 | 1 | 2 | 3 | 4 | 5 | Disease incidence (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 45 | 5 | 40 | 1 | 2 | 1 | 1 | 0 | 11.11 |
| 2. | 47 | 5 | 42 | 0 | 1 | 2 | 1 | 1 | 10.64 |
| 3. | 39 | 6 | 33 | 0 | 0 | 1 | 3 | 2 | 15.38 |
| 4. | 37 | 2 | 35 | 0 | 0 | 1 | 1 | 0 | 5.41 |
| 5. | 41 | 13 | 28 | 2 | 4 | 3 | 2 | 2 | 31.71 |
| 6. | 32 | 10 | 22 | 3 | 3 | 2 | 0 | 2 | 31.25 |
| 7. | 28 | 5 | 23 | 5 | 0 | 0 | 0 | 0 | 17.8 |
| 8. | 42 | 7 | 35 | 1 | 2 | 1 | 2 | 1 | 16.67 |
| 9. | 37 | 14 | 23 | 4 | 5 | 2 | 3 | 0 | 37.84 |
| 10. | 39 | 5 | 34 | 0 | 1 | 1 | 2 | 1 | 12.82 |
| 11. | 41 | 9 | 32 | 0 | 1 | 1 | 3 | 4 | 21.95 |
| 12. | 37 | 3 | 34 | 1 | 0 | 0 | 0 | 2 | 8.11 |
| 13. | 42 | 2 | 40 | 1 | 1 | 0 | 0 | 0 | 4.76 |
| 14. | 51 | 10 | 41 | 1 | 2 | 2 | 3 | 2 | 19.61 |
| 15. | 39 | 5 | 34 | 0 | 1 | 2 | 1 | 1 | 12.82 |
| 16. | 49 | 12 | 37 | 2 | 2 | 3 | 3 | 2 | 24.49 |
| 17. | 54 | 3 | 51 | 1 | 1 | 0 | 1 | 0 | 5.56 |
| 18. | 57 | 10 | 47 | 3 | 1 | 0 | 2 | 4 | 17.54 |
| 19. | 61 | 23 | 38 | 9 | 3 | 2 | 5 | 4 | 37.70 |
| 20. | 37 | 14 | 23 | 1 | 2 | 4 | 2 | 5 | 37.84 |
| 21. | 47 | 9 | 38 | 0 | 2 | 2 | 2 | 3 | 19.15 |
| 22. | 28 | 11 | 17 | 5 | 0 | 1 | 3 | 2 | 39.29 |
| 23. | 35 | 9 | 26 | 3 | 0 | 2 | 2 | 3 | 25.71 |
| 24. | 29 | 7 | 22 | 5 | 0 | 1 | 0 | 1 | 20.69 |
| 25. | 31 | 10 | 21 | 3 | 2 | 0 | 1 | 4 | 32.25 |
| 26. | 51 | 8 | 43 | 1 | 3 | 1 | 3 | 0 | 15.69 |
| 27. | 21 | 2 | 19 | 2 | 0 | 0 | 0 | 0 | 9.52 |
| 28. | 27 | 2 | 25 | 1 | 1 | 0 | 0 | 0 | 7.41 |
| 29. | 58 | 21 | 37 | 9 | 3 | 0 | 5 | 1 | 36.20 |
| 30. | 62 | 7 | 55 | 2 | 0 | 0 | 3 | 2 | 11.29 |
| 31. | 29 | 6 | 23 | 5 | 0 | 0 | 0 | 1 | 20.69 |
| 32. | 31 | 6 | 25 | 1 | 2 | 0 | 1 | 2 | 19.35 |
| 33. | 29 | 3 | 26 | 0 | 0 | 1 | 2 | 0 | 10.34 |
| 34. | 38 | 14 | 24 | 10 | 4 | 0 | 0 | 0 | 36.84 |
| 35. | 34 | 8 | 26 | 2 | 1 | 1 | 3 | 1 | 23.53 |
| 36. | 51 | 10 | 41 | 3 | 0 | 3 | 3 | 1 | 19.61 |
| 37. | 54 | 6 | 48 | 1 | 0 | 0 | 4 | 1 | 11.11 |
| 38. | 43 | 6 | 37 | 2 | 1 | 1 | 0 | 2 | 13.95 |
| 39. | 39 | 1 | 38 | 0 | 0 | 1 | 0 | 0 | 2.56 |
| 40. | 43 | 4 | 39 | 0 | 0 | 1 | 1 | 2 | 9.30 |

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|---|---|---|---|---|---|---|---|---|---|
| 41. | 48 | 17 | 31 | 0 | 0 | 5 | 5 | 7 | 35.42 |
| 42. | 65 | 6 | 59 | 2 | 3 | 1 | 0 | 0 | 9.23 |
| 43. | 31 | 5 | 26 | 3 | 0 | 0 | 0 | 2 | 16.13 |
| 44. | 26 | 14 | 12 | 3 | 1 | 2 | 3 | 5 | 53.85 |
| 45. | 43 | 2 | 41 | 0 | 0 | 1 | 1 | 0 | 4.65 |
| 46. | 41 | 3 | 38 | 0 | 1 | 1 | 1 | 0 | 7.32 |
| 47. | 39 | 8 | 31 | 1 | 1 | 1 | 1 | 4 | 20.51 |
| 48. | 53 | 9 | 44 | 0 | 1 | 1 | 3 | 4 | 16.98 |
| 49. | 41 | 3 | 38 | 0 | 0 | 0 | 2 | 1 | 7.32 |
| 50. | 46 | 4 | 42 | 1 | 2 | 0 | 0 | 1 | 8.70 |
| 51. | 41 | 3 | 38 | 0 | 1 | 1 | 1 | 0 | 7.32 |
| 52. | 28 | 3 | 25 | 0 | 2 | 0 | 0 | 1 | 10.71 |
| 53. | 49 | 4 | 45 | 0 | 1 | 0 | 0 | 3 | 8.16 |
| 54. | 41 | 4 | 37 | 1 | 2 | 0 | 1 | 0 | 9.76 |
| 55. | 39 | 9 | 30 | 0 | 2 | 3 | 3 | 1 | 23.08 |
| 56. | 40 | 3 | 37 | 0 | 1 | 1 | 1 | 0 | 7.50 |
| 57. | 27 | 4 | 23 | 0 | 1 | 1 | 2 | 0 | 14.81 |
| 58. | 47 | 8 | 39 | 1 | 3 | 1 | 2 | 1 | 17.02 |
| 59. | 49 | 16 | 33 | 2 | 2 | 4 | 4 | 4 | 32.65 |
| 60. | 44 | 6 | 38 | 0 | 2 | 0 | 1 | 3 | 13.64 |
| 61. | 37 | 3 | 34 | 0 | 0 | 0 | 0 | 3 | 8.11 |
| 62. | 51 | 7 | 44 | 3 | 1 | 0 | 1 | 2 | 13.73 |
| 63. | 37 | 6 | 31 | 1 | 0 | 1 | 2 | 2 | 16.22 |
| 64. | 31 | 2 | 29 | 0 | 0 | 1 | 1 | 0 | 6.45 |
| 65. | 59 | 8 | 51 | 3 | 1 | 0 | 3 | 1 | 13.56 |
| 66. | 33 | 3 | 30 | 0 | 1 | 1 | 0 | 1 | 9.09 |
| 67. | 44 | 2 | 42 | 0 | 0 | 1 | 0 | 1 | 4.55 |
| 68. | 37 | 7 | 30 | 0 | 4 | 2 | 1 | 0 | 18.92 |
| 69. | 41 | 2 | 39 | 0 | 1 | 1 | 0 | 0 | 4.88 |
| 70. | 39 | 3 | 36 | 0 | 1 | 1 | 1 | 0 | 7.69 |
| 71. | 37 | 5 | 32 | 0 | 1 | 1 | 1 | 2 | 13.51 |
| 72. | 44 | 4 | 40 | 0 | 3 | 1 | 0 | 0 | 9.09 |
| 73. | 39 | 4 | 35 | 0 | 0 | 1 | 1 | 2 | 10.26 |
| 74. | 37 | 9 | 28 | 0 | 1 | 3 | 2 | 3 | 24.32 |
| 75. | 34 | 3 | 31 | 0 | 1 | 1 | 1 | 0 | 8.82 |
| 76. | 31 | 5 | 26 | 1 | 1 | 1 | 0 | 2 | 16.13 |
| 77. | 37 | 3 | 34 | 1 | 0 | 0 | 0 | 2 | 8.11 |
| 78. | 41 | 9 | 32 | 0 | 1 | 2 | 3 | 3 | 21.95 |
| 79. | 41 | 2 | 39 | 0 | 1 | 1 | 0 | 0 | 4.88 |
| 80. | 47 | 14 | 33 | 1 | 2 | 4 | 5 | 2 | 29.79 |
| 81. | 37 | 4 | 33 | 1 | 3 | 0 | 0 | 0 | 10.81 |
| 82. | 34 | 4 | 30 | 4 | 0 | 0 | 0 | 0 | 11.76 |
| 83. | 44 | 4 | 40 | 0 | 0 | 1 | 2 | 1 | 9.09 |
| 84. | 43 | 4 | 39 | 1 | 1 | 0 | 2 | 0 | 9.30 |

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|---|---|---|---|---|---|---|---|---|---|
| 85. | 22 | 5 | 17 | 3 | 2 | 0 | 0 | 0 | 22.72 |
| 86. | 11 | 6 | 5 | 3 | 1 | 1 | 1 | 0 | 54.54 |
| 87. | 21 | 14 | 7 | 3 | 1 | 3 | 2 | 5 | 66.67 |
| 88. | 22 | 8 | 14 | 1 | 5 | 2 | 0 | 0 | 36.36 |
| 89. | 28 | 11 | 17 | 6 | 4 | 0 | 0 | 1 | 39.28 |
| 90. | 37 | 3 | 34 | 0 | 1 | 1 | 0 | 1 | 8.11 |
| 91. | 47 | 6 | 41 | 1 | 4 | 0 | 1 | 0 | 12.77 |
| 92. | 48 | 3 | 45 | 1 | 1 | 1 | 0 | 0 | 6.25 |
| 93. | 33 | 2 | 31 | 1 | 1 | 0 | 0 | 0 | 6.06 |
| 94. | 31 | 2 | 29 | 1 | 1 | 0 | 0 | 0 | 6.45 |
| 95. | 37 | 3 | 34 | 2 | 0 | 0 | 0 | 1 | 8.11 |
| 96. | 31 | 4 | 27 | 0 | 1 | 1 | 1 | 1 | 12.90 |
| 97. | 43 | 6 | 37 | 0 | 2 | 1 | 1 | 2 | 13.95 |
| 98. | 39 | 3 | 36 | 1 | 1 | 0 | 0 | 1 | 7.69 |
| 99. | 38 | 3 | 35 | 0 | 1 | 1 | 1 | 0 | 7.89 |
| 100. | 29 | 3 | 26 | 0 | 1 | 1 | 0 | 1 | 10.34 |
| 101. | 43 | 3 | 40 | 1 | 1 | 0 | 1 | 0 | 6.98 |
| 102. | 41 | 3 | 38 | 1 | 1 | 1 | 0 | 0 | 7.32 |
| 103. | 39 | 3 | 36 | 2 | 1 | 0 | 0 | 0 | 7.69 |
| 104. | 33 | 3 | 30 | 0 | 1 | 2 | 0 | 0 | 9.09 |
| 105. | 27 | 3 | 24 | 0 | 0 | 0 | 1 | 2 | 11.11 |
| 106. | 48 | 2 | 46 | 1 | 1 | 0 | 0 | 0 | 4.17 |
| 107. | 53 | 2 | 51 | 1 | 0 | 1 | 0 | 0 | 3.77 |
| 108. | 28 | 3 | 25 | 1 | 1 | 0 | 1 | 0 | 10.71 |
| 109. | 31 | 4 | 27 | 0 | 1 | 1 | 1 | 1 | 12.90 |
| 110. | 29 | 5 | 24 | 3 | 0 | 2 | 0 | 0 | 17.24 |
| 111. | 33 | 2 | 31 | 0 | 1 | 1 | 0 | 0 | 6.06 |
| 112. | 17 | 3 | 14 | 0 | 0 | 0 | 1 | 2 | 17.65 |
| 113. | 37 | 3 | 34 | 0 | 2 | 1 | 0 | 0 | 8.11 |
| 114. | 34 | 3 | 31 | 1 | 0 | 0 | 1 | 1 | 8.82 |
| 115. | 35 | 2 | 33 | 0 | 1 | 0 | 1 | 0 | 5.71 |
| 116. | 36 | 4 | 32 | 0 | 0 | 0 | 4 | 0 | 11.11 |
| 117. | 33 | 7 | 26 | 3 | 1 | 1 | 1 | 1 | 21.21 |
| 118. | 28 | 11 | 17 | 6 | 4 | 0 | 0 | 1 | 39.28 |
| 119. | 47 | 9 | 38 | 2 | 1 | 1 | 2 | 3 | 19.15 |
| 120. | 39 | 2 | 37 | 0 | 1 | 1 | 0 | 0 | 5.13 |
| 121. | 43 | 3 | 40 | 1 | 0 | 2 | 0 | 0 | 6.98 |
| 122. | 41 | 3 | 38 | 0 | 1 | 2 | 0 | 0 | 7.32 |
| 123. | 47 | 3 | 44 | 1 | 1 | 1 | 0 | 0 | 6.38 |
| 124. | 39 | 2 | 37 | 1 | 1 | 0 | 0 | 0 | 5.13 |
| 125. | 27 | 1 | 26 | 1 | 0 | 0 | 1 | 0 | 3.70 |
| 126. | 6 | 1 | 5 | 0 | 1 | 0 | 0 | 0 | 16.67 |
| 127. | 21 | 4 | 17 | 1 | 1 | 0 | 1 | 1 | 19.05 |
| 128. | 31 | 2 | 29 | 0 | 1 | 1 | 0 | 0 | 6.45 |
| 129. | 39 | 4 | 35 | 1 | 3 | 0 | 0 | 0 | 10.2 |

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|---|---|---|---|---|---|---|---|---|---|
| 130. | 27 | 2 | 25 | 1 | 0 | 0 | 1 | 0 | 7.41 |
| 131. | 19 | 4 | 15 | 1 | 2 | 0 | 1 | 0 | 21.05 |
| 132. | 33 | 2 | 31 | 1 | 1 | 0 | 0 | 0 | 6.06 |
| 133. | 27 | 3 | 24 | 0 | 1 | 1 | 1 | 0 | 11.11 |
| 134. | 8 | 5 | 3 | 0 | 0 | 2 | 2 | 1 | 62.50 |
| 135. | 6 | 2 | 4 | 0 | 0 | 0 | 1 | 1 | 33.33 |
| 136. | 13 | 4 | 9 | 1 | 0 | 2 | 0 | 1 | 30.77 |
| 137. | 12 | 2 | 10 | 0 | 0 | 0 | 1 | 1 | 16.67 |
| 138. | 21 | 2 | 19 | 0 | 0 | 1 | 0 | 1 | 9.52 |
| 139. | 31 | 9 | 22 | 0 | 1 | 2 | 2 | 4 | 29.03 |
| 140. | 29 | 3 | 26 | 0 | 1 | 2 | 0 | 0 | 10.34 |
| 141. | 28 | 9 | 19 | 0 | 1 | 3 | 2 | 3 | 32.14 |
| 142. | 19 | 4 | 15 | 1 | 1 | 2 | 0 | 0 | 21.05 |
| 143. | 44 | 1 | 43 | 1 | 0 | 0 | 0 | 0 | 2.27 |
| 144. | 31 | 6 | 25 | 2 | 1 | 1 | 2 | 0 | 19.35 |
| 145. | 37 | 4 | 33 | 2 | 0 | 2 | 0 | 0 | 10.81 |
| 146. | 45 | 9 | 36 | 4 | 0 | 0 | 0 | 5 | 20.00 |
| 147. | 43 | 2 | 41 | 0 | 2 | 0 | 0 | 0 | 4.65 |
| 148. | 37 | 2 | 35 | 2 | 0 | 0 | 0 | 0 | 5.41 |
| 149. | 42 | 3 | 39 | 1 | 2 | 0 | 0 | 0 | 7.14 |
| 150. | 41 | 3 | 38 | 2 | 1 | 0 | 0 | 0 | 7.32 |
| 151. | 37 | 2 | 35 | 0 | 2 | 0 | 0 | 0 | 5.41 |
| 152. | 43 | 2 | 41 | 0 | 2 | 0 | 0 | 0 | 4.65 |
| 153. | 41 | 2 | 39 | 1 | 0 | 1 | 0 | 0 | 4.88 |
| 154. | 37 | 4 | 33 | 4 | 0 | 0 | 0 | 0 | 10.81 |
| 155. | 42 | 3 | 39 | 1 | 2 | 0 | 0 | 0 | 7.14 |
| 156. | 41 | 3 | 38 | 2 | 1 | 0 | 0 | 0 | 7.32 |
| 157. | 43 | 10 | 33 | 5 | 3 | 2 | 0 | 0 | 23.25 |
| 158. | 38 | 7 | 31 | 0 | 0 | 2 | 2 | 3 | 18.42 |
| 159. | 43 | 7 | 36 | 2 | 1 | 2 | 0 | 2 | 16.27 |
| 160. | 42 | 2 | 40 | 0 | 1 | 0 | 1 | 0 | 4.76 |
| 161. | 48 | 2 | 46 | 0 | 1 | 1 | 0 | 0 | 4.17 |
| 162. | 41 | 3 | 38 | 1 | 1 | 1 | 0 | 0 | 7.32 |
| 163. | 39 | 3 | 36 | 2 | 1 | 0 | 0 | 0 | 7.69 |
| 164. | 19 | 2 | 17 | 0 | 2 | 0 | 0 | 0 | 10.53 |
| 165. | 17 | 2 | 15 | 0 | 0 | 2 | 0 | 0 | 11.76 |
| 166. | 34 | 7 | 27 | 0 | 1 | 1 | 2 | 3 | 20.59 |
| 167. | 39 | 2 | 37 | 2 | 0 | 0 | 0 | 0 | 5.13 |
| 168. | 28 | 3 | 25 | 3 | 0 | 0 | 0 | 0 | 10.71 |
| 169. | 43 | 4 | 39 | 2 | 0 | 1 | 0 | 1 | 9.30 |
| 170. | 41 | 2 | 39 | 0 | 1 | 1 | 0 | 0 | 4.88 |
| 171. | 32 | 10 | 22 | 3 | 3 | 2 | 0 | 2 | 31.25 |
| 172. | 28 | 5 | 23 | 5 | 0 | 0 | 0 | 0 | 17.8 |
| 173. | 21 | 3 | 18 | 1 | 1 | 1 | 0 | 0 | 14.29 |
| 174. | 49 | 12 | 37 | 2 | 2 | 3 | 3 | 2 | 24.49 |

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|---|---|---|---|---|---|---|---|---|---|
| 175. | 54 | 3 | 51 | 1 | 1 | 0 | 1 | 0 | 5.56 |
| 176. | 57 | 10 | 47 | 3 | 1 | 0 | 2 | 4 | 17.54 |
| 177. | 61 | 23 | 38 | 9 | 3 | 2 | 5 | 4 | 37.70 |
| 178. | 43 | 2 | 41 | 0 | 0 | 1 | 1 | 0 | 4.65 |
| 179. | 27 | 2 | 25 | 1 | 1 | 0 | 0 | 0 | 7.41 |
| 180. | 58 | 21 | 37 | 9 | 3 | 0 | 5 | 1 | 36.20 |
| 181. | 62 | 7 | 55 | 2 | 0 | 0 | 3 | 2 | 11.29 |
| 182. | 29 | 6 | 23 | 5 | 0 | 0 | 0 | 1 | 20.69 |
| 183. | 31 | 6 | 25 | 1 | 2 | 0 | 1 | 2 | 19.35 |
| 184. | 57 | 9 | 48 | 1 | 1 | 2 | 2 | 3 | 15.79 |
| 185. | 62 | 7 | 55 | 3 | 0 | 0 | 4 | 0 | 11.29 |
| 186. | 47 | 2 | 45 | 0 | 0 | 0 | 2 | 0 | 4.26 |
| 187. | 51 | 3 | 48 | 0 | 2 | 1 | 0 | 0 | 5.88 |
| 188. | 31 | 8 | 23 | 1 | 3 | 2 | 2 | 0 | 25.81 |
| 189. | 11 | 2 | 9 | 1 | 1 | 0 | 0 | 0 | 18.18 |
| 190. | 33 | 3 | 30 | 0 | 1 | 2 | 0 | 0 | 9.09 |
| 191. | 39 | 3 | 36 | 2 | 0 | 0 | 1 | 0 | 7.69 |
| 192. | 37 | 2 | 35 | 0 | 0 | 0 | 2 | 0 | 5.41 |
| 193. | 35 | 2 | 33 | 0 | 1 | 1 | 0 | 0 | 5.71 |
| 194. | 23 | 3 | 20 | 1 | 0 | 1 | 0 | 1 | 13.04 |
| 195. | 20 | 2 | 18 | 0 | 0 | 2 | 0 | 0 | 10.00 |
| 196. | 23 | 2 | 21 | 0 | 0 | 2 | 0 | 0 | 8.70 |
| 197. | 33 | 2 | 31 | 0 | 1 | 1 | 0 | 0 | 6.06 |
| 198. | 38 | 7 | 31 | 2 | 2 | 2 | 1 | 0 | 18.42 |
| 199. | 41 | 2 | 39 | 0 | 1 | 1 | 0 | 0 | 4.88 |
| 200. | 44 | 3 | 41 | 0 | 1 | 2 | 0 | 0 | 6.82 |
| 201. | 39 | 2 | 37 | 0 | 1 | 0 | 1 | 0 | 5.13 |
| 202. | 38 | 3 | 35 | 0 | 1 | 0 | 2 | 0 | 7.89 |
| 203. | 31 | 2 | 29 | 0 | 2 | 0 | 0 | 0 | 6.45 |
| 204. | 37 | 3 | 34 | 0 | 1 | 0 | 1 | 1 | 8.11 |
| 205. | 29 | 4 | 25 | 0 | 1 | 2 | 1 | 0 | 13.79 |
| 206. | 37 | 4 | 33 | 0 | 1 | 2 | 1 | 0 | 10.81 |
| 207. | 41 | 3 | 38 | 0 | 0 | 1 | 1 | 1 | 7.32 |
| 208. | 24 | 2 | 22 | 0 | 0 | 0 | 2 | 0 | 8.33 |
| 209. | 19 | 2 | 17 | 0 | 0 | 0 | 0 | 2 | 10.53 |

# 4. DISCUSSION

## 4.1 Screening Of Karnal Bunt Resistance

*Triticumaestivum* breeders have improved wheat adaptation to stress prone environments around the globe that synthetic hexaploids are immune to Karnal bunt infection and 49% of them show 0% infection after artificial inoculation (Villareal*et al.*, 1994). One or both parents of synthetic hexaploids can be resistant to a disease or a pest,

but the level of resistance in synthetic hexaploids against biotic and abiotic stresses may vary. It is reported in previous findings that the resistance to Karnal bunt in synthetic hexaploids is due to *Aegilopstauschii*(DD), the D-genome donor. *Triticum durum* (BBAA) is also found to be resistant to Karnal bunt under field conditions but when inoculated artificially, they may become susceptible to the disease. On the other hand, *Aegilopstauschii*is resistant to Karnal bunt under both natural and artificial inoculation (Mujeeb-Kazi *et al.*, 2006). Since synthetic hexaploids per se cannot become varieties due to their tall height and non-free threshing character, they are used as genetic stocks and their valued primitive traits can be transferred to elite wheat varieties via recombination breeding. Our study is contrasting to Mujeeb-Kazi *et al.*, (2006) as Karnal bunt susceptibility of synthetic hexaploids in this case is highly prevalent.

The development of infection is directly proportional to moisture content and optimum temperature required for the development of disease is 25ºC (Srivastava *et al.*, 2011; Zhang *et al.*, 1984). Environmental conditions were favorable for the development of disease during the present study. Fuentes-Davilla, 1992 discovered that the inoculation of *Tilletiaindica* inoculum in booting stage results in higher level of infection. Our results correlate with his study as higher incidence of disease could probably be due to favorable environmental condition after inoculation for disease progression.

The identification of only two resistant lines from 209 derivatives fall on the poor output side. It probably happened due to advancing bread wheat/synthetic hexaploid $F_1$ combinations that were focused for yield maximization and rust resistance; whereas Karnal bunt screening was not integrated with the breeding generation advances from $F_2$ to $F_9$. Hence, Karnal bunt resistance was not selected for, leading to the present $F_9$ generation derivatives that are devoid of resistance in a high frequency. We suggest that in future, Karnal bunt screening may be integrated with each breeding generation advance from $F_2$ onwards and resistant plants selected.

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**Editorial Office**

Journal of Natural Sciences

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

Sector H-12, Islamabad, Pakistan