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)

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)

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)

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

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

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

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

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

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

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

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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