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# Molecular Characterizations Of BYDV Genes In CIMMYT Elite Wheat-Genotypes

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**ABSTRACT**
Barley Yellow Dwarf (BYD) is an important disease of crop plants that is detrimental to small grains including wheat. Management practices and the application of insecticides can control BYD; however, breeding for resistant cultivars is the most effective and environmentally sound alternative to conventional methods to prevent yield losses. The SSR marker *Xgwm37* is a reliable marker that greatly assists in selecting BYDV-resistant wheat genotypes. Significant variation in the number of lines showed high susceptibility to BYDV between the years (24 in Year 2020 vs. 62 in Year 2021). PCR with specific primers of the marker *Xgwm37* resulted in amplification of products of 175 bp for the *null*, 198 bp for *bdv3* and 290 bp for *bdv2* genes. Molecular marker assisted amplification indicated that the wheat lines with *null* allele was noted in maximum frequency (f = 0.543) followed by *bdv3* allele (f = 0.470). The number of wheat lines containing the *bdv2* allele was 89 (f = 0.297). Similarly, a total of 84 wheat lines (28%) were heterozygous containing different allelic combinations. The visual scoring indicated BYD associated symptoms ranged between 10-65% in the lines containing *null* allele (Mean = 40.0%), whereas minimum infection was noted in lines carrying the *Bdv2* allele (Range = 0-40%; Mean = 22.5%). Similarly, lower BYDV susceptibility was noted in lines containing both *Bdv2*/*Bdv3* alleles, confirming the polygenic nature of resistance. In conclusion, it was observed that predicting years with high disease pressure is difficult; incorporating years with consistent BYD disease data in the selection could increase prediction ability for BYDV resistance and using *Bdv2* as a covariate had increased prediction performance.

**Key Words:** CIMMYT, elite Wheat, BYDV genes, PCR, SSR marker, Resistance.

## INTRODUCTION

Barley Yellow Dwarf (BYD), viruses are detrimental to small grains including wheat and leads to serious economic problems in crop production (Kaddachi *et al*., 2014; Beoni *et al*., 2016), confined to the plant phloem that are transmitted from infected to healthy plants in a persistent circulative non-propagative manner by at least 25 aphid species with varying efficiencies (Gray and Gildow, 2003).

Wheat (*Triticum aestivum*), Oat (*Avena sativa*), Barley (*Hordeum vulgare*), Rye-grass, Rice, and Maize, all belong to the family *Poaceae* are commonly infected with the Barley yellow dwarf virus (BYDV) and observed with significant yield losses (D'Arcy and Domier, 2005). BYDV like symptoms were observed on wheat in the area near Pakistan-Afghanistan border since 1964. However, the infections were confirmed for the first time during 1987 (Aslam and Ahmad, 1987). In Pakistan the most prevalent strain BYDV-PAV was first confirmed serologically during 1997, and was found occurring frequently in the cereal crop during peak aphid BYD activity period in various studies (Bashir *et al*., 1997; Siddiqui, 2011; Ibrahim and Shah, 2015). Due to BYDV infection in a single, variable yield losses have been reported ranging from 5% to 80% depending on the management practices, the prevailing physical environment, the crop and its genetic background (Perry *et al.* 2000; Gaunce and Bockus, 2015). The wide host range and the complex life-style of its vectors make BYD very difficult to manage different management strategies such as culture practices (alternate cropping, sowing date, and removal of virus titer) and the application of insecticides to control vector populations) can help control BYD disease partially, but breeding for resistant cultivars is the most effective and environmentally sound alternative in comparison to the conventional methods (MA and Tomita, 2013; Goutam *et al*., 2015; Jarosova *et al*., 2016).

The most typical symptoms of leaves discoloration (yellow, red, orange and purple) and plants stunting or dwarfing commonly observed in wheat crop caused by BYD viral infection (Bockus *et al*., 2016). Phenotyping barley yellow dwarf in wheat fields is extremely challenging due to symptoms' similarities to other biotic and abiotic stresses (Silva *et al*., 2022). Therefore, identification of molecular markers that could tag the presence of important resistance genes can be used by breeders for marker-assisted selection (MAS), a potential tool that revolutionized the process of plant breeding with acceleration and accuracy to identify the resistance genes rapidly and accurately (Varshney *et al*., 2006; Todorovska *et al*., 2009; Hasan *et al*., 2021). Different types of markers such as random DNA markers, gene targeted markers (Gupta *et al*., 2009) and functional markers (Liu *et al*., 2012) are being used for identification of genes responsible for individual traits. Ayala *et al.* (2001) reported that the SSR marker gwm37 is a reliable marker that greatly assists in selecting BYDV-resistant wheat genotypes. In wheat, two genes (*Bdv1* and *Bdv2*) have been identified for BYDV tolerance (singh *et al*., 1993; Ayala *et al*., 2002). The *Bdv 1* gene is reported to confer tolerance only to BYDV MAV serotype not to all BYDV serotypes. The genes *Bdv 2* and *Bdv 3* originated from *Thinopyron intermedium*, *Bdv 2* confer high level of resistance to GAV, GPV and PAV serotype and the gene *Bdv 3* is thought to confer moderate resistance to BYDV-PAV, and MAV and the gene *Bdv 4* located on chrosome 2 is known to confers resistance to BYDV GAV, GPV and PAGV serotypes (Aradottir and Crespo-Herrera, 2021). In Pakistan no commercial wheat variety is

known to have resistance against BYDV under natural and controlled environment. Therefore, the present study was conducted to track resistance genes (*bdv1* and *bdv2*) in CIMMYT ELITE wheat genotypes along with two local check resistant (Tatara) and susceptible (Fakhr-e-Sarhad) through PCR amplification of *gwm37*SSR marker.

## MATERIALS AND METHODS

For molecular tagging of BYDV resistant genes total 288 CIMMYT wheat genotypes (51st IBWSN) and two local check varieties (Fakhre-sarhad and Tatara) were planted through hand drill planting during the year 2021-2022 in a single row of 2.5m length and separated by 3cm The wheat seeds with pedigree information presented in (Table 1) were obtained from the section of Plant Breeding and Genetics section, the Agriculture University, Peshawar. The seedlings were grown for extraction of DNA.

**Table 1. Pedegree information of CIMMYT International Bread Wheat Screening Nursery (51st IBWSN)**

| Accession# | Pedigree | Accession# | Pedigree |
|---|---|---|---|
| IBWSN-1001 | KACHU # 1 | IBWSN-1042 | KUTZ//KFA/2*KACHU |
| IBWSN-1002 | KUTZ | IBWSN-1043 | KUTZ//KFA/2*KACHU |
| IBWSN-1003 | MUCUY | IBWSN-1044 | FRNCLN/BECARD//KACHU/KINDE |
| IBWSN-1004 | KACHU/SAUAL/4/ATTILA*2/PBW65//PIHA /3/ATTILA/… | IBWSN-1045 | BOKOTA//KFA/2*KACHU |
| IBWSN-1005 | NELOKI//SOKOLL/EXCALIBUR | IBWSN-1046 | BOKOTA//KFA/2*KACHU |
| IBWSN-1006 | WBLL1*2/SHAMA//KACHU/3/KINGBIRD #1//…INGBIRD # 1//… | IBWSN-1047 | QUELEA//MUTUS/AKURI |
| IBWSN-1007 | SAUAL/MUTUS/4/KACHU#1//WBLL*2/KU KNA/3/…CHU #1// WBLL1*2 / KUKUNA/3/… | IBWSN-1048 | CHIPAK//KFA/2*KACHU |
| IBWSN-1008 | ATTILA*2/PBW65*2//KACHU/3/FRNCLN*2/ TECUE #1 | IBWSN-1049 | KACHU//WBLL1*2/BRAMBLING/3/KACHU /KIRITATI |
| IBWSN-1009 | KFA/2*KACHU/3/KINGBIRD #1//INQALAB91*2 /…GBIRD # 1//INQALAB 91*2/… | LOCAL-CHECK | (Fakhr-e-Sarhad)<br>(Tatara) |
| IBWSN-1010 | CN079//PF70354/MUS/3/PASTOR/4/BAV92/ 5/… | IBWSN-1051 | WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRA MBLING/4/… |
| IBWSN-1011 | MUCUY//MUTUS*2/TECUE#1ECUE#1 | IBWSN-1052 | WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRA MBLING/4/… |
| IBWSN-1012 | MUCUY/3/PBW343*2/KUKUNA*2//FRTL/PI FED | IBWSN-1053 | SUP152/AKURI//SUP152/3/MUCUY |
| IBWSN-1013 | BL2064//SW89-5124*2/FASAN/3/TILHI*2/5/KAUZ//… | IBWSN-1054 | SUP152/AKURI//SUP152/3/MUCUY |
| IBWSN-1014 | ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/ 5/… | IBWSN-1055 | SUP152/AKURI//SUP152/3/MUCUY |
| IBWSN-1015 | SUP152/QUAIU #2//BECARD/QUAIU #1UAIU # 2//BECARD/QUAIU # 1ECARD/QUAIU # 1AIU # 1 | IBWSN-1056 | GRACK/CHYAK/6/ROLFO7*2/5/FCT/3/GO V/AZ//MUS/… |
| IBWSN-1016 | KRL 19/QUAIU # 1//BECARD/QUAIU # 1AIU # 1//BECARD/QUAIU # 1 | IBWSN-1057 | BECARD//ND643/2*WBLL1/3/KSW/SAUAL/ /SAUAL |
| IBWSN-1017 | MUNAL*2//WAXWING*2/TUKURU/3/MUC UY | IBWSN-1058 | BABAX/LR42//BABAX*2/3/KUKUNA/4/CR OSBILL #1/… |
| IBWSN-1018 | ND643/2*TRCH//BECARD/3/BECARD/4/SU P152*2/… | IBWSN-1059 | BABAX/LR42//BABAX*2/3/KUKUNA/4/CR OSBILL #1/… |
| IBWSN-1019 | MUTUS*2/KINGBIRD#1/3/KSW/SAUAL//SA UAL# 1/3/KSW/SAUAL//SAUAL | IBWSN-1060 | BABAX/LR42//BABAX*2/3/PAVON7S3, + LR47/4/… |
| IBWSN-1020 | WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2* WBLL1/4/… | IBWSN-1061 | ROLFO7*2/3/ATTILA*2/PBW65//MURGA |
| IBWSN-1021 | ATTILA/3*BCN//BAV92/3/PASTOR/4/… | IBWSN-1062 | BAJ #1*2/BORL14 |
| IBWSN-1022 | BABAX/LR42//BABAX*2/3/KUKUNA/4/CR OSBILL#1/…UNA/4/CROSBILL # 1OSBILL # 1/… | IBWSN-1063 | SAUAL/MUTUS//2*KACHU/KIRITATI |
| IBWSN-1023 | KACHU#1/YUNMAI47//KACHU/4/MUU # 1 // … | IBWSN-1064 | COPIO/3/ATTILA*2/PBW65*2//KACHU/4/N ELOKI |
| IBWSN-1024 | KFA/2*KACHU/3/KINGBIRD#1//INQALAB 91*2/…GBIRD # 1//INQALAB 91*2/… | IBWSN-1065 | SAUAL/YANAC//SAUAL/3/2*KFA/2*KACH U |
| LOCAL-CHECK | PFAU S/SERI/ BOWS (Fakhr-e-Sarhad) JUP/ALD "S" // KLT "S"/3VEE" S" (Tatara) | IBWSN-1066 | ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOV E/BUC*2/6/.. |

| | | | |
|---|---|---|---|
| IBWSN-1026 | TRCH/SRTU//KACHU/3/BORLI14 | IBWSN-1067 | SUP152/BOUK#1/3/PRL/2*PASTOR*2//VORB /4/… |
| IBWSN-1027 | TRCH/SRTU//KACHU/3/BORLI14 | IBWSN-1068 | SUP152/BOUK#1/3/PRL/2*PASTOR*2//VORB /4/… |
| IBWSN-1028 | PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/4/… | IBWSN-1069 | TACUPETOF2001*2/BRAMBLING//WBLL1*2/… |
| IBWSN-1029 | NELOKI//KFA/2*KACHU | IBWSN-1070 | FRANCOLIN#1//WBLL1*2/BRAMBLING/6/WBLL1*2/… |
| IBWSN-1030 | NELKOI//KFA/2*KACHU | IBWSN-1071 | BOKOTA//BECARD/QUAIU #1/3/BOKOTA |
| IBWSN-1031 | BORL14/MUNAL # 1 | IBWSN-1072 | ALTAR84/AE.SQUARROSA (221)//3*BORL95/3/… |
| IBWSN-1032 | BORL14/MUNAL # 1UNAL # 1 | IBWSN-1073 | TRCH/HUIRIVIS#1/3/2*ATTILA*2/PBW65//MURGA |
| IBWSN-1033 | BORL14/FITIS | IBWSN-1074 | SUP152/HUIRIVIS #1//2*BORL14 |
| IBWSN-1034 | BORL14/FITIS | LOCAL-CHECK | Fakhr-e-Sarhad and Tatara |
| IBWSN-1035 | BORL14/FITIS | IBWSN-1076 | SUP152/HUIRIVIS #1//2*BORL14 |
| IBWSN-1036 | BORL14//MUNAL #1/FRANCOLIN # | IBWSN-1077 | SUP152/HUIRIVIS #1//2*BORL14 |
| IBWSN-1037 | BORL14//KFA/2*KACHU | IBWSN-1078 | BECARD/AKURI/3/KACHU//WBLL1*2/BRAMBLING/4/… |
| IBWSN-1038 | BORL14//BECARD/QUAIU #1 | IBWSN-1079 | KSW/SAUAL//SAUAL/3/TRCH/HUIRIVIS #1/5/… |
| IBWSN-1039 | FITIS/3/KACHU #1/KIRITATI//KACHU | IBWSN-1080 | KSW/SAUAL//SAUAL/3/TRCH/HUIRIVIS #1/5/… |
| IBWSN-1040 | KUTZ//KFA/2*KACHU | IBWSN-1081 | KSW/SAUAL/SAUAL/3/2*BORL14 |
| IBWSN-1041 | KUTZ//KFA/2*KACHU | IBWSN-1082 | KSW/SAUAL/SAUAL/3/2*BORL14 |
| | | IBWSN-1083 | KSW/SAUAL/SAUAL/3/2*BORL14 |
| IBWSN-1084 | KFA/2*KACHU*2//SUP152 | IBWSN-1126 | KACHU #1/3/T. DICOCCONP194624/… |
| IBWSN-1085 | KFA/2*KACHU*2//SUP152 | IBWSN-1127 | MERCATO/BECARD//BOKOTA |
| IBWSN-1086 | KFA/2*KACHU*2//SUP152 | IBWSN-1128 | NGL/FRANCOLIN#1//FRNCLN/ROLF07 |
| IBWSN-1087 | KFA/2*KACHU*2//MISR 1 | IBWSN-1129 | FD07072/FRANCOLIN#1/4/ATTILA*2/PBW65//… |
| IBWSN-1088 | KFA/2*KACHU*2//MISR 1 | IBWSN-1130 | BAJ#1/3/SUP152//WBLL1*2/BRAMBLING |
| IBWSN-1089 | KFA/2*KACHU*2/3/ATTILA*2/PBW65//MURGA | IBWSN-1131 | VILLAJUAREZF2009/6/ATTILA/3*BCN//BAV92/3/… |
| IBWSN-1090 | KFA/2*KACHU*2/3/ATTILA*2/PBW65//MURGA | IBWSN-1132 | WBLL1/KUKUNA//TACUPETO F2001/3/BAJ #1/4/… |
| IBWSN-1091 | KFA/2*KACHU*2/3/ATTILA*2/PBW65//MURGA | IBWSN-1133 | ATTILA*2/PBW65//PIHA/3/ATTILA/2*PASTOR/5/… |
| IBWSN-1092 | BORL14*2//KFA/2*KACHU | IBWSN-1134 | FRET2*2/SHAMA//KACHU/3/MUTUS*2/MUU |
| IBWSN-1093 | BORL14*2//MUNAL #1/FRANCOLIN #1 | IBWSN-1135 | PAURAQ/MUCUY |
| IBWSN-1094 | BORL14*2/7/MUU/5/WBLL1*2/4/YACO/PBW65/3/… | IBWSN-1136 | SW89.5277/BORL95//SKAUZ/3/PRL/2*PASTOR/4/… |
| IBWSN-1095 | CN079//PF70354/MUS/3/PASTOR/4/BAV92*2/5/… | IBWSN-1137 | TACUPETOF2001/BRAMBLING//KACHU/8/REH/HARE//… |
| IBWSN-1096 | ROLF07*2DIAMONDBIRD//TRCH/HUIRIVIS #1/3/… | IBWSN-1138 | BORL14/4/BAJ#1/3/KIRITATI//ATTILA*2/PASTOR |
| IBWSN-1097 | WBLL1*2/CHAPIO*2//MURGA/3/2*MIS 1 | IBWSN-1139 | COPIO//KFA/2*KACHU |
| IBWSN-1098 | WBLL1*2/CHAPIO*2//MURGA/3/BECARD/QUAIU #1/4/… | IBWSN-1140 | PBW343*2/KUKUNA*2//FRTL/PIFED/5/ATTILA/… |
| IBWSN-1099 | WBLL1/FRET2//PASTOR*2/3/MURGA/6/KSW/5/… | IBWSN-1141 | PBW343*2/KUKUNA*2//FRTL/PIFED/5/ATTILA/… |
| LOCAL-CHECK | Fakhr-e-Sarhad and Tatara | IBWSN-1142 | BAJ #1/TECUE #1//MUTUS*2/TECUE #1 |
| IBWSN-1101 | WBLL1/FRET2//PASTOR*2/3/MURGA/4/WBLL1*2/… | IBWSN-1143 | KINGBIRD #1//INQALAB 91*2/TUKURU/3/BECARD/… |

| | | | |
|---|---|---|---|
| IBWSN-1102 | KUTZ*2//KFA/2*KACHU | IBWSN-1144 | UP2338*2/SHAMA//2*BAJ # 1/3/BECARD//ND643/… |
| IBWSN-1103 | KUTZ*2//KFA/2*KACHU | IBWSN-1145 | KFA/2*KACHU/3/ATTILA*2/PBW65*2//MURGA |
| IBWSN-1104 | KUTZ*2//KFA/2*KACHU | IBWSN-1146 | KFA/2*KACHU//KACHU/KIRITATI |
| IBWSN-1105 | KUTZ*2//KFA/2*KACHU | IBWSN-1147 | SUP152/BAJ #1/3KINGBIRD # 1//INQALAB 91*2/… |
| IBWSN-1106 | WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/… | IBWSN-1148 | SUP152/BAJ # 1//KFA/2*KACHU |
| IBWSN-1107 | SAUAL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUAL*2/5/… | IBWSN-1149 | SUP152/BAJ # 1//KFA/2*KACHU |
| IBWSN-1108 | KACHU#1/3/380.13/C80.1/3*BATAVIA//2*WBLL1/4/KACHU/… | LOCAL-CHECK | Fakhr-e-Sarhad and Tatara |
| IBWSN-1109 | WBLL1*2/SHAMA//BAJ #1*2/3/BORL14 | IBWSN-1151 | SUP152/BAJ # 1//KFA/2*KACHU |
| IBWSN-1110 | WBLL1*2/SHAMA//BAJ #1*2/3/BORL14 | IBWSN-1152 | SUP152/BAJ#1/3/KACHU///WBLL1*2/BRAMBLING |
| IBWSN-1111 | NADI//TRCH/HUIRIVIS #1/3/NADI | IBWSN-1153 | SUP152/BAJ # 1/5/ATTILA/3*BCN*2//BAV92/3/… |
| IBWSN-1112 | NADI/3/WBLL1*2/SHAMA//BAJ #1/4/NADI | IBWSN-1154 | WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRAMBLING/4/… |
| IBWSN-1113 | WBLL1*2/KURUKU//HEILO/3/WBLL1*2/KURUKU/4/… | IBWSN-1155 | FRANCOLINE#1/3WBLL4/KUKUNA//WBLL1/4/MUNAL/… |
| IBWSN-1114 | WBLL1*2/KURUKU//HEILO/3/WBLL1*2/KURUKU/4/… | IBWSN-1156 | VILLA JUAREZ F2009/CHYAK//MUSTUS/AKURI |
| IBWSN-1115 | MUU/5/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//… | IBWSN-1157 | WBLL1*2/BRAMBLING//CHYAK/3/ONIX/KBIRD |
| IBWSN-1116 | KACHU//WBLL1*2/BRAMBLING/3/2*KFA/2*KACHU | IBWSN-1158 | SUP152*2/TECUE#1/3/ATTILA*2/PBW65*2//MURGA |
| IBWSN-1117 | SUP152/BAJ#1/4/BAJ#1/3/KIRITATI//ATTILA*2/… | IBWSN-1159 | WBLL1*2/BRIMBLING//VORB/FISCAL/3/BECARD/5/… |
| IBWSN-1118 | SUP152/BAJ#1/4/BAJ#1/3/KIRITATI//ATTILA*2/… | IBWSN-1160 | KLEIN DON ENRIQUE * 2 / 3 / FRET2/ WBLL1//… |
| IBWSN-1119 | BAJ#1/3/KIRITATI//ATTILA*2/PASTORS*2/4/… | IBWSN-1161 | BECARD/QUAIU #1//ONIX/KBIRD |
| IBWSN-1120 | BAJ#1/3/KIRITATI//ATTILA*2/PASTORS*2/4/… | IBWSN-1162 | BECARD/FRNCLN//BORL14 |
| IBWSN-1121 | WBLL1*2/BRAMBLING//WBLL1*2/BRAMBLING/3/… | IBWSN-1163 | ATTILA/3*BCN//BAV92/3/PASTOR/4/… |
| IBWSN-1122 | WBLL1*2/BRAMBLING//WBLL1*2/BRAMBLING/3/… | IBWSN-1164 | KACHU/BECARD//WBLL1*2/BRAMBLING/3/MUNAL #1/… |
| IBWSN-1123 | FRANCOLIN#1*2/HAWFINCH#1//2*MUCUY | IBWSN-1165 | KACHU/BECARD//WBLL1*2/BRAMBLING/4/FRET2/… |
| IBWSN-1124 | MUTUS/3/HUW234+LR34/PRINIA//PFAU/WEAVER/4/… | IBWSN-1166 | KACHU/BECARD//WBLL1*2/BRAMBLING/3/FRNCLN*2/… |
| LOCAL-CHECK | Fakhr-e-Sarhad and Tatara | IBWSN-1167 | KACHU/BECARD//WBLL1*2/BRAMBLING/3/FRNCLN*2/… |
| IBWSN-1168 | KACHU/BECARD//WBLL1*2/BRAMBLING/3/KACHU/… | IBWSN-1216 | BORL14*2//KFA/2*KACHU |
| IBWSN-1169 | KACHU/BECARD//WBLL1*2/BRAMBLING/3/KACHU/… | IBWSN-1217 | BORL14*2//KFA/2*KACHU |
| IBWSN-1170 | OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO/5/… | IBWSN-1218 | BORL14*2//KFA/2*KACHU |
| IBWSN-1171 | KUTZ/BORL14 | IBWSN-1219 | BORL14*2//KFA/2*KACHU |
| IBWSN-1172 | FRAME//MILAN/KAUZ/3/PASTOR/4/SOKOLL/5/KACHU/… | IBWSN-1220 | BORL14*2//KFA/2*KACHU |
| IBWSN-1173 | PREMIO//P1 610750/PIFED/4/VORB/FISCAL//… | IBWSN-1221 | BORL14*2//KFA/2*KACHU |
| IBWSN-1174 | WORRAKATTA/2*PASTOR/6/KAUZ/5/PAT10/ALD//… | IBWSN-1222 | BORL14*2//KFA/2*KACHU |
| LOCAL-CHECK | Fakhr-e-Sarhad and Tatara | IBWSN-1223 | BORL14*2//KFA/2*KACHU |
| IBWSN-1176 | WBLL1/KUKUNA//TACUPETO F2001/3/BAJ #1*2/4/… | IBWSN-1224 | BORL14*2//BECARD/QUAIU # 1 |
| IBWSN-1177 | TRCH/SRTU//KACHU/3/2*BORL14 | LOCAL-CHECK | Fakhr-e-Sarhad and Tatara |

| | | | |
|---|---|---|---|
| IBWSN-1178 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1226 | BORL14*2//BECARD/QUAIU # 1 |
| IBWSN-1179 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1227 | CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/… |
| IBWSN-1180 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1228 | CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/… |
| IBWSN-1181 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1229 | COPIO*2/MUCUY |
| IBWSN-1182 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1230 | KACHU#1/KIRITATTI//KACHU*2/3/GRACK/CHYAK |
| IBWSN-1183 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1231 | SAUAL/YANAC//SAUAL/3/2*KUTZ |
| IBWSN-1184 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1232 | SAUAL/YANAC//SAUAL/3/2*KUTZ |
| IBWSN-1185 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1233 | PRL/2*PASTOR*2//FH617*2/3/KFA/2*KACHU |
| IBWSN-1186 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1234 | FITIS*2/3/ATTILA*2/PBW65*2//MURGA |
| IBWSN-1187 | TRCH/SRTU//KACHU/3/2*BORL14 | IBWSN-1235 | FITIS*2/3/ATTILA*2/PBW65*2//MURGA |
| IBWSN-1188 | NELOKI1*2//KFA/2*KACHU | IBWSN-1236 | ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOVE/BUC*2/7/… |
| IBWSN-1189 | NELOKI1*2//KACHU/KIRITATI | IBWSN-1237 | SAUAL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUAL*2/5/… |
| IBWSN-1190 | NELOKI1*2//KACHU/KIRITATI | IBWSN-1238 | MUNAL #1/FRANCOLIN #1*2//KUTZ |
| IBWSN-1191 | BORL14*2/MUNAL # 1 | IBWSN-1239 | MUNAL #1/FRANCOLIN #1*2//KUTZ |
| IBWSN-1192 | BORL14*2/MUNAL # 1 | IBWSN-1240 | SUP152/BLOUK#1*2/4/TUKURU//BAV92/RAYON*2/3/… |
| IBWSN-1193 | BORL14*2/MUNAL # 1 | IBWSB-1241 | WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/… |
| IBWSN-1194 | BORL14*2/MUNAL # 1 | IBWSN-1242 | NADI*2/3/MUTUS/AKURI #1//MUTUS |
| IBWSN-1195 | BORL14*2/MUNAL # 1 | IBWSN-1243 | NADI*2/3/MUTUS/AKURI#1//MUTS |
| IBWSN-1196 | BORL14*2/MUNAL # 1 | IBWSN-1244 | KACHU//WBLL1*2/BRAMBLING*2/3/KACHU/KIRITATI |
| IBWSN-1197 | BORL14*2/MUNAL # 1 | IBWSN-1245 | KACHU//WBLL1*2/BRAMBLING*2/3/KACHU/KIRITATI |
| IBWSN-1198 | BORL14*2//MUNAL#1/FRANCOLIN#1 | IBWSN-1246 | KACHU//WBLL1*2/BRAMBLING*2/3/KACHU/KIRITATI |
| IBWSN-1199 | BORL14*2//MUNAL#1/FRANCOLIN#1 | IBWSN-1247 | KACHU//WBLL1*2/BRAMBLING*2/3/KACHU/KIRITATI |
| LOCAL-CHECK | Fakhr-e-Sarhad and Tatara | IBWSN-1248 | KACHU//WBLL1*2/BRAMBLING*2/3/KACHU/KIRITATI |
| IBWSN-1201 | BORL14*2//MUNAL # 1/FRANCOLIN # 1 | IBWSN-1249 | KACHU//WBLL1*2/BRAMBLING*2/3/KACHJU/KIRITATI |
| IBWSN-1202 | BORL14*2//MUNAL # 1/FRANCOLIN # 1 | LOCAL-CHECK | Fakhr-e-Sarhad and Tatara |
| IBWSN-1203 | BORL14*2//MUNAL # 1/FRANCOLIN # 1 | IBWSN-1251 | SUP152/BAJ #1*2//KFA/2*KACHU |
| IBWSN-1204 | BORL14*2//MUNAL # 1/FRANCOLIN # 1 | IBWSN-1252 | TUKURU//BAV92/RAYON/3/MUNAL#1/4/2*KFA/... |
| IBWSN-1205 | BORL14*2//MUNAL # 1/FRANCOLIN # 1 | IBWSN-1253 | WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRAMBLING*2/4/…BLL1/3/WBLL1*2/BRAMBLING*2/4/… |
| IBWSN-1206 | BORL14*2/3/KBRID//WBLL1*2/KURUKU | IBWSN-1254 | ONIX/KBIRD//BORL14/3/ONIX/KBIRD |
| IBWSN-1207 | BORL14*2/3/KBRID//WBLL1*2/KURUKU | IBWSN-1255 | ONIX/KBIRD//BORL14/3/ONIX/KBIRD |
| IBWSN-1208 | BORL14*2/3/KBRID//WBLL1*2/KURUKU | IBWSN-1256 | STLN/MUNAL #1//2*BORL14 |
| 1BWSN-1209 | BORL14*2/3/KBRID//WBLL1*2/KURUKU | IBWSN-1257 | STLN/MUNAL #1//2*BORL14 |
| IBWSN-1210 | BORL14*2/3/WBLL1*2/TUKURU//CROSBILL #1 | IBWSN-1258 | STLN/MUNAL #1//2*BORL14 |
| IBWSN-1211 | BORL14*2/3/WBLL1*2/TUKURU//CROSBILL #1 | IBWSN-1259 | STLN/MUNAL #1//2*BORL14 |

| | | | |
|---|---|---|---|
| IBWSN-1212 | BORL14*2/3/WBLL1*2/TUKURU//CROSBILL #1 | IBWSN-1260 | STLN/MUNAL #1//2*BORL14 |
| IBWSN-1213 | BORL14*2//KFA/2*KACHU | IBWSN-1261 | STLN/MUNAL #1//2*BORL14 |
| IBWSN-1214 | BORL14*2//KFA/2*KACHU | IBWSN-1262 | STLN/MUNAL #1//2*BORL14 |
| IBWSN-1215 | BORL14*2//KFA/2*KACHU | IBWSN-1263 | STLN/MUNAL#1//KFA/2*KACHU/3/MUNAL #1/… |
| IBWSN-1264 | FRANCOLIN#1/MESIA//MUNAL#1/3/SUP152/… | IBWSN-1283 | WBLL1*2/BRAMBLING//CHYAK*2/3/KING BIRD #1//… |
| IBWSN-1265 | FRANCOLIN#1/MESIA//MUNAL#1/3/SUP152/… | IBWSN-1284 | WBLL1*2/BRAMBLING//CHYAK*2/3/KING BIRD #1//… |
| IBWSN-1266 | ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/5/… | IBWSN-1285 | CHIBIA//PRLII/CM65531/3/MISR 2*2/4/QUAIU/5/… |
| IBWSN-1267 | ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/5/… | IBWSN-1286 | FRET2*2/BRAMBLING//BECARD/3/WBLL1*2/… |
| IBWSN-1268 | ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/5/… | IBWSN-1287 | FRET2*2/BRAMBLING//BECARD/3/WBLL1*2/… |
| IBWSN-1269 | ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/5/… | IBWSN-1288 | INIA CHURRINCHE/KIRITATI*2//KACHU/KINDI |
| IBWSN-1270 | ATTILA/3*BCN//BAV92/3/TILHI/4/SUP152/5/… | IBWSN-1289 | INIA CHURRINCHE/KIRITATI*2//KACHU/KINDI |
| IBWSN-1271 | FRET2*2/BRAMBLING//MESIA/3/BECARD/4/… | IBWSN-1290 | ATTILA/3*BCN*2//BAV92/3/HEILO/4/CHIBIA//… |
| IBWSN-1272 | KIRITATI/WBLL1//MESIA/3/KIRITATI/WBLL1*2/4/… | IBWSN-1291 | MUNAL/HEILO//MUNAL/3/2*BORL14 |
| IBWSN-1273 | KIRITATI/WBLL1//2*BLOUK #1*2/3/BECARD/… | IBWSN-1292 | MUNAL/HEILO//MUNAL/3/2*BORL14 |
| IBWSN-1274 | KIRITATI/WBLL1//2*BLOUK #1*2/3/BECARD/… | IBWSN-1293 | MUNAL/HEILO//MUNAL/3/2*BORL14 |
| LOCAL-CHECK | Fakhr-e-Sarhad and Tatara | IBWSN-1294 | BLOUK#1/MUNAL/3/WBLL1*2/SHAMA//BAJ #1/4/… |
| IBWSN-1276 | SUP152*2/HAWFINCH #1/3/2*KACHU #1/KIRITATI//… | IBWSN-1295 | KACHU#1//WBLL1*2/KUKUNA/3/BRBT1*2/KIRITATI/… |
| IBWSN-1277 | SUP152*2/HAWFINCH #1/3/2*KACHU #1/KIRITATI//… | IBWSN-1296 | OASIS/5*BORL95/5/CNDO/R143//ENTE/MEX175/3/… |
| IBWSN-1278 | SUP152*2/HUIRIVIS #1/3/WBLL1*2/SHAMA//… | IBWSN-1297 | BABAX/KS93U76//BABAX/3/2*SOKOLL*2/4/BECARD/… |
| IBWSN-1279 | SUP152/TECUE #1//SUP152/3/2*BORL14 | IBWSN-1298 | MERCATO//JNRB.5/PIFED*2/3/SAUAL/YANAC//SAUAL |
| IBWSN-1280 | KACHU/KINDE//NELOKI/3/BORL14 | IBWSN-1299 | WHEAR/VIVITSI//WHEAR/3/WHEAR/SOKOLL/4/BORL14 |
| IBWSN-1281 | BAJ #1/PAURAQ/3/KACHU #1/KIRITATI//KACHU/4/… | LOCAL-CHECK | Fakhr-e-Sarhad and Tatara |
| IBWSN-1282 | WBLL1*2/BRAMBLING//CHYAK/3/WAXBI/4/WBLL1*2/… | | |

**Collection of Plant Samples**

During the early growth stages, two to three wheat leaves from each row were ramdomly collected in the zip-lock plastic bags and tagged. The samples were transported to the laboratory in a container filled with ice and stored at -80°C until DNA extraction and PCR analyses were performed.

**DNA Extraction**

DNA extraction was performed by freezing fresh leaves in liquid nitrogen and grinding them to obtain fine powder (Imtiaz *et al.*, 2008). The leaves' powder was homogenized with 1 ml of CTAB (Cetyl trimethyl ammonium Bromide) and followed by phase separation through addition of 1 ml chloroform isoamyl alcohol. The homogenate was shaken for 20 minutes and the upper aqueous phase was transferred to new Eppendorf tubes after centrifugation for 20 minutes. The DNA wasfinally precipitated with isopropanol (1mL) as reported by Shah *et al.* (2010).

**Amplification of BYDV resistant genes**

Standard CIMMYT protocol was used to quantify DNA, prepare agarose gel, perform electrophoresis, perform PCR, and score BYDV resistance gene (CIMMYT, 2005). The SSR marker*Xgwm37* that co segregates with *Bdv2* derived from *Thinopyrum intermedium* (Ayala *et al.*, 2001), further modified using sequence specific information (Ohm and Kong, 2006), was used to identify the translocated lines of wheat. Specific sequence of the primers was Bdv2/Forward: 5'- CTT AAC TTC ATT GTT GAT CTT A -3' and Bdv2/Reverse: 5'- CGA CGA ATT CCC AGC TAA ACT AGA CT -3'. The PCR product with these primers was yield a product of 290 bp and 198 bp respectively linked to the *Bdv* resistance genes, *Bdv2* (on chromosome 7St)

and *Bdv3* (on 7E) from *T. intermedium*. Similarly, a fragment of ~175 bp was amplified in the non-BDV genotypes of wheat. Approximately 100 ng of wheat DNA was amplified in a PCR reaction containing 1 X PCR buffer, 200 µM dNTP each, and 0.2 µM primers each, 1.5 mM $MgCl_2$ and 1 U *Taq*DNA polymerase. The PCR conditions were and initial denaturing step of 94°C for 2 min, 35 cycles of amplification at 94°C for 30 sec, 52°C for 40 sec and 72°C for 60 sec and a final extension step of 72°C for 7 minutes. The PCR product was separated through 2.0 % agarose gel, stained with ethidium bromide and visualized through UV light. Good quality gel pictures were used to score the *bdv2*, *bdv3* or non-bdv resistant genotypes.

**Statistical analysis of the Genotypic Data**

The Presence or absence of the *Thinopyrum intermedium* translocation region in the wheat genotypes were scored manually from the gel and used to calculate alleles and genotype frequencies. Association of the genomic region with BYDV resistance was determined through odd ratios and their corresponding confidence intervals. A p-value of 0.05 or less was considered statistically significant.

**Results**

**Molecular Analysis of BYDV resistance Genes in selected Wheat lines**

So, for four resistance genes, *Bdv1*, *Bdv2*, *Bdv3* and *Bdv4* have been reported in wheat, but the introduction of these genes in commercial cultivars has not been effective. Furthermore, evaluation of resistant sources carrying the *bdv1* and *bdv2* genes suggests a polygenic nature for BYDV resistance. PCR with specific primers of the marker Xgwm37 resulted in amplification of a null allele (A1) of ~ 175 bp, *bdv3* allele (A2) of ~ 198 bp and *bdv2* allele (A3) of ~ 290 bp (Figure 1). Agarose gel pattern of the PCR amplified products indicated that the wheat lines with null allele was noted in maximum frequency (f = 0.543) followed by bdv3 allele (f = 0.470). The number of wheat lines containing the bdv2 allele was 89 (f = 0.297). Similarly, a total of 84 wheat lines (28%) were heterozygous, including 53 containing the A1/A2 alleles (18%), 27 containing A2/A3 alleles (9%) and only 4 lines containing A1/A3 alleles (1%) combinations (Figure 1). Both the check varieties, Tatara and Fakhr-e-Sarhad were noted to carry the null allele (A1).

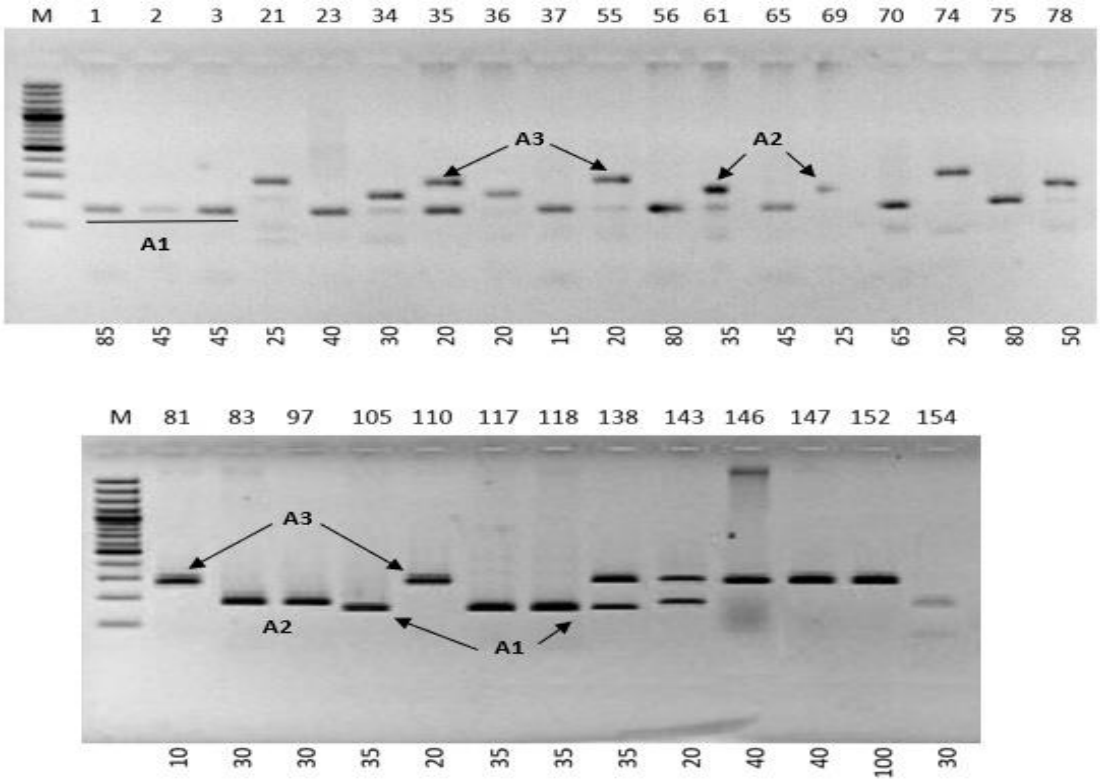


**Fig. 1 Agarose Gel Electrophoresis separated PCR products of the selected BYDV resistant and susceptible IBWSN lines. M= 1000 bp molecular weight marker. The numbers above the gel indicate IBWSN lines/entries from IBWSN-1001 to 1300. Numbers below the gel picture indicate average BYDV susceptibility score.**

**Response of Wheat lines with different Allelic Combination to BYDV infection**

Developing resistant or tolerant wheat cultivars is essential for sustainable control of BYDV infection. The BYDV resistance genes in wheat are lost as they are chosen for other traits, and therefore, careful selection for the presence of resistant genes is required in the early generations. The BYDV infection severity was measured in wheat lines containing different alleles of resistant genes (Figure 2 and 3). The presence of A1 allele was associated with higher BYDV infection. The susceptibility of wheat lines ranged between 10-65% in the lines containing A1 allele (Mean = 40.0%), whereas it ranged between 0-55% in lines without the A1 allele. (Mean = 27.5%). It can be further noted from the data that the lines containing the A2 and A3 allele have a lower BYDV infection (Figure 5.4). Minimum susceptibility to BYDV infection was noted in lines carrying the A3 allele (Range = 0-40%; Mean = 22.5%). In contrast, the BYDV infection in the wheat lines without the A3 allele ranged

between 15-65% (Mean = 37.5%). Similarly, the BYDV infection was significantly lower in lines carrying the A2 allele (Range = 0-60%; Mean = 30.0%) compared with lines without the A2 allele (Range = 0-85%; Mean = 37.5%).

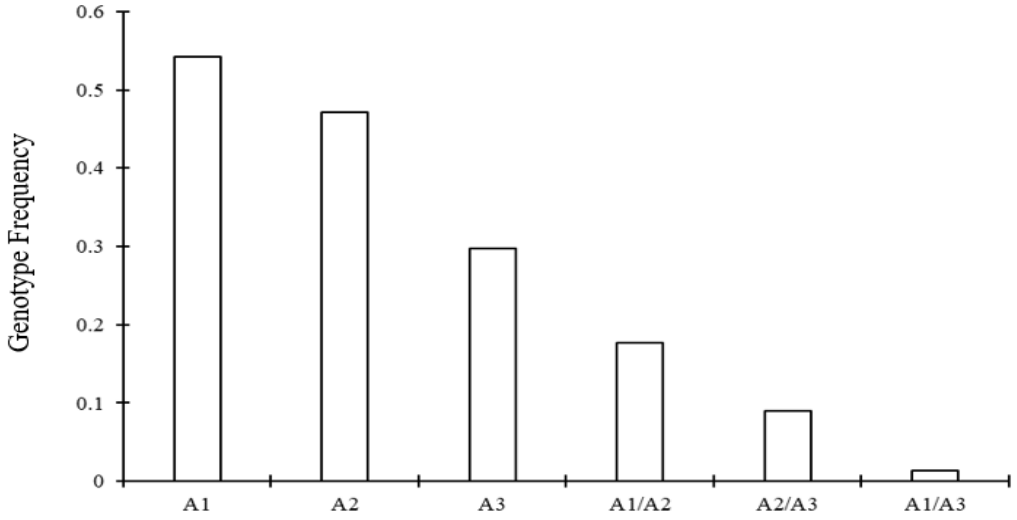


**Fig. 2 Genotypic frequency of the null (A1), *bdv3*(A2) and *bdv2*(A3) loci in the IBWSN lines used in this study**

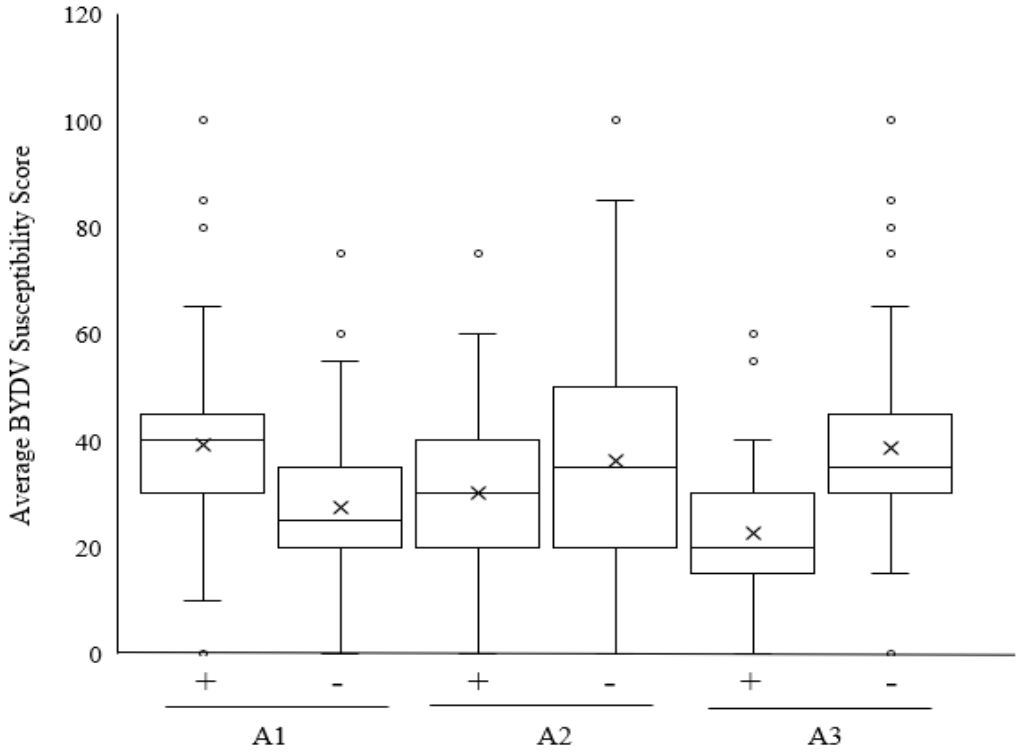


**Fig. 3 Average BYDV resistance score (0-100%) of the IBWSN–wheat lines homozygous for different bdv resistance loci (2021/2022)**

Similarly, significant variation was recorded when the *bdv* gene loci were present in heterozygous combinations. The BYDV susceptibility was higher in the absence of A2 and A3 alleles. The average BYDV susceptibility ranged between 0-60 percent (Mean 27.5%) in lines with A1/A2 genotype, whereas, it ranged between 0-77.5% (Mean = 37.5%). Similarly, lower BYDV susceptibility was noted in A2/A3 and A1/A3 genotypes. The lowest BYDV susceptibility was noted in wheat lines with the A1/A3 genotype, however, fewer genotypes with this allelic arrangement may be making the susceptibility rating biased (Figure 4).

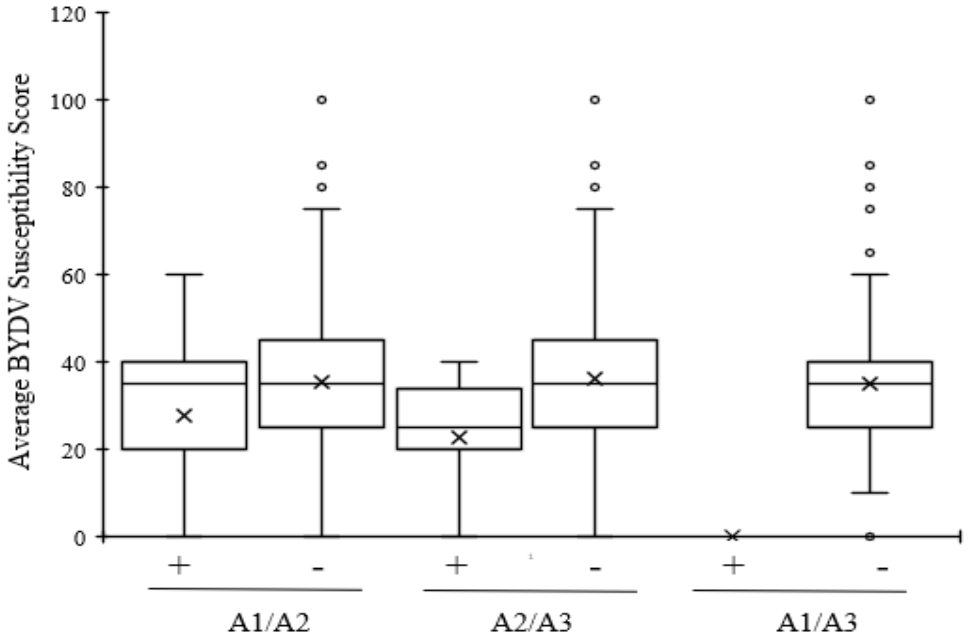


**Fig. 4 Average BYDV resistance score (0-100%) of the IBWSN–wheat lines heterozygous with different alleles of resistant genes (2021/2022).**

**Discussion**

During this study genotypic analysis through molecular markers was conducted to determine BYDV response of wheat genotypes. This study was conducted with the objective to track resistance genes (*bdv1* and *bdv2*) in selected wheat genotypes along with two local check resistant (Tatara) and susceptible (Fakhr-e-Sarhad) varieties through PCR amplification of gwm37 SSR marker.

PCR amplification with *Xgwm37* marker indicated that the wheat lines with null allele (A1) was noted in maximum frequency, followed by *bdv3* allele (A2). The number of wheat lines containing the *bdv2* allele (A3) was 30% of the total screening population. Similarly, 28% of the wheat lines were heterozygous, including 18% containing the A1/A2 alleles, 9% containing A2/A3 alleles and only 1% of the lines containing A1/A3 allele's combinations. During this study, the BYDV infection severity was measured in wheat lines containing different alleles of resistant genes. Minimum susceptibility to BYDV infection was noted in lines carrying the *bdv2* gene (A3 allele). In contrast, the BYDV infection in the wheat lines without the *bdv2* gene significantly higher. Similarly, significant variation was noted when the bdv genes were present in heterozygous combinations and lower BYDV susceptibility was noted in wheat lines with the *bdv2*/*bdv3* genotype. The BYDV susceptibility was higher in the absence of *bdv3* and *bdv2* alleles.

According to (Zhou *et al*., 1990) only four resistance genes have been described for BYD, and as of right now, no significant gene conferring immunity or a high resistant phenotype has been found in bread wheat. A largely effective and dominant gene called *bdv1* is responsible for the tolerance of wheat types like Anza, as shown by Singh *et al.* (1993). Specifically, *Bdv1* has been integrated into CIMMYT germplasm by means of its tolerance gene linkage with the ubiquitous durable leaf rust gene *Lr34* (Ginkel and Henry, 2002). However, it was noted that the gene *Bdv1* only confers tolerance to the BYDV-MAV serotype in specific environments based on field investigations. Wheat germplasm from different geographic origins has also demonstrated some resistance to MAV and BYDV-PAV serotypes, indicating that wheat's resistance is multigenic (Ayala *et al*., 2002). Through extensive crossing from intermediate wheatgrass, the three additional genes-*bdv2*, *bdv3*, and *bdv4*-were introduced into wheat. The initial BYDV resistance gene, *bdv2*, was imported into wheat as an alien chromosome from *Th. Intermedium* (Choudhury *et al*., 2018). But *bdv2* does not offer total protection; it can also have an impact on grain yield and quality (Zhou *et al*., 2015). These findings, which show that complete resistance does not manifest even in the presence of the *bdv2* gene, support our findings. Additionally, the multigenic character of tolerance is consistent with the results of this investigation, which showed that the presence of the *bdv2* and *bdv3* genes increased resistance. Apart from the four resistance genes that are known, genetic mapping has revealed additional genomic areas linked to BYD resistance on almost every wheat chromosome (Jarosova *et al*., 2016; Choudhury *et al*., 2019a, 2019b, 2019c). Genetic characterization of the genes linked to BYDV resistance is still pending. Furthermore, several of these new genomic areas show cumulative effects, according to recent studies (Choudhury *et al*., 2019a, 2019b).

**Conclusions and Recommendations**

In conclusion, this study demonstrated that *bdv2*, which likely mediated the defense response to BYDV, has a major effect controlling resistance in the IBWSN breeding lines and the presence of *bdv3* has an additive effect, resulting in increasing resistance. However, both the local check varieties, Fakhre Sarhad (Susceptible) and Tatara (Resistant) contained the A1 allele, demonstrating that an alternate mechanism is also present in conferring resistance.

Overall, it was observed that predicting Bdv resistance by making selection on data from a single year is complicated; however, incorporating consistent phenotyping over the years against BYDV disease could increase resistance forecasting. In addition, using *bdv2*, as a covariate could further increase selection prediction for BYDV resistance, signifying superior power of the *bdv2* presence as predictor of resistant genotype at early growth stages.

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