Identification of high yielding inbred lines resistant to late wilt disease caused by *Harpophora maydis* in maize

K . Aruna*1, E. Gangappa1, S. Ramesh1 and D. S. Swamy2

1Department of Genetics and Plant Breeding, University of Agricultural Sciences, Bengaluru, Karnataka-560065, India
2Pathology phenotyping, Monsanto India Ltd, Chikkaballapura, Karnataka -561213, India

*E-Mail: arunamysoregpb@gmail.com

**Abstract**

Diseases are the major constraints in realizing the yield potential of maize. Late wilt disease (LWD) caused by *Harpophora maydis* one of the recently reported and widely spreading diseases across the world. Identification of LWD resistant source is an economical and eco-friendly approach. An experiment was conducted to identify LWD resistant inbred lines by subjecting 290 inbred lines to artificial screening. The same set of lines were evaluated for yield and yield attributing traits separately. Inbred lines were subjected to screening by inoculating *Harpophora maydis* spore suspension to stalks. Disease severity and intensity were recorded in split opened stalks using a 1 - 9 scale. Estimates of yield and yield attributing traits were also recorded and 14 inbred lines with the disease score ≤4 were identified as resistant/tolerant. Two inbred lines namely, 78 and 32589 are both tolerant to LWD and best yielding lines which can serve as potential parents for developing hybrids.

**Key words**

Maize, inbred line, late wilt disease, artificial screening, disease score

**INTRODUCTION**

Maize stands third in production among cereal crops. Maize is grown in a wide range of agro-ecologies of the world. It has the highest genetic potential among the cereals. Biotic and abiotic stresses are the major constraints in realizing the yield potential in maize. About 9 per cent yield losses in maize are attributed to diseases alone (Oerke, 2006) which vary from 4 per cent in northern Europe to 14 per cent in West Africa and South Asia. Diseases cause severe yield loss in both quantity and quality of the grain and also increasing the cost of production. In Southeast Asia, hot, humid conditions have favoured disease development while economic constraints prevent the deployment of effective protective measures.

The post flowering stalk rot (PFSR) complex is one of the destructive and widespread groups of diseases in maize (Khokar *et al*., 2014). The disease is known to be associated with many pathogens, majorly, *Fusarium moniliforme*, *Macrophomin aphpelseolina* and *Harpophora maydis* (Shekhar *et al*., 2010). The disease causes internal decay and discoloration of stalk tissue, directly reducing yield by blocking translocation of water, nutrient and can result in death and lodging of the plant. PFSR is a complex disease and involves a number of fungi, bacteria and nematodes in decaying the pith (Cook, 1978). *Harpophora maydis* is one of the fungi involved in PFSR complex. When the maize crop is infected by *H. maydis* alone, it causes late wilt disease (LWD) which is seed-borne and soil-borne (Michail *et al*., 1999; Degani and Cernica, 2014) causing loss upto 51 per cent (Johal *et al*., 2004).

Late wilt disease is characterized by relatively rapid wilting of maize plants typically at the age of 70 to 80 days, before tasselling and until shortly before maturity (Chalkey, 2016). It is considered as endemic in major maize growing areas (Degani and Cernica, 2014). The LWD was first reported...
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in Egypt in 1963 (Samra et al., 1963), subsequently it was reported from different maize growing countries such as Tanzania, Pakistan, Hungary and Kenya (Freeman and Ward, 2004), Egypt and India (Ward and Bateman, 1999), Portugal and Spain (Moliner-Ruíz et al., 2010), Romania (Bergstorn et al., 2008) and Israel (Drori et al., 2013). The disease is distributed widely in the Iberian Peninsula (Ortiz-Bustos et al., 2015).

This nature of disease misleads farmers from taking up plant protection measures. Later, the disease becomes severe leading to yield loss. Among the various methods to address the losses due to diseases, the use of resistant cultivars gains priority (El-shafey et al., 1988). Hence, breeding for resistant cultivar is the need of the hour to combat the losses caused by LWD. In any breeding program, it is pre-requisite to identifying the resistant/tolerant source of disease. With this background, research was conducted to identify inbred lines resistant/tolerant to LWD with high yield.

MATERIALS AND METHODS

The material for this study consisted of 290 inbred lines (Table 1) procured from CIMMYT, IIMR, Zonal Agricultural Research Station (ZARS), Mandya and University of Agricultural Sciences (UAS), Bengaluru along with a resistant check (DKC 9141) and a susceptible check (DKC 9081) procured from Monsanto India Ltd. The same set of 290 inbred lines were grown separately for recording yield and its attributes.

Inbred lines were grown along with a resistant check (DKC 9141) and a susceptible check (DKC 9081) for identifying LWD resistant/tolerant lines during kharif-2016. Whereas, SKV-50, MAI-105 and MAI-137 were used as a check for evaluating yield parameters. Separate experiments were conducted for yield and disease screening. Each inbred line was planted in a single row of 3 m length, with a spacing of 0.6 m between the rows and 0.3 m between the plants within a row. The crop was raised by applying a recommended dose of nitrogen (two split doses) and phosphorous. Potassium was not applied in order to rule out the possibility of ‘Potassium’ conferred resistance of inbred lines. All other production practices were followed as per the recommended package of practice. However, all the recommended practices were followed for the experiment carried out to identify high yielding inbred lines.

Isolation and mass multiplication of H. maydis: Maize stalks showing symptoms typical of LWD were collected from the infected field and were split into small fibrous pieces and surface sterilized using 4 per cent sodium hypochlorite solution. The stalks were then washed twice in sterile distilled water, air dried and plated on 39 per cent Potato Dextrose Agar (PDA) medium in petri plates. Petri plates were incubated for five days in Biological Oxygen Demand (BOD) incubator for the development of the pathogen. The pathogen colonies developed in petri plates were examined for morphology and fruiting body characteristics of H. maydis. Characteristics of typical mycelia of the late wilt pathogen are olivaceous brown with radiating hyphae at borders and the conidia are cylindrical, curved, borne in slimy heads (Gam, 2000). Once the characteristics were confirmed, the mycelia was then placed on PDA for pure culture and sub culturing. The mycelia were aseptically transferred to sterile 24 per cent Potato Dextrose Broth (PDB) in a conical flask for mass multiplication and incubated for 15 days for mycelial mat development. On the 15th day, the mycellial mat was ground and filtered to obtain a pathogen spore suspension.

Table 1. List of inbred lines, their pedigree and source of collection

| Sl No. | Inbred line | Pedigree | Source | Sl No. | Inbred line | Pedigree | Source |
|--------|-------------|----------|--------|--------|-------------|----------|--------|
| 1      | MAI-415     | IIMR     | 17     | MAI-429 | IIMR        |
| 2      | MAI-416     | IIMR     | 18     | MAI-430 | IIMR        |
| 3      | MAI-417     | IIMR     | 19     | MAI-204 | 1232-2 IIMR|
| 4      | MAI-418     | IIMR     | 20     | MAI-431 | IIMR        |
| 5      | MAI711      | Mandya   | 21     | MAI714  | IIMR        |
| 6      | MAI49      | IIMR     | 22     | MAI437  | IIMR        |
| 7      | MAI-420     | IIMR     | 23     | MAI-433 | IIMR        |
| 8      | MAI-421     | IIMR     | 24     | MAI-434 | IIMR        |
| 9      | MAI-422     | IIMR     | 25     | MAI-435 | IIMR        |
| 10     | MAI-423     | IIMR     | 26     | MAI-436 | IIMR        |
| 11     | MAI-424     | IIMR     | 27     | MAI-437 | IIMR        |
| 12     | MAI-425     | IIMR     | 28     | MAI-438 | IIMR        |
| 13     | MAI712     | Mandya   | 29     | MAI714  | IIMR        |
| 14     | MAI-426     | IIMR     | 30     | MAI318  | 2354-1 IIMR|
| 15     | MAI-427     | IIMR     | 31     | MAI-439 | IIMR        |
| 16     | MAI-428     | IIMR     | 32     | MAI-440 | IIMR        |
EJPB

33  40130  MAI-441  IIMR  86  13  MAI-556  IIMR
34  40085b  MAI-442  IIMR  87  MAI334  2570-4  IIMR
35  MAI7  INDIMYT-345  Mandya  88  29  MAI-557  IIMR
36  18715  MAI-443  IIMR  89  32076  MAI-558  IIMR
37  32702  MAI-444  IIMR  90  40  MAI-559  IIMR
38  32225  MAI-445  IIMR  91  MAI215  ZS9-3  CIMMYT
39  40483  MAI-446  IIMR  92  40067  MAI-560  IIMR
40  79  MAI-447  IIMR  93  102  MAI-453  IIMR
41  40369  MAI-448  IIMR  94  40378  MAI-454  IIMR
42  MQ43  MAI-449  IIMR  95  1  MAI-455  IIMR
43  40490  MAI-450  IIMR  96  T20-45  MAI-456  IIMR
44  MAI712  INDIMYT-345  Mandya  97  MAI21  INDIMYT-345  Mandya
45  106b  MAI-451  IIMR  98  24  MAI-457  IIMR
46  40364  MAI-452  IIMR  99  MQPM2  MAI-458  IIMR
47  33018  MAI-531  IIMR  100  40058  MAI-459  IIMR
48  46  MAI-532  IIMR  101  62  MAI-460  IIMR
49  32850  MAI-533  IIMR  102  33154  MAI-461  IIMR
50  MAI380  Z49-102  CIMMYT  103  MAI133  CML-172  IIMR
51  MAI319  Z44-1-4  IIMR  104  40421  MAI-462  IIMR
52  40375  MAI-534  IIMR  105  15  MAI-463  IIMR
53  MA20  MAI-535  IIMR  106  MAI168  SOOTLYQ-HG-B-B-B-36-B-B  IIMR
54  72  MAI-536  IIMR  107  65  MAI-464  IIMR
55  MAI142(w)  CML-338  CIMMYT  108  103a  MAI-465  IIMR
56  MAI224  Z63-16  CIMMYT  109  106a  MAI-466  IIMR
57  18092  MAI-537  IIMR  110  MAI740  INDIMYT-345  Mandya
58  MAI725  INDIMYT-345  Mandya  111  40099  MAI-467  IIMR
59  MAI261  Z49-102  CIMMYT  112  20  MAI-468  IIMR
60  32871  MAI-538  IIMR  113  MAI760  INDIMYT-145  Mandya
61  82  MAI-539  IIMR  114  40073  MAI-469  IIMR
62  85  MAI-540  IIMR  115  31890  MAI-470  IIMR
63  51  MAI-541  IIMR  116  34  MAI-471  IIMR
64  MAI135  CML-41  CIMMYT  117  MAI214  249-87  IIMR
65  MAI298  1554  IIMR  118  40013  MAI-472  IIMR
66  31830  MAI-542  IIMR  119  94a  MAI-473  IIMR
67  31  MAI-543  IIMR  120  32084  MAI-474  IIMR
68  60  MAI-544  IIMR  121  40083  MAI-475  IIMR
69  10269  MAI-545  IIMR  122  MAI138  CML-326  IIMR
70  40058  MAI-546  IIMR  123  MAI170  (CML-165/AMATLCOHS71-1-1-1-1-1-1-B-B-B-B-36-B-B) B-2-2-B-B-CIMMYT
71  MAI724  INDIMYT-345  Mandya  124  22  MAI-476  IIMR
72  97a  MAI-547  IIMR  125  40522  MAI-477  IIMR
73  31810  MAI-548  IIMR  126  26  MAI-478  IIMR
74  96  MAI-549  IIMR  127  2  MAI-479  IIMR
75  10235.27  MAI-550  IIMR  128  MAI726  INDIMYT-345  Mandya
76  32575  MAI-551  IIMR  129  40489  MAI-480  IIMR
77  104  MAI-552  IIMR  130  40376  MAI-481  IIMR
78  MAI13  INDIMYT-345  Mandya  131  67  MAI-482  IIMR
79  31734  MAI-553  IIMR  132  64  MAI-483  IIMR
80  MAI175  CM-132  IIMR  133  MAI250  Z50-3  CIMMYT
81  MAI262  Z49-49  CIMMYT  134  40155  MAI-484  IIMR
82  31956  MAI-554  IIMR  135  40480  MAI-485  IIMR
83  MAI755  INDIMYT-345  Mandya  136  40292  MAI-486  IIMR
84  MAI137  CML-359  IIMR  137  28  MAI-487  IIMR
85  12262  MAI-555  IIMR  138  MAI746  INDIMYT-345  Mandya

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| No. | Variety          | Location | Rating | Variety Code | District | Source |
|-----|------------------|----------|--------|--------------|----------|--------|
| 139 | MAI-562          | IIMR     | 195    | MAI-602      | IIMR     |
| 140 | MAI754           | IIMR     | 196    | MAI-196      | IIMR     |
| 141 | MAI-489          | IIMR     | 197    | MAI-19104    | IIMR     |
| 142 | MAI-490          | IIMR     | 198    | MAI-32541    | IIMR     |
| 143 | MAI491           | IIMR     | 199    | MAI-18       | IIMR     |
| 144 | MAI-492          | IIMR     | 200    | MAI-40065    | IIMR     |
| 145 | MAI-562          | IIMR     | 201    | MAI-3911     | IIMR     |
| 146 | MAI-563          | IIMR     | 202    | MAI-40040    | IIMR     |
| 147 | MAI-564          | IIMR     | 203    | MAI-59       | IIMR     |
| 148 | MAI-565          | IIMR     | 204    | MAI-43210    | IIMR     |
| 149 | MAI-566          | IIMR     | 205    | MAI-40230    | IIMR     |
| 150 | MAI-338          | CIMMYT   | 206    | MAI-3891     | IIMR     |
| 151 | MAI715           | IIMR     | 207    | MAI-501      | IIMR     |
| 152 | MAI-567          | IIMR     | 208    | MAI-280      | IIMR     |
| 153 | MAI-568          | IIMR     | 209    | MAI-502      | IIMR     |
| 154 | MAI-569          | IIMR     | 210    | MAI-202      | IIMR     |
| 155 | MAI-570          | IIMR     | 211    | MAI-758      | IIMR     |
| 156 | MAI-587          | IIMR     | 212    | MAI-93       | IIMR     |
| 157 | MAI322           | IIMR     | 213    | MAI-32809    | IIMR     |
| 158 | MAI-572          | IIMR     | 214    | MAI-53       | IIMR     |
| 159 | MAI-573          | IIMR     | 215    | MAI-316      | IIMR     |
| 160 | MAI338           | IIMR     | 216    | MAI-75       | IIMR     |
| 161 | MAI764           | IIMR     | 217    | MAI-13       | IIMR     |
| 162 | MAI-574          | IIMR     | 218    | MAI-31838    | IIMR     |
| 163 | MAI-769          | IIMR     | 219    | MAI-4002     | IIMR     |
| 164 | MAI211           | CIMMYT   | 220    | MAI-108      | IIMR     |
| 165 | MAI-575          | IIMR     | 221    | MAI-3b       | IIMR     |
| 166 | MAI-576          | IIMR     | 222    | MAI-230      | CIMMYT   |
| 167 | MAI-577          | IIMR     | 223    | MAI-40396    | IIMR     |
| 168 | MAI-578          | IIMR     | 224    | MAI-25       | IIMR     |
| 169 | MAI182           | CML-238-B-B | 225  | MAI-32931    | IIMR     |
| 170 | MAI-579          | IIMR     | 226    | MAI-393      | IIMR     |
| 171 | MAI-580          | IIMR     | 227    | MAI-32865    | IIMR     |
| 172 | MAI-581          | IIMR     | 228    | MAI-18005    | IIMR     |
| 173 | MAI-582          | IIMR     | 229    | MAI-18758    | IIMR     |
| 174 | MAI-267          | CML-238-B-B | 230  | MAI-729      | IIMR     |
| 175 | MAI-583          | IIMR     | 231    | MAI-32645    | IIMR     |
| 176 | MAI-584          | IIMR     | 232    | MAI-23       | IIMR     |
| 177 | MAI-585          | IIMR     | 233    | MAI-40458    | IIMR     |
| 178 | MAI-586          | IIMR     | 234    | MAI-134      | IIMR     |
| 179 | MAI-587          | IIMR     | 235    | MAI-268      | CIMMYT   |
| 180 | MAI-588          | IIMR     | 236    | MAI-56       | IIMR     |
| 181 | MAI-589          | IIMR     | 237    | MAI-275      | CIMMYT   |
| 182 | MAI-590          | IIMR     | 238    | MAI-57       | IIMR     |
| 183 | MAI-591          | IIMR     | 239    | MAI-20       | IIMR     |
| 184 | MAI-592          | IIMR     | 240    | MAI-329      | IIMR     |
| 185 | MAI-593          | IIMR     | 241    | MAI-31888    | IIMR     |
| 186 | MAI-594          | IIMR     | 242    | MAI-751      | IIMR     |
| 187 | MAI276           | CML-238-B-B | 244  | MAI-40333    | IIMR     |
| 188 | MAI-595          | IIMR     | 245    | MAI-77       | IIMR     |
| 189 | MAI-596          | IIMR     | 246    | MAI-35       | IIMR     |
| 190 | MAI-597          | IIMR     | 247    | MAI-40363    | IIMR     |
| 191 | MAI-598          | IIMR     | 248    | MAI-41       | IIMR     |
| 192 | MAI-599          | IIMR     | 249    | MAI-54       | IIMR     |
| 193 | MAI-600          | IIMR     | 250    | MAI-40250    | IIMR     |
| 194 | MAI-601          | IIMR     | 251    | MAI-69       | IIMR     |

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Inoculum load and inoculation technique: Since the pathogen suspension is inoculated to stalks, the spore load plays a critical role in causing the disease. The spore suspension was observed under the microscope and the desired spore concentration of $4 \times 10^6$ spores ml$^{-1}$ was adjusted using Haemocytometer. Whenever the concentration of spore was more, sterile distilled water was used for dilution to obtain the desired spore concentration. Spore concentration @ $4 \times 10^6$ spores ml$^{-1}$ of $H. maydis$ culture was injected into the stalks at the second inter-nodal region from the base of the inbred lines using a medical syringe. Each inbred line was poked to hole and approximately 2 ml of spore suspension was dispensed to stalks of each inbred line at 55 days after sowing (1$^{st}$ inoculation) and 65 days after sowing (2$^{nd}$ inoculation). As a control, one row was injected with water blank and one row was left poked without injecting to have a comparative study.

Responses of inbred lines to LWD: 20-25 days after inoculation, LWD symptoms were observed on the inbred lines. For disease phenotyping, 30 days after inoculation, the stalks of the inbred lines were split opened and disease severity and intensity were recorded on an individual plant basis using a 1-9 scale which takes into account both discoloration of tissues and disintegration of fibres (Rakesh et al., 2016 a). Further, inbred lines were categorized into different response groups (Table 2).

Observations on yield attributing characters viz., days to 50% silking, days to 50% tasseling, anthesis-silking interval, plant height, cob length, cob diameter, kernel rows per cob, kernels per row, grain yield per plant, 100 seed weight and cob shelling per cent were recorded on five randomly selected plants of each inbred line based on counting/measurement using appropriate scale depending on the traits.

Table 2. Classification of inbred lines into different response groups based on their scores of responses to late wilt disease

| Score | Response group of inbred lines |
|-------|-------------------------------|
| 1     | Highly Resistant               |
| >1 to 3 | Resistant                     |
| >3 to 6 | Tolerant                      |
| >6 to 7 | Susceptible                   |
| >7 to 9 | Highly Susceptible             |

RESULTS AND DISCUSSION
The response scores of 290 inbred lines to LWD were subjected to ANOVA. Mean squares attributable to inbred lines, checks and inbred line vs. check were found significant. Out of 290 inbred lines, 7 lines were found resistant; 241 were tolerant; 30 were susceptible and 12 were found to be highly susceptible. However, none of the lines was found to be highly resistant. These inbred lines with the disease score of ≤4 are useful in the breeding programme, as they show lower infection (Mohamed et al., 1966; Rakesh et al., 2016 b). The lack of highly resistant sources among the inbred lines screened indicates the need for creating variability to identify inbred lines resistant to LWD for their commercial exploitation through heterosis breeding. Inbred lines with contrasting responses to LWD could be used to unravel the genetics of LWD resistance by classical phenotype-based and/or marker assisted methods. Based on these results, 14 inbred lines with the ≤4 LWD response score were identified as a resistant source to LWD (Table 3).
In the experiment conducted to evaluate yield and yield attributing traits, considerably good performance was recorded for 14 inbred lines identified as LWD resistant/tolerant (Table 4). Anthesis-silking interval, one of the important traits ranged between -2.60 and 4. Inbred lines, 18092, 32850, 78, 32589 and 76 exhibited least ASI estimates of 0, -0.15, 0.6, 1.2 and 1.2, respectively. ASI is one of the major surrogate traits for drought resistance. Lower the magnitude ASI value irrespective of direction, genotype is said to be more resistant to drought as those types will surpass the flowering, one of the critical stages quickly causing less damage to plant. Hence, inbred lines with resistance/tolerance to LWD and with lower ASI estimates are the valuable inbred lines that can be used in breeding programs.

Table 4. Estimates of grain yield and its component traits of LWD resistant/tolerant inbred lines

| Sl. No. | Genotype | DAS | DAT | ASI | PH | CL | CD | KRC | KR | GYP | 100SW | CS% | LWD Score |
|---------|----------|-----|-----|-----|----|----|----|-----|----|-----|-------|-----|-----------|
| 1       | 78'      | 59.60 | 59.00 | 0.60 | 218.00 | 17.88 | 15.50 | 16.00 | 38.50 | 179.00 | 30.53 | 82.30 | 4.00 |
| 2       | 40105    | 68.00 | 64.00 | 4.00 | 205.00 | 16.60 | 13.90 | 14.00 | 31.20 | 95.00 | 25.50 | 78.13 | 3.87 |
| 3       | 32589'   | 57.60 | 56.40 | 1.20 | 216.00 | 18.32 | 15.04 | 14.80 | 41.80 | 168.40 | 29.10 | 86.98 | 2.75 |
| 4       | MAI-740  | 55.20 | 53.60 | 1.60 | 174.00 | 16.00 | 14.83 | 16.00 | 31.67 | 108.67 | 24.10 | 86.93 | 3.40 |
| 5       | 8a       | 67.50 | 64.50 | 3.00 | 189.00 | 15.50 | 11.75 | 12.50 | 29.75 | 61.00 | 19.50 | 75.54 | 4.00 |
| 6       | 18092    | 57.00 | 57.00 | 0.00 | 195.00 | 15.84 | 13.40 | 14.40 | 40.00 | 89.00 | 20.61 | 79.07 | 4.00 |
| 7       | 30a      | 55.50 | 56.75 | -1.25 | 150.60 | 14.40 | 12.00 | 15.20 | 27.20 | 75.20 | 22.40 | 87.04 | 3.80 |
| 8       | 40376    | 59.00 | 55.75 | 3.25 | 144.60 | 13.08 | 12.58 | 16.50 | 18.80 | 55.60 | 20.34 | 78.09 | 4.00 |
| 9       | MAI-261  | 64.60 | 67.20 | -2.60 | 179.20 | 15.90 | 12.00 | 10.80 | 25.40 | 75.00 | 29.21 | 79.20 | 3.66 |
| 10      | 97b      | 72.20 | 69.80 | 2.40 | 184.00 | 13.10 | 12.70 | 15.60 | 21.40 | 68.20 | 25.80 | 75.78 | 3.00 |
| 11      | 40423    | 60.75 | 58.75 | 2.00 | 161.20 | 15.04 | 14.02 | 15.60 | 31.40 | 122.80 | 26.30 | 85.52 | 4.00 |
| 12      | 76       | 60.00 | 58.80 | 1.20 | 215.50 | 14.63 | 11.50 | 12.50 | 30.25 | 62.75 | 20.60 | 79.43 | 4.00 |
| 13      | 32850    | 58.25 | 58.40 | -0.15 | 176.20 | 19.67 | 13.83 | 11.33 | 41.00 | 142.67 | 32.10 | 82.31 | 3.75 |
| 14      | 40496    | 57.60 | 56.00 | 1.60 | 227.00 | 19.50 | 15.67 | 15.33 | 37.67 | 169.67 | 31.10 | 80.54 | 3.60 |

* - High yielding and LWD resistant/tolerant; Inbred lines DAS- days to silking; DAT – days to tassel; ASI – anthesis-silking interval; PH – plant height; CL – cob length; CD – cob diameter; KRC – kernel rows cob; KR – kernels row; GYP – Grain yield plant; 100SW – seed weight; CS% - cob shelling %.

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genotypes were reported to be resistant among the genotypes screened for LWD response (Sabet et al., 1972; Singh et al., 1986; Satyanarayana, 1995).

Inbred lines identified as resistant/tolerant with disease score ≤4 are the most promising source of resistance to LWD. Further, estimates of yield and yield attributing components of those inbred lines indicate that they are considerably good yielders. Two inbred lines namely, 78 and 32589 are both tolerant to LWD and best yielding lines serve as potential parents for developing hybrids.

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