

## Effectiveness of One Cycle of Phenotype-Based Backcross Breeding for Resistance to Late Wilt Disease in Maize (*Zea mays* L.)

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### ABSTRACT

Late Wilt Disease (LWD) caused by *Harpophora maydis* is one of the serious biotic constraints of maize leading to considerable yield losses. Host-plant resistance breeding is widely accepted as the ideal, eco-friendly and economical strategy to minimize losses attributable to LWD. Backcross selection is widely used method for introgressing LWD resistance into elite agronomic genetic background. LWD tolerant donor parent, CV138811 and susceptible recurrent parent, CV143587 were used to develop BC<sub>1</sub>F<sub>1</sub> plants at Monsanto breeding station. The BC<sub>1</sub>F<sub>1</sub> plants were screened for responses to LWD infection under artificial inoculation condition. Forty three BC<sub>1</sub>F<sub>1</sub> plants significantly different from susceptible parent but similar to resistant parent for LWD tolerance were selected. BC<sub>1</sub>F<sub>2</sub> families derived from 43 selected BC<sub>1</sub>F<sub>1</sub> plants differed significantly. Forty nine per cent of BC<sub>1</sub>F<sub>2</sub> families and 40 per cent of BC<sub>1</sub>F<sub>2</sub> plants transgressed resistant parent for LWD tolerance. Among the transgressed BC<sub>1</sub>F<sub>2</sub> families, four best families homogeneous for LWD tolerance with narrow range and low Phenotypic Coefficient of Variation (PCV) were selected. These results provided adequate evidence for the effectiveness of one-cycle of phenotype-based back cross selection.

*Keywords* : LWD, Back cross, Effectiveness, Phenotype-based selection

MAIZE is an important contributor to the world's agriculture catering to the agro-based industries of food, fodder and fuel. Its production is known to be constrained by around 112 diseases across the globe. Of these, 35 of them threaten maize production in India (Kumar *et al.*, 2013). In particular, Post-Flowering Stalk Rot (PFSR) complex disease is reported to cause production losses of economic proportions in India and other tropical countries (Khokar *et al.*, 2014). *Harpophora maydis* (*H. maydis*) is one among the three devastating pathogens causing PFSR. Despite inducing PFSR complex, *H. maydis* causes Late Wilt Disease (LWD). LWD is characterised by rapid premature wilting of infected plants after flowering. Since the symptoms do not appear until later stages of the crop, it is given the name 'Late Wilt' (Samra *et al.*, 1963). The pathogen is reported to interfere with the seed filling, thus, affecting the seed yield to a considerable extent (Drori *et al.*, 2013). Seed yield losses as high as 70 per cent in Egypt (Galal *et al.*, 1979) while 51 per cent in India (Johal *et al.*, 2004) attributable to LWD have been reported. Genetic

options are considered as the most viable and cost-effective strategy to mitigate production losses attributable to LWD. Susceptible inbred CV143587 has been identified as one of the best general combiners and is being used as parent for some commercial hybrids. Thus, introgression of LWD resistance to its background would enhance its utility. In this regard, objective of the investigation was to introgress genomic regions controlling resistance to LWD from a proven resistant donor to agronomically desired genetic background through one-cycle of phenotype-based backcross breeding procedure and to assess its effectiveness.

### MATERIAL AND METHODS

#### Experimental material

The basic material consisted of one LWD resistant inbred, CV138811 and one LWD susceptible inbred, CV143587. These resistant and susceptible inbreds were crossed to derive F<sub>1</sub> (CV138811 × CV143587) during 2017 rainy season. The F<sub>1</sub> plants were backcrossed to susceptible parent CV143587 during

2017 post-rainy season to derive BC<sub>1</sub>F<sub>1</sub> plants. The BC<sub>1</sub>F<sub>1</sub> plants were screened for responses to LWD infection under artificial inoculation condition during 2018 rainy season.

### Inoculum preparation and artificial inoculation

The LWD infested stalk specimens collected from maize production fields were used for inoculum preparation (Rakesh *et al.*, 2016). The inoculum concentration was assessed using Haemocytometer and adjusted to  $4 \times 10^6$  spores ml<sup>-1</sup>. A hole was made at the second internode of the BC<sub>1</sub>F<sub>1</sub> plants using screw driver at 45 days after sowing (DAS). Two ml of the *H. maydis* inoculum with  $4 \times 10^6$  spores ml<sup>-1</sup> concentration was injected to the holes using syringe. The plants were inoculated twice i.e. at 45 and 55 days after sowing (DAS) at Kallinayakanahalli, Chikkaballapur. The inoculated stalks BC<sub>1</sub>F<sub>1</sub> plants were split opened at 110 DAS and the LWD response was recorded using modified 1-9 scale (Rakesh *et al.*, 2016), where 1=no infection and 9=highly susceptible. 43 BC<sub>1</sub>F<sub>1</sub> plants with scores <5.00 were selected as LWD tolerant and forwarded to BC<sub>1</sub>F<sub>2</sub> during 2018 rainy season. The number of plants in 43 BC<sub>1</sub>F<sub>2</sub> families ranged from 4 to 154 plants. A total of 1570 BC<sub>1</sub>F<sub>2</sub> plants belonging to 43 BC<sub>1</sub>F<sub>2</sub> families were also screened for responses to LWD infection under artificial inoculation condition during 2018 post-rainy season following the protocol (Rakesh *et al.*, 2016) described above.

### Statistical analysis

#### Criteria to assess the effectiveness of backcross selection

The significance of differences between group of LWD tolerant (43) BC<sub>1</sub>F<sub>1</sub> plants and their resistant and susceptible parents were examined using one-way Analysis of Variance (ANOVA) implemented in SPSS software version 16.0. After ascertaining significant differences between 43 LWD tolerant BC<sub>1</sub>F<sub>1</sub> plants and their resistant and susceptible parents, significance of difference between BC<sub>1</sub>F<sub>1</sub> plants and their susceptible parent for LWD scores was determined using critical difference estimated based on error mean squares of one-way ANOVA. The significantly lower

LWD scores of BC<sub>1</sub>F<sub>1</sub> plants (*i.e.*, in favour of tolerance) than those of their susceptible recurrent parent was considered as a criterion to assess the effectiveness of phenotype-based one cycle backcross. Further, one-way ANOVA was performed to detect significance of differences between and within BC<sub>1</sub>F<sub>2</sub> families implemented in SPSS version 16.0. Based on lower mean, narrow standardized range (highest score - lowest score/mean disease score) and low phenotypic coefficient of variation (PCV) of LWD scores, tolerant BC<sub>1</sub>F<sub>2</sub> families were identified. The BC<sub>1</sub>F<sub>2</sub> families and plants with LWD scores < 4.0 (mean LWD score of resistant parents) were considered as transgressive segregants in favour of tolerance.

### RESULTS AND DISCUSSION

#### Effectiveness of phenotype-based backcross selection

One-way ANOVA suggested significant variation between 43 selected LWD tolerant BC<sub>1</sub>F<sub>1</sub> plants and their resistant and recurrent susceptible parents (Table 1). Further, while selected BC<sub>1</sub>F<sub>1</sub> plants were

TABLE 1  
ANOVA BC<sub>1</sub>F<sub>1</sub> with parents

Source of variation	Degrees of freedom	Mean sum of squares	F	P-value
Between BC <sub>1</sub> F <sub>1</sub> plants & their parents	02.00	23.75	47.97	$1.06 \times 10^{-12}$
Within BC <sub>1</sub> F <sub>1</sub> plants & their parents	54.00	0.50		
Total	56.00			

comparable to its resistant parent (CV138811), they were significantly different from recurrent susceptible parent (CV143587) for mean LWD score as evident from critical difference as well as from box-plot (Fig. 1). These results provide reasonably good evidence for effectiveness of phenotype-based one-cycle of back cross followed by selection for resistance to LWD in the genetic background of CV143587. Abalo *et al.* (2009), Korinsak *et al.* (2011) and Willcox

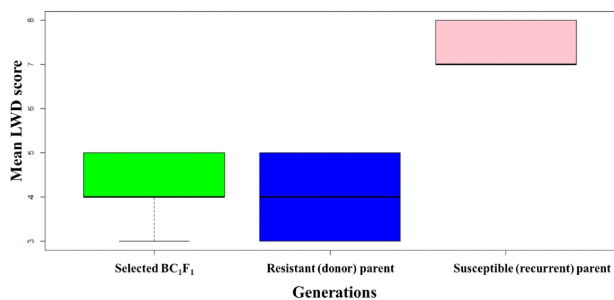


Fig. 1 : Comparison of selected LWD tolerant BC<sub>1</sub>F<sub>1</sub> plants with their susceptible recurrent parent for mean LWD score

*et al.* (2002) also documented the effectiveness of phenotype-based back cross selection for resistance to maize streak virus, rice leaf blast and south-western corn borer, respectively.

With an objective of stabilising and identifying BC<sub>1</sub>F<sub>2</sub> families homogeneous for LWD tolerance, 43 selected tolerant BC<sub>1</sub>F<sub>1</sub> plants were selfed. The 43 BC<sub>1</sub>F<sub>2</sub> families derived from BC<sub>1</sub>F<sub>1</sub> plants differed significantly among themselves for LWD response as suggested by significant mean squares between families (Table 2). As high as 49 per cent of the BC<sub>1</sub>F<sub>2</sub> families transgressed mean LWD score of resistant parent

TABLE 3  
Frequency of BC<sub>1</sub>F<sub>2</sub> plants and families with LWD scores less than resistant parent

Disease score	On individual plant basis	On family basis
< 4	0.40	0.49
< 4 d ≤ 3	0.24	0.40
< 3	0.16	0.09

(4.00) (Table 3). Of these, nine per cent of BC<sub>1</sub>F<sub>2</sub> families showed mean LWD scores less than 3.00. These results offered further scope for selection for LWD tolerance in BC<sub>1</sub>F<sub>2</sub>. Among transgressed BC<sub>1</sub>F<sub>2</sub> families, four best BC<sub>1</sub>F<sub>2</sub> families homogeneous for LWD tolerance were selected based on mean LWD scores less than those of resistant parent, low

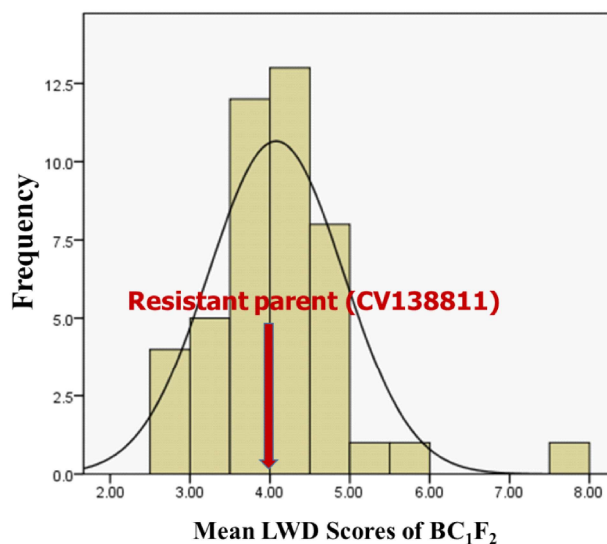


Fig. 2 : Frequency of BC<sub>1</sub>F<sub>2</sub> plants that surpassed limits of resistant parent for response to LWD infection

TABLE 2  
ANOVA for LWD response of BC<sub>1</sub>F<sub>2</sub> families

Source of variation	Degrees of freedom	MSS	'F' statistics	P-value
Between families	40	11.98	5.53	1.29×10 <sup>-24</sup>
Within families	1416	2.16	-	-
Total	1456	-	-	-

TABLE 4  
The best four BC<sub>1</sub>F<sub>2</sub> families with low mean, narrow range and low PCV for LWD scores

BC <sub>1</sub> F <sub>1</sub> ID	BC <sub>1</sub> F <sub>1</sub> LW score	BC <sub>1</sub> F <sub>2</sub> ID	BC <sub>1</sub> F <sub>2</sub> family mean	PCV	Range		Standardised range
					Lowest	Highest	
1_5	5.00	197	2.86	1.97	2.00	4.00	0.70
1_63	5.00	1216	2.90	2.54	2.00	4.00	0.69
1_64	5.00	1241	3.50	2.02	3.00	4.00	0.29
1_67	5.00	1250	2.50	1.77	2.00	4.00	0.80

standardised range and low PCV (Table 4). Among the transgressed BC<sub>1</sub>F<sub>2</sub> families that were homogeneous for LWD tolerance, as high as 40 per cent of the plants surpassed the resistant parent for LWD tolerance and 16 per cent of the plants showed fairly higher tolerance levels than the resistant parent with < 3.00 LWD score (Table 3; Fig. 2). These 16 per cent transgressive segregants are being selfed to identify homozygous and homogeneous BC<sub>1</sub>F<sub>3</sub> plants for further evaluation and confirmation of LWD tolerance.

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