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RESEARCH ARTICLE

Genetic characterization and comparative assessment of response of biparental and multi-parental populations to northern corn leaf blight in maize (*Zea mays* L.)

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Abstract

To compare the efficiency of multiparental and biparental progenies to northern corn leaf blight (NCLB) resistance, F_3 progenies were evaluated during two seasons. A total of 418 F_3 progenies derived from multiple founder parents and 248 F_3 progenies derived from two parents, namely, SKV50 (Resistant) and CM202 (Susceptible) were phenotyped for their response to the NCLB during summer and *kharif* 2020. A wide range of disease severity was observed among F_3 progenies derived from multiple parents ranging from 28.12 to 62.93%. The difference in the variances between multiparent and biparental progenies was indicated by the significance of Levene's test. High genotypic and phenotypic coefficient of variation was observed among multi-parental progenies than those derived from the biparental population. Reaction to NCLB showed moderate heritability of 57% and high genetic advance as per cent mean (20.70%) in the case of biparental population, whereas, multi-parental population exhibited a high heritability (68%) and high genetic advance as per cent mean (28.57%). The distribution of F_3 progenies was found to be positively skewed and platykurtic indicating that the trait was under the control of large number of genes with complementary gene action.

Keywords: Maize, northern corn leaf blight, biparental population, multi-parental population, variance

Introduction

Maize (*Zea mays* L.) is the most productive crop worldwide with a global production of 1116.21 million tonnes and average productivity of around 5.78 t/ha. In India, the crop is cultivated in an area of 9.47 million hectares with production and productivity of 28.72 million tonnes and 3032 kg/ha, respectively. The difference in maize productivity between India and the world is 190%. The production of maize is constrained by an increased incidence of various biotic and abiotic stresses in recent years. Among various biotic stresses, Northern Corn Leaf Blight (NCLB), also known as *Turcicum* Leaf Blight (TLB), is the most important disease of maize caused by hemi-biotrophic pathogen, *Setosphaeria turcica* anamorph *Exserohilum turcicum* formerly known as *Helminthosporium turcicum* [Pass] Leonard and Suggs. Maize plants have evolved with both qualitative and quantitative resistance to combat this pathogen.

Populations that were developed for deriving elite inbred lines and phenotypic characterization have primarily used biparental populations derived from two founder parents. Biparental populations are often chosen due to their ease of development. Major weakness of biparental populations includes limited opportunity for effective recombination, which may lead to an insufficient amount of genotypic and phenotypic variation (Mahan et al. 2018). Hence, there is a need to develop more sophisticated and systematic design to create and capture the greater amount of variation for NCLB disease resistance.

The limited genetic variation of bi-parental populations

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was addressed via the concept of multi-parental intercross populations to increase allelic diversity and to capture more recombination fraction (Huang et al. 2015). Multi-parental population provides greater phenotypic variability than biparental populations due to additional alleles and an additional round of intermating to reshuffling of alleles from breaking of more linkages (Della'Aqua et al. 2015; Mahan et al. 2018). Recent studies on multi-parental populations have revealed that they can contribute significantly in the development of elite inbred lines and improvement in genetic advance (Han et al. 2019; Zaw et al. 2019). With this background, an attempt was made to develop multi-parental population employing eight elite inbred lines and to assess and compare the efficiency of multi-parental population with biparental population in terms of amount of phenotypic variability released for resistance to NCLB.

Materials and methods

Plant material and field experiments

Our investigation was based on eight elite maize inbred lines (Table 1) differing in their response to NCLB. The funnel approach was followed to develop a multi-parental population (Huang et al. 2015). Initially, the eight parents were crossed to derive four single cross hybrids and obtain two 4-way cross hybrids. Finally, A total of 36 crosses were made between the 60 individuals of two different 4-way cross hybrids to obtain 8-way cross hybrid. Further plants in the 8-way cross hybrid were inter crossed in pairs among themselves to capture one more round of recombination and intercrossed seed bulked. Then these intercrossed progenies were selfed to obtain 450 F_2 plants, and thereby 418 F_3 progenies were obtained (Fig. 1).

Simultaneously, the biparental population was also developed comprising SKV50 (Resistant to NCLB) and CM202 (Susceptible to NCLB). Initially, two parents were crossed during Summer-2017 to obtain F_1. In subsequent seasons, the F_1 was selfed to obtain F_2 and finally to obtain 248 F_3 progenies. Then multi-parental and biparental populations were screened for two seasons for their response to the NCLB during Summer-2020 and *Kharif*-2020. The alpha-lattice design (Patterson and Williams 1976) with two replications was employed to constitute the field trials at the National disease screening nursery for Northern corn leaf blight, V.C. Farm, Mandya, Karnataka, India, by artificial inoculation at 30 days after sowing. Each F_3progeny was sown in a single row of 2 m length with an interrow spacing of 0.6 m and interplant spacing of 0.2 m. The parental inbreds, CM 202 (Susceptible check) and SKV 50 (Resistant check), were planted after every 10th row of test entries to ensure disease uniformity.

Mass multiplication and inoculation of Exserohilum turcicum

The artificial inoculation method followed was using heavily infected leaves with *Exserohilum turcicum* collected in the previous season. These leaves were stored in loosely packed gunny bags in a cool and dry place. Prior to inoculations, the leaves were ground coarse by milling. A pinch of the leaf powder was dropped into the whorl of each plant at 30 DAS. This was followed by a spray with water using a knapsack sprayer, while directing the spray to the whorl (Hooda et al. 2018).

Another effective artificial inoculation method followed was by using infected sorghum grains 10 days after the first inoculation to ensure uniform disease development. About an inch layer of sorghum grains (nearly 40 to 45 g) was soaked in water in a conical flask (500 mL) for about 3-4 hours and excess water was drained off. The flasks containing sorghum grains were autoclaved and inoculated with fungus under aseptic conditions and kept for incubation at 25–27°C. The flasks were shaken once in 2-3 days to facilitate uniform growth of mycelium on sorghum grains. After incubation for about a fortnight the sorghum grains were completely covered with mycelium and the material was ready for inoculation. The above-impregnated sorghum grains were dried on a clean paper sheet in the shade at room temperature. After drying, these grains were ground into a fine powder with the help of mixer-grinder. A pinch of powder was directed into the plant's whorl followed by spraying 10-12 mL of water in the whorls by means of sprayer to maintain adequate moisture for longer period to permit spore germination. Inoculation was carried out in late afternoon (3-6 PM) to avoid the maximum day temperature during the incubation period (Hooda et al. 2018).

Phenotyping of multi-parental and biparental populations for their response to NCLB

The NCLB symptoms started developing after a week of artificial inoculation. However, symptoms became prominent only after reproductive phase. Disease development was recorded twice; the first score was taken at 60 days of crop, and the second or final score was taken on 75th day. The NCLB scoring was recorded using 1–9 scale (Chung et al. 2010; Mitiku et al. 2014) (Table 2) and per cent disease severity was calculated using the formula,

$$\text{Per cent disease severity} = \frac{\text{Sum of the individual disease ratings}}{\text{Total no. of plants observed} \times \text{Maximum grade}}$$

Statistical analysis

The disease response data obtained on 248 F_3 biparental progenies and 418 F_3 multi-parental progenies along with two parents for NCLB over two seasons (Summer and *Kharif* 2020) were subjected to arcsine transformation (Little and

Hills 1978) to make the means and variances independent and normally distributed. The analysis of variance was performed on transformed phenotypic data using PBIB package (Kaur et al. 2017) in R-Studio. Before pooling the data of two seasons, Bartlett's test was conducted to test for homogeneity between environments (Gomez and Gomez 1984). Pooled analysis of variance was performed in R-Studio using METAN (Multi Environment Trials Analysis) package (Olivoto et al. 2020).

Transformed values were subjected to calculate the descriptive statistical parameters like mean and range as per Patterson and Williams (1976). Phenotypic and genotypic coefficient of variation were assessed as per Burton and DeVane (1953) and classified based on Robinson et al. (1949); heritability (broad sense) was calculated based on Lush (1945) and classified based on Robinson et al. (1949); Genetic advance as per cent of mean was assessed and classified based on Johnson et al. (1955). Skewness, the third-degree statistic and kurtosis, the fourth-degree statistic were estimated as per Snedecor and Cochran (1994) using the SPSS software program to understand the nature of distribution of F_3 progenies for NCLB disease reaction. Levene's test (Levene, 1960) was performed to test the significance of variance between the multi-parental and biparental populations.

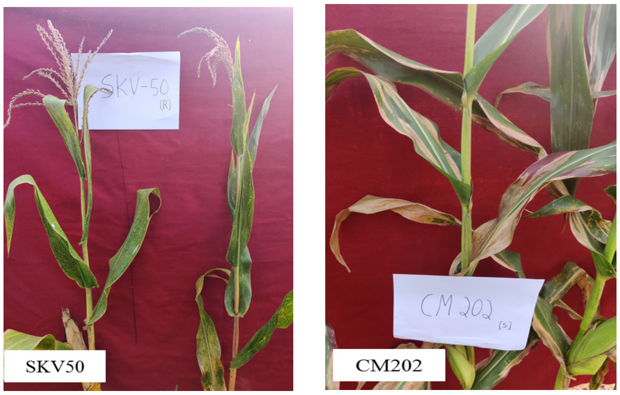

Fig. 1. Disease reaction of resistant (SKV50) and susceptible (CM202) founder parents against NCLB

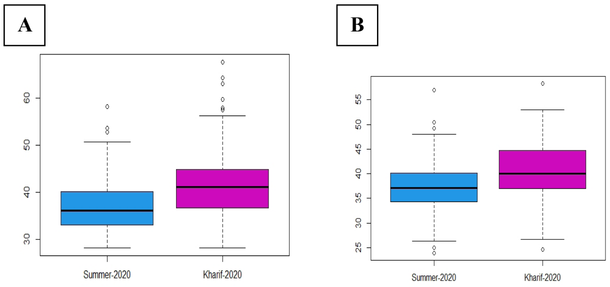

Fig. 2. Boxplot depicting the per cent disease severity of NCLB across two seasons in multi-parental population (A) and bi-parental population (B)

Results and discussion

In maize breeding programme, the use of a multi-parental population offers several advantages over traditional method of deriving inbreds using two founder parents like, increased allelic diversity and captures more recombination fraction, which in turn generates more variability. To combat the emerging disease namely Northern corn leaf blight, which causes grain yield losses ranging from 50% when infection occurs at the grain-filling stage (Human et al. 2016) to 100% when infected at the seedling stage (Hooda et al. 2016). In this backdrop, 418 multi-parental along with 248 biparental F_3 progenies were developed and subjected to artificial screening against Northern corn leaf blight disease across two seasons.

Weather conditions in the National disease screening nursery at Mandya favored the development of NCLB disease. *Kharif* 2020 season was more favorable for disease development compared to summer 2020. Susceptible check (CM202) was completely infected by the NCLB, indicating the effectiveness of inoculation method. The Analysis of variance (ANOVA) was performed on the arcsine transformed mean% disease severity as a diagnostic step to detect the presence of genetic variability among the F_3 progenies. Mean squares attributable to F_3 progenies

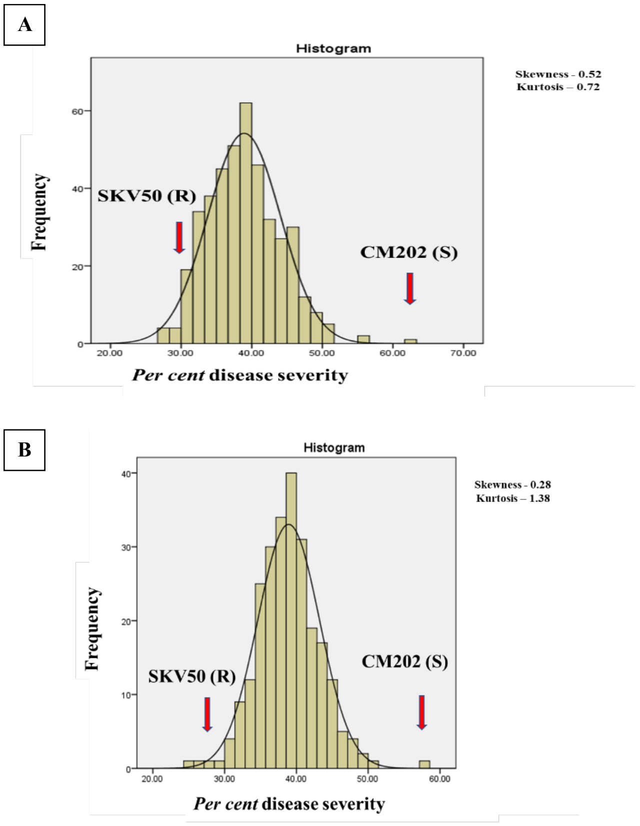

Fig. 3. Frequency distribution of per cent disease severity for NCLB disease across two seasons in multi-parental (A) and bi-parental (B) F_3 progenies

Table 1. List of maize inbred lines used in the development of multi-parental population

S. No.	Inbred	Pedigree details	Characteristic features
1	PDM-4341	(Comp8551 X Comp 8527 x Ageti76 X MDR) -9- 4-2-8-7-1-1-2-1-L-1-1	Significant GCA effects for grain yield (Kg/ha)
2	VL109545	[CL-G2501xCML170]-B-2-3-2-BB-3-BB	Resistant to Northern corn leaf blight (NCLB)
3	CML451	((NPH-28-1/G25)/NPH-28-1)-1-2-1-1-3-1-B	Drought tolerant
4	CAL1443	(CTS013008/AMATLC0HS71-1-1-2-1-1-1-B*5/Nei402020)-B*5	Resistant to NCLB
5	CM 212	USA/ACC No.2132 (Alm)-3-2-f-#-13-#- -bulk	Resistant to Fusarium stalk rot (FSR)
6	SKV50	SKV-50 (population 147-F2 # 89-3-2-B-1-B)	Resistant to NCLB, Sorghum downy mildew (SDM), Polysora rust (PR)
7	CAL1518	SW5-10-B*5-2	Significant GCA effects for test weight
8	CM 202	C121 (EARLY)	Susceptible to NCLB, SDM but resistant to FSR

Table 2. Scoring of disease severity of Northern corn leaf blight (0-9 scale)

Rating scale	Degree of infection (% diseased leaf area)	PDI	Reaction
1.	Nil to very slight infection (≤10%).	≤ 11.11	Resistant (Score: ≤ 3.0) (PDI: ≤ 33.33)
2.	Slight infection, a few lesions scattered on two lower leaves (10.1-20%)	22.22	
3.	Light infection, moderate number of lesions on four lower leaves (20.1-30%)	33.33	
4.	Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%)	44.44	Moderately Resistant (Score: 3.1-5.0) (PDI: 33.34-55.55)
5.	Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%)	55.55	
6.	Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and few lesions on two leaves above the cob (50.1-60%)	66.66	Moderately susceptible (Score: 5.1-7.0) (PDI: 55.56-77.77)
7.	Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%)	77.77	
8.	Very heavy infection, lesions abundantly scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%)	88.88	Susceptible (Score: > 7.0) (PDI: > 77.77)
9.	Very heavy infection, lesions abundantly scattered on almost all leaves, plants prematurely dried or killed (>80%)	99.99	

Table 3. Pooled ANOVA of mean NCLB disease severity in multi-parental population

Source	df	MSS	Pr > F
Env	1	6998.8***	<0.001
F_3 Progenies	419	106.2***	<0.001
F_3 Progenies × Seasons	419	36.0***	<0.001
Error	820	20.2	

***Significant at P = 0.001

Table 4. Pooled ANOVA of mean NCLB disease severity in bi-parental population

Source	df	MSS	Pr > F
Env	1	2364.0***	<0.001
F_3 Progenies	249	74.3***	<0.001
F_3 Progenies × Seasons	249	32.0***	<0.001
Error	480	19.9	

***Significant at P = 0.001

Table 5. Genetic estimates of multi-parental and biparental F_3 progenies for NCLB disease across two seasons

Genetic parameters	Multi-parental population	Biparental population
Mean	38.92	39.00
Range	34.81	32.85
Phenotypic coefficient of variation	20.42	17.59
Genotypic coefficient of variation	16.84	13.37
Broad sense heritability	0.68	0.57
Genetic advance as per cent Mean	28.57	20.70
Skewness	0.52	0.28
Kurtosis	0.72	1.38

were found to be highly significant (p-value = <0.001) in both summer 2020 and *kharif* 2020 seasons, indicating significant differences among the F_3 progenies for NCLB disease reaction and this variability can be further utilized in maize breeding programme. Before performing the pooled ANOVA, we performed Bartlett's test to verify the homogeneity between seasons, and the test was found to be non-significant with Bartlett's K-squared = 6.108 and p-value = 0.013, which confirmed the homogeneity of error variances between summer and *kharif* 2020 seasons. In the pooled ANOVA mean squares attributable to F_3 progenies, seasons and their interactions were found to be highly significant (Tables 3 and 4), indicating that the expression of disease incidence significantly varied among F_3 progenies and disease incidence depends upon testing season (Wang et al. 2018).

The response of F_3 multi-parental and biparental progenies for per cent disease severity across two seasons is depicted in box plot (Fig. 2). The range estimation denotes the occurrence of NCLB disease with extreme expression in F_3 progenies. The highest per cent disease severity was found to be 62.93 per cent in multi-parental population and 57.67% for biparental population and the lowest disease severity was found to be 28.12% and 24.82% in multi-parental population and biparental population, respectively. The range was comparatively higher in multi-parental population (34.81) than biparental population (32.85) (Table 5), which indicated the higher variation in the response of F_3 progenies to NCLB disease.

The phenotypic value is attributable to the sum of genotypic value and the environmental deviation across and within seasons. Genotypic coefficient of variation (GCV) was found to be 16.84% and 13.37% for multi-parental and biparental populations, respectively (Table 5). The estimates of phenotypic coefficient of variation (PCV) were found to be high (20.42%) in multi-parental populations and moderate (17.59%) in biparental population. Levene's test was performed to test the homogeneity of variance released by the multi-parental and biparental populations. The test was significant, confirming that the variances were not equal (Table 6). Both GCV and PCV were higher in multi-parental population than biparental population because the inclusion of multiple parents which incorporated additional alleles and subsequent generations of intermating, resulted in greater genotypic and phenotypic variation (Mahan et al. 2018).

The extent of contribution of genotype to the phenotypic variation for a trait in a population is expressed as the ratio of genotypic variance to the total variance known as heritability (Rojas and Sprague 1952). Heritability values help predict the expected progress to be achieved through selection process. Genetic advance explains the degree of the gain obtained in a character under particular selection pressure. Moderate heritability (57%) and high genetic advance as per cent mean (20.70%) were obtained for% disease severity in biparental population (Table 5) whereas, in the case of multi-parental population, high heritability (68%) coupled with high genetic advance as per cent mean (28.57%) were observed. The heritability for resistance to NCLB was reported to be moderate to high (Kistner et al. 2022; Welz and Geiger 2000; Ding et al. 2015). High genetic advance coupled with high heritability estimates offers the most suitable condition for selecting NCLB-resistant progenies (Asea et al. 2012). It also indicated the predominance of additive effects in the genetic control of resistance to NCLB and further suggested the effectiveness of selection in breeding for NCLB resistance.

The theoretical basis of the usefulness of skewness for explaining the genetic causes for variation in a trait was outlined by Fisher et al. (1932). The distribution study using skewness and kurtosis provides information about the nature of gene action (Fisher et al. 1932) and the number of genes controlling the trait (Robson, 1956), respectively. Positive skewness is associated with complementary gene interactions, while negative skewnessis associated with duplicate gene interactions. The distribution of F_3 progenies for the NCLB disease reaction was found to be positively skewed with the coefficient of skewness value 0.52 in multi-parental population and 0.28 in biparental population (Fig. 3), indicating that the resistance to NCLB was found to be controlled by complimentary gene action (Wang et al. 2018; Xia et al. 2020; Ranganatha et al. 2021).. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait.

In both populations, platykurtic distribution (< 3.0) with the value of 0.72 in multi-parental population (Fig. 3) and 1.38 in biparental population (Fig. 5) was observed, which suggested the involvement of large number of genes in the disease expression. Similar results were obtained in biparental $F_{2:3}$ progenies by Ranganatha et al. (2021) and in recombinant inbred lines (RILs) by Wang et al. (2018). The skewness and kurtosis together reveal interaction gene effects and provide more comprehensive picture of understanding the genetic architecture of resistance to the NCLB. Positively skewed platykurtic distribution indicated that the polygenes govern the expression of NCLB disease resistance with complementary epistasis. It also suggested that the genes at the corresponding trait loci exhibit decreasing effects and indicated that the genetic gain could be rapid with mild selection and less rapid with intense selection for resistance to the NCLB.

In maize, several multi-parental populations have been developed, which highlighted the importance of generating more variability for the selection of traits like resistance to Mediterranean corn borer (Jiménez-Galindo et al. 2019), Fusarium seedling rot resistance (Septiani et al. 2019) and Fusarium ear rot resistance (Butron et al. 2019). In the present study, multi-parental population served the purpose of generating large range of phenotypic variation for NCLB resistance compared to the biparental population, as evidenced by the significance of Levene's test. The inclusion of multiple diverse founder parents in multi-parental population provides the opportunity to target greater number of segregating traits and resistant progenies identified will be forwarded to derive NCLB-resistant inbreds. Furthermore, these 418 $F_{2:3}$ progenies to will be used to derive recombinant inbred lines with multiple disease resistance and can be used as a next generation mapping resource for identifying the QTLs conferring resistance to NCLB and other useful traits.

Authors' contribution

Conceptualization of research (HCL, SPD); Designing of the experiments (HCL, NM); Contribution of experimental materials (HCL); Execution of field/lab experiments and data collection (SPD, HCL, NM); Analysis of data and interpretation (SPD, HCL); Preparation of the manuscript (SPD, HCL).

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