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RESEARCH ARTICLE

Genetic characterization of maize doubled haploid lines for Fusarium stalk rot caused by *Fusarium verticillioides*

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Abstract. Fusarium stalk rot disease (FSR) of maize caused by *Fusarium verticillioides* (Sacc.) Nirenberg is becoming an important biotic production constraint in many of the major maize growing areas causing substantial yield losses. Inbreds are preferred as parents in hybrid development owing to homozygous nature and high heterotic ability. Double haploid (DH) technology has emerged as a significant milestone. A total of 339 DH lines were generated from two inbred lines, VL1043 (susceptible) and CM212 (resistant), through *in vivo* haploid induction method. The 339 DH lines along with parents were phenotyped for their response to the FSR at the College of Agriculture, V. C. Farm, Mandya, India during summer, kharif and rabi seasons of the 2019–2020. Best linear unbiased predictors (BLUPs) were estimated for the FSR disease scores over three seasons. A wide range of BLUP scores of three to nine indicated the presence of higher variation for response of DH lines to FSR disease. The higher estimates of standardized range (1.31) and phenotypic coefficient of variation (19.80) also displayed higher variability. Nine lines were moderately resistant and 188 exhibited moderately susceptible reaction. The distribution of DH lines was positively skewed (1.34) and platykurtic (2.31) which suggested complementary epistasis and involvement of large number of genes in the disease expression.

Keywords. maize; Fusarium stalk rot; doubled haploid lines; resistance; platykurtic; complementary epistasis.

Introduction

Maize is an important cereal crop worldwide, which contributes to food security in most of the developing countries. The production of maize is constrained by increased incidence of pests and diseases in recent years. Among various biotic factors limiting yield level in maize, Fusarium stalk rot (FSR) disease, caused by soil borne fungi *Fusarium verticillioides* (Sacc.) Nirenberg (1976) is one of the important constraints. In India, the disease is prevalent in most of the maize growing areas, namely Jammu and Kashmir, Punjab, Haryana, Delhi, Rajasthan, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Tamil Nadu and Karnataka. The disease incidence ranges from 10 to 42% (Desai *et al*. 1991; Kumar *et al*. 1998; Harlapur *et al*. 2002) in Karnataka. The FSR usually occurs after flowering stage and prior to physiological maturity, reducing yields in two ways; (i) affected plants die prematurely, thereby producing light weight ears having poorly filled kernels, (ii) plants with stalk rot easily lodge, which make harvesting difficult and ears are left in the field while harvesting. The disease was also reported to cause a reduction of 18.7% in cob weight and 11.2% in 1000-grain weight in the infected plants (Cook 1978).

Genetic resistance is the most economical and eco-friendly approach to mitigate production losses caused by FSR. Development of maize hybrids resistant to FSR requires identification of inbreds with resistance. Since doubled haploid plants are 100% homozygous and developed in two to three seasons, they have several advantages over near homozygous inbred lines which usually require eight to nine seasons. In this study, 339 doubled haploid lines derived from the cross VL 1043 (susceptible) × CM 212 (resistant) and two parental inbred lines were screened

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for their reaction to the FSR and to work out the descriptive statistics to understand the inheritance pattern in maize.

Materials and methods

The inbred lines VL 1043 and CM 212 with contrasting response to FSR were crossed during kharif, 2017 at the College of Agriculture, Mandya, India. The F_1 seeds were planted in 25 rows of 4 m length at M/s Corteva Agriscience Research Farm, Gauribidanur, India and crossed with male haploid inducer inbred (Chaikam *et al*. 2019). The progenies of the cross had both haploid and diploids kernels. The dominant grain purple colour marker gene (R1-nj marker) was used for the identification of haploid kernels. The kernels with pigmentation were regular diploids and those without pigmentation were haploids. The haploid kernels were germinated on paper towels until the coleoptiles were about 2-cm long. The coleoptile tip was cut-off before submergence in colchicine solution with DMSO to facilitate uptake of the doubling chemicals. Subsequently, seedlings were washed under tap water, planted in biodegradable Jiffy peat pellets (http://www.jiffypot.com/), in a shade house for recovery until the three-leaf stage, and then transplanted to a DH nursery net house. The plants were selfed and the harvested cobs (D_0) were doubled haploids. The D_0 plants were advanced to D_1 nursery with strict selfing to screen and rogue out the haploids, off types and false positives. Approximately 15% successful establishment rate of the DH lines was observed in the field. It was possible to develop 339 doubled haploid lines (DHs). These lines were screened for their response to the FSR. The augmented design (Federer 1961) was employed to constitute the field trials at the College of Agriculture, during summer, kharif and rabi seasons of 2019–20 by artificial inoculation of pathogen spores at 65 days after sowing (DAS) (Shekhar and Sangit Kumar 2012; Archana *et al.* 2019). Each DH line 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 inbred lines VL 1043 and CM 212 were planted as checks after every 10th row of test entries.

Isolation and mass multiplication of F. verticillioides

Maize stalks showing symptoms typical of FSR were collected from the field. Infected stalks were cut into small tissue and surface sterilized in 4% sodium hypochlorite solution. The same were washed twice in sterile distilled water, dried and plated on potato dextrose agar (PDA) medium. The Petri plates were incubated for five days in Biological Oxygen Demand (BOD) incubator for the development of pathogen colonies. The pathogen colonies were examined for morphological and fruiting body characteristics typical of *F. verticillioides*. The mycelia were aseptically transferred to sterile potato dextrose broth (PDB) in conical flasks for mass multiplication. These conical flasks were incubated for 15 days for the development of mycelia. On the 15th day, the mycelia were grounded and filtered to obtain pathogen spore suspension.

Preparation of inoculum

The spore suspension was observed under microscope and the concentration was adjusted to 1×10^6 spores per mL using haemocytometer. Whenever spore concentration was high, it was diluted with sterile distilled water to maintain desired concentration of spores.

Phenotyping of DH lines for their response to FSR

To all the 339 DH lines, 2 mL of the inoculum was injected diagonally using the syringe after pricking and making 2-cm hole with the help of a jabber to the second internode from the base at 65 DAS. For disease phenotyping, the stalks were split opened after 30 days inoculation. Disease severity and intensity was recorded on individual plants in each lines using 1–9 rating scale in individual seasons. The scoring pattern was developed based on spread of internode discolouration inside the maize stalks from the point of inoculation (table 1). Higher the discolouration, higher is the rating.

Table 1. Disease rating scale for FSR: (IIMR, New Delhi; Hooker 1956 and Payak and Sharma 1983).

Disease score	Symptoms	Disease reaction
1	Healthy or slight discolouration at the site of inoculation	Highly resistant
2	Up to 50% of the inoculated internode is discoloured	Resistant
3	Inoculated internode 51–75% were discoloured	Moderately resistant
4	Inoculated internode 76–100% were discoloured	Moderately susceptible
5	Less than 50% discolouration of the adjacent internode	Susceptible
6	More than 50% discolouration of the adjacent internode	Highly susceptible
7	Discolouration of three internodes	Highly susceptible
8	Discolouration of four internodes	Highly susceptible
9	Discolouration of five or more internodes and premature death of plant	Highly susceptible

Statistical analysis

The disease response data obtained on 339 doubled haploid lines to FSR over three seasons (summer, kharif and rabi, 2019–2020) were subjected to pooled augmented analysis of variance (ANOVA). The pooled ANOVA was carried out to detect DH line × season interaction, if any. After ascertaining the existence/nonexistence of DH line × season interaction, best linear unbiased predictors (BLUPs) were estimated by considering blocks and DH lines as random effects and seasons as fixed effects with restricted maximum likelihood (REML) estimation mixed model procedure (PROC MIXED) in SAS v. 9.3 software programme to estimate genetic and nongenetic variances across seasons. On the basis of BLUP scores, the 339 doubled haploid lines were classified as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS). The BLUP values were further subjected to calculate the descriptive statistical parameters like mean, range and standardized range as per Sundarraj *et al.* (1972). Genotypic and phenotypic variance was estimated as per Lush (1945), phenotypic and genotypic coefficient of variation was assessed as per Burton and DeVane (1953) and were 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 and genetic advance as per cent mean were 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 DH lines for FSR disease reaction.

Results and discussion

In maize breeding development, use of DH lines offer several advantages over conventionally derived inbred lines through reducing the breeding cycle per unit time, enhancing the genetic gain (Xu *et al*. 2017) and lowering the costs in line development and maintenance (Prasanna *et al.* 2012). Faster product cycle times are important for countering emerging diseases (sorghum downy mildew, Turcicum leaf blight, banded leaf and sheath blight, Fuarium stalk rot) and pests, (stem borer and Fall armyworm). The FSR is one such most destructive disease of maize causing yield loss up to 38% in India (Kenganal *et al*. 2017). In this backdrop, 339 DH lines were developed by *in vivo* haploid induction method and were subjected for screening against FSR disease response across three seasons. The mean scores of responses of 339 DH lines to the FSR across three seasons namely, summer, kharif and rabi (2019–2020) were subjected to ANOVA since it is the diagnostic step to detect the presence of genetic variability among the doubled haploid lines. Mean squares attributable to blocks, seasons, checks and DH lines were found significant (table 2) thereby indicating significant differences among the DH lines for FSR disease reaction. The DH lines vs seasons was found nonsignificant which revealed that the DH lines disease response was uniform across seasons and the method of artificial inoculation was found effective in establishing uniform disease condition across the seasons.

Table 2. ANOVA of mean FSR disease scores of 339 DH lines over three seasons.

Source of variation	Degrees of freedom	Mean sum of squares	Probability
Block (ignoring treatments)	12	2.14	0.0001
Seasons	2	31.31	< 0.0001
Check	1	324.61	< 0.0001
Doubled haploids	338	1.42	< 0.0001
Seasons vs doubled haploids	678	0.47	0.7305
Error	62	0.52	< 0.0001

BLUP scores were computed since it involves simultaneous prediction of genetic effects and estimation of genetic and nongenetic variances. It shows great potential and can be used in classifying the disease response and further can be efficiently utilized for interpreting the results. The mean BLUP score computed from each DH line across the seasons was used to classify DHs into different response groups. Of the 339 DH lines, nine were found moderately resistant with a BLUP score of 3. They are useful in breeding programme as they show lower disease infection (Mohamed *et al.* 1966). Even Archana *et al*. (2019) found four moderately resistant inbreds among 115 inbred lines screened for their reaction to the FSR. Moderately susceptible reaction was exhibited by 188 DH lines, 91 showed susceptible disease reaction and 51 were found highly susceptible (table 3). The majority of 339 DH lines belonged to moderately susceptible response group and none were found in highly resistant and resistant. The reason is because of moderate resistance exhibited by genotype CM212.

Knowledge on relative contribution of genetic and nongenetic sources on the trait variability is useful in formulating appropriate selection strategies to breed improved maize cultivars. In DH lines, the mean FSR disease

Table 3. Classification of doubled haploid lines into different groups based their Fusarium stalk rot BLUP values across three seasons.

Score	Response	No. of doubled haploid lines
1	Highly resistant	00
2	Resistant	00
3	Moderately resistant	9
4	Moderately susceptible	188
5	Susceptible	91
> 6 to 9	Highly susceptible	51

incidence across seasons was 4.59. The estimates of the range provide clues about the occurrence of DH lines with extreme expression. The highest incidence BLUP score was 9 and the lowest incidence BLUP score was 3 in DHs over seasons (summer, kharif and rabi, 2019–2020) was used to estimate the range (highest–lowest trait mean) and the same was taken as standardized range. The estimate of standardized range was 1.31 (table 4) which indicated the higher variation for response of DH lines to FSR disease and reflected on better discriminating ability of the method of inoculation followed in the present study.

The FSR disease phenotypic value of an individual DH line is the result of genotypic value and environmental deviation. Variation in this phenotypic value is therefore determined by variance attributable to genotypic values and environment deviation across and within seasons. Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are the standard estimates of variability at genotypic and phenotypic levels, respectively. The estimate of PCV was moderately higher with a BLUP score of 19.80 (table 4) which provide true reflection of variability among DH lines. The difference between PCV and GCV estimates was less indicating the effectiveness of artificial disease screening employed and selection practiced for resistant genotypes based on phenotypic performance. Thus, the estimates of standardized range and PCV complemented each other in revealing the variability in DH lines for FSR disease reaction.

The extent of contribution of genotype to the phenotypic variation for a trait in a population is expressed as the ratio of genetic variance to the total variance known as heritability (Rojas and Sprague 1952). Estimates of heritability serve as a guide to the breeder indicating the effectiveness of selection. Higher estimates of broad-sense heritability (0.37) and expected GAM (15.17) also indicated the effectiveness of phenotypic selection for improving the FSR disease resistance in maize.

Table 4. Genetic estimates of doubled haploid lines in maize for FSR disease across three seasons.

Genetic parameters	Genetic estimates (BLUP scores)
Mean	4.59
Range	3–9
Standardized range	1.31
Phenotypic coefficient of variation	19.80
Genotypic coefficient of variation	12.07
Heritability	0.37
Genetic advance	0.70
Genetic advance as per cent mean	15.17
Skewness	1.34
Kurtosis	2.31

Genetic analysis based on skewness (the third degree statistic and kurtosis (the fourth degree statistic) is more powerful and useful than first (mean) and second (variance) degree statistics (Choo and Reinbergs 1982), especially for detecting and characterizing the nature of epistasis. Fisher *et al.* (1932) also outlined the theoretical basis of usefulness of skewness for explaining the genetic causes for variation in a trait. The distribution of DH lines for the FSR disease response was positively skewed with a disease score of 1.34 (figure 1). The skewed distribution suggested the importance of nonadditive gene action (Pooni *et al.* 1977; Kimbeng and Bingham 1998; Roy 2000). Positive skewness is caused by complementary gene interactions predominantly in the same direction (Snape and Riggs 1975). The genes controlling the FSR disease reaction with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effects on the expression of FSR disease response. The positively skewed distribution also suggested that the genes at the corresponding trait loci exhibited decreasing effects.

Kurtosis, the fourth degree statistic, indicates the degree of peak of distribution. Kurtosis also indicates the relative number of genes controlling the trait under investigation (Robson 1956). The DH lines exhibited platykurtic distribution (< 3.0) with BLUP score of 2.31, which suggested the involvement of large number of genes in the disease expression. Skewness and kurtosis together reveal interaction gene effects and provide more comprehensive picture of understanding the genetic architecture of resistance to the FSR. Positively skewed platykurtic distribution indicated that the polygenes govern the expression of FSR in DH lines with complementary epistasis. It also suggested that the genes at the corresponding trait loci exhibit decreasing effects and indicate that the genetic gain could be rapid with mild selection and less rapid with intense selection for resistance to the FSR.

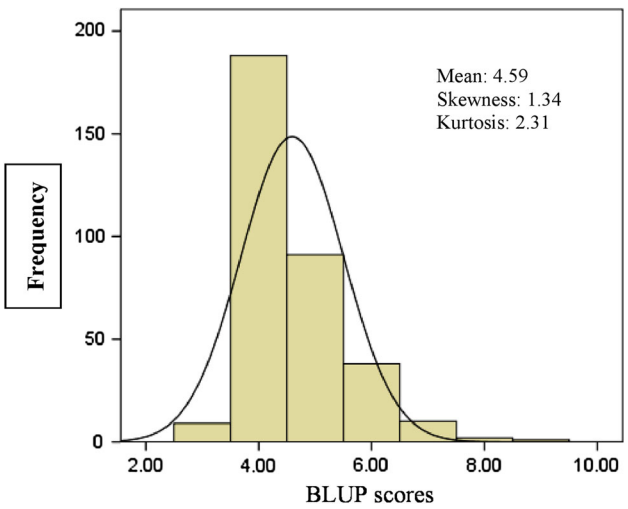

Figure 1. Frequency distribution of BLUP scores for FSR disease across three seasons in DH lines of maize.

The information elicited from genetic analysis of FSR at lower order (third and fourth) levels in DH lines and the identified moderately resistant and moderately susceptible DHs to FSR in our study are good candidates for deriving potential hybrids. These DH lines could also be used for mapping resistance to the FSR. Once markers are identified and validated, linked markers could be used as surrogates of FSR resistance while breeding for resistant maize hybrids.

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References

Archana R., Lohithaswa H. C., Uma M. S., Shivakumar K. V., Sanathkumar V. B. and Pavan R. 2019 Genetic analysis of Fusarium stalk rot resistance in maize (*Zea mays* L). *J. Pharmacognosy Phytochem*. **SP1**, 58–61.

Burton G. W. and DeVane E. M. 1953 Estimating heritability in tall Fescue (*Festuca circunelinaceae*) from replicated clonal material. *J. Agron.* **45**, 478–481.

Chaikam V., Molenaar W., Melchinger A. E. and Prasanna B. M. 2019 Doubled haploid technology for line development in maize: technical advances and prospects. *Theor. Appl. Genet.* **132,** 3227–3243.

Choo T. M. and Reinbergs E. 1982 Analysis of skewness and kurtosis for detecting gene interaction in a double haploid population. *Crop Sci.* **22**, 231–235.

Cook R. J. 1978 The incidence of stalk rot (*Fusarium* spp.) on maize hybrids and its effect on yield of maize in Britain. *Ann. Appl. Biol.* **88**, 23–30.

Desai S., Hegde R. K. and Desai S. 1991 A preliminary survey of incidence of stalk rot complex of maize in two districts of Karnataka. *Indian Phytopathol.* **43**, 575–576.

Federer W. T. 1961 Augmented design with one-way elimination of heterogeneity. *Biometrics* **17**, 447–473.

Fisher F. A., Immer F. R. and Tedin O. 1932 The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. *Genetics* **17**, 107–124.

Harlapur S. I., Wali M. C., Prashanth M. and Shakuntala N. M. 2002 Assessment of yield losses in maize due to charcoal rot in Ghataporabha Left Bank Cannal (GLBC) command area of Karnataka. *Karnataka J. Agric. Sci.* **15**, 590–591.

Hooker A. L. 1956 Association of resistance to several seedling, root, stalk, and ear diseases of corn. *Phytopathology* **46**, 379–384.

Johnson H. W., Robinson H. F. and Comstock R. E. 1955 Estimates of genetic and environmental variability in soybean. *Agron. J.* **47**, 314–318.

Kenganal M., Patil M. B. and Nimbarag Y. 2017 Management of stalk rot of maize caused by *Fusarium moniliforme* (Sheldon). *Int. J. Curr. Microbiol. App. Sci.* **6,** 3546–3552.

Kimbeng C. A. and Bingham E. T. 1998 Population improvement in Lucerne (*Medicago sativa* L.): components of inbreeding depression are different in original and improved populations. *Aust. J. Exp Agric.* **38**, 831–836.

Kumar M., Lal H. C. and Jha M. M. 1998 Assessment of yield loss due to post-flowering stalk rots in maize. *J. Appl. Biol.* **8**, 90–92.

Lush J. L. 1945 Heritability of quantitative characters in farm animals. Proc. 8th Genetics Congress. *Hereditas* **35**, 356–375.

Mohamed H. A., Moneim A. T. A. and Fathi S. M. 1966 Reaction of corn inbred lines, varieties and hybrids to four fungi causing stalk rots. *Plant Dis. Rep.* **50**, 401–402.

Nirenberg H. 1976 Untersuchungen iiber die morphologische und biologische Differenzierung in der *Fusarium* Sektion Liseola. *Mitt. Biol. Bund. Anst. Ld-u. Forstw.* 169.

Payak M. M. and Sharma R. C. 1983 Disease rating scale in Maize in India. Techniques of scoring for resistance to important diseases of maize. All India Coordinated Maize Improvement Project, pp 1–5. IARI, New Delhi.

Pooni H. S., Jinks J. L. and Cornish M. A. 1977 The causes and consequences of non-normality in predicting the properties of recombinant inbred lines. *Heredity* **38**, 329–338.

Prasanna B. M., Chaikam V. and Mahuku G. 2012 *Doubled haploid technology in maize breeding: theory and practice*. CIMMYT, Mexico.

Robinson H. F., Comstock R. E. and Harney P. H. 1949 Estimates of heritability and degree in corn. *J. Agron.* **41**, 353–359.

Robson D. S. 1956 Application of K4 statistics to genetic variance component analysis. *Biometrics* **12**, 433–444.

Rojas B. A. and Sprague G. F. 1952 A comparison of variance components in corn yield trials III. General and specific combining ability and their interaction with locations and years. *Agron. J.* **44**, 462–466.

Roy D. 2000 *Plant breeding—the analysis and exploitation of variability*, pp. 198. Narosa Publishing House. New Delhi, India.

Shekhar M. and Sangit Kumar 2012 Inoculation methods and disease rating scales for maize diseases. Directorate of Maize Research, Indian Council of Agricultural Research, Pusa Campus, pp. 20–28. New Delhi, India.

Snape J. W. and Riggs T. J. 1975 Genetical consequences of single seed descent in the breeding self-pollinated crops. *Heridity* **35**, 211–219.

Snedecor G. W. and Cochran W. G. 1994 *Statistical methods*, fifth edition. Iowa State University Press, Ames.

Sundarraj N., Nagaraj S., Venkataramu M. N. and Jagannath M. K. 1972 *Design and analysis of field experiments*. University of Agricultural Sciences, Bengaluru.

Xu Y., Li P., Zou C., Lu Y., Xie C., Zhang X. *et al*. 2017 Enhancing genetic gain in the era of molecular breeding. *J. Exp. Bot.* **68**, 2641–2666.

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