Genome wide association mapping for identification of QTL(S) underlying resistance to *Alternaria* blight (*Alternaria brassicae* (Berk) Sacc.) in Indian mustard (*Brassica juncea* L. Czern. & Coss.)"

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Abstract

Disease screening against *Alternaria* blight under field condition showed that, none was free from disease only 13 genotypes were found MR and 152 genotypes were found MS, 252 S and 3HS. The high estimate of phenotypic and genotypic coefficient of variation was recorded for YPP and SB., high estimate of broad sense heritability was recorded all characters except YPP. The genetics advance in per cent of mean was found high for PB, high heritability along with high genetic advance was observed for HPF. At phenotypic and genotypic level both YPP shows highly significant and positive correlation. At phenotypic and genotypic level path coefficient revealed that in HPF, PL. The genotypes were grouped into eight different non overlapping cluster (five 115, three 100, six 84, one 78, seven 37 and three, two and four 1, eight 36 genotypes). The maximum and minimum inter cluster distance was between six, eight and one, three. One highest cluster mean for PH, MAT, and HPF and two (MAT, PH, HPF). Three (MAT, PH, HPF) four (MAT, PH, HPF) five (PH, MAT, HPF) six (MAT, PH, HPF) seven (MAT, HPF, PH), eight had no values due to reason no germinated or un survived plant and GWAS analysis of identified six significant association for *Alternaria* blight resistance on chromosome (A07, A09, A03, B07, B04, B03) respectively, This study showed that the diversity panel of Indian mustard identified QTLs for controlling of disease resistance against *Alternaria* blight in Indian mustard.

Introduction

Brassica juncea is a predominant oilseed crop of Indian subcontinent occupying an area of more than 6.0 mh (Chauhan et al. 2011). It is one of the three allopolyploids which form the U's triangle (Nagaharu 1935) that consists of three diploids and their respective allopolyploid *Brassica* species. All the 6 (six) species of U's triangle are economically important as major oilseed crops (D'Antuono et al 2007, Shekhawat et al 2012). So far, the genome sequencing of rapeseed mustard reveals two diploids and two allopolyploids of the U's triangle: *Brassica rapa* (AA) (Wang et al 2011), *Brassica oleracea* (CC) (Liu et al 2014), *Brassica napus* (AACC) (Chalhoub et al 2014) and *Brassica juncea* (Indian mustard) (AABB) (Yang et al. 2016) Respectively. *Brassica* species play an importance role in world agriculture as oilseeds, vegetables, fodder, green manure and condiments Mustard seed oil is mainly used for edible purposes (Bhowmik 2003). Mustard is a good source of protein (28–36%) and phenolic antioxidants like sinapic acid and sinapine (Thiyam et al 2006) These crops require cool growing season and moderate temperature throughout, hence are sown in the month of October and November in northern India (Das et al 2009). Despite considerable increase in the productivity, a wide gap exists between the potential yield and the yield realized at the farmers field, which is largely due to a number of abiotic and biotic stresses to which rapeseed-mustard crop is exposed (Chattopadhyay 2008). Among the biotic stresses, *Alternaria* blight disease caused by fungus species *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world and is one of chronic and important diseases of Indian mustard causing up to 47% yield losses (Meena et al 2010). The other strain, *A. brassicicola* and *A. brassicae*, of this pathogen infect host plants (Indian mustard) at all developmental stages (Kolte et al 1987). And disease symptoms appears like dark brown lesions/spots with characteristic concentric circumferences, often with a yellowish chlorotic halo on stems, leaves, and siliquae or spread whole plant (Meena et al 2012, Humpherson-Jones 1992). The enlarge necrotic lesions drastically reduce the photosynthetic efficiency has ten plants senescence and lead to collapse and death of plants under high “pathogen” pressure (Kolte 1985). Crop yield is a complex quantitative traits loci (QTLs) governed by various yield component traits and highly influenced by genotype-environment interaction (Ozer et al. 1999). Various yield related traits such as plant height, main shoot length, biomass, harvest index, phenology and adaptation indirectly influence the yield (Quijada et al 2006). Therefore, discovery of QTLs governing yield and yield related traits under diverse environments is important to make rapid genetic gains in the canola breeding programs (Shi et al 2014). Genome wide association study (GWAS) using an array of markers based on SNP and INDELS have been successfully applied in plants to discover causative SNP in a gene to be responsible for the natural variation in phenotypes (Flint-Garcia et al 2003). Ecke et al (2010) investigated extent and structure of linkage disequilibrium in canola quality *Brassica napus* and suggested association mapping as a valuable approach for QTL discovery with significant higher resolution owing to the rapid decay of linkage disequilibrium (LD) with distance between linked markers. Several studies for fine mapping and candidate gene discovery through association analysis have been reported in *Brassica napus* for various yield traits such as shatter resistance (Raman et al 2008) days to flowering (Wang et al. 2016) plant height, branch initiation height and number of branches (Zheng et al 2017). GWAS have greatly altered the approach to studying complex phenotype genetics. It is utilized to map > 50,000 unique SNP phenotype associations in plant to date (MacArthur et al 2017). It is applied to study complex phenotypes in several animals and plants. As a reference plant, *Arabidopsis thaliana* is an ideal organism for GWAS because its inbreeding nature allows the preservation of the genotypic information of samples (Korte and Farlow 2013) and applied to various crop as well due to one major reason for missing true phenotype-associated genes is the very strict significance thresholds applied in GWAS to reduce false positives when testing the associations of numerous SNPs simultaneously (Huang and Han 2014). The strict P-value thresholds after adjustment for multiple hypothesis tests such as the Bonferroni correction generally allow only a handful of SNPs to be significant. Genetic variants associated with complex phenotypes are distributed across many genes in the “phenotype-involved pathways”, which results in “genetic heterogeneity” (McClellan and King 2010) where phenotype-associated variants occur in only a subset of a population for the phenotype, consequently reducing the “statistical power” of the association between a “single variant” and “phenotype”. Presumably, this statistical limitation may be overcome somewhat by increasing the population size; but at a much higher cost. The pathway nature of complex phenotypes also provides an opportunity for augmenting GWAS by integrating phenotype-association data via the “co-functional gene” network, which maps the functional couplings between “genes”. (Lee et al 2011) high linkage disequilibrium with the associated SNPs identified by GWAS and directly involved in “disease resistance” (Stagnati et al 2018).

Materials And Methods

Collection of germplasm of *Brassica juncea*

A total collection of 456 genotypes was selected for this study. These genotypes were chosen as they were genetically diverse collected from of the species. Some of the vary genotypes for which few seed were available, had poor germination efficiency and some plant were not survives. A total number of 420 genotypes were used for disease screening and for measurement of economic agronomic traits.

Methods

The experiment material consisted of diverse stock of Indian mustard genotypes were evaluated in alpha lattice design with two replications under field condition in *rabi* session 2018-19. The character studies were days to 50% flowering days, days to 100% flowering to days to maturity, plant height (cm), primary branches, secondary branches, main shoot length (cm), pods per main shoot, pod length, yield per plant and disease severity, and QTL(s) identification of *Alternaria* blight.

Statistical Analysis

The analysis of variance for each characteristics was performed using Microsoft Excel, MSTAT software and correlation and path co-efficient analysis was performed using R-STAT (Agricole) software.

Estimation of variation and its heritable components in genetic parameters: Phenotypic and Genotypic variance

The phenotypic and genotypic variances were estimated according to (Johnson *et al.*, 1955). The error mean square (EMS) was considered as error variance (2e). Genotypic variances (2g) were calculated by $GMS-EMS/r$, where, GMS = Genotypic mean square, EMS = Error mean square r =replication. The phenotypic variances (2p) were derived by adding genotypic variances with the error variances (2e) as given by the following formula: $2p = 2g + 2e$. The genotypic and phenotypic components of variance were computed according to formulae given by (Lush, 1940).

Genotypic and phenotypic Co-efficient of variation (GCV and PCV)

Genotypic and phenotypic co-efficient of variations were computed by the formula suggested by (Burton and Devane, 1953) as given below:

Where, g = Phenotypic standard deviation, σ_p

population mean.

Heritability

Heritability in broad sense was estimated according to (Johnson *et al.* 1955).

Genetic advance

The expected genetic advance for different characters was estimated as per the formula given by (Johnson *et al.* 1955).

Where,

K = Selection differential, the value of which is 2.06 at 5% selection intensity,

p = Phenotypic standard deviation.

Character association by correlation study and path analysis

Correlation and path-coefficient analysis were estimated by the association of characteristics and cause effect relationship studied for yield and component characteristics.

Estimation of correlation

Association of different characteristics under the study was analyzed by the working out genotypic and phenotypic degree of correlation and simple correlation coefficient for all the possible parts of characteristics combination by the method of (Hayes *et al.* 1955) and (Singh and Chowdhury 1985)

Estimation of direct and indirect effect of different characters on yield

In order to find a clear picture of the inter-relationship between yield and other components, path analysis splits the correlation coefficient into the measure of the direct and indirect effect of each contributing characteristics towards yield at genotypic level was done following by (Dewey and Lu 1959).

Calculation of residual effect

After calculating the direct and indirect effect of different characteristics, the residual effect was calculated using the formula suggested by (Singh and Choudhury, 1985)

Development of *Alternaria* blight in Indian mustard genotypes under field conditions

Screening of *Alternaria* blight- epiphytotic developed from the natural inoculum was observed at 30, 45 and 50 days after sowing in the first true leaves. Following the first appearance of the *Alternaria* blight, the leaves of the plants were scored for disease severity. Scoring was done during the epiphytotic conditions by selecting five leaves per plant on five plants from each genotype on a random basis in each replication. The blighting was assessed as per cent leaf area covered (Kumar and Singh, 2006, Kumar 2008).

$$\text{Average severity score} = \frac{(N - 1X0) + (N - 2X1) + (N - 3X3) + (N - 4X5) + (N - 5X7) + (N - 6X9)}{\text{No. of leaf samples}}$$

Where

N-1 to N-6 represents frequencies of leaves in the respective score.

Genomic DNA Isolation

DNA isolation from Indian mustard leaves was carried out using a rapid extraction protocol (Murray and Thompson (1980). Briefly, leaves (<100 mg) were crushed in 500 mL of extraction buffer [200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA (pH 8.0), 0.5% SDS] and centrifuged at 10,000 rpm for 10 min to remove the debris. An equal amount of isopropanol was added to the supernatant to precipitate the DNA and centrifuged again at 10,000 rpm for 10 min. The pellet was washed with 70% ethanol, air-dried, and resuspended in 50 mL of 2 mM Tris (pH 8.0). Genomic DNA extraction for SNP genotyping was carried out using MDI (Advanced Microdevices, Pvt. Ltd, India) plant genomic DNA miniprep kit according to the manufacturer's instructions.

Evaluation of genetic materials and their statistical analysis

DNA isolated from leaves of association mapping panel genotypes from leaves sample. A reduced representation GBS will be outsourced. The TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) GBS (Genotyping By Sequence) pipeline (Glaubitz et al 2014) and reference *B. juncea* genome assembly was used (at default parameters) to call SNPs from uniquely aligned reads and generate a hapmap file. Identified SNPs will then be filtered based on missing data points and minor allele frequency (MAF) to identify informative SNPs. The mean values of target traits from each accession grown in two environments and informative SNPs was used for association analysis. Significant marker-trait association for various phenotypic traits recorded. The informative SNPs was used to carry out association mapping using software Farm CPU (www.zzzlab.net/FarmCPU/; Liu et al (2016) that takes care of population structure through kinship and PCA analysis. Significant associations were inferred using an adjusted p-value (Bonferroni correction).

Results

Screening of Indian mustard genotypes against *Alternaria* blight in field condition.

A total number of 420 genotypes of Indian mustard were screening against *Alternaria* blight under field condition. Among 420 genotypes Indian mustard, none was free from *Alternaria* blight. Only thirteen genotypes i.e., PRG-909, CN-107719, CM-8, PRG-2001-62, LM-114-6, Bio-179, Bio-169-95, Consult-1, BM-2, CRL-1359-18-19, BLM-2-5, B-272, and CSR-943 were found moderately resistant. 152 genotypes moderately susceptible, 252 genotypes were categorized as susceptible and three genotypes were found as highly susceptible.

Coefficient of variation

The PCV was found higher than the genotypes coefficient of variation (GCV) for all character similarly σ^2_p and the PCV give larger values than the σ^2_g and the GCV for all traits. Kashif et al (2003) also indicated same results slightly higher value of PCV than GCV and similarly higher value of σ^2_p compared to σ^2_g shows environmental effect on all characters. (Table 3). The high estimate (>20%) of phenotypic and genotypic of variation was recorded for recorded for SB, YPP, MSL, DIS, PB, PH, MSP, PL, FPF, MAT and HPF similar result reported that phenotypic and the genotypic coefficient of variations for seed yield. High GCV, PCV were stated for seed yield and number of pods per plant. height and pods per plant in *Brassica juncea*. (Table -3).

Heritability in broad sense and genetics advance in percent of mean

High estimate of broad sense heritability (h^2_b) >75% was recorded for the HPF, FPF, MAT, PB, MSP and MSL, PL, SB and PH, The moderate estimates of broad sense heritability (h^2_b) >75% were recorded for DIS and YPP. Higher estimates of heritability coupled with high genetic advance were found for the indicating additive effect of concern gene(s) for a trait of interest and selection could be effective for these traits (Table 3).

The genetic advance in per cent of mean was found high (>20%) for PB, MSP, DIS, PH, FPF, HPF, MAT, PL, YPP, SB, and MSL. High heritability coupled with moderate genetic advance for FPF indicated of both additive and non-additive gene action. Moreover, higher estimates of genetic advance role expressed as percent of mean revealed relative differences among the studied quantitative traits like FPF, number of primary branches per plant, number of secondary branches per plant, seed yield per plant and biological yield per plant. Moderate estimates of genetic advance in percent of mean were recorded for plant height, length of siliqua, number of seeds per siliqua, 1000 seed weight and harvest index and remaining all other characters showed lower estimates of genetic advance in percent of mean (Table 3).

Pod length, yield per plant and secondary branches exhibited to high level of heritability along with low estimation of genetic advance, whereas, low values of both the parameter was observed for disease severity, main shoot length and pods per main shoot. Heritability and genetic advance together prove more useful in predicting the resultant effect of selection on phenotypic expression of character (Table 3).

Correlation coefficient analysis

At phenotypic level, YPP showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP, PL and DIS. (Table 4a). DIS showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP, PL. PL showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL and MSP. MSP showed positive and highly significant correlation with FPF, HPF, MAT, PL, PB, SB and MSL. MSL showed positive and highly significant correlation with FPF, HPF, MAT, PH, SB and negative highly significant correlation with correlation PB. seed yield per plant had positive and significant interrelationship with PL, PB SB. SB showed positive and highly significant correlation with FPF, HPF, MAT, PH and negative highly significant correlation PB. PB showed positive and highly significant correlation with FPF, HPF, MAT, PH. PH showed positive and highly significant correlation with FPF, HPF, MAT, at phenotypic level highly significant correlation with FPF, HPF to flowering showed positive and highly significant correlation with days FPF at

genotypic level in genotypes YPP showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP, PL and DIS, DIS showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP and PL. and PL showed positive and highly significant correlation with, FPF, HPF, MAT, PH, PB, SB, MSL, and MSP. MSP showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB MSL., MSL showed positive and highly significant correlation with FPF, HPF, MAT, PL, PB and SB. At genotypic level, SB showed positive and highly significant correlation with FPF, HPF, MAT, PH, and PB. PB showed positive and highly significant correlation with FPF, HPF, MAT and PL and PL showed positive and highly significant correlation with FPF, HPF and MAT and MAT correlation with positive highly significant FPF, HPF, and HPF showed positive and highly significant correlation with FPF (Table 4b).

Path coefficient analysis

Phenotypic path coefficient analysis

The highest positive direct effect on grain YPP plant was exhibited by HPF, PL, SB, PB, PL, MSL, and MSP. The direct effects of remaining characters were too low to be considered important. HPF exhibited indirect positive effect on grain YPP *via* PL, PH, PB, SB, MSP, and MSL, while negative indirect effect *via* FPF, MAT and DIS. PL exhibited indirect positive effect on grain YPP *via* HPF, PH, PB, SB, DIS, MSP, and MSL while negatively direct effect *via* MAT, FPF. And SB exhibited indirect positive effect on YPP *via* HPF, PL, MSL, PH, MSP, and negatively direct effect *via* FPF, MAT, and DIS (Table 5a). These characters are very important in indirect selection for yield.

Genotypic path coefficient analysis

The highest positive direct effect on YPP was exhibited by PL, SB, PH. The direct effect of remaining character was too low to be consider important. PL exhibited indirect positive effect on YPP *via* PH, SB, HP, FPF, PB, MSL while, negatively indirect on YPP *via* DIS, MAT, MSP and next SB exhibited positive and high indirect effect on YPP *via* PL, PH, HPF, FPF, MSL, PB, while negatively indirect on YPP *via* DIS, MAT, MSP. And PH exhibited positive and high indirect effect on YPP *via* PL, SB, HPF, FPF, PB, MSL, while negatively indirect on YPP *via* DIS, MAT, MSP (Table 5b).

Genetic Divergence analysis

The genetic divergence analysis was performed by employing Non-hierarchical Euclidean cluster analysis. The genotypes were grouped in to eight different non-overlapping clusters, cluster five, having one hundred fifteen genotypes, emerged with highest number of genotypes followed by cluster three with one hundred genotypes, cluster six with eighty four cluster one with seventy eight genotypes, cluster seven with thirty seven genotypes and cluster (Table 6).

three, cluster two and cluster four with one genotype only, while all genotypes having all characters is zero due to some genotypes not germinated and some plant not survives, so will be data not recorded these showed in cluster no. eight (0.00). respectively (Table 6).

Cluster analysis assigns genotypes into qualitative homogenous groups based on response similarities and also assists to classify genotypes. Greater heterotic effect is generated when clusters are divergent the investigated is aimed to evaluate variability, heritability, relationship among yield associated traits for improving seed yield of rapeseed classify the genotypes via cluster analysis.

Estimate of average inter cluster D²

The estimates of inter cluster distances for eight clusters. The maximum inter-cluster distance was recorded between cluster six and eight followed by cluster six and seven, cluster two and six, cluster four and six, cluster one and six. The minimum estimate for the inter-cluster distance was recorded between cluster one and three followed by cluster three and five, cluster three and seven, cluster five and seven and cluster one and five. And intra cluster eight showed values zero, they indicate the all not germinated and unsurvive plant between no distance in genotypes (Table 7)

Cluster means analysis

The cluster mean for FPF was highest in cluster four and the lowest cluster three. In case of HPF, was highest in cluster six and the lowest cluster two. The cluster mean for MAT was highest in cluster three and four and the lowest cluster two. In case of PH was highest in cluster five and the lowest cluster seven. The cluster mean for PB was highest in cluster five and the lowest cluster one. In case of SB was highest in cluster one and the lowest cluster seven. The cluster mean for MSL was highest in cluster one the lowest cluster four. In case of MSP was highest in cluster six and the lowest cluster seven. The cluster mean for PL was highest in cluster two the lowest cluster six. In case of DIS was highest in cluster six and the lowest cluster one and the cluster mean for PL was highest in cluster four the lowest cluster seven (Table 7).

Genome wide association study (GWAS)

A total of 1.7 to 6.1 million raw reads was obtained through GBS of 51 genotypes. GC per cent ranged from 39.8 to 44.5 with Q ≥ 30 values ranging from 90.75 to 94.29. Overall, 72.23 to 82.42 per cent of reads aligned to reference genome for all the genotypes. A total 30673 SNPs were obtained with SNP calling using (samtools version 0.1.18). Out of which, 18258 SNPs having less than 20% missing data and MAF ≥ 5% were obtained after filtering. MAF in the filtered SNP data ranged from 0.05 to 0.5. These filtered SNPs were used for genome wide association analysis incorporating phenotypic data. SNPs were uniformly distributed covering the whole genome of 18 chromosomes of *B. juncea*. Chromosome wise distributions of all SNPs were given in supplementary with the highest SNPs obtained from A07 chromosome whereas the least numbers from B03 chromosome.

A Mixed linear model (Farm CPU) detected six SNP with significant associated with Alternaria blight. The greatly reduced positive, as illustrated in quartile – quartile plate. There were a total of six SNP Significant associated with Alternaria blight resistance were identified at threshold of $P < 5.31 \times 10^{-5}$ (1/18429: -

$\log_{10} 1/18429) > 6.5$) With situated on different 6 chromosome (A07, A09, A03, B07, B04, B03) respectively. Among the six SNPs, found to be significant associated with *Alternaria* blight traits lateral (CA07.20522907, chromosome A07, $P = 12.90 \times 10^{-5}$, SNP effect -5.36156), and CA09.1560419 SNP (chr, A09, Effect -3.955693, $-\log_{10}(p)$ 8.291579), CA03.24022418 (Chromosome A03 Effect -4.012085, $-\log_{10}(p)$ 7.530178), (CB07.3169967, Chromosome, B07 Effect -6.71656, $-\log_{10}(p)$ 7.097997), CB04.3377365, Chromosome, B04, Effect -4.18757, $-\log_{10}(p)$ 6.876148) (CB03.15428252, Chromosome, B03, Effect -3.00002, $-\log_{10}(p)$ 6.50724) (Table 8 and Figure 2).

Discussion

Indian mustard were screening against *Alternaria* blight under field condition not found disease resistance only thirteen genotypes were found moderately resistant. 152 genotypes moderately susceptible, 252 genotypes were categorized as susceptible and 3 genotypes were found as highly susceptible were found moderately resistant (Khan et al 1991; Rahman et al 2010; Summuna et al 2012; (Khan et al 2007). The PCV was found higher than the genotypes coefficient of variation (GCV) for all character similarly σ^2_p and the PCV give larger values than the σ^2_g and the GCV. Kashif et al (2003) slightly higher value of PCV than GCV and similarly higher value of σ^2_p compared to σ^2_g shows environmental effect on all characters. The high estimate of phenotypic and genotypic of variation was recorded for recorded for SB, YPP, MSL, DIS, PB, PH, MSP, PL, FPF, MAT and HPF phenotypic and the genotypic coefficient of variations for seed yield. High GCV, PCV (Giessen et al 2003; Akbar et al 2007; Kardam and Singh 2005; Yadava et al 2011) for different yield contributing traits pertaining to occurrence of high genetic variability (Singh 2004) for different traits including seed yield. High estimate of $h^2 b$ was recorded for the HPF, FPF, MAT, PB, MSP and MSL, PL, SB and PH, The moderate estimates of $h^2 b$ were recorded for DIS and YPP. Higher estimates of heritability coupled with high genetic advance were found for the traits indicating additive effect of concern gene(s) for a trait of interest and selection could be effective for these traits (Malik, et al 2018) Singh et al (2004). The genetic advance in per cent of mean was found high for PB, MSP, DIS, PH, FPF, HPF, MAT, PL, YPP, SB, and MSL. (Singh et al 2003b; Arifllah et al 2013; Singh et al 2013) High heritability coupled with moderate genetic advance indicated of both additive and non-additive gene action. Moreover, higher estimates of genetic advance role expressed as percent of mean revealed relative differences among the studied quantitative traits (Singh et al 2003; Mahala et al 2003; Malik, et al 2018) PL, YPP and SB exhibited to high level of heritability along with low estimation of genetic advance, whereas, low values of both the parameter was observed for disease severity, MSL and MSP heritability and genetic advance together prove more useful in predicting the resultant effect of selection on phenotypic expression of character (Johnson et al 1955), analysis on phenotypic basis YPP showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP, PL and DIS. (Verma et al 2008; Yadava et al 2011; Singh et al 2013b). DIS showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP, PL. (Dawar et al 2018) PL showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL and MSP. (Khan et al 2006; Lohia et al 2013) MSP showed positive and highly significant correlation with FPF, HPF, MAT, PL, PB, SB and MSL (Kumar et al 1984; Kardam and Singh, 2005) MSL showed positive and highly significant correlation with FPF, HPF, MAT, PH, SB and negative highly significant correlation PB. (Singh et al 2009c) SB showed positive and highly significant correlation with FPF, HPF, MAT, PH and negative highly significant correlation PB. (Arifullah et al 2013; Lohia et al 2013). PB showed positive and highly significant correlation with FPF, HPF, MAT, PH for positive and significant correlation with PB, SB (Lodhi et al 2014) PH showed positive and highly significant correlation with FPF, HPF, MAT, at phenotypic level highly significant correlation with FPF, HPF to flowering showed positive and highly significant correlation with days FPF, (Srivastava and Singh, 2002; Verma et al 2008; Gangapur et al 2009; Singh et al 2010; Misra, 2012; Singh et al 2013b) at genotypic basis YPP showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP, PL and DIS. high positive significant genotypic correlation with grain yield per plant (Zang and Zhou, 2006; Jeromela et al 2007; Devi 2018), DIS showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB, MSL, MSP and PL. and PL showed positive and highly significant correlation with, FPF, HPF, MAT, PH, PB, SB, MSL, and MSP. (Rao et al 2014; Singh et al 2013b), MSP showed positive and highly significant correlation with FPF, HPF, MAT, PH, PB, SB MSL, Khan et al (2008), MSL showed positive and highly significant correlation with FPF, HPF, MAT, PL, PB and SB. Correlation on genotypic basis, SB showed positive and highly significant correlation with FPF, HPF, MAT, PH, and PB. (Zang and Zhou 2006; Jeromela et al 2007). PB showed positive and highly significant correlation with FPF, HPF, MAT and PH and PL showed positive and highly significant correlation with FPF, HPF and MAT and MAT correlation with positive highly significant FPF, HPF, and HPF showed positive and highly significant correlation with FPF, (Shekhawat et al 2014). The highest positive direct effect on YPP was exhibited by HPF, PL, SB, PB, PL, MSL, and MSP. The direct effects of remaining characters were too low to be considered important. HPF exhibited indirect positive effect on grain YPP *via* PL, PH, PB, SB, MSP, and MSL, while negative indirect effect *via* FPF, MAT and DIS. PL exhibited indirect positive effect on grain YPP *via* HPF, PH, PB, SB, DIS, MSP, and MSL while negatively direct effect *via* MAT, FPF. And SB exhibited indirect positive effect on YPP *via* HPF, PL, MSL, PH, MSP, and negatively direct effect *via* FPF, MAT, and DIS. These characters are very important in indirect selection for yield. (Dastidar and Patra 2004; Rai et al 2005; Tusar et al 2006; Singh and Singh 2010) Genotypic path analysis The highest positive direct effect on YPP was exhibited by PL, SB, PH. The direct effect of remaining character was too low to be consider important. PL exhibited indirect positive effect on YPP *via* PH, SB, HPF, FPF, PB, MSL while, negatively indirect on YPP *via* DIS, MAT, MSP and next SB exhibited positive and high indirect effect on YPP *via* PL, PH, HPF, FPF, MSL, PB, while negatively indirect on YPP *via* DIS, MAT, MSP. And PH exhibited positive and high indirect effect on YPP *via* PL, SB, HPF, FPF, PB, MSL, while negatively indirect on YPP *via* DIS, MAT, MSP (Devi et al 2018, Kumar et al 2016; Singh and Singh 2010). The genetic divergence analysis were grouped in to eight different non-overlapping clusters, cluster five, having one hundred fifteen genotypes, emerged with highest number of genotypes followed by cluster three with one hundred genotypes, cluster six with eighty four cluster one with seventy eight genotypes, cluster seven with thirty seven genotypes and cluster three, cluster two and cluster four with one genotype only, while all genotypes having all characters is zero due to some genotypes not germinated and some plant not survives, so will be data not recorded these showed in cluster no. eight (0.00). Cluster analysis assigns genotypes into qualitative homogenous groups based on response similarities and also assists to classify genotypes. Greater heterotic effect is generated when clusters are divergent (Mahasi and Kamundia, 2007; Kumar and Mishra, 2007; Rauf and Rahim, 2018). The estimates of inter cluster distances for eight clusters. The maximum inter-cluster distance was recorded between cluster six and eight followed by cluster six and seven, cluster two and six, cluster four and six, cluster one and six. The minimum estimate for the inter-cluster distance was recorded between cluster one and three followed by cluster three and five, cluster three and seven, cluster five and seven and cluster one and five. And intra cluster eight showed values zero, they indicate the all not germinated and unsurvive plant between no distance in genotypes. (Kumar et al 2013; Singh et al 2018). The cluster mean for FPF was highest in cluster four and the lowest cluster three. In case of HPF, was highest in cluster six and the lowest cluster two. The cluster mean for MAT was

highest in cluster three and four and the lowest cluster two. In case of PH was highest in cluster five and the lowest cluster seven. The cluster mean for PB was highest in cluster five and the lowest cluster one. In case of SB was highest in cluster one and the lowest cluster seven. The cluster mean for MSL was highest in cluster one the lowest cluster four. In case of MSP was highest in cluster six and the lowest cluster seven. The cluster mean for PL was highest in cluster two the lowest cluster six. In case of DIS was highest in cluster six and the lowest cluster one and the cluster mean for PL was highest in cluster four the lowest cluster seven. (Aytaç and Kinacı, 2009) Singh et al (2018) (Kumar et al 2013) There were a total of six SNP Significant associated with *Alternaria* blight resistance were identified, with situated on different 6 chromosome (A07, A09, A03, B07, B04, B03) respectively. Among the six SNPs, found to be significant associated with *Alternaria* blight traits lateral (CA07.20522907, chromosome A07, $P = 12.90 \times 10^{-5}$, SNP effect - 5.36156), and CA09.1560419 SNP (chr, A09, Effect - 3.955693, $-\log_{10}(p)$ 8.291579), CA03.24022418 (Chromosome A03 Effect-4.012085, $-\log_{10}(p)$ 7.530178), (CB07.3169967, Chromosome, B07 Effect - 6.71656, $-\log_{10}(p)$ 7.097997), CB04.3377365, Chromosome, B04, Effect - 4.18757, $-\log_{10}(p)$ 6.876148) (CB03.15428252, Chromosome, B03, Effect-3.00002 - $\log_{10}(p)$ 6.50724).

Conclusion

Considerable genetic variability and disease screening was present among the mustard genotypes selected in the study. The highest range of variation was recorded in average HPF, FPF, MAT, PB, MSP, MSL, PL, SB, PH, DIS, YPP and genome wide association mapping, Both correlation and path co efficient analysis, cluster analysis carried in this study suggested that HPF, FPF, MAT, PB, MSP, MSL, PL, SB, PH, DIS, YPP and SNP resistance against *Alternaria* blight. The improvement in marketable yield per plant will be efficient if the selection is based HPF, FPF, MAT, PB, MSP, MSL, PL, SB, PH, DIS, on marketable resistance of *Alternaria* a disease. The above all estimation of heritability, correlation and path-coefficient analysis and SNP of mustard genotypes indicated that individual plant selection would be effective for varietal improvement of this crop.

Declarations

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Tables

Table-1. 0-9 scale for rating disease severity of Alternaria blight in Indian mustard ICRP on R&M- 2011)

Rating scale	Disease Intensity (%)	Pathogen Reaction
0	0	Near immune/highly resistant (I)
1	<5	Resistant (R)
3	5-10	Moderately Resistant (MR)
5	11-25	Moderately Susceptible (MS)
7	26-50	Susceptible (S)
9	>50	Highly Susceptible (HS)

Table-2 Analysis of variance (ANOVA) for eleven characters in Indian mustard genotypes.

Sources of variation	DF	FPF	HPF	MAT	PH	PB	DIS	SB	MSL	MSP
Replication	1	271.93	15.79	91.58	1376.24	953.46	279.29	60.84	146.08	0.10
Blocks(rep.)	18	499.06*	470.81 ^{ns}	2973.60**	24694.26 ^{ns}	1628.05**	2256.10 ^{ns}	3.65 ^{ns}	1007.63 ^{ns}	568.51
Treatments	437	503166.25**	628932.02**	1577247.56**	2425994.24**	355585.342**	149183.56**	17233.52**	148328.19**	19428
Error	436	11406	10515.36	46213.31	655167.21	15946.50	53329.00	4757.48	18063.33	9139.9

** = Significant at 1% level, * =Significant at 5% probability level, NS = Non significant

Table 3: Coefficient of variability, (GCV, PCV) heritability in broad sense and genetic advance in per cent of mean, in Indian mustard genotypes.

Traits	FPF	HPF	MAT	PH	PB	DIS	SB	MSL	MSP	PL	YPP
Genotypic Variance	567.91	707.54	1751.75	2026.11	388.60	109.53	21.19	148.99	211.83	1.44	1.58
Phenotypic Variance	594.01	731.66	1857.50	3525.35	425.09	231.84	32.07	190.42	232.75	1.90	17.76
GCV	32.42	32.26	31.90	34.79	44.99	40.01	74.72	59.35	40.21	31.89	27.24
PCV	33.16	32.81	32.85	45.89	47.06	58.21	91.93	67.10	42.15	36.58	91.05
Heritability (Broad)	97.77	98.33	97.11	75.81	95.61	68.73	81.27	88.45	95.40	87.19	29.91
Genetic Advance GA	49.09	54.79	86.21	92.72	40.60	21.55	9.48	25.14	29.98	2.47	2.59
Genetic Advance As % Mean	66.80	66.47	65.71	71.67	92.69	82.43	153.92	122.26	82.84	65.70	56.11

Table -4. (a) Estimation of phenotypic correlation coefficient between eleven characters in Indian mustard genotypes.

Traits	HPF	MAT	PH	PB	SB	MLS	MSP	PL	DIS	YPP
FPF	0.96**	0.91**	0.68**	0.41**	0.34**	0.25**	0.67**	0.82**	0.53**	0.41**
HPF		0.92**	0.70**	0.39**	0.35**	0.26**	0.67**	0.81**	0.53**	0.42**
MAT			0.72**	0.43**	0.36**	0.27**	0.70**	0.83**	0.56**	0.41**
PH				0.47**	0.35**	0.26**	0.68**	0.68**	0.45**	0.38**
PB					-0.19**	-0.49**	0.45**	0.55**	0.37**	0.19**
SB						0.62**	0.38**	0.26**	0.15**	0.28**
MSL							0.28**	0.14**	0.01**	0.19**
MSP								0.65**	0.47	0.36**
PL									0.59**	0.42**
DIS										0.23**

Table -4 (b) Estimation of genotypic correlation coefficient between eleven characters in Indian mustard

Traits	HPF	MAT	PH	PB	SB	MLS	MSP	PL	DIS	YPP
FPF	0.95**	0.92**	0.77**	0.91**	0.60**	0.46**	0.66**	0.83**	0.58**	0.99**
HPF		0.93**	0.79**	0.89**	0.60**	0.43**	0.66**	0.81**	0.58**	0.99**
MAT			0.83**	0.94**	0.62**	0.46**	0.69**	0.84**	0.62**	0.99**
PH				0.87**	0.72**	0.57**	0.82**	0.84**	0.60**	0.99**
PB					0.36**	0.25**	0.99**	0.75**	0.41**	0.89**
SB						0.49**	0.68**	0.57**	0.46**	0.82**
MSL							0.49**	0.51**	0.33**	0.63**
MSP								0.71**	0.56**	0.82**
PL									0.70**	0.99**
DIS										0.67**

Table 5 (a) Direct and indirect effect (Phenotypic) of eleven characters on grain yield per plant in Indian mustard genotypes.

Traits	FPF	HPF	MAT	PH	PB	SB	MSL	MSP	PL	DIS	YPP
FPF	-0.083	0.254	-0.058	0.043	0.0318	0.052	0.013	0.008	0.172	-0.021	0.415
HPF	-0.080	0.264	-0.058	0.044	0.030	0.030	0.014	0.008	0.170	-0.021	0.427
MAT	-0.076	0.244	-0.063	0.046	0.033	0.054	0.015	0.008	0.173	-0.0224	0.414
PH	-0.057	0.185	-0.045	0.064	0.036	0.054	0.014	0.008	0.142	-0.018	0.384
PB	-0.034	0.105	-0.027	0.030	0.077	-0.030	-0.026	0.005	0.114	-0.015	0.2
SB	-0.028	0.093	-0.022	0.022	-0.015	0.152	0.033	0.004	0.055	-0.006	0.289
MLS	-0.021	0.071	-0.017	0.016	-0.038	0.094	0.055	0.003	0.029	-0.0005	0.193
MSP	-0.055	0.178	-0.044	0.044	0.034	0.058	0.015	0.012	0.136	-0.018	0.361
PL	-0.069	0.216	-0.052	0.043	0.042	0.040	0.007	0.008	0.208	0.023	0.422
DIS	-0.044	0.140	-0.035	0.029	0.029	0.023	0.0007	0.005	-0.039	-0.04	0.233

Residual are- 0.77006

Table 5 (b): Direct and indirect effect (Genotypic) of eleven characters on grain yield per plant in Indian mustard genotypes.

Traits	FPF	HPF	MAT	PH	PB	SB	MSL	MSP	PL	DIS	YPP
FPF	0.027	0.172	-0.053	0.204	0.009	0.192	0.005	-0.022	0.804	-0.339	0.99**
HPF	0.025	0.172	-0.053	0.204	0.009	0.192	0.005	-0.022	0.804	-0.339	0.99**
MAT	0.024	0.160	-0.057	0.214	0.009	0.199	0.006	-0.023	0.828	-0.362	0.99**
PH	0.021	0.139	-0.048	0.253	0.008	0.212	0.006	-0.026	0.770	-0.337	0.999**
PB	0.025	0.158	-0.056	0.216	0.01	0.108	0.003	-0.032	0.699	-0.239	0.894**
SB	0.016	0.108	-0.037	0.175	0.003	0.306	0.005	-0.021	0.511	-0.242	0.826**
MLS	0.012	0.081	-0.028	0.141	0.002	0.140	0.012	-0.014	0.447	-0.158	0.636**
MSP	0.018	0.118	-0.041	0.204	0.009	0.207	0.005	-0.032	0.639	-0.309	0.820**
PL	0.023	0.150	-0.051	0.212	0.007	0.170	0.006	-0.022	0.919	-0.414	1.000**
DIS	0.018	0.117	-0.042	0.172	0.004	0.149	0.003	-0.020	0.767	-0.497	0.675**

Residual are - 0.22057

Table 6: Estimation of average inter and intra cluster D² values

Cluster	1	2	3	4	5	6	7	8
1	0.00	320.42	40.31	166.54	83.28	1066.82	94.11	254.14
2		0.00	312.93	342.27	310.50	1117.21	314.77	411.61
3			0.00	156.20	55.78	1073.23	65.19	245.64
4				0.00	150.12	1100.64	150.57	188.69
5					0.00	1060.58	70.28	253.36
6						0.00	1124.03	1228.26
7							0.00	219.73
8								0.00

Table 7: Cluster mean for different characters for Indian mustard genotypes

Cluster	FPF	HPF	MAT	PH	PB	SB	MSL	MSP	PL	DIS	YPP
1	80	91	145	146.35	5.77	13.2	70.37	40.51	3.84	23.77	5.53
2	80	85	142	135	30.7	6.7	14.8	43.6	4.34	33.8	6.25
3	79	94	149	138.16	14.33	7.91	32.77	44.08	3.79	26.23	5.56
4	91	99	149	119.23	40.38	5.68	4.74	28.17	4.5	28.18	7.63
5	80	89	144	149.48	64.07	5.44	11.84	41.67	4.25	29.7	4.93
6	82	97	143	121	65	5.8	15.8	45.8	3.52	36	5.5
7	86	94	147	86.51	38.77	4.22	8.45	25.91	4.04	27.89	4.84
8	0	0	0	0	0	0	0	0	0	0	0

Table 8: Significant SNP markers associated with disease of *Alternaria* blight in *B. juncea*.

Modal	SNP	Chromosome	Position	Allele	maf	Effect	$-\log_{10}(p)$
FarmCPU	CA07.20522907	A07	20522907	A/G	0.45098	-5.36156	12.90658
FarmCPU	CA09.1560419	A09	1560419	T/A	0.284314	3.955693	8.291579
FarmCPU	CA03.24022418	A03	24022418	T/C	0.186275	4.012085	7.530178
FarmCPU	CB07.3169967	B07	3169967	C/T	0.058824	-6.71656	7.097997
FarmCPU	CB04.3377365	B04	3377365	C/G	0.098039	-4.18757	6.876148
FarmCPU	CB03.15428252	B03	15428252	G/A	0.166667	-3.00002	6.50724

Figures

Figure 1

Manhattan plots of single nucleotide polymorphism (SNP) association with disease resistance phenotype (disease severity) *Alternaria* blight Indian Mustard. SNPs along each chromosome are represented along the x-axis, and the $-\log_{10}(P)$ value is shown along the y-axis. Indian mustard chromosomes 1–18 are shown in contrasting colours on the x-axis.

Figure 2

Quantile-Quantile (QQ) plot of the P-values observed and expected from the association analysis. Colour online