# GENETIC DIVERSITY IN AUSTRALIAN,INDIAN AND CHINESE OILSEED BRASSICA GERMPLASM AGAINST SCLEROTINIA- ROT RESISTANCE

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Sclerotinia stem rot (SSR) has been recorded with incidences upto 80% in the Punjab and Haryana states (Kang and Chahal 2001). Being a ubiquitous necrotrophic pathogen with many different hosts Sclerotinia stem rot is difficult to manage. For this reason, using stem and soil inoculation techniques, we evaluated oilseed *Brassica* germplasm from *Brassica napus* and *B. juncea* from India, China and Australia for resistance to *S. sclerotiorum*.The multivariate analysis is an important tool for the assessment of genetic divergence against Sclerotinia rot. Thus it is utilized to assess genetic divergence against Sclerotinia rot along with relative importance of different traits in the total divergence.

## MATERIALS AND METHODS

Seed of accessions of *B. napus* and *B. juncea* was obtained from Australia, China and India through an ACIAR collaborative programas listed in Tables 1. Ninety eight genotypes (*29 B. napus* and 69 *B. juncea)* were tested in the field at the CCS HAU, Hisar, India.The germplasm was hand sown on 23.10.2008 and 27.10.2009 in plot size of 5mx2m. Row to row and plant to plant spacing of 45 cm x 15 cm was maintained throughout the experiment.All recommended agronomic practices were followed.Thirty plants within each replication(3) were arbitrarily picked and inoculated at the flowering stage.Stem inoculation was carried out according to the method used by Buchwaldt *et al.* (2005) in both crop seasons. A single 5 mm diameter agar plug disc (Hisar isolate) was used to inoculate each plant.Agar plug was secured on the first internode above middle internode of each stem by twisting the ends of the parafilm strip around the stem. A wet cotton swab was also wrapped around the stem just above the top of parafilm strip to maintain high humidity during the infection period.Stem diameter was measured by Vernier callipers as reported by Li *et al.* (2006) and Buchwaldt *et al*. (2005).Stem lesion length (mm) was measured daily with ruler upto 9 weeks after inoculation (wai) but data presented are of 3 weeks after inoculation were categorized resistant and susceptible as this has been shown to be the time most ideal to demonstrate differences in host responses to the pathogen (Li *et al.* 2007).The dates of inoculation and of flowering for each genotype were recorded.The pooled data of two years were subjected to D2 analysis (Mahalanobis, 1930) as elaborated by Murty and Arunachalam (1966). The genotypes were grouped into different clusters by following Tocher's method as described by Rao (1952).

## RESULTS AND DISCUSSION

All 99 genotypes were grouped into 8 clusters, using the Tocher's method; in such a way that the genotypes within the cluster had smaller $D^2$ values among themselves than those belonging to different clusters (Table 1).Cluster I and IV each comprised of maximum number of genotypes, 31 and 16 followed by cluster VIII with twelve genotypes. Cluster II, III, VI and VI each included eight genotypes.Cluster V consisted of five genotypes.Most of the Indian accessions fell in cluster I II, III and IV, whereas, Australian genotypes existed in all clusters. China genotypes belong to cluster IV, VI, VII and VIII. Cluster VII had genotypes from both the China as well as Australian collections. The grouping of genotypes indicated that geographical distribution need not necessarily be the indicator of genetic divergence as reported byVerma and Sachan (2000), Jeena and Sheikh (2003). Based on stem lesion length (<3 cm) progressed after 3 weeks of inoculation and percentage of plant dead/wilted (<10%) as observed by Li *et al.*(2006),cluster VII genotypes of *B. juncea* included 'RH13', 'Ringot', 'Manasihuang', 'Brassica juncea 1', 'Brassica juncea 2', 'Brassica juncea 3' from China and 'JM 018' from Australia. For *B. napus* germplasm tested, 'Ag Outback', 'Monty', 'RR002', 'Ag Spectrum' from Australia showed useful resistance for breeding programme (Table1). None of the tested genotype of Indian origin was observed resistant to Sclerotinia rot. Genotypes from India, China and Australia tested expressed varying levels of resistance to SSR (Table 1). Genotypes from cluster I, II, III, IV, V, VI and VIII were susceptible. Plant age at the time of inoculation , stem diameter, stem lesion length and plant dead and wilted were the parameters determining Sclerotinia rot resistance with their contribution 5.7, 21.3, 28.4 and 44.6 per cent respectively

towards the total divergence (Table 2). Following stem inoculation by the mycelia disc technique, stem lesion length, stem diameter and incidence of plant death were important parameters to measure comparative resistance in genotypes has been reported by Buchwaldt *et al.* (2005), Li *et al.* (2004) and Li *et al.* (2006),which corroborate present study. The variance for the cluster means were calculated for these 4 quantitative characters. Maximum variance for cluster mean was observed for stem lesion length, plant wilted /dead, plant age at the time of inoculation and stem diameter, which suggested that these characters were highly responsible for genetic divergence in the present material (Table 4). This indicated that the parents selected for hybridization for Sclerotinia rot resistance on the basis of these characters are represented to be genetically diverse. The above results were supported by Kumar *et al*., 2007 and Yu-cheng *et al*., 2007. The $D^2$ analysis showed intra and inter-cluster distance (Table 3). Cluster VII and III were observed genetically most divergent with inter-cluster distance of 5.3 followed by cluster V and VII. The lowest inter-cluster distance (1.91) was found between cluster I and III,followed by cluster IV and VI indicating a close relationship between them. The highest intra-cluster distance (1.95) was observed in cluster V and lowest in cluster VI (fig 1). The genotypes grouped into same cluster displayed the lowest degree of divergence from one another, and in case crosses are made between genotypes belonging to same clusters, no transgressive segregant is expected from such combinations.Genotypes for hybridization may be chosen from widely separated cluster having maximum inter-cluster distance and belonging to the different cluster(fig 2).It is observed that there are several genotypes of cluster VII having Sclerotinia rot resistance included in the crossing programme of breeding having low stem lesion length and plant wilt. Although, for final selection of the parents for breeding programme, the genotypes to be used may be selected almost without exception or its proven performance in the areas of intended use including quantitative characters (Table 4) could be utilized in the quantitative characters and included in crossing with hybridization programme for getting desirable transgressive segregants and high heterotic response (Allard 1960, Murty and Arunachalam, 1966).

**Table-1** Grouping of Australian,Indian and Chinese oilseed Brassica germplasm into different cluster under field condition at CCS HAU-Hisar (India) evaluated by mycelial agar plug disc inoculation technique

| Cluster | Genotype | Mean plant age at time of inoculation and range (days) | Mean stem diameter and range (mm) | Mean stem lesion length and range (mm) | Percent dead /wilted plants and range |
|---|---|---|---|---|---|
| 1 | JN004, JO008, JN031, JN033, JM06002, JM06012, JM06013, JM06021, JM06026, JM06006, JM06014, JM06015, JM06011, JM016, JM06010, JO009, JR042, JN010, JN032, JM06020 (A,Bj)*, Vasundera, RGN13, Bio902, Aravali, Jagannath, Swaran jyoti, Kranti, GM2, Geeta, JM 1, Pusa mahak,(I,Bj) | 81 (76-84) | 11 (8-16) | 92 (70-118) | 38.16 (26.66-53.33) |
| 11 | JR049, JM06001, JN028 (A,Bj), CS 54, Ashirwad, GM 3, Basanti, CS 52 (I,Bj) | 79 (78-81) | 9 (8-18) | 73 (48-85) | 25.33 (20-30) |
| 111 | Laxmi,Narender ageti,JM 2,JM 3,Maya,Urvashi (I,Bj),JM06004,JM06003 (A,Bj) | 78 (77-81) | 10 (8-12) | 118 (108-150) | 60.00 (53.33-73.33) |
| IV | Trigold,RR013,Trilogy,RR009 (A,Bn), Datonghuangiie, RL, Loiret, Montana, AmoraIII, Jinshahuang, Tunliuhuangiie, TQ0055-02W2, Yilihuang, Qianxianjiecai (C,Bj),Teri 9903,GSL 1 (I,Bn) | 103 (99-105) | 16 (11-20) | 104 (78-140) | 43.56 (30-56.66) |
| V | Surpass 400, Scar, Mystic (A, Bn), JM06018, JM06019 (A, Bj) | 88 (81-102) | 20 (18-24) | 122 (95-150) | 64.46 (56.66-70) |
| V1 | Rivette, RR005, Lantern, RQ 001-02M2, Purler, Skipton, RR001(A,Bn), Hatianyoucai (C,Bj) | 102 (101-105) | 22 (19-25) | 91 (74-105) | 41.11 (26.66-46.66) |
| V11 | Monty, RR002, Ag Outback, Ag Spectrum (A,Bn), Manasihuang, Brassica juncea 2, Brassica juncea 1, Brassica juncea 3,RH 13,Ringot (C,Bj), JM018(A,Bj) | 102 (85-106) | 15 (11-21) | 20 (2-30) | 3.00 (0-10.00) |
| V111 | Rainbow, Tranby, RQ011, Av Sapphire, Charlton, BST-702N2 (A,Bn) Ekla, RK 2, Haoyou II, Berry (C,Bj), GSC 5, HNS0501 (I,Bj) | 101 (96-102) | 16 (14-23) | 70 (42-110) | 24.5 (10-36.66) |

Fig in parenthesis (*) indicate A=Australia,C=China,I=India,Bj=*Brassica juncea*,Bn=*Brassica napus*

**Table-2** Contribution of different characters determining genetic divergence in oilseed Brassica germplasm against Sclerotinia rot

| Characters | percent contribution |
|---|---|
| Plant age at the time of inoculation (days) | 5.7 |
| Stem diameter (mm) | 21.3 |
| Stem lesion length(mm) | 28.4 |
| Percent plant mortality/dead | 44.6 |

**Table -3** Average intra and inter-cluster distance $D^2$ values in oilseed Brassica gemplasm

| | 1 Cluster | 2 Cluster | 3 Cluster | 4 Cluster | 5 Cluster | 6 Cluster | **7 Cluster** | **8** |
|---|---|---|---|---|---|---|---|---|
| 1 Cluster | 1.17 | 1.95 | 1.91 | 2.58 | 3.33 | 3.29 | 3.92 | 2.50 |
| 2 Cluster | | 1.50 | 3.16 | 3.38 | 4.48 | 3.85 | 3.16 | 2.51 |
| 3 Cluster | | | 1.08 | 2.92 | 3.07 | 3.95 | 5.26 | 3.55 |
| 4 Cluster | | | | 1.25 | 2.40 | 1.92 | 3.75 | 1.99 |
| 5 Cluster | | | | | 1.95 | 2.56 | 5.24 | 3.55 |
| 6 Cluster | | | | | | 1.02 | 3.46 | 2.22 |
| 7 Cluster | | | | | | | 1.14 | 2.26 |
| 8 Cluster | | | | | | | | 1.10 |

**Table-4** Cluster means of different characters determining Sclerotinia rot resistance

| | **X** | **X** | **X** | **X** |
|---|---|---|---|---|
| 1 Cluster | 79.7 | 11.6 | 91.6 | 38.3 |
| 2 Cluster | 79.8 | 10.0 | 59.3 | 22.6 |
| 3 Cluster | 78.6 | 10.1 | 116.1 | 60.4 |
| 4 Cluster | 102.8 | 16.0 | 105.2 | 43.5 |
| 5 Cluster | 93.6 | 20.4 | 122.0 | 64.7 |
| 6 Cluster | 102.1 | 22.2 | 90.4 | 36.2 |
| 7 Cluster | 103.4 | 15.5 | 24.3 | 2.5 |
| 8 Cluster | 101.2 | 14.7 | 70.1 | 24.8 |

$X_1$=plant at time of inoculation  $X_2$=Stem diameter  $X_3$ =Stem lesion length  $X_4$=plant dead/wilted

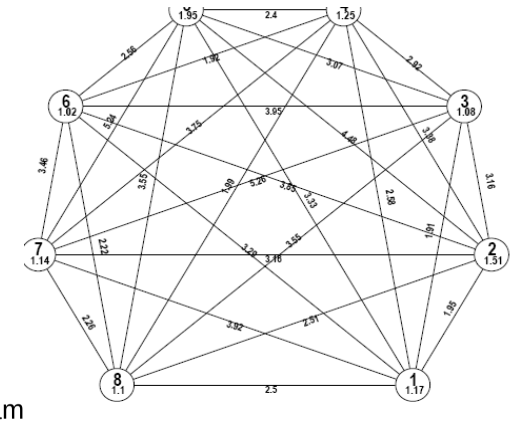


Fig-1 Euclidean cluster distance diagram