

**Abstract No: BO-28**

***In-vitro* screening of *Punica granatum* breeding lines for anthracnose disease resistance by detached leaf inoculation technique**

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The economic losses of *Punica granatum* cultivation are significantly reduced due to several factors, including anthracnose caused by several *Colletotrichum* species, notably *Colletotrichum gloeosporioides*. Primary inoculum disseminated by wind or rain and infects the host using diverse strategies and causes biotrophic and necrotrophic infections. It can lead to significant economic losses in *Punica granatum* orchards by causing fruit rot, premature fruit drop, and decreased yield. The current study aimed to screen the pathogenicity of *Colletotrichum* sp. against six new *Punica granatum* lines to evaluate their level of resistance against anthracnose disease. Detached healthy, young seven leaves of six *Punica granatum* breeding lines and three varieties were collected for in vitro screening. These leaves were wounded and subjected to drop inoculation with  $1 \times 10^5$  conidia/ml spore suspension, with five replicates per treatment, one leaf was used as negative control with sterile distilled water drop and one leaf was used as physical control which was repeated thrice. After incubation of inoculated leaves for two days in a moist chamber, leaf and lesion areas were measured using ImageJ software. Then, the disease severity was calculated as the percentage of infected leaf area over total leaf area, breeding lines were assigned to 1-5 rating scale (0–5% = highly resistance, >5–10% = resistance, >10–25% = moderate resistance, >25–50% = susceptible, >50% highly susceptible). Significance of disease severity among varieties and breeding lines were analyzed by ANOVA and Duncan's post-hoc test using SAS statistical software. According to the ANOVA test, the effect of variety line on disease severity was significant. Thus there were significant differences in disease severity among the six lines and three varieties ( $F(8,70) = 4.03$ ,  $p < 0.0001$ ). Average disease severity percentages of breeding lines, 67, 78, TC11, 46, A1 and A2 were  $0\% \pm 0.00$ ,  $2.0\% \pm 3.99$ ,  $4.8\% \pm 1.81$ ,  $15.93\% \pm 2.49$ ,  $24.16\% \pm 9.69$  and  $25.76\% \pm 13.18$ , respectively. According to disease severity estimates, 67, 78 and TC11 lines were rated as highly resistant lines and whereas 46, A1 were moderate resistant and A2 was susceptible. Average disease severity percentages of the recommended varieties Nimali, Daya and Kalpitiya hybrid were  $4.27\% \pm 5.76$ ,  $4.57\% \pm 6.84$  and  $36.18\% \pm 21.11$ , respectively. Nimali and Daya varieties were rated as highly resistant varieties and whereas KH was susceptible. Duncan's Multiple Range Test further identified distinct groups of variety and line based on the disease severity, thus there is no statistically significant difference among the resistance levels of 67, 78 and TC11 new lines and Nimali and Daya varieties and indicate higher resistance to *Colletotrichum* sp. infection. 46, A1 and A2 new lines have no statistically significant difference among the resistance level and KH variety indicates a significantly higher disease severity compared to all other lines and varieties at  $\alpha = 0.05$ . Based on the current study and according to the results *Punica granatum* breeding lines 67, 78 and TC11 have high level of resistance to *Colletotrichum* sp. infection causing anthracnose disease.

**Keywords:** Breeding lines, *Colletotrichum*, Detached leaves, Fruit rot, Resistant varieties