# RESULTS

## Collection and maintenance of *Pyricularia grisea* isolate

A total of 70 monoconidial *Pyricularia grisea* isolates were obtained from infected weed and rice leaves and panicles collected in Central part Laos. They were collected from five districts of Savanakhet Province namely, Khanthabuly, Champhone, Xonbuly, Xayphuthong and Xaybuly (Table 2).

Table 2. *Pyricularia grisea* monoconidial isolates collected from diseased samples from five districts of Savanakhet Province.

| District | sampled fields | Weed isolates | Rice isolates | Total isolates |
|---|---|---|---|---|
| Khanthabuly (SKH) | 11 | 0 | 8 | 8 |
| Champhone (SCH) | 15 | 3 | 30 | 33 |
| Xonbuly (SXB) | 5 | 0 | 10 | 10 |
| Xayphuthong (SXT) | 2 | 0 | 7 | 7 |
| Xaybuly (SXB) | 7 | 11 | 1 | 12 |
| Total | 40 | 14 | 56 | 70 |

## Parental lines and test varieties screening

Data from parental screening using 50 isolates showed that JHN is resistant to all isolates with RI value of 1 obtained from leaf blast screening. The RI value of JHN is 62% higher than KDML 105 as shown in Figure 3.1.

Result of parental lines and test varieties screening using 22 isolates showed that resistance index of JHN, IR62266 and CT9993 were resistant to all isolates with RI value of 1 obtained for leaf blast screening. The RI value was 80% higher than KDML105 as shown in Figure 3.2.

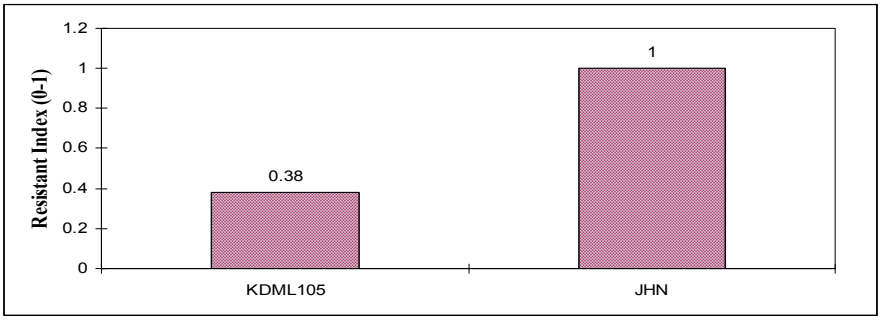


Figure 3.1. Resistance index (RI) of KDML105 and JHN screened for leaf blast using 50 isolates.

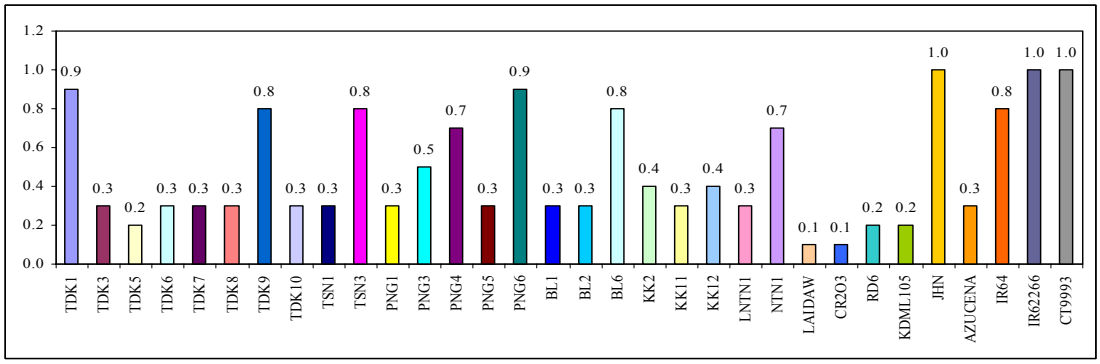


Figure 3.2. Resistance index (RI) of 32 parental lines and test varieties screened for leaf blast using 22 isolates.

## Pathogenicity Analysis

*Pathotypic similarity and cluster analysis of 50 blast isolates*

The data were analyzed using cluster analysis by sampling matching followed by UPGMA in NTSYS-pc. Dendrogram was derived from the virulence pattern of 50 blast isolates (8 weed and 42 rice isolates) using JHN and KDML105 cultivars as shown in Figure 4.1. The 50 isolates used to screen the parental lines showed pathotypic similarity. The 8 weed isolates were grouped as one using similarity index of 65% while the 42 rice isolates were grouped into two using the same similarity index. Using similarity index of 70%, the rice isolates were grouped into three based on the isolates virulence pattern.

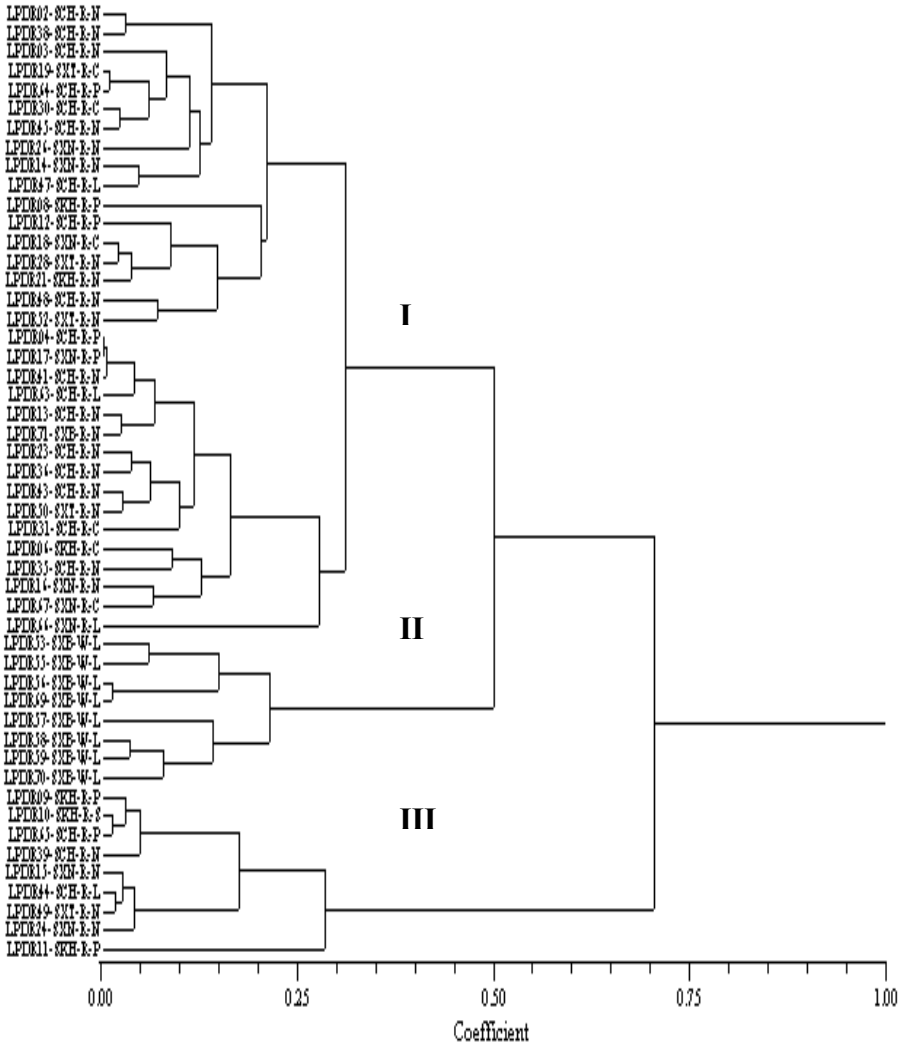


Figure 4.1. Dendrogram derived from virulence pattern of 50 blast islates using KDML105 and JHN cultivars by sampling matching followed by UPGMA NTSYS-pc.

The 22 rice isolates selected out of the 50 showed pathotypic similarity as shown in Figure 4.2. The isolates were clustered into two groups at similarity index level of 45% and into three small groups at 62%. 3 isolates from 3 groups were chosen to screen RILs for the virulence pattern of the isolates.

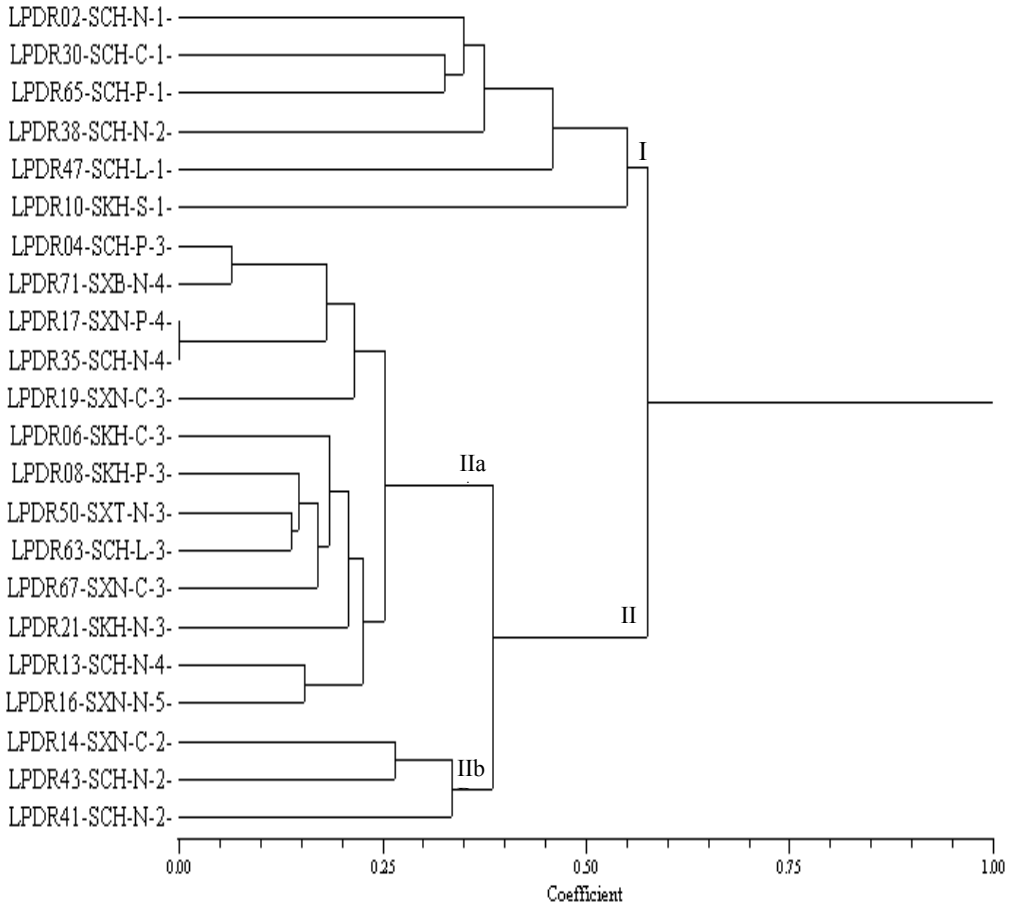


Figure 4.2.Dendrogram derived from virulence pattern of 22 blast isolates using 32 test varieties including KDML105 and JHN cultivars by CANBERRA similarity coefficient and the UPGMA NTSYS-pc.

Similarity and clustering analysis of the 22 Lao isolates and 5 Thai isolates based on their virulence pattern showed that 5 Lao isolates belong to THL318 were in group I. 16 Lao isolates were similar to group II that is more aggressive (THL557, THL899, TH16 and THL191) at similarity index of 50% (Figure 4.3). LPDR10 was out of group that was considered avirulent and intermediate reaction on some susceptible varieties.

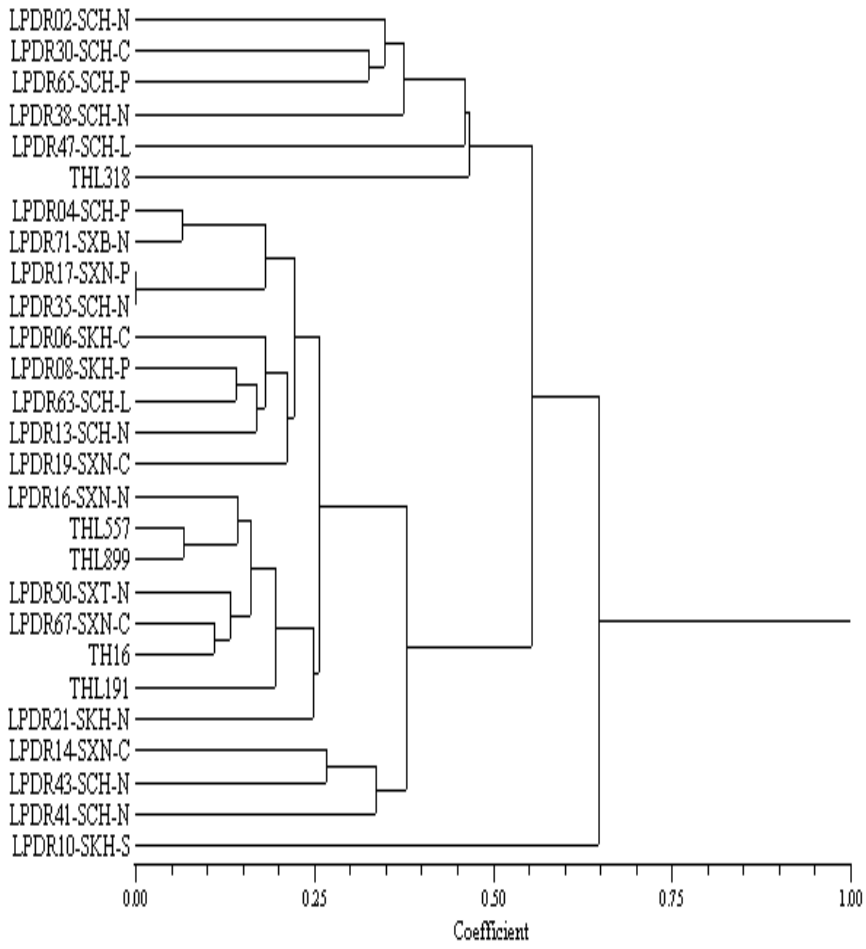


Figure 4.3. Dendrogram derived from virulence pattern of 22 isolates from Laos and 5 isolates from Thailand using 32 test varieties including KDML105 and JHN cultivars by CANBERRA similarity coefficient and the UPGMA NTSYS-pc.

**Virulent Analysis**

The results of virulence testing of 22 isolates with 32 test varieties are summarized in Table 3. The data were classified into 2 groups, Avirulent (0, 1, 2, 3) and Virulent (4, 5, 6). From 22 blast isolates, 12 were virulent (55%) and 10 were not against the 32 test varieties. None of the Lao blast isolates were virulent on JHN and CT9993 and only two isolates infected IR64 and IR62266.

Table 3. Summary of 22 *Pyricuria grisea* isolates virulence reaction on 32 parental lines and test varieties

| No. | Isolate | Avirulent | virulence | % of virulence |
|---|---|---|---|---|
| 1 | LPDR02 | 29 | 3 | 9 |
| 2 | LPDR04 | 12 | 19 | 61 |
| 3 | LPDR06 | 16 | 16 | 50 |
| 4 | LPDR08 | 13 | 19 | 59 |
| 5 | LPDR10 | 32 | 0 | 0 |
| 6 | LPDR13 | 11 | 21 | 66 |
| 7 | LPDR14 | 26 | 6 | 19 |
| 8 | LPDR16 | 3 | 28 | 90 |
| 9 | LPDR17 | 10 | 21 | 68 |
| 10 | LPDR19 | 12 | 20 | 63 |
| 11 | LPDR21 | 22 | 10 | 31 |
| 12 | LPDR30 | 30 | 2 | 6 |
| 13 | LPDR35 | 10 | 21 | 68 |
| 14 | LPDR38 | 30 | 2 | 6 |
| 15 | LPDR43 | 32 | 0 | 0 |
| 16 | LPDR41 | 27 | 5 | 16 |
| 17 | LPDR47 | 28 | 3 | 10 |
| 18 | LPDR50 | 11 | 21 | 66 |
| 19 | LPDR63 | 16 | 16 | 50 |
| 20 | LPDR65 | 31 | 1 | 3 |
| 21 | LPDR67 | 10 | 22 | 69 |
| 22 | LPDR71 | 12 | 19 | 61 |
| | Total | 10 | 12 | 55 |

**Phenotypic Reaction to Leaf Blast Resistance**

The 587 RILs of the cross KDML105/JHN were used for QTL mapping using 3 blast isolates. These isolates were selected from 22 isolates based on the differential virulence spectrum of 32 parental lines and test varieties and used as the representative isolates of each pathotypic grouping. After inoculation, disease severity groups for leaf blast were obtained as shown in Table 4 and Figure 5. The disease severity index showed abnormal distribution. 71% of RILs were resistant to LPDR16, 75% were resistant to LPDR47 and only 52% were resistant to LPDR41. Phenotypic distribution of leaf blast severity revealed discrete classes of resistant and susceptible reactions.

Table 4. Ratio between resistant and susceptible reactions and RI values of 587 RILs screened for leaf blast resistance using 3 selected isolates.

| Isolate | Disease severity index | | | | | | | Total | Ratio | | AVI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | | R:S | R:S | |
| LPDR16 | 108 | 101 | 58 | 115 | 84 | 38 | 35 | 539 | 382:157 | 2.4:1 | 0.70 |
| LPDR41 | 16 | 109 | 78 | 85 | 94 | 113 | 64 | 559 | 288:271 | 1.0:1 | 0.51 |
| LPDR47 | 84 | 198 | 81 | 59 | 74 | 50 | 14 | 560 | 422:138 | 3.0:1 | 0.75 |

However, if the data were to be classified into 2 groups, resistant (0, 1, 2, 3) and susceptible (4, 5, 6), the chi-square ($X^2$) test revealed that 2 out of 3 sets of data had fitted a ratio of approximately 3:1 and one set fitted 1:1 ratio of resistant to susceptible population. The ratio of 3:1 was observed using LPDR16 (382:157) and LPDR47 (422:138) while ratio of 1:1 was observed using LPDR41 (288:271). When Avirulent Index (AVI) was calculated, results showed that an AVI value of 0.51 using LPDR41 was lower than the two isolates (0.70 and 0.75) for LPDR16 and LPDR47 respectively.

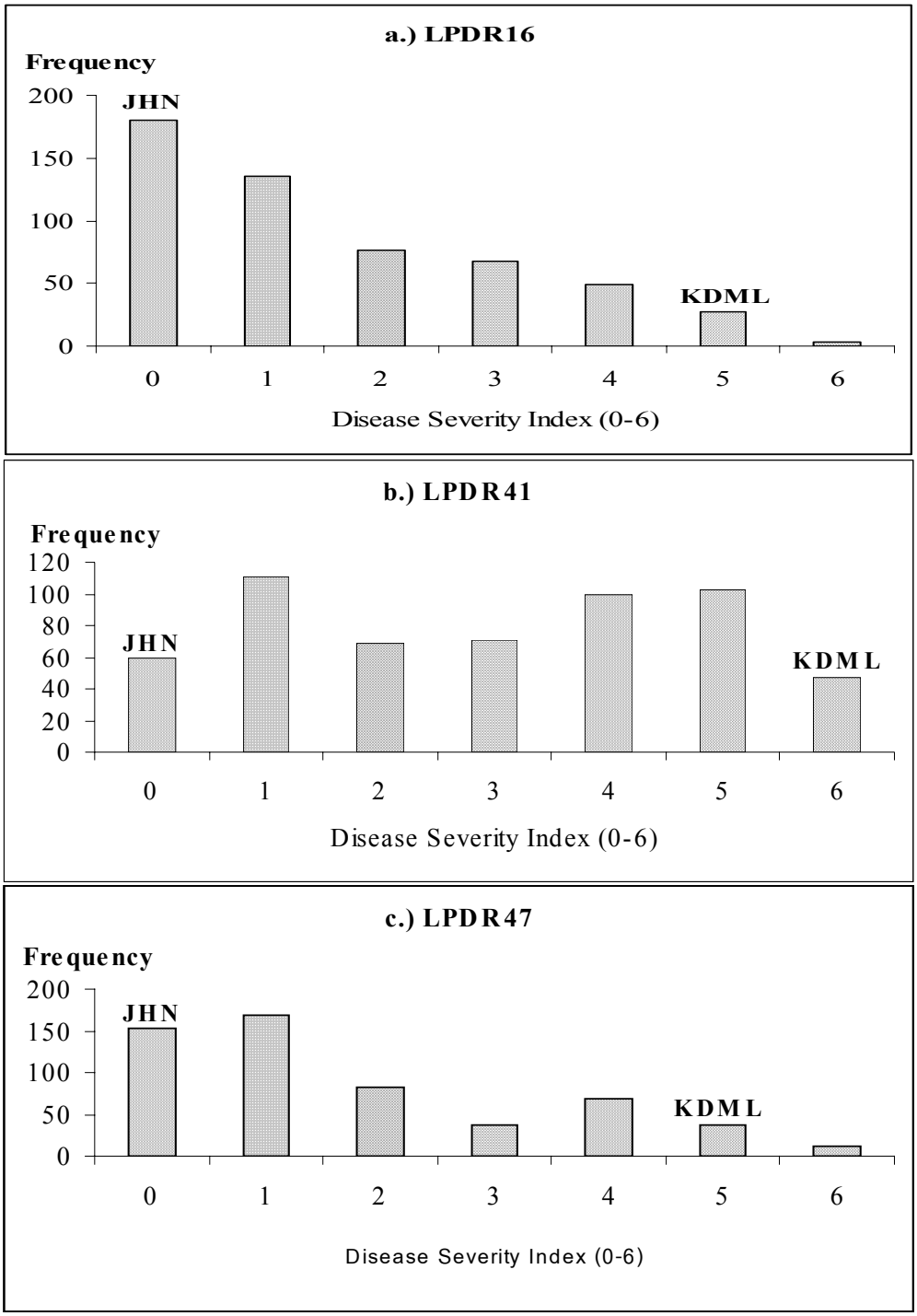


Figure 5. Distribution of disease severity index of 587 RILs screened for leaf blast resistance using 3 selected isolates

**Quantitative Trait Loci (QTL) Analysis**

Intervals and peaks of QTL for leaf blast resistance and linkage map of 587 RILs were shown in Table 5 with details as follows.

After inoculation with LPDR16, the six markers designated as RM5, RM246, RM237, RM319, RM212 and RM104 were mapped on chromosome1. One QTL for leaf blast resistance was detected with the peak between RM237 – RM319 markers having LOD of 8.82. Results from the same isolate revealed the other QTL location on chromosome11 mapped with seven markers designated as RM21, RM206, RM254, AC113249, RM224, RM139 and RM144. The peak was found at RM139 with LOD of 58.63. The coefficient of determination ($R^2$) was 45.10 % with JHN as sole contributor of all resistant alleles.

When the second isolate LPDR41, was inoculated onto the same population, one QTL was also detected on chromosome1 between RM246 – RM104 markers having the peak between RM237 – RM319 with LOD of 10.09. The other QTL was found on chromosome11 located between RM21 – RM224 with the peak at RM139 having LOD of 86.02. The total coefficient of determination ($R^2$) was 58.06 %. Similarly, JHN had contributed resistant alleles to these QTLs.

After the third isolate, LPDR47 was used; three QTLs were located on all three chromosomes 1, 11 and 12. The first one was detected on chromosome1 between RM246 – RM104 with the peak at RM319 and LOD of 6.71. The second one was found on chromosome11 between RM21 – RMRM224 with the peak at RM139 having LOD of 24.24. For two QTLs, JHN was the sole contributing parent of resistant alleles. The third QTL location was detected on chromosome12 between OSR32 – RM309 having the peak between OSR32 - RM179 and with LOD of 19.54. The total coefficient of determination ($R^2$) was 39.96 % for leaf blast.

Table 5. Intervals and peaks of QTLs on chromosome1, 11 and 12 located on 16 SSR markers.

| Isolate | Chromosome | Contributor | Interval | Peak | LOD | Total $R^2$ % |
|---|---|---|---|---|---|---|
| LPDR16 | 1 | JHN | RM246-RM104 | RM237-RM319 | 8.82 | 45.10** |
| | 11 | JHN | RM21-RM244 | RM139 | 58.63 | |
| LPDR41 | 1 | JHN | RM246-RM104 | RM237-RM319 | 10.09 | 58.06** |
| | 11 | JHN | RM21-RM244 | RM139 | 86.02 | |
| LPDR47 | 1 | JHN | RM246-RM104 | RM319 | 6.71 | 39.96** |
| | 11 | JHN | RM21-RM244 | RM139 | 24.24 | |
| | 12 | KDML105 | OSR32-RM309 | OS32-RM179 | 19.54 | |

Data from sixteen SSR markers were analyzed for the order of markers by MAPMARKER/QTL developed by Lander *et al.* (1987). Distance between markers was analyzed using Kosambi. The order of six markers on chromosome1 started from RM5, RM246, RM237, RM319, RM212, and RM204 and the distance between markers are 9.3, 7.6, 12.3, 1.5 and 22.7 cM, respectively. The total distance on chromosome1 was 51 cM while that on chromosome11 was as long as 84.9 cM. There were seven markers mapped on chromosome11 in the following order: RM21, RM206, RM254, AC113249, RM144, RM139 and RM244. Distances between these markers were 22.7, 20.7, 9.4, 13.3, 2.9 and 10.6 cM, correspondingly. The total distance on chromosome 12 appeared to be the shortest among the three chromosomes with distances between markers at 12.7 and 27.4 cM, respectively (Figure 6).

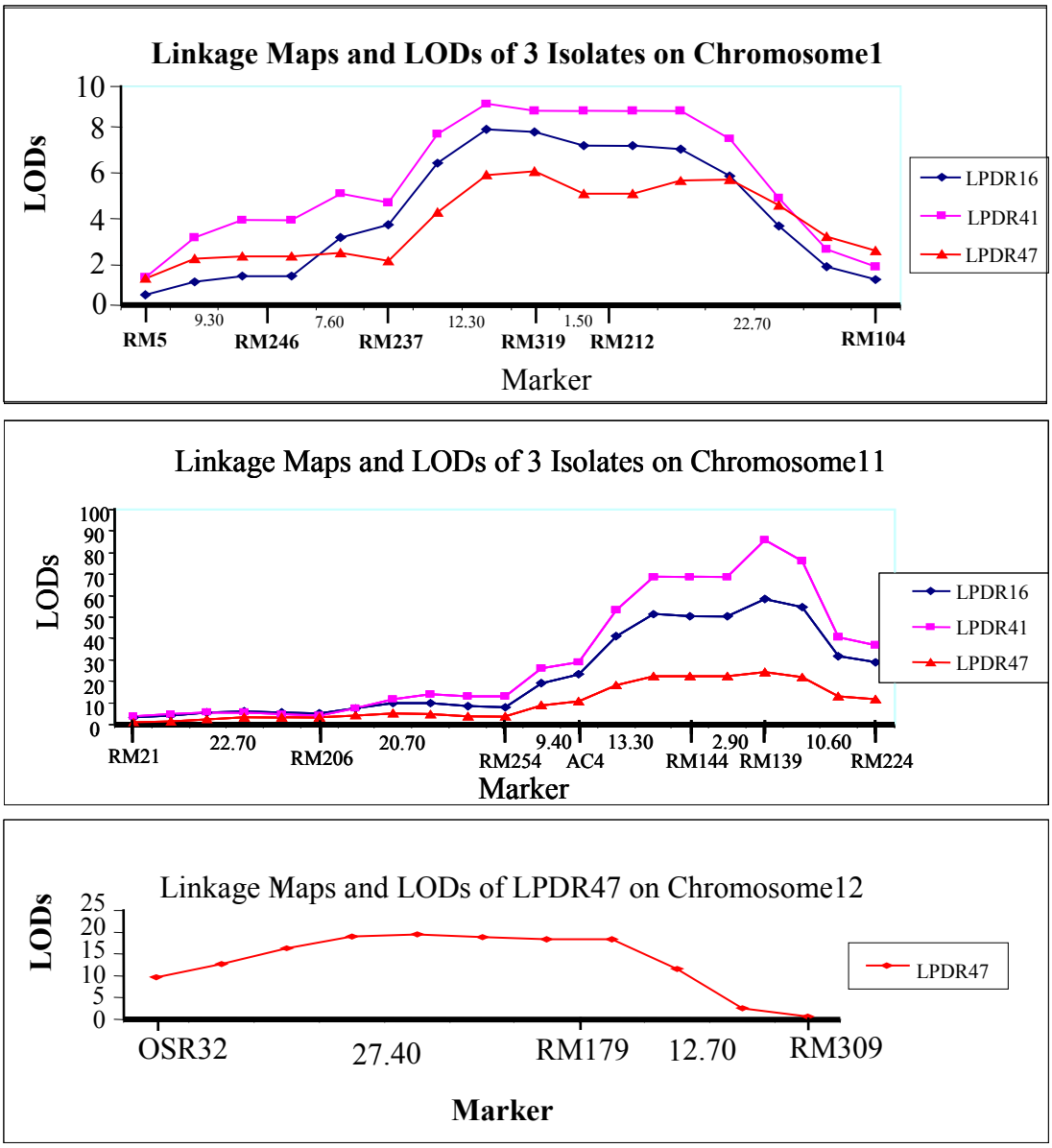


Figure 6. Linkage map of 587 RILs from KDML 105 and JHN population. Sixteen SSR markers were used to locate QTL following inoculation with LPDR16, LPDR41 and LPDR47 respectively. QTL likelihood map for leaf blast resistance on chromosome 1, 11 and 12 constructed with LOD of 6, 7 and 3.

On chromosome1, a likelihood of the presence of QTLs for leaf blast was found between RM246 and RM104 with the peaks between RM237 and RM319 using all three blast isolates LPDR16, LPDR41 and LPDR47. The presence of QTL on chromosome11 was found between RM21 and RM244 with the peak at RM139 following the inoculation of LPDR16 and LPDR41. On chromosome 12, QTLs were found between OSR32 - RM309 with the peaks between OSR32 - RM179 using LPDR47.

**Main Effects and Interactions of Genes**

Results from phenotypic reaction and analysis have supported those of the genetic analysis. Using NQTL analysis, the main effect was detected on chromosome11 between RM21 and RM224 using LPDR16 and LPDR41 with JHN as contributor of all resistant alleles. This was reflected by lower mean scores for disease severity ranging from 0.38 – 0.43 whenever JHN alleles were present on chromosome11 in comparison with 2.85 as recorded for the homozygous alleles of KDML105 (Figure 7). The minor effect was detected on chromosome1, 11 and 12 following inoculation of LPDR47. Results showed that resistant alleles on chromosome1 and 11 were obtained from JHN while those on chromosome12 were obtained from KDML105 as reflected by a low mean score of disease severity (0.53) for heterozygous population with JHN alleles on chromosome1 and 11 and KDML105 on chromosome12 . There were additive interactions between three QTLs on chromosome1, 11 and 12 following inoculation with LPDR47. Whenever JHN alleles were present, mean scores of disease severity were low ranging from 0.53 - 3.82. The lowest mean score of 0.53 was recorded when JHN was the sole contributor of all resistant alleles on chromosome1, 11 and 12 suggesting a high degree of resistance index of JHN.

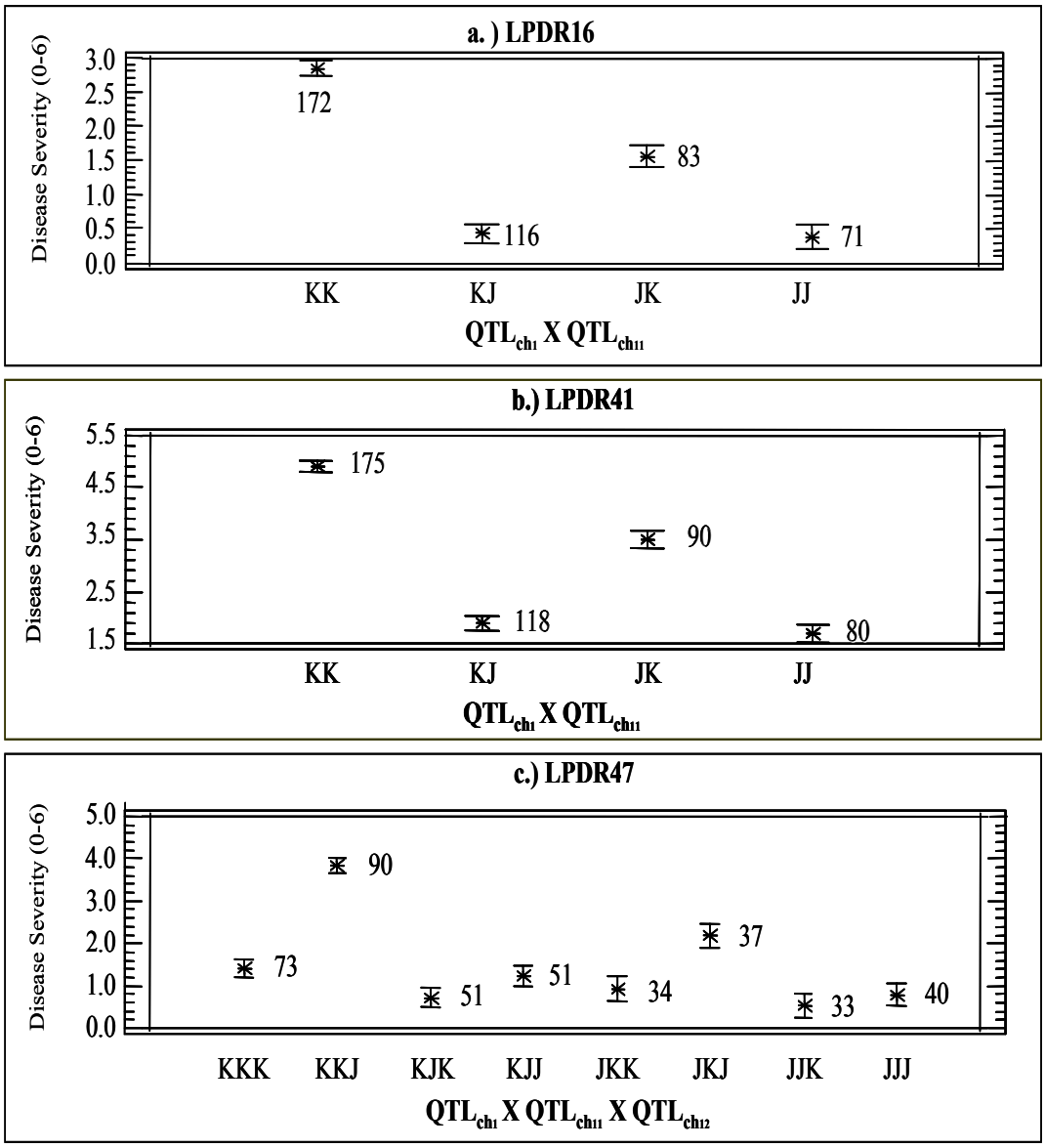


Figure 7. Interactions between QTLs for leaf blast resistance on chromosome1, 11 and 12 obtained after inoculation with LPDR16, LPDR41 and LPDR47 having 95.0 % LSD interval.