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# Genetic molecular diversity in interspecific peanut lines differing in temporal resistance to peanut bud necrosis disease

**Jignesh H. Kamdar, Bindu R. Goswami and Sandip Kumar Bera***

Directorate of Groundnut Research (ICAR), Junagadh 362001, Gujarat, India.

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**Peanut bud necrosis disease is one of the major diseases in peanut. Interspecific pre-breeding lines were identified as resistant to bud necrosis disease. Molecular diversity analysis in 115 lines resistant to bud necrosis disease using simple sequence repeat markers revealed wide genetic diversity among lines. Out of 219 bands amplified, 205 were found polymorphic. Polymorphism information content (PIC) value ranged from 0.5 to 0.94, with an average of 0.82. The cluster analysis and PCoA grouped 115 resistant lines and one susceptible cultivar into three major clusters sharing 58% similarity. Susceptible cultivar KRG-1 was distantly related to resistant lines NRCGCS-28 and NRCGCS-86. AMOVA predicted 96% variation within population and 4% among populations. NRCGCS-28 and NRCGCS-86 were found to be moderately resistant and KRG-1 as highly susceptible under artificially challenged inoculation conditions. The incubation period for appearance of disease symptoms were longer in NRCGCS-28 and NRCGCS-86 than KRG-1 under artificially challenged inoculation conditions. Thus, the present study reports additional sources for resistance to peanut bud necrosis disease (PBND).**

**Key Words:** Peanut, peanut bud necrosis disease (PBND), simple sequence repeat (SSR), genetic diversity.

## INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important legume grown in approximately 24 m ha throughout the world (FAO statistical database, 2010). Peanut bud necrosis disease (PBND) caused by peanut bud necrosis virus (PBNV) vectored by Thrips palmiKarny (Vijayalakshmi et al., 1995) has emerged as a serious yield constraint in South and Southeast Asia. Up to 80% yield losses were reported due to PBND in India (Dwivedi et al., 1993; Basu, 1995; Singh and Srivastava, 1995). The yield loss due to PBND in groundnut mainly depends on the time of infection. If the infection occurs on young plants (before 60 days after sowing) pod yield loss will be 100% (Gopal and Upadhyaya, 1988). If infection occurs after the plants start to produce pods, losses are minimal. Sometimes low disease incidence in certain lines could be because of vector non-preference (Buiel, 1993). Although progress has been made in breeding peanut for resistance to PBND and several high-yielding peanut lines resistant to PBND have been generated over the years (Reddy et al., 1995; Bera et al., 2010a, 2010b), complete host plant

*Corresponding author. E-mail: berask67@yahoo.co.in.

**Abbreviations: PBND,** Peanut bud necrosis disease; **PBNV,** peanut bud necrosis virus; **RAPD,** random amplification of polymorphic DNA; **ISSR,** inter-simple sequence repeat; **AFLP,** amplified fragment length polymorphism; **SSR,** simple sequence repeat; **CTAB**, cetyle trimethyl ammonium bromide; **PCR,** polymerase chain reaction; **PIC,** polymorphism information content; MI, marker Index; **PCoA,** principal coordinate analysis; **AMOVA,** analysis of molecular variance.

resistance to PBNV is scarce. Identification of peanut genotype that can tolerate the disease during early stages of crop growth is more desirable in mitigating yield loss.

Thus, it is imperative to identify genetically diverse source of resistance to PBNV for widening the genetic base of cultivated peanut. Better knowledge on the genetic similarity of breeding materials could help maintain genetic diversity and sustain long-term selection gain. Several previous studies have used molecular markers for identification of genetically diverse parents which can be used in cultivar improvement programme. However, cultivated peanut has been characterized with narrow genetic base and exhibits a low level of variation at the DNA level using random amplification of polymorphic DNA (RAPD) (Halward et al., 1991; Subramanian et al., 2000; Mondal et al., 2005), inter-simple sequence repeat (ISSR) (Raina et al., 2001), amplified fragment length polymorphism (AFLP) (Herselman, 2003) and simple sequence repeat (SSR) markers (Halward et al., 1991; Kochert, 1991; Paik-Ro, 1992; Hilu and Stalker, 1995; Kochert et al., 1996; Hopkins et al., 1999; He and Prakash, 1997; Subramanian et al., 2000; Herselman, 2003). Of the major DNA marker types, SSR marker has been the most successful in identifying molecular variation within the cultivated peanut species (Hopkins et al., 1999; Ferguson et al., 2004; Mace et al., 2006). How-ever, no information is available on genetic diversity in peanut to resistance to PBND. Hence 435 interspecific pre-breeding lines were screened for resistance to PBND over 2 seasons under field conditions. Besides, the lines were found to be resistant and highly-resistant along with susceptible cultivar were subjected to polymorphism analysis of SSR marker for identification of genetically diverse pre-breeding lines.

## MATERIALS AND METHODS

Directorate of Groundnut Research (DGR), Junagadh, India has developed a large number of interspecific breeding lines over a period of time to introgress desirable genes from wild *Arachis* species to cultivated peanut. Among them, a set of 435 interspecific peanut breeding lines developed using cultivated peanut as female parent and wild *Arachis* species viz, *Arachis diogoi, Arachis correntina, Arachis helodes, Arachis pusilla, Arachis cardenasii, Arachis duranensis, Arachis batizocoi*, *Arachis stenosperma*, *Arachis monticola, Arachis villosa*, *Arachis* kempff-mercadoi, *Arachis pintoi, Arachis kretschmeri, Arachis oteroi* and *Arachis villosulicarpa* were screened for resistance to PBND during kharif 2010 (June to October) and also during rabi 2011 (January to May). The lines were screened under field conditions in the farm of University of Agricultural Sciences (UAS), Raichur, Karnataka, a hot spot for PBND. Raichur is situated between 16°15'N latitude and 77°20'E longitude at an elevation of 389 m above mean sea level with an average rainfall of 621.33 mm. The monthly mean maximum and minimum temperature of 38.0 and 16.2°C were recorded in the month of April and December, respectively. The mean relative humidity varies between 52.96% in April and 83.86% in August (http://www.uasraichur.edu.in). The screening for resistance to PBND was done under natural disease incidence condition. Lines were sown in Randomised Complete Block Design (RCBD) with three replications. The crop was raised as per the recommended package of practices except for the plant protection measures against PBND. Each genotype was sown in 2 rows of 5 m length and at every 4th row, a susceptible check KRG-1 was planted with a spacing of 45 cm between rows and 10 cm between plants. Crop grown during rabi (February - May) season was irrigated at regular interval whereas life saving irrigation was provided to kharif (June - October) season crop to maintain healthy growth of the crop.

Initial plant count was recorded in all lines at 20 days after sowing while the number of healthy and diseased plants were recorded one week before harvest of the crop and expressed in terms of percent disease incidence. The percent PBND incidence was calculated by using the formula "Percent disease (%) = (Number of PBND infected plants/ Total number of plants) (X) 100" and was pooled over two seasons. Based on pooled disease incidence, lines were grouped into different categories following standard (0-5) disease rating scale (Sunkad et al., 2012). Genomic DNA was extracted from the leaf samples collected from field grown plants following cetyl trimethyl ammonium bromide (CTAB) method with modifications (Doyle and Doyle, 1987). The concentration of DNA was checked in Nanodrop spectrophotometer model-ND1000 and the DNA samples were diluted to 100 ng/µl prior to polymerase chain reaction (PCR). The quality of DNA was checked in 0.8% (W/V) agarose gel electrophoresis. The DNA samples were stored at -20°C for downstream use.

The PCR mixtures (15 µl) contained 0.5 µl (50 ng) genomic DNA, 0.5 µl Taq DNA polymerase, 1.5 µl of Taq Buffer (Genei, Banglore, India), 1 µl dNTPS (Genei, Bangalore, India), 9.5 µl Mili-Q water, 1.0 µl forward primer, 1.0 µl reverse primer (25 pmoles). PCR amplification was performed in C1000 thermal cycler (BIO-RAD, USA). 30 cycles of 30 s at 94°C for denaturation of template, 1 min at 54°C for primer annealing followed by 30 s at 72°C for primer extension was used. The amplified DNA fragments along with 100 bp DNA marker were size separated on 8% polyacrylamide gel stained in ethidium bromide and run in 1X TBE buffer at 200 V for 1-2 h (0.1%). The resolved amplification of bands was scanned using laser scanner (Fujifilm FLA 5100, Japan).

The numbers of bands were scored manually as '1' (presence) and '0' (absence) across the lanes comparing their respective sizes. Only strong, reproducible and clearly distinguished bands were considered for the following analysis. A dendrogram was constructed by NTSYS-pc 2.1 program (Rohlf, 2000) using the unweighted pair-group method of arithmetic average (UPGMA) cluster analysis based on Jaccard's similarity coefficient. The polymorphism information content (PIC) was calculated by applying the formula PIC = $[1-\sum fi^2]$, where, f is the frequency of the allele averaged across loci (Powell et al., 1996) and polymorphism per cent was calculated by the formula 'Polymorphism per cent = number of polymorphic bands/total number of bands in that assay unit'. Marker Index (MI) for each SSR primer was calculated by applying the formulae 'MI = polymorphism per cent (X) PIC value' (Powell et al., 1996; Smith et al., 1997). Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) were calculated using GenAlEx 6.5 software (Peakall and Smouse, 2012).

## RESULTS AND DISCUSSION

The screening of 435 peanut breeding lines over two seasons resulted in identification of additional sources of resistance to PBND. More than 60% disease incidence was observed in the susceptible check variety, KRG-1 confirming high disease pressure under normal field conditions at experimental location. Basu (1995) also reported Raichur, Karnataka, India as natural hot spot for PBND. Based on pooled disease incidence over two seasons, 42 lines were found to be highly resistant and 73

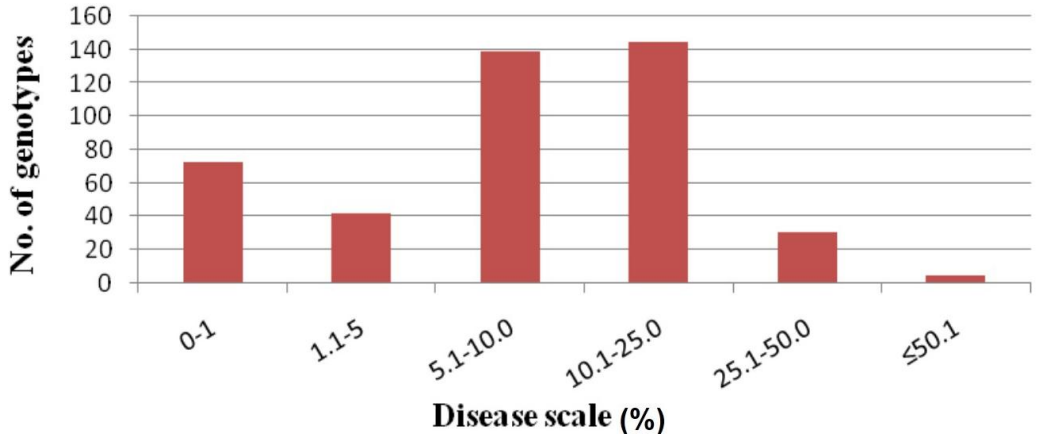


**Figure 1.** Frequency distribution of 435 interspecific peanut breeding lines based on PBND incidence under field conditions at hot spot location.

**Table 1.** PBND incidence of 116 breeding lines which have been used in genetic diversity analysis.

| PBND incidence (%) | Reaction | Lines (NRCGCS numbers) |
|---|---|---|
| 0.0-1.0 | Highly resistant | 2, 7, 8, 36, 46, 51, 55, 57, 58, 75, 79, 81, 82, 85, 86, 102, 103, 108, 153, 159, 161, 177, 244, 246, 262, 267, 268, 269, 271, 275, 277, 281, 282, 285, 286, 300, 301, 319, 327, 328, 417, 421 |
| 1.1-5.0 | Resistant | 10, 27, 28, 37, 40, 41, 43, 45, 54, 59, 73, 76, 77, 83, 92, 94, 96, 97, 98, 99, 104, 105, 107, 113, 116, 120, 137, 144, 151, 156, 167, 184, 186, 187, 190, 192, 196, 201, 202, 203, 205, 206, 211, 212, 215, 220, 227, 228, 230, 232, 250, 263, 264, 293, 308, 311, 337, 338, 339, 375, 377, 378, 379, 381, 396, 398, 402, 412, 420, 422, 423, 425, 431 |
| > 50.0 | Highly susceptible | KRG-1 |

lines as resistant. The rest of the lines were moderately resistant to highly susceptible (Figure 1). Screening under field conditions at hot spot location has been used earlier for selection of peanut lines resistant to PBND (Pensuk et al., 2002a, 2002b, 2004; Sunkad et al., 2012). Selected highly resistant, resistant and one susceptible elite cultivar were subjected to genetic diversity analysis (Table 1). SSR primer pairs (Table 2) reported earlier (Hopkins et al. 1999; He et al., 2005; Moretzsohn et al., 2005) were screened among lines and yielded a total of 219 fragments. Out of which 205 were polymorphic with an average of 10.25 fragments per primer. All 20 SSRs were highly polymorphic (> 50%) in cultivated peanut and produced higher number of alleles. Polymorphism percent ranged from 57 to 100% with an average of 92.2% (Table 3).

The number of fragments amplified by these SSRs ranged from 2 to 34 per locus. Out of 20 primers, eight produced more than average number of alleles per locus. Of which the primer TC2C07 produced highest (34) number of alleles. Primers amplified more than one locus in peanut breeding lines indicating locus duplication. This may be attributed to the presence of A-genome and B-genome in allotertraploid cultivated peanut. Amplification of more than one fragments by one pair of primers in tetraploid peanut accessions have been reported in previous studies (Hopkins et al., 1999; Gimenes et al., 2007; Varshney et al., 2009).

The PIC value ranged from 0.50 to 0.91 with an average of 0.80 and high PIC value (> 0.5) was found in 19 out of 20 SSRs. The MI value of SSR primers ranged from 43.3 to 92.0 with an average of 75.8. The PIC values derived from allelic diversity and frequency among the lines was not uniform among the SSR loci tested. The higher PIC value of primers could reveal maximum genetic information from lines under investigation. In this study, 19 out of 20 SSRs had higher PIC value (> 0.5).

Such higher PIC value could be due to marker preselection with higher GC/CT repeats. Hence, caution must be taken to verify the diversity revealed based on PIC value by combining additional parameters like polymorphic per cent, MI value and number of alleles amplified per locus since quantitative estimation of marker utility and detection of polymorphism have been depicted in terms of mean heterozygosity and MI (Powel et al.,

**Table 2.** List of SSR markers used for diversity analysis among 116 peanut breeding lines.

| Primer | Sequence | bp | Temperature (°C) |
|---|---|---|---|
| AC2H11 | F-TCCTTTACTTGTGCAGTTGTGC | 41 | 55.9 |
| | R-AAAACGCCATGTGGTGGAT | | 55.3 |
| AC3DO7 | F-TAGCTTCGATAACCAGGGAGAC | 43 | 55.8 |
| | R-CCCAACACTCGTTCATTCCTC | | 55.8 |
| TC3BO4 | F-GAAGAAGAAGTCACTGCGGC | 45 | 56.2 |
| | R-AAGCTAGTTTCTGATTAAAGCACCA | | 54.8 |
| AC2C12 | F-TATCGAGCCGAATATGAAT | 40 | 48.0 |
| | R-GCAGGATTTTGTAATTGAGAG | | 49.4 |
| TC3GO3 | F-ATCTGCAGCCTCAAGCTGAT | 40 | 56.7 |
| | R-GCCGGTATGAGAGATTGGAG | | 54.9 |
| TC2B01 | F-TTGCAGAAAAGGCAGAGACA | 44 | 54.6 |
| | R-GAAAGAAGCTAAGAAGGACCCATA | | 54.2 |
| TC2E05 | F-GAATTTATAAGGCGTGGCGA | 40 | 53.0 |
| | R-CCATCCCTTCTTCCTTCACA | | 54.3 |
| TC2CO7 | F-CACCACACTCCCAAGGTTTT | 40 | 55.5 |
| | R-TCAAGAACGGCTCCAGAGTT | | 56.2 |
| TC4FO1 | F-GAACAACCGGGAGCAATTTA | 45 | 53.2 |
| | R-CGTCCAGTTCCTATAGAACCTATCA | | 55.3 |
| TC1AO2 | F-GCAATTTGCACATTATCCGA | 42 | 51.6 |
| | R-CATGTTCGGTTTCAAGTCTCAA | | 53.4 |
| TC7C06 | F-GGCAGGGGAATAAAACTACTAACT | 46 | 54.5 |
| | R-TTTTCCTTCCTTCTCCTTTGTC | | 52.7 |
| TC4DO9 | F-TTGTGCTCTGCTCTTGGTTG | 40 | 55.7 |
| | R-CTTGCTGGAGGAAACACACA | | 55.4 |
| TC2DO6 | F-AGGGGGAGTCAAAGGAAAGA | 40 | 55.6 |
| | R-TCACGATCCCTTCTCCTTCA | | 55.2 |
| TC11AO4 | F-ACTCTGCATGGATGGCTACAG | 43 | 56.9 |
| | R-CATGTTCGGTTTCAAGTCTCAA | | 53.4 |
| TC9F10 | F-ATCACAATCACAGCTCCAACAA | 44 | 54.9 |
| | R-GGCAAGTCTAATCTCCTTTCCA | | 54.5 |
| TC11E04 | F-ACGACACCCTGAAATCAAGTTT | 42 | 54.8 |
| | R-CCGAAGGCACCAAAAAGTAT | | 53.2 |
| PMC-588 | F-CCATTTTGGACCCCTCAAAT | 42 | 53.1 |
| | R-TGAGCAATAGTGACCTTGCATT | | 54.7 |
| PM-375 | F-CGGCAACAGTTTTGATGGTT | 39 | 54.2 |
| | R-GAAAAATATGCCGCCGTTG | | 52.7 |
| PM-15 | F-CCTTTTCTAACACATTCACAC | 40 | 53.7 |
| | R-GGCTCCCTTCGATGATGAC | | 55.4 |
| PM-402 | F-CCGCCCTAAAAACTGTATTCG | 40 | 53.9 |
| | R-CCTAAGAGTACACGCGACGA | | 56.2 |

1996). In this study, AC2C12, TC2E05, TC2C07, TC1A02, TC7C06, TC4D09, TC9F10 and PM375 were found highly informative in revealing genetic diversity and partitioning genetic variation. These primers had higher number of alleles per locus along with higher polymorphic per cent, PIC content and MI value. The polymorphism observed in this study was much higher than the polymorphism revealed by earlier studies in cultivated peanut (Mondal et al., 2009). The average number of bands produced per primer in this set of lines was similar to previous studies (Krishna et al., 2004; He et al., 2005).

Cluster analysis based on Jaccard's similarity coefficient classified the peanut lines into three major clusters (Figure 2). The dendrogram indicated that majority of the lines clustered in the range of 0.59 to 0.92 similarity coefficient indicating the presence of wide variability among the lines under study. The cluster 'I', 'II' and 'III' housed 33, 22 and 61 lines, respectively (Table 4). Both

**Table 3.** Numbers of total bands per locus, PIC values and MI of 20 SSRs based on diversity revealed across 116 breeding lines.

| Primer | Number of allele amplified | Polymorphism percent | PIC Value | MI value |
|---|---|---|---|---|
| AC2H11 | 7 | 100 | 0.85 | 85.5 |
| AC3DO7 | 10 | 100 | 0.89 | 89.9 |
| TC3BO4 | 5 | 80 | 0.79 | 63.9 |
| AC2C12 | 11 | 100 | 0.89 | 89.0 |
| TC3GO3 | 7 | 57 | 0.75 | 43.3 |
| TC2301 | 5 | 100 | 0.79 | 79.9 |
| TC2E05 | 12 | 100 | 0.91 | 91.2 |
| TC2CO7 | 34 | 97 | 0.64 | 62.2 |
| TC4FO | 10 | 90 | 0.88 | 79.2 |
| TC1AO2 | 20 | 95 | 0.55 | 55.2 |
| TC7C06 | 14 | 100 | 0.92 | 92.0 |
| TC4DO9 | 16 | 100 | 0.87 | 87.2 |
| TC2DO6 | 8 | 100 | 0.86 | 86.7 |
| TC11AO4 | 9 | 89 | 0.89 | 79.2 |
| TC9F10 | 17 | 94 | 0.93 | 88.3 |
| TC11E04 | 5 | 100 | 0.79 | 79.3 |
| PMC588 | 7 | 71 | 0.85 | 60.9 |
| PM375 | 11 | 82 | 0.90 | 73.9 |
| PM15 | 9 | 89 | 0.88 | 78.2 |
| PM402 | 2 | 100 | 0.5 | 50 |
| Total | 219 | 1844 | 16 | 1515 |
| Mean | 10.95 | 92.2 | 0.80 | 75.75 |

clusters ‘II’ and ‘III’ shared about 0.58 similarity with cluster ‘I’. Lines NRCGCS-46, NRCGCS-99, NRCGCS-201, NRCGCS-308 and NRCGCS-398 were closely related to each other. In contrast, genotype NRCGCS-268 belong to cluster ‘III’ was distantly related to the genotype NRCGCS-28 belong to cluster ‘I’. Further, susceptible cultivar KRG-1 was distantly related to NRCGCS-28 and was followed by NRCGCSCS-86. These 2 lines could be most diverse parents for use in the peanut breeding programme for improving resistance to PBND.

The grouping of lines based on Jaccard’s similarity coefficient was also further confirmed by PCoA. PCoA is one of the multivariate approaches of grouping based on similarity coefficients or variance/ covariance values and expected to be more informative about differentiation of major groups. PCoA can be used in combination with cluster analysis to extract maximum information from the molecular marker data, particularly when the first two or three PCs explain > 25% of the original variation (Messmer et al., 1992). In this study, grouping of lines identified by PCoA was comparable to those identified by UPGMA cluster analysis (Figure 3). More than 26% of genetic variations were explained by first and second coordinates. The first and second coordinates explained 16.6 and 10.3% variations with Eigen value of 1.76 and 1.09, respectively. Similar to UPGMA cluster analysis, PCoA also indicated that these peanut lines could be roughly divided into three groups.

AMOVA was done to know the variability among the groups identified by UPGMA and PCoA analysis. The three populations showed significant variation ($P < 0.001$); with 96% of the variation attributed to within populations and 4% attributed to among populations indicating that these three groups are genetically diverse (Table 5).

Reaction of the lines NRCGCS-28, NRCGCS-86 and KRG-1 to PBND were further confirmed under glass house conditions using sap inoculation method followed by enzyme linked immunosorbent assay (ELISA). The lines NRCGCS-28 and NRCGCS-86 were though found positive to PBNV; they were foundmoderately resistant to PBND with 8 and 9% disease incidence, respectively. In contrast KRG-1 was found to be highly susceptible with 70% disease incidence (Table 6). Incubation period for appearance of disease symptoms was used as a criterion for tolerance to disease. Lines with longer incubation periods are more resistant to disease than lines with shorter incubation periods. Incubation period for appearance of disease symptom was longer in case of NRCGCS-28 and NRCGCS-86, while it was shorter in case of KRG-1. This indicates that the incubation period for appearance of disease symptoms is negatively correlated with PBND. Such negative correlation between incubation period for appearance of disease symptom and disease scoring has been reported by Subrahmanyam et al. (1993).

Collecting data on genetic diversity in parents and progeny, however, is time consuming and expensive (Maughan

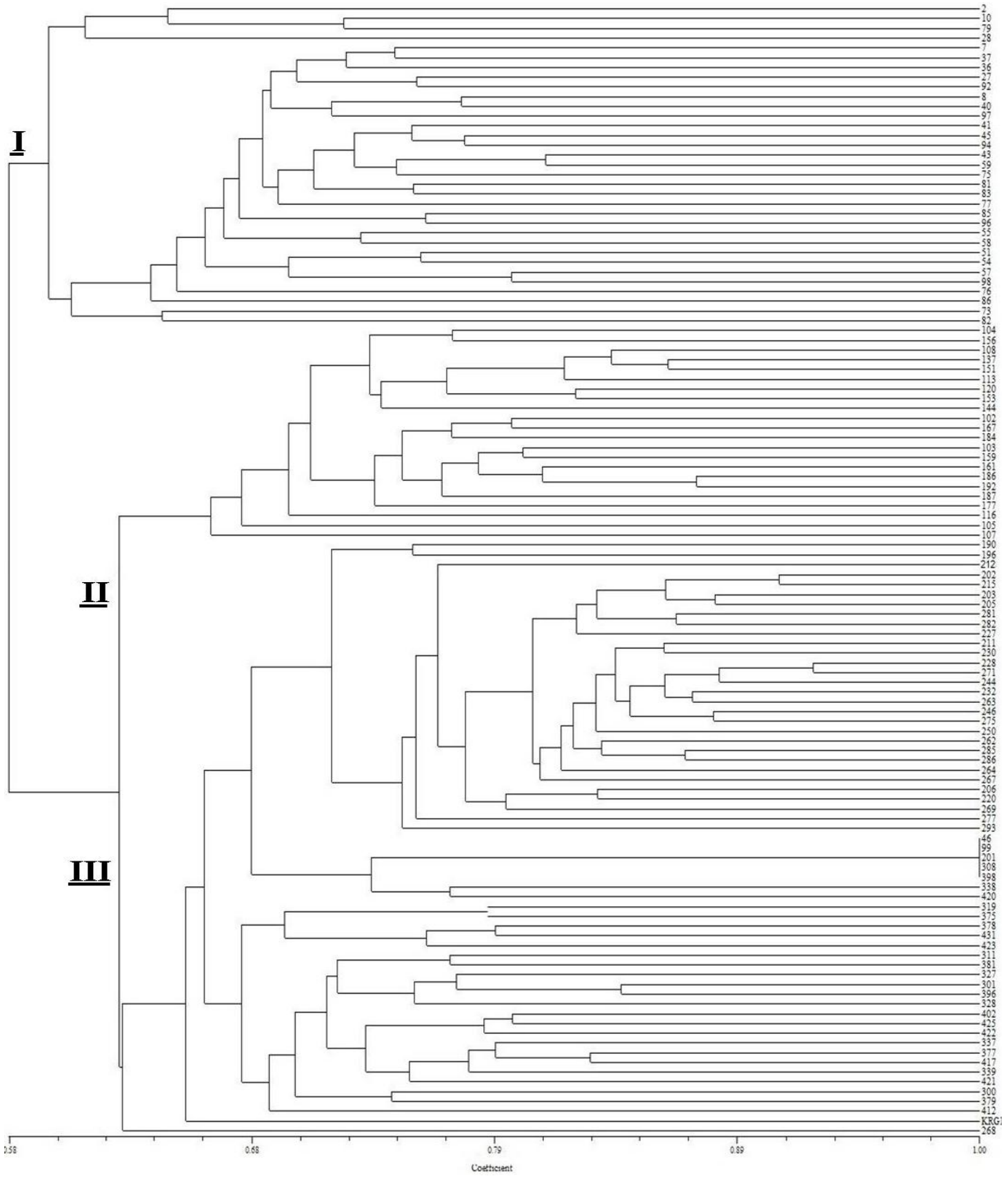


**Figure 2.** UPGMA dendrogram of 116 peanut breeding lines grouping into three major clusters based on Jaccard's coefficient using SSR markers.

**Table 4.** Peanut breeding lines grouped into three clusters based on cluster analysis and PCA.

| Cluster | Number of lines | Line (NRCGCS number) |
|---|---|---|
| Cluster-I | 33 | 2,10,79, 28, 7, 37, 36, 27, 92, 8, 40, 97, 41, 45, 94, 43, 59, 75, 81, 83, 77, 85, 96, 55, 58, 51, 54, 57, 98, 76, 86, 73, 82 |
| Cluster-II | 22 | 104, 156, 108, 137, 151, 113, 120, 153, 144, 102, 167, 184, 103, 159, 161, 186, 192, 187, 177, 116, 105, 107 |
| Cluster-III | 61 | 190, 196, 212, 202, 215, 203, 205, 281, 282, 227, 211, 230, 228, 271, 244, 232, 263, 246, 275, 250, 262, 285, 286, 264, 267, 206, 220, 269, 277, 293, 46, 99, 201, 308, 398, 338, 420, 319, 375, 378, 431, 423, 311, 381, 327, 301, 396, 328, 402, 425, 422, 337, 377, 417, 339, 421, 300, 379, 412, KRG-1, 268 |

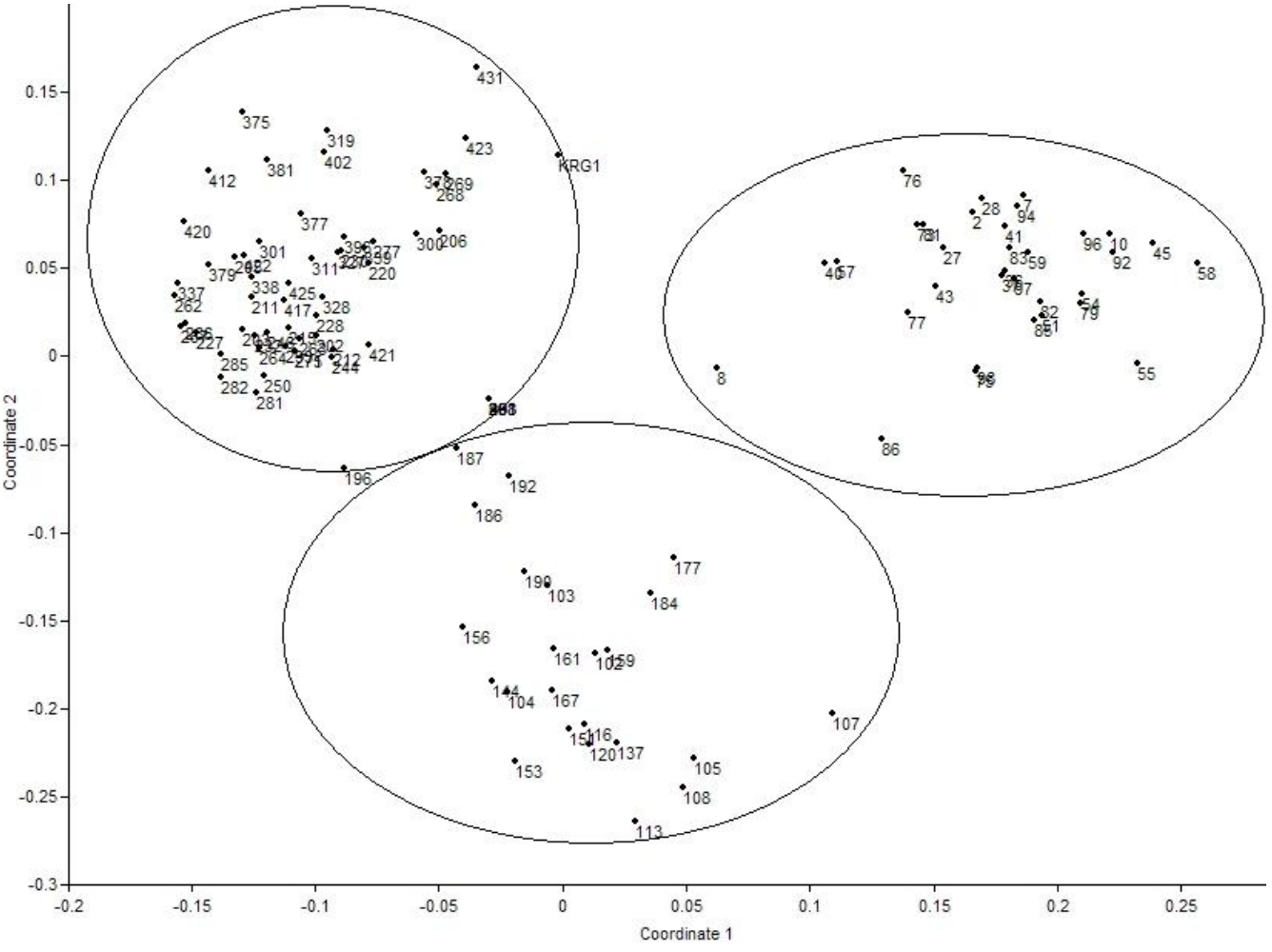


**Figure 3.** Principal co-ordinate analysis of the 116 peanut breeding lines grouped into three distinct clusters.

et al., 1996). Hence the present studies made potential germplasm resources resistant to PBND available to the peanut breeders that can be used for mapping and introgression of resistant genes into elite otherwise suscep-

**Table 5.** Summary of AMOVA within and among 116 peanut breeding lines.

| Source | df | SS | MS | Est. Var | % | Stat. | Value | Probability |
|---|---|---|---|---|---|---|---|---|
| Among populations | 2 | 17.063 | 8.532 | 0.143 | 4% | | | |
| Within populations | 113 | 394.006 | 3.487 | 3.487 | 96% | PhiPT | 0.040 | 0.010 |

**Table 6.** Selected peanut breeding lines showing differential level of resistance to PBND under challenged inoculation in glass house conditions.

| S/N | Line | Incubation period | Days to first symptoms appearance | Days to last symptoms appearance | Disease incidence (%) |
|---|---|---|---|---|---|
| 1 | NRCGCS-28 | 14 | 11 | 14 | 8 |
| 2 | NRCGCS-86 | 11 | 8 | 14 | 9 |
| 3 | KRG-1 | 8 | 7 | 20 | 70 |

tible cultivars. Thus, identifying genetically diverse parents having desirable traits based on molecular markers would be a good approach for the desirable progeny. The registration of genotype NRCGCS-86 has already been secured in the Indian Council of Agricultural Research (ICAR), India as multiple diseases resistant (MDR) Pre-breeding peanut genotype (Bera et al., 2010). Literature reports indicate that there is a relationship between marker diversity of parents and genetic variance of the resulting progeny. This approach has already been used for production of improved progeny in peanut (Holbrook, 2001).

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