DISCUSSION

5.1 Characterization of simple sequence repeats (SSRs) in pigeonpea

The codominant microsatellite markers have varied uses in crop plants from cultivar identification to marker assisted breeding in crop improvement programmes. Microsatellites have been developed in several legumes such as lentil, common bean, Vigna, chickpea, pigeonpea, Medicago and soybean. Although, a large number of SSR markers have been reported in pigeonpea, only a small percentage of SSR markers turn out to be polymorphic (Burns et al. 2001; Odeny et al. 2009). In species where BAC (Bacterial Artificial Chromosome)-end sequences (BESs) are available, the development of SSR markers from BESs is very cost effective (Temnykh et al. 2001; Shultz et al. 2007). In pigeonpea, Bohra et al. (2011) generated a set of 88,860 BESs after constructing two BAC libraries by using HindIII and BamHI restriction enzymes. A total of 3,072 novel SSR primer pairs were synthesized out of which only 842 were found to be polymorphic. They developed the first genetic map comprising 239 loci for pigeonpea. However, there is a need to analyze the new SSR markers for their ability to characterize and fully exploit the genetic diversity of pigeonpea germplasm. Hence the results of the present study are valuable to users while selecting SSR markers for genetic diversity analyses, cultivar identification and marker assisted breeding.
5.1.1 Abundance of SSRs in pigeonpea genome

In the present study, a total of 22,358 (25.39%) repeats were identified from 88,066 pigeonpea BAC-end sequences in public domain (National Centre for Biotechnology Information) out of which 2773 (12.4%) repeats were having mono- to 10-mer repeat motifs. Dinucleotide repeats were the most abundant class of SSRs (1559), followed by trinucleotide (366) and hexanucleotide repeats (236). The most abundant motif found by Odeny et al. (2007) were also AT based (AT, AAT, TTAT) followed by TC class of repeats. In the present study also majority of the repeats identified were A/T rich using TRF search module. AT-based motifs have been reported to be the most abundant in plants (Morgante and Olivieri, 1993, Cardle et al. 2000, Morgante et al. 2002). Dinucleotide repeats have been reported to reside outside coding regions of genes (Temnykh et al. 2001) and are characterized by higher repeat numbers (Li et al. 2004) making them the best source of highly polymorphic SSR markers. At ICRISAT, BESs were surveyed by means of MISA (MIcroSAtellite) search module for the presence of SSRs (Bohra et al. 2011) which resulted in identification of 18,149 SSRs with mononucleotide (49% of total) and 42% of total was di-nucleotide repeats. The patterns similar to present results have been reported in the rice genome sequence data of Monsanto (Barry, 2001). BAC-end sequences have been very useful to develop SSR markers in several plant species including the legumes, soybean (Shultz et al. 2007), common bean (Schlueter et al. 2008) and Medicago (Mun et al. 2006) and pigeonpea (Bohra et al. 2011). In pigeonpea sufficient numbers of polymorphic markers
were not available earlier to allow molecular mapping and gene tagging. It has been reported that the pattern and number of SSR motifs from the genomic and EST (Expressed Sequence Tag) sequences were quite different with the predominance of AT in the genomic sequence while AG was predominant in the EST sequence in *Arabidopsis* (Cardle et al. 2000). Recently, Dutta et al. (2011) also identified dinucleotides (TC/GA, AG/CT and TA/TA) as the most common repeat motif in the genic SSR markers developed in pigeonpea by deep transcriptome sequencing.

### 5.1.2 Diversity analysis in pigeonpea germplasm

A set of 139 primer pairs were selected from the 22,358 SSRs maintaining a minimum cut-off value of >5 repeat for the core motif. The higher tandem repeat number was selected with a logic that these are expected to be more polymorphic compared to the microsatellites with lower number of repeats. Out of 139 primer pairs screened, 67 polymorphic primer pairs with very good and scorable amplifications were selected for diversity analysis in a set of 58 pigeonpea accessions from different eco-geographic regions including seven wild relatives. A total of 251 alleles were detected in the genotypes analysed with an average of 3.75 alleles per locus which is comparable to previous studies in pigeonpea (3.1 alleles per locus by Burns et al. 2001; 3.1 alleles per locus by Odeny et al. 2009 and 3.4 alleles per locus by (Saxena et al. 2010). Bohra et al. (2011) obtained an average of 5.65 alleles per marker in 22 pigeonpea genotypes including one wild species using 842 polymorphic BES-SSR markers. The average number of alleles per marker observed by Dutta et al. (2011) was 3.75 alleles per locus for *C. cajanu*
cultivars and 4.1 alleles per locus for wild species using genic-SSR markers. Barbosa de Sousa (2011) obtained an average of 5.1 alleles in 77 pigeonpea genotypes adapted to South American regions but low levels of genetic diversity using 16 polymorphic SSR markers. The clustering of pigeonpea accessions based on simple matching coefficients and Nei and Li’s genetic distances using SSR data did not show any clear distinction between the released varieties and germplasm. Recently, Kassa et al. (2012) have also reported no clear distinction between landraces and modern cultivars in pigeonpea using SNP (Single Nucleotide Polymorphism) markers. However the average Nei’s genetic distance for released varieties (0.290) was higher in comparison to cultivated germplasm (0.245). Hence the genetic diversity for released varieties was higher than that of the cultivated germplasm. The clustering pattern obtained in this study failed to indicate any relationship between genetic similarity and geographical distribution. This is in agreement with the earlier findings in pigeonpea based on morphological data (Asawa 1979; Dumbre and Deshmukh 1984; Murthy and Dorairaj 1990; Rekha et al. 2011). Estimation of genetic variability in pigeonpea using diversity arrays technology also reported no clear differentiation among the genotypes from various geographic areas of pigeonpea cultivation (Yang et al. 2006).

5.1.3 Differentiation and partitioning of genetic variation

Analysis of molecular variance (AMOVA) was conducted to test the degree of differentiation of the accessions from different regions of India. The AMOVA indicated that variation among groups accounted for -2.36% of total
variation and 14.44% of variation was contributed by differences among sub-groups within groups. The percentage of variation among individuals within sub-group was 48.47% and variation within individuals contributed 39.45% of total variation (Table 5). Hence most of the variation was contributed by variation within sub-groups and variation within individuals. The theoretical expectations from AMOVA were used to calculate F-statistics namely, $F_{IS}$, $F_{SC}$, $F_{CT}$ and $F_{IT}$. The value of $F_{SC}$ was observed to be 0.141 indicating moderate differentiation of the accessions analyzed. The value of $F_{CT}$ was observed to be -0.024 indicating very low individual to individual variation among the groups. The $F_{IT}$ value of 0.605 indicates presence of substantial within group intermating and inbreeding for the genotypes from different regions. The moderate value of $F_{IS}$ (0.551) supports this conclusion as it indicates moderate differences for allelic frequencies between the populations. A comparison of pairwise $F_{ST}$ among seven populations indicated that the varieties from north have differentiated very little from other sub-groups (Fig. 10). The pairwise $F_{ST}$ values were greater than 0.2 for varieties from west with varieties from peninsular region and germplasm from east and peninsular regions; varieties from peninsular region with germplasm from north; germplasm from central region with germplasm from east and between germplasm from north and east (Table 6). The results obtained here indicate the need to sample more localities as well as the need to collect samples of higher sizes within a region for assembling diverse germplasm. These patterns of diversity partitioning is along expected
lines since pigeonpea is an often cross-pollinated plant and differentiation between sub-groups appear to be greater than within sub-groups.

5.1.4 Allelic diversity and population sub-structure

The population diversity measures calculated using software POPGEN32 clearly indicated the presence of higher genetic diversity in varieties from peninsular and northern regions and germplasm from east. When the two groups (varieties and germplasm) were considered, the highest number of alleles per locus, effective number of alleles, Shannon’s information index, Nei’s gene diversity and the number of polymorphic loci showed slightly higher values for released varieties. But the value of gene flow for cultivated germplasm was higher than that of varieties which may be due to controlled pollination practiced while maintaining released varieties which restrict gene flow. It is to be considered that the genetic diversity estimates are influenced greatly by sample sizes; hence for sub-groups such as varieties from west and germplasm from peninsular region with small number of samples, the diversity values are not indicative.

Bar plots from the population sub-structure analyses at k=9 which was found to be statistically well supported, indicated large scale sharing of alleles among varieties from north and west region and germplasm from north region. The varieties from west region have substantial genetic contributions from the germplasm from north. The varieties from peninsular region and germplasm from central region have several unique alleles though these genotypes share some allelic variations with germplasm from peninsular region. Similarly, the germplasm from east also harbours several
unique alleles. The results indicated the presence of substantial amount of unutilized genetic variation which might be of immense use in crop improvement programmes.

5.2 Isolation of *Fusarium udum* from pigeonpea seeds

In the present study pigeonpea seeds were used for isolation of *Fusarium udum*. Seeds were placed on moistened blotters as well as potato dextrose agar medium. The pathogen was isolated and cultured on PDA medium. *F. udum* was observed only in seeds collected from plants sown on infested soil. *Fusarium* wilt is mainly soil borne, but the pathogen may be carried as contaminant of pigeonpea seed and may show presence of the pathogen but if the seeds have been collected from healthy plants they may not contain *Fusarium* infection. Earlier workers have reported isolation of *Fusarium* from plant parts like stalk and roots of infected pigeonpea plants (Kiprop et al. 2005; Mahesh et al. 2009).

5.3 Screening of pigeonpea germplasm for *Fusarium* wilt

Various techniques have been used for glasshouse screening for resistance to soil-borne pathogens, including root dipping in inoculum (Phipps and Stipes, 1973), growing seed or seedlings in infested soils (Russell, 1978), soaking seed in inoculum (Sakar et al. 1982) and injecting inoculums into plants (Jindal et al. 1982). The effectiveness of these techniques depends on various factors, including spore concentration, plant age at inoculation, and environmental conditions such as temperature and humidity (Ribeiro and Hagedorn, 1979). Okiror (1998) compared five techniques, namely, sowing seeds or transplanting seedlings into infested soil, dipping roots or
soaking seed in a spore suspension and stem injection under glasshouse conditions on four cultivars of pigeonpea with different levels of resistance. It was observed that sowing seeds in infested soils (sick plots) gave the highest mortality and allowed for easy differentiation of resistant and susceptible plants. Moreover, the technique is simple, reliable, easy to apply, and cost-effective. The other techniques either give severe wilting, inconsistent results or low wilting or were considered unreliable (Hillocks, 1984). Hence, in the present study, sowing seeds in wilt sick plots was used for wilt screening. Out of 51 pigeonpea accessions, 13 were found to be resistant. Pigeonpea has traditionally been screened for wilt resistance in wilt-infested fields (Butler, 1908; Deshpande et al. 1963). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad also uses field screening but has reported cases of inconsistent results (Nene et al. 1981). Hence, screening in wilt-sick plots appears to be the preferred procedure in pigeonpea.

5.4 Identification and Characterization of RGAs in pigeonpea

The present study represents a comprehensive analysis of resistance gene analogs (RGAs) for *Fusarium* wilt resistance in three diverse groups of pigeonpea namely wild species, cultivated germplasm and released varieties. Characterization of wilt resistance genes is essential for deployment of those genes in the crop improvement programmes through marker assisted selection procedures. Sequences of cloned plant disease resistance genes from a wide range of plant taxa reveal significant similarities in sequence homology and structural motifs. This is observed
among genes conferring resistance to viral, bacterial and fungal pathogens (Kanazin et al. 1996). Majority of resistance genes (R-genes) encode cytoplasmic receptor like proteins that contain a leucine rich repeat (LRR) domain and a nucleotide binding site (NBS). The conserved domains of resistance genes provide opportunities for designing degenerate primers and isolating resistance gene analogs by polymerase chain reaction (PCR) strategy from plant genomes. Although not all resistance genes have been demonstrated to reside in clusters, tight linkage associations of many resistance genes have been well established (Kanazin et al., 1996). Clustering of resistance genes suggests that a common genetic mechanism has been involved in their evolution (Sudapak et al. 1993). However the occurrence of clustered multigene families is a major obstacle in the cloning of R-genes (Dixon et al. 1996; Ori et al. 1997 and Shen et al.1998). It also makes difficult to determine the functional copy of these genes. In view of this, RGAs mapped on different chromosomes would be helpful in the identification of multigene families, which in turn might lead to the establishment of correlation between the chromosomal position of known R-genes and R-gene analogues (Sharma et al. 2009). The results of the present study indicated the presence of substantial ‘bottlenecks’ during the course of domestication which may be responsible for presence of a fraction of these variations for RGAs in cultivated genepool.

5.4.1 Phylogenetic relationship between wild and cultivated pigeonpea
A total of 58 accessions of pigeonpea were used in the molecular analysis by using different degenerate primers based on conserved sequences of R-
genes. Among these, 13 accessions were known to be resistant to *Fusarium* wilt. Out of the 50 primers screened, only seven primers were finally selected since they gave reproducible and single amplification product without any mixture. Other primers were rejected either due to no amplification or producing multiple bands since it was difficult to ascertain the homology of the products. The selected PCR products were purified, sequenced and subjected to multiple sequence alignment using CLUSTAL X software. The RGA sequences amplified in pigeonpea were analyzed using BLAST before further analyses. The sequences amplified by primers RGA6, RGA1FCG, RGA1FCC, gi28190624 and gi28190626 showed high similarity with soybean tobacco mosaic disease resistance protein mRNA and the sequences amplified by primer gi13111699 showed similarity with partial coding sequence of soybean NBS type putative resistance protein. GM15 showed similarity to soybean uncharacterized protein related to resistance. The software MEGA was used to estimate the parameters of evolutionary divergence. All RGA sequences revealed higher diversity within wild species. The minimum evolution trees based on nucleotide diversity in 58 pigeonpea accessions for the products from RGA primers GM15 and primer gi13111699 grouped 5-6 wilt resistant genotypes together. This suggests that these two RGAs may be having some role in imparting resistance to wilt in these pigeonpea cultivars although the evidences are not unequivocal.

**5.4.2 Polymorphism within and between wild and cultivated pigeonpea**

Genetic diversity analysis was carried out using DnaSP software. The analysis of DNA polymorphism is a powerful approach to understand the
evolutionary process and to establish the functional significance of particular genomic regions (Begun et al. 2007; Nielsen, 2005; Rosenberg and Nordborg, 2002). In this context, estimating the impact of natural selection (both positive and negative) is of major interest. DnaSP is a software package for a comprehensive analysis of DNA polymorphism data. The program estimates the most common DNA polymorphism and divergence summary statistics (such as the nucleotide and haplotype diversity, the population mutation parameter, the number of nucleotide substitutions per site, etc.), and neutrality tests (such as Tajima’s, Fu and Li’s and Fu’s tests).

The two common measures used to describe sequence variation or nucleotide diversity are π, the expected heterozygosity per nucleotide site (Tajima, 1983); and θ, the number of polymorphic sites in a genotypic sample corrected for sample size (Watterson, 1975). In the present study although, there was locus-to-locus variation in terms of π and θ values, substantial levels of nucleotide diversity was clearly indicated within wild species. Indeed, wild pigeonpea appeared to harbor fifteen times more diversity (π=0.03 and θ=0.04) compared to that of many other crops including Glycine soja (π=0.00217 and θ=0.00235; Hyten et al. 2006), wild Arabidopsis thaliana for PgiC locus (π=0.003 and θ=0.0065; Kawabe et al. 2000). In contrast, cultivated pigeonpea varieties included in the present study showed less nucleotide variation (π=0.00131 and θ=0.00219). Generally the loss in genetic diversity occurred in a specific order i.e. highest in elite open pollinated cultivars or inbred lines. Among various categories of germplasm, generally wild germplasm and landraces showed the highest
genetic diversity and thus can contribute towards the broadening of genetic base of cultivated germplasm and/or inbred line/hybrids. The present study has revealed higher genetic diversity in released varieties as compared to the cultivated germplasm. Among evaluated plant breeding methods, plant introduction was found to add up the genetic diversity when local germplasm was partially substituted or supplemented by the introduced germplasm. Therefore genetic diversity in many parts of the world was found high due to time to time plant introductions. Among various plant selection schemes participatory plant selection was found the most effective in generating an allelic rich and broad genetic based plant material (Rauf et al. 2010). The low genetic variability amongst cultivars when compared with the wild suggests that natural and artificial selection has contributed to the selection of specific alleles and to changes of allelic frequencies at specific loci. Similar findings have been reported in groundnut (Moretzsohn et al. 2004) and mulberry (Zhao et al. 2005).

5.4.3 Domestication Bottleneck

The marked reduction in nucleotide diversity recorded in cultivated pigeonpea germplasm and varieties compared to that of wild species suggested that a domestication bottleneck effect has occurred. This is because domestication process which involves selection of a few morphological types preferred by prehistoric man creates genetic bottleneck that can decrease genetic diversity in the resulting population (Halliburton, 2004). Previous studies using SSR (Odeny et al. 2007) and DArT (Yang et al. 2006) markers also detected a reduction in levels of genetic diversity in
domesticated pigeonpea compared to wild relatives though the degree of bottleneck effect was not quantified. Compared to these earlier studies, here clear quantification of the domestication bottleneck effect has been made. Crop cultivars are developed from wild progenitors through domestication and intensive selection for various features during breeding. It has been well documented that selection targeted at individual loci will reduce genetic diversity within and around the selected loci which is indicated by the selection sweeps in populations. In this study the domestication appears to have led to reduction of genetic diversity in germplasm and cultivars as compared to wild population. Similar results were reported while studying domestication history of soybean (Hyten et al. 2006). In their study the cultivars vs. wild resulted in sequence diversity losses of 49% of $\pi$, 65% of $\theta$ and 44% of $H_d$. However the pigeonpea germplasm showed less nucleotide diversity as compared to released varieties ($\pi=0.00101$ and $\theta=0.00216$). The $H_d$ value also suggests a loss of genetic diversity in germplasm and cultivars (0.2480 and 0.3136 respectively) as compared to wild species (0.7598). In an earlier study, genetic patterns of domestication in pigeonpea (\textit{Cajanus cajan} (L.) Millsp.) and wild \textit{Cajanus} relatives was revealed with 752 SNPs derived from 670 low copy orthologous genes to clarify the evolutionary history of pigeonpea (79 accessions) and its wild relatives (31 accessions). It was observed that domesticated \textit{C. cajan} possess 75% less allelic diversity than the progenitor clade of wild Indian species, indicating a severe ‘domestication bottleneck’ during pigeonpea domestication (Kassa \textit{et al.} 2012). It was reported that landraces (primitive cultivars) and improved
(elite) cultivars that comprise the domesticated portion of genotype panel contained similar levels of polymorphic SNPs, indicating that much of the diversity that survived through the incipient stages of domestication was retained in current day cultivars and breeding lines. Despite the genetically narrow base of pigeonpea, the cultigen is noted for high levels of morphological diversity. Similar genetic bottleneck effects have also been observed in other crop species such as soybean (Guo et al. 2010; Hyten et al. 2006), sunflower (Liu and Burke, 2006), and lima beans (Motta-Aldana, 2010).

It has been seen that the process of domestication leads to increase in linkage disequilibrium (LD). $ZnS$ (average of $R^2$ over all pairwise comparisons), $Za$ (the average of $R^2$ over all pairwise comparisons between adjacent polymorphic sites) and $ZZ$ ($Za-ZnS$) values decreased in germplasm and cultivars as compared to wild species in all loci except gi28190626. The same has been observed in case of values of Wall’s B (proportion of pairs of adjacent segregating sites that are congruent) and Wall’s Q (measure of linkage disequilibrium between adjacent pairs of segregating sites). The main reason for this increased LD may be due to presence of frequent cross pollinations in cultivated germplasm which may result in recombination between loci and thereby increased LD. LD is a result of the interplay of many factors, such as mutation and recombination rates, mating system, selection, population size, structure and history of population. The estimation of recombination requires that considerably larger segments of contiguous DNA be sequenced and more data should be
collected to fully address the problem. As domestication is a human-driven process, it can also be influenced by random gene flow from wild relatives. Moreover, when crops are grown in the vicinity of locally adapted wild species, there is great potential for both intentional and accidental genetic admixture, which would further impact allele content in the cultivated gene pool. Cultivated pigeonpea would be particularly prone to such admixture, depending on its significant out-crossing rates and cross-compatibility with local wild species. The potential for gene flow is significant, as insect-aided natural outcrossing in pigeonpea may be up to 70% (Bhatia et al. 1981; Ratnaparkhe et al. 1995; Souframanien et al. 2003; Saxena and Kumar, 2010; Saxena and Kumar, 2003) and recently up to 17% natural out-crossing has been reported for wild species (Saxena and Kumar, 2010), with the highest out-crossing rate recorded for *C. lineatus*. The present study indicated higher diversity in cultivars as compared to germplasm. This is probably because most of the varieties have been developed through hybridization followed by selection and hence the varieties appear to have regained higher diversity compared to the cultivated germplasm. Similar findings have been reported in wheat (Jain and Yadav, 2009). The genetic diversity analysis among pre-green revolution, post-green revolution era cultivars and wheat landraces by microsatellite markers revealed that diversity among the released varieties in the post-green revolution era has widened rather than narrowing down. The traditional tall varieties released during the pre-green revolution era were clustered along with some of the landraces, indicating that they had possibly been developed through
selection among the landraces. Molecular variance analysis showed that variance was mainly distributed within (91.9%) rather than among (8.01%) the bread wheat varieties and landraces (Jain and Yadav, 2009). In rice also modern cultivars showed higher diversity than those of landraces (Prashanth et al. 2002) as revealed by AFLP analysis.

5.4.4 Effect of selection on diversity

The results of the present study revealed that all the loci analysed depicted similar diversity loss in cultivars and germplasm compared to wild relatives except RGA1FCC locus. This indicates that there was substantial effect of domestication and selection on the germplasm and selection was targeted for related genes. Earlier, Maynard and Haigh (1974) stated that a strong positive selection leads to loss of nucleotide diversity. Overall results indicated that, despite domestication and selection, the Indian cultivars retained considerable diversity in genes or traits to an extent to be suitable for cultivation in diverse agro climatic regions of the country. This trend was also evident from earlier reports (Bhat et al. 1999; Duhoon et al. 2004). One of the possible reasons for this trend was that the majority of Indian cultivars were developed by selection from local collections or landraces to suit diverse local agro climatic regions of the country.

The nucleotide diversity retained by cultivated germplasm was of the magnitude of 3.33% (π) and 5.35% (θ) of wild species diversity while the varieties retained 4.33% (π) and 5.42% (θ) of the wild species. These results indicate that the development of varieties does not appear to have caused significant genetic erosion in pigeonpea instead they have regained some
diversity lost during domestication since the breeding process might have infused greater diversity in the form of exotic germplasm in breeding material. Similar results were observed in rice in which genetic diversity analyses in cultivars and landraces of *Oryza sativa* subsp. *indica* by AFLP markers revealed that modern cultivars showed more diversity than the landraces from the same region, and yet clustered closer to the traditional cultivars. Genetic diversity was slightly higher in the modern cultivars than in the traditional cultivars from Tamil Nadu (Prashanth *et al.* 2002). Nucleotide diversity may be higher due to mutation after domestication followed by farmer’s selection for desirable traits. The haplotype diversity and the average number of pairwise differences also showed higher values in wild species for all loci. Tajima’s D, Fu and Li’s D and Fu and Li’s F along with Fu’s Fs statistics were performed for the present investigation for studying neutrality in detail. Tajima’s D is often used to determine allele frequency changes by comparing two populations before and after a genetic bottleneck (Wright *et al.* 2005). The value of Tajima’s D is negative for synonymous and non-synonymous sites for RGA6 locus indicating increased variation in rare alleles/sites. The negative values of the neutrality tests suggest that they are mostly under negative selection or reflect a recent population expansion. Also the Tajima’s D (Nonsyn/Syn) ratio of 8.54812 indicates that the contribution towards variation through synonymous sites is greater than through non synonymous sites. Tajima’s D, Fu and Li’s D and Fu and Li’s F tests gave negative values for the germplasm. The varieties also showed negative values for all neutrality tests for all loci except RGA6 and
gi28190626. The Fu’s Fs analysis for varieties showed negative values for all loci except gi28190626 implying that there was an excess of alleles due to population expansion or genetic hitchhiking.

The analysis indicates presence of severe bottlenecks in the process of domestication and higher diversity in Indian released varieties in comparison to native germplasm due to utilization of exotic germplasm in breeding programmes. The results of analyses of seven RGA regions and comparison of SNP variation pattern with host plant resistance helped in identification of GM15 and gi13111699 and to some extent RGA6 as putative loci imparting resistance to *Fusarium* wilt in pigeonpea. Further confirmation of the relationship between the loci and resistance needs to be ascertained through analyses of biparental mapping populations.

**Future line of work**

1. Availability of whole genome sequence in pigeonpea and its gene annotation has opened several possibilities for the use of genomics information and tools in crop improvement programmes. There is a need to conduct extensive SNP haplotype analyses for the candidate RGA s coupled with genome wide association studies to identify SNP haplotypes contributing towards resistance in the host plant. These may then be validated through biparental mapping and used in marker assisted selection programmes.

2. There is a need to analyse the variability in the *Fusarium* pathogen for the levels of virulence so that specific breeding strategies can be devised.
3. The world core collection designated in pigeonpea must be screened for characterizing levels of resistance against diverse *Fusarium* wilt pathogen isolates so that specific resistance genes can be identified and utilized in crop improvement programmes.