Agronomic and quantitative trait loci analyses of yield and yield-related traits in pigeonpea

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Thesis abstract

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important multi-purpose crop widely grown in the tropics and sub-tropics. However, pigeonpea productivity has been stagnant with grain yield of 750 kg ha^{-1}due to several biotic, abiotic and socio-economic constraints. Narrow genetic diversity present in the cultivated species has further hampered progresses in the development and deployment of improved cultivars and genomic resources.

The main objective of this study was to develop improved cultivars of pigeonpea through agronomic characterization and by identifying genomic regions associated with yield and yield-related traits for efficient marker-assisted selection programs. The specific objectives of this study were: 1) to assess the phenotypic variability and to identify promising genotypes among F_2 segregants of pigeonpea populations derived from crosses of six parental lines with diverse genetic backgrounds, 2) to apply correlation and path coefficient analyses and identify most useful yield and yield-related components of newly developed pigeonpea mapping populations, 3) to determine the genetic control of eight yield and yield-related traits involving a total of 460 F_2 pigeonpea progenies derived from three families of varied genetic backgrounds and 4) to identify quantitative trait loci (QTL) associated with eight yield and yield-related traits using 420 F_2 progenies developed from three divergent pigeonpea families.

The following six parental lines: AL 201, ICPL 20325, ICP 8863, ICPL 87119, ICP 5529 and ICP 7035 were crossed using a bi-parental mating design. Parents and a total of 611 F_2 individuals were phenotyped. Data collected included days to flowering, number of primary branches $plant^{-1}$ number of secondary branches $plant^{-1}$ number of pods $plant^{-1}$, number of seeds pod^{-1}, seed weight and seed yield $plant^{-1}$. ICP 7035 and ICP 5529, which are long maturity lines had desirable attributes such as increased seed weight, reduced plant height, greater number of primary branches and better seed yield $plant^{-1}$ followed by medium maturity lines, ICPL 87119 and ICP 8863. Short maturity lines ICPL 20325 and AL 201 had the least preferred traits. Results suggested that number of pods $plant^{-1}$, followed by seed yield $plant^{-1}$, number of secondary branches $plant^{-1}$ and number of

primary branches plant^{-1} exhibited higher variation across all the studied geneotypes. The highest phenotypic variability was exhibited by ICP 5529 × ICP 7035, followed by ICP 8863 × ICPL 87119 and AL 201 × ICPL 20325.Phenotypic correlations of eight yield and yield-related characters were investigated to determine desired characters for selection of progenies with improved seed yield. Significantly positive correlations were found between seed yield and number of pods plant^{-1}, followed by the number of secondary branches plant^{-1}, number of primary branches plant^{-1} and 100-seed weight across all populations. Partitioning of the correlation coefficients into direct and indirect effect, revealed number of pods plant^{-1} and 100-seed weight to have the highest direct effect on seed yield plant^{-1}. Selection of number of pods plant^{-1},100-seed weight, and number of secondary branches enhances seed yield in the present populations of pigeonpea.

A study on the genetic control of yield and yield-related traits involving a total of 460 F_2 pigeonpea individuals was conducted in three mapping populations. The skewness and kurtosis of evaluated characters were tested. The results indicated predominance of additive gene action affecting the studied characters. The estimated coefficients of skewness and kurtosis revealed there were no gene interactions found for some of the traits implying that there were no influences among the genes for the phynotypic expression of the respective traits. The traits were days-to-50% flowering in the cross AL 201 × ICPL 20325, plant height for crosses ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035, the number of primary branches, secondary branches and 100-seed weight for cross ICP 5529 × ICP 7035. Number of seeds pod^{-1} in cross AL 201 × ICPL 20325, days-to-50 % flowering, number of seed pod^{-1} and 100-seed weight in population ICP 8863 × ICPL 87119, and 100-seed weight in ICP 5529 × ICP 7035, recorded negative skewness values indicating the presence of duplicate epistatic gene interaction. All other remaining characters in all the crosses recorded positive skewness indicating the presence of complementary epistasis interaction among loci.

QTL analyses were conducted for the above eight yield and yield-related traits using three mapping populations with composite interval mapping (CIM). A total

of 42 QTLs were detected of which 5 were in AL 201 × ICPL 20325, 7 in ICP 5529 × ICP 7035 and 30 in ICP 8863 × ICPL 87119. QTLs ranged from 1 to 4 per trait and the phenotypic value explained (PVE %) ranged between 10.35 to 16.27% in AL 201× ICPL 20325, 10.44 to 17.9% in ICP 5529 × ICP7035 and 10.71 to 89.12% in ICP 8863 × ICPL 87119. The detected QTLs were co-localized within the same genomic regions indicating the presence of pleiotropic effect or linkage.

In summary, the present study identified useful genetic resources for further breeding, determined the most influential traits in pigeonpea breeding to improve seed yield and yield components and developed mapping populations segregating for yield and yield-related traits in the crosses of AL 201 × ICPL 20325, ICP 5529 × ICP 7035 and ICP 8863 × ICPL87119. The 42 putative QTLs identified in the study associated with seed yield and yield-related traits are useful for strategic marker-assisted breeding of pigeonpea.

Declaration

I, Seleman Rashid Kaoneka, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) Their words have been re-written but the general information attributed to them has been referenced.

b) Where their exact words have been used, their writing has been placed in italics and inside quotation marks and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference sections.

Signed: ..... Date: ...05/04/2017.....

Seleman. R. Kaoneka (Candidate)

As the candidate's supervisors we agree to the submission of this thesis

Signed: ..... Date: ...05/04/2017.....

Professor Hussein Shimelis (Principal supervisor)

Signed: ..... Date: 07/04/2017.....

Dr. Rajeev Varshney (Co- supervisor)

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I would like to extend my special thanks to my beloved wife Halima Nassor as her passion, love and care. She has been the source of encouragement that made my study successful.

Dedication

This thesis is dedicated to my late parents, Rashid Kaoneka and Halima Ally Nguzo who laid the foundation of my education.

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Abbreviations

ºC	Degrees Celsius
cm	Centimetre
CTAB	Cetyl trimethyl ammonium bromide
CIM	Composite Interval Mapping
CV	Coefficient of Variation
DNA	Deoxyribonucleic acid
ESA	Eastern and Southern Africa
F_2	Second filial generation
GAB	Genomic assisted breeding
GBS	Genotyping-by-Sequencing
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LOD	Logarithm of odds
MAS	Marker assisted selection
ng/µl	Nano-grams per microlitre
NARS	National Agricultural Research Systems
%	Percent
PVE	Phenotypic Variance Explained
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
SMA	Single marker analysis
SSA	Sub-Saharan Africa
SNP	Single Nucleotide Polymorphism

Background

Pigeonpea (*Cajanus cajan* L. Millsp.) is an important grain legume of the tropical and subtropical regions of the world. Pigeonpea is the least widely grown crop among the six major tropical legumes (Abate et al. 2012). It accounts for less than 5% of total world pulse production (Josh et al.2001). Pigeonpea is grown extensively in India, South-East Asia, East Africa, Latin America and the Caribbean, where it plays significant role in the food and nutritional security. The protein content of commonly grown pigeonpea has been reported to range between 18–26% (Odeny 2007). Further, it has significant usage as animal feed, fodder, firewood, thatching material and for improving soil structure and fertility (Odeny 2007).

There is a large variation in its maturity that helps in its wide adaptation including diverse locations and cropping systems (Saxena 2010). Generally, the short-duration (100-140 days) cultivars of pigeonpea are grown as a sole crop, while the medium (160-180 days) and long-duration (> 200 days) types are invariably grown as intercrop or mixed crop with other short-duration cereals and legumes (Saxena 2010). Pigeonpea has self-compatible flowers, but it is often cross-pollinated by bees with 25-35% outcrossing (Saxena 2008). The estimated size of pigeonpea genome packed in 11 chromosomes is 833Mbp (Varshney et al. 2012).

Pigeonpea is constrained by inadequate genomic resources, such as genome sequence or molecular markers for improving the productivity of this crop. Its yield gain has lagged behind due to limited breeding progress and lack of basic information on the genomics and genes associated with yield and yield components (Kumawat et al. 2012).

Constraints to pigeonpea production

Pigeonpea production has shown an increasing trend worldwide with harvested area of 2.7 million hectare (Mha) during 1961 to 4.6 Mha (2009) (FAO 2009, http://faostat.fao.org/). However, no increase has been observed in its productivity, which in the past five decades remained stagnated at around 750 kg/ha (Bohra et al. 2012). Average yields are approximately 730 and 840 kg per ha for Sub-Saharan Africa (SSA) and Southern Asia (SA), respectively, compared to 885 kg per ha of the world average productivity (Abate et al. 2011). Pigeonpea productivity is challenged by several biotic and abiotic stresses under marginal field conditions, and as a result there is a huge yield gap between realizable yield (2500 kg ha^{-1}) and the yield in farmer's fields (866 kg ha^{-1}in Asia and 736 kg ha^{-1}in Africa) (Abate et al. 2011).

A number of factors are responsible for the poor productivity of the crop in Sub-Saharan Africa (SSA), including lack of improved cultivars, poor crop husbandry, pests, and diseases. Major diseases include *Fusarium* wilt caused by *Fusarium udum* Butler, sterility mosaic disease caused by sterility mosaic virus and phytophthora blight (*Phytophthora drechsleri*). The major pests include gram pod borer (*Helicoverpa armigera*), maruca (*Maruca vitrata*), pod fly (*Melanagromyza obtusa*), plume moth (*Exelastis atomosa*) causing substantial yield reduction to pigeonpea production annually (Varshney et al. 2010a).

To increase the productivity of pigeonpea, identification of candidate genes responsible for desired traits and subsequent breeding efforts to transfer the desired trait(s) in the elite background is a prerequisite (Varshney et al. 2012; and Kumawat et al. 2012). New breeding approaches using genome-wide information with high precision phenotyping can assist in harnessing the genetic diversity present in the gene pools for crop improvement programmes in a number of crop species. However, this has not been the case in pigeonpea especially in SSA.

Problem statement

In the past, conventional breeding of pigeonpea had limited success in overcoming biotic and abiotic challenges and in boosting productivity of the crop (Varshney et al. 2007; and Saxena 2008). Lack of intensive breeding efforts and unavailability of high yielding cultivars, among others, have significantly contributed to the stagnant productivity of the crop (Zaveri and Pathank 1998).

Narrow genetic diversity in cultivated germplasm, caused by domestication and breeding from a small number of genotypes (Bohra et al. 2012). has further hampered the effective utilization of conventional breeding as well as development and utilization of genomic tools, resulting in pigeonpea being often referred to as an 'orphan crop legume' (Varshney et al.2010b).

Rationale of the study

Genomic-assisted breeding has the potential of accelerating cultivar development in conventional breeding programs (Varshney et al. 2005; Varshney et al. 2010). In addition, genomic tools can help in selecting suitable parents and promising segregants to develop elite breeding lines (Pazhamala et al. 2015). Linkage and marker-trait association analyses and efficient phenotyping of pigeonpea populations may enhance genetic gains and cultivar development.

Therefore, this study was conducted for the purpose of selecting suitable parents and promising segregants, and availing the information on genomic resources for pigeonpea, needed by conventional breeders in accelerating the pigeonpea breeding of elite cultivars through marker-assisted transfer.

Overall objective

The overall goal of the project was to identify quantitative trait loci (QTLs) associated with yield and yield-related traits in newly developed pigeonpea populations for marker-assisted breeding of the crop.

The specific objectives

The specific objectives of the study were:

1. To assess the phenotypic variability and identify promising genotypes among F_2 segregants of pigeonpea populations derived from crosses of six parental lines with diverse genetic backgrounds.
2. To apply simple correlation and path analyses and identify most useful yield and yield-related components of newly developed pigeonpea mapping populations.
3. To determine the genetic control of yield and yield-related traits involving a total of 460 F_2 pigeonpea progenies derived from three families of varied genetic backgrounds.
4. To identify quantitative trait loci associated with yield and yield-related traits using 420 F_2 progenies developed from three diverse pigeonpea families for marker-assisted breeding.

Outline of the thesis

This thesis consists of five distinct chapters in accordance with a number of activities related to the above objectives. All chapters were written in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). As such, there is some unavoidable repetition of references and some introductory information between chapters. The contents of Chapter 1 have been published in the Journal of Plant Breeding.

Table 0.1: Structure of the Thesis

Chapter	Title
-	Thesis introduction
1	Review of Literature
2	Phenotypic variability among F_2 individuals of pigeonpea derived from three genetic backgrounds
3	Correlation and path-coefficient analyses of seed yield and related traits in newly developed pigeonpea populations
4	Prediction of gene action controlling yield and yield-related traits in pigeonpea
5	Quantitative trait loci mapping for yield and yield- related traits in pigeonpea

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CHAPTER ONE

A review of the literature [1]

Abstract

Pigeonpea (*Cajanus cajan* [L.] Millspaugh) is an important multipurpose grain legume crop primarily grown in tropical and subtropical areas of Asia, Africa and Latin America. In Africa, the crop is grown for several purposes including food , income generation, livestock feed and in agroforestry. Production in Eastern and Southern Africa (ESA) is however faced with many challenges including limited use of high-yielding cultivars, diseases and pests, drought, under-investment in research and lack of scientific expertise. The aim of this review is to highlight the challenges facing pigeonpea breeding research in ESA and the existing opportunities for improving the overall pigeonpea subsector in the region. We have discussed the potential of the recently available pigeonpea genomic resources for accelerated molecular breeding, the prospects for conventional breeding and commercial hybrid pigeonpea, and the relevant seed policies, among others, which are viewed as opportunities to enhance pigeonpea productivity.

Key words: Africa — *Cajanus cajan* — climate change — food security

[1] This literature review was published as: Kaoneka, S.R., Saxena, R.K., Silim, S.N., Odeny, D.A., Ganga Rao, N.V.P.R., Shimelis, H.A., Siambi, M., and Varshney, R.K. 2016. Pigeonpea breeding in eastern and southern Africa: challenges and opportunities. Plant Breeding 135 (2): 148–154, doi: 10.1111/pbr.12340.

1.0. Introduction

Climate change and nutritional food security have attracted global concerns in the recent years. Generally, the resource- poorer developing countries are more vulnerable to climate change because of their low incomes and dependence on climate sensitive sectors such as agriculture (IPCC 2007). Although African countries have recorded improved economic growth over the last 5 years, the continent is still considered most susceptible to climate change due to its vulnerability and inability to cope with the physical, human and socio-economic consequences of climate extremes (Kabasa and Sage 2009). Furthermore, an estimated 30% of children under the age of five in sub-Saharan Africa (SSA) are underweight, mainly due to malnutrition (Mula and Saxena 2010). Sustainable solutions to agriculture and food security in Africa must consider more focused research efforts on locally adapted, highly nutritious and stress-tolerant crops alongside sustainable government support to agricultural research and development. One such crop with potential to cope with climate change and provide nutritional food security is pigeonpea (*Cajanus cajan [L*.] Millspaugh).

Pigeonpea, a diploid legume crop species (2n = 2x = 22), belongs to *Cajaninae* sub-tribe of the economically most important leguminous tribe *Phaseoleae* (Van der Maesen 1990). The crop derives its name from Barbados, where the seeds were once used to feed pigeons (Van der Maesen 1990). It is generally grown under risk-prone marginal lands with low inputs (Mula and Saxena 2010). Pigeonpea is increasingly gaining importance in Africa, especially in ESA, where it occupies an area of about 990 000 ha (Table 1.1) (TIA/IAI 2012; FAO 2013). Both local and export demand for this multipurpose legume crop continue to rise, presenting an opportunity for faster productivity enhancement and strengthening of seed delivery systems, as well as improvement of existing value chains. Pigeonpea is likely to become a major player in ESA's agriculture, especially with increased research investment. The aim of this review is to highlight the challenges affecting pigeonpea production and improvement and the existing opportunities for improving pigeonpea research and overall subsector in ESA.

Table.1.1. Area, yield and production of pigeonpea in five countries between 1990 to 2011

Country	Area ('000 ha)			Yield (Kg/ha)			Production ('000 t)		
	1990-92	2000-02	2011	1990-92	2000-02	2011	1990-92	2000-02	2011
Kenya	159.8	166.7	182.3	409	465.4	608	65.2	77.4	111
Malawi	142.3	137.4	196.5	683.8	752.9	1103	97.3	103.4	217
Mozambique	-	68.8	193.2	-	465.1	504	-	32.0	97.4
Tanzania	56.0	134.0	288.1	673.2	650	946	37.7	87.1	273
Uganda	61.3	80.0	92.5	827.1	1000	1025	50.7	80.0	94.8
Total	**419.4**	**586.9**	**952.6**	**598.2**	**647.3**	**832**	**250.9**	**379.9**	**793**

1.1 Historical perspectives of pigeonpea genetic diversity and breeding in Africa

The centre of origin of pigeonpea has been a subject of discussions in the past. For instance, some studies (Leslie 1976; Purseglove 1976; Singh et al. 2001) suggest the origin of pigeonpea to be in Africa. Many other studies (Van der Maesen 1990; Fuller and Harvey 2006; Saxena et al. 2014) suggest India as the origin of the crop. The presence of several wild relatives, the diverse genepool of the crop in the Indian subcontinent and some recent molecular studies provide a stronger evidence of the latter group. Africa harbours only two wild species of pigeonpea: *C. kerstingii* (Harms) and *C. scarabaeiodes* (L.) Thouars (Van der Maesen 1990). It is most likely that pigeonpea was introduced by immigrants in the 19th century who moved to Africa to become railway workers and storekeepers (Odeny 2007).

From eastern Africa, pigeonpea spread over the African continent, albeit without acquiring a prominent position. In Africa and the Far East, pigeonpea has been

grown for at least 4000 years (Van der Maesen 1980) and therefore considerable agro-ecological adaptation has been obtained locally.

The traditional African pigeonpea genotypes are long-duration, cream- and large-seeded (Remanandan 1990). In Uganda, medium-duration, cream to mottle small-medium seeded type (Manyasa et al. 2009) have been part of the traditional cropping system (Silim et al. 1991; Kimani 2001). Uganda was the first country in ESA to implement a pigeonpea breeding programme in 1968 at Makerere University (Saxena 2008). However, there is a scope to expand further under sustainable intensification of cropping systems with pigeonpea as one of the component crops.

1.2. Challenges facing pigeonpea production and improvement

Challenges facing pigeonpea production and improvement in ESA are divided into two main categories, namely technical and institutional challenges.

1.2.1. Technical challenges

1.2.1.1. Limited use of high-yielding varieties

Low realized productivity in pigeonpea remains one of the major constraints despite past and ongoing breeding efforts. In ESA, the yield of green pods varies from 1000 to 9000 kg/ha and that of dry grain may reach 2500 kg/ha in pure stands with modern cultivars. Present regional yields are about 800 kg/ha under intercropping systems which is much lower than the realizable yield potential. Malawi is the major producer of pigeonpea in the region with productivity of about 1327 kg/ha at present (FAO 2013). Although several improved varieties are now available, adoption is limited and most farmers grow traditional landraces that are prone to soil borne fungal diseases and grain yields are of low quality (Høgh-Jensen et al. 2007).

Short-duration varieties are much more susceptible to insect pest attack, necessitating the use of insecticides, which most ESA farmers cannot afford,

therefore opting to grow traditional long-duration types (Jones et al. 2002). However, recent trend was on cultivation of medium-duration varieties that can fit very well into existing cropping systems. More breeding efforts are needed to focus on developing farmer- and market preferred genotypes with high yield, *Fusarium* wilt resistance and pest tolerance.

1.2.1.2. Biotic stresses

Biotic stresses significantly reduce the pigeonpea yield in ESA (Reddy et al. 1990). The most important fungal diseases of pigeonpea in ESA are Fusarium wilt, *Cercospora* leaf spot and powdery mildew (Brink and Belay 2006). Fusarium wilt is the most serious disease in all major pigeonpea-producing countries in the region (Silim et al. 1995). Surveys carried out in 1980 estimated wilt incidences to be 60% in Kenya, 36% in Malawi and 24% in Tanzania with annual losses of US$ 5 million in each of these countries (Kannaiyan et al. 1984). Accessions with less wilt incidences and high yield, which are potential donors in resistance breeding, have been identified, such as ICEAP 00926, ICEAP 00576-1, ICEAP 00933, ICEAP 00040 and ICP 9145 (Rao et al. 2012). They have been identified as potential in terms of yield and resistance traits. Insects that are serious, widely distributed and cause heavy economic losses in pigeonpea in ESA are pod and seed boring Lepidoptera (*Helicoverpa armigera* (H€ubner), *Maruca vitrata* (=testulalis) Geyer, *Etiella zinkenella* (Treitschke), and pod fly (*Melanagromyza chalcosoma* (Spencer) (Johansen et al. 1993; Minja et al. 1999). *H. armigera* is the major biotic constraint to pigeonpea production (Lateef and Reed 1990), with yield loss estimated at 42% (Abate and Orr 2012). Reports on the seed damage due to pod-sucking bugs in Kenya, Malawi, Tanzania and Uganda have shown it ranges from 3 to 32% and varies among locations within and between countries (Minja 1997).

Pigeonpea lines with resistance to *H. armigera* have been reported, but little progress has been made in incorporating resistance in cultivars that are acceptable to farmers (Shanower et al. 1999). The development of insect-

resistant and/or -tolerant pigeonpea cultivars has been a high priority in the research programmes, but the progress is hindered by high variation in pest populations (within and across seasons) and the high degree of out-crossing in pigeonpea (Shanower et al. 1999).

1.2.1.3. Abiotic stresses

In ESA, pigeonpea is grown purely under rainfed conditions with varying temperatures, altitudes and latitudes (Silim et al. 2006). Pigeonpea encounters various abiotic stresses during its life cycle such as moisture stress (drought), temperature, photoperiod and mineral (salinity/acidity) stress (Choudhary et al. 2011). Among these stresses, moisture stress is most prevalent (Silim and Omanga 2001). The medium and long-duration genotypes that are commonly grown in ESA depend on residual moisture for the reproductive phase development. In some cases, this leads to terminal drought stress which is causing substantial yield reduction (Kimani 2001). In a study concerned with field evaluation of pigeonpea germplasm, a high (>50%) yield loss was attributed to a combination of a severe mid-season drought and high temperatures (Gwata 2010).

Through multi-locational and multiyear evaluations, medium-duration genotypes such as ICEAP 00673, ICEAP 01170 and ICEAP 01179, as well as long-duration genotypes such as ICEAP 01423 and ICEAP 01202 possessing drought tolerance coupled with high yield have been identified (Rao et al. 2012).

1.2.2. Institutional challenges

1.2.2.1. Shortage of improved seed

Access to improved seeds and markets is particularly limited in sub-Saharan Africa (ICRISAT 2009). Inadequate supply of the breeder seeds by the public sector (Rao et al. 2012), limited involvement of the private sector (Jones et al. 2002) and non-existence of the commercial pigeonpea seed markets (Tripp

2000) are the major challenges facing the pigeonpea seed industry in ESA. In addition, lack of access to quality seeds (Abate and Orr 2012) and poor extension services (Abate et al. 2012) significantly contribute to the poor adoption of the improved pigeonpea seeds in ESA.

1.2.2.2. Under-investment in research

Most of the research on pigeonpea in ESA to date has been through donor funding to the National Agricultural Research Systems (NARS) and ICRISAT (Jones et al. 2001). Despite positive growth in the 1980s, public investment in agricultural research and development (AR&D) in ESA has declined (Beintema and Stads 2010). For instance, in Malawi, the major pigeonpea producer in ESA, the government currently invests only 4% of the agricultural budget in research (Phiri et al. 2012). In Tanzania, for the past decade, the government budget approved for the Department of Research and Training has been in the average of 24% of the total actual budget requirement for all agricultural crops (ESAFF 2013).

1.2.2.3. Lack of human resource capacity

In ESA, all major producers of pigeonpea have limited capacity to carry out effective research and development on pigeonpea, which have traditionally received less attention than cereals and cash crops (Abate and Orr 2012).

Information from the Uganda National Agricultural Research Organization (NARO) revealed that is within the national programme, currently there is only one scientist who is actively involved in pigeonpea breeding (Yuventino Obong personal communication 2015). The same applies to Malawi where only one pigeonpea breeder and one agronomist within the national programme are working (Esnart Nyirenda Personal communication 2015).

There is also still a huge gap in scientific capacity left by retired scientists, due to failure by the national governments to continue hiring and support agricultural scientists for a long time (Beintema and Stads 2006). For instance, in Tanzania, the situation is most extreme at Ilonga Agricultural Research Institute, a country

pigeonpea mandate, where most of the posts for senior research officers are vacant (Coulson and Diyamett 2012).

1.3. Opportunities for Pigeonpea Production and Breeding in ESA

1.3.1. Increased adoption for pigeonpea production as a strategy in climate smart agriculture

The agricultural system in ESA is characterized by low productivity, low use of external inputs, traditional management practices and limited capacity to respond to environmental shocks (Tabo et al. 2007). Pigeonpea has a huge untapped potential for improvement both in quantity and in quality of production in ESA (Odeny 2007). Besides its ability to tolerate droughts and availing water and soil mineral nutrients (Valenzuela and Smith 2002; Mathews and Saxena 2005; and Adu-Gyamfi et al. 2007), pigeonpea is also a multipurpose crop (Boehringer and Caldwell 1989; Kimani 2001; Snapp et al. 2003; and Saxena et al. 2010).

Unreliable rainfall received in many parts of the sub-Saharan Africa has reduced cereal production especially maize and wheat, and made farmers to shift to legumes production especially pigeonpea which is drought tolerant, and in most cases intercropped with cereals mainly maize or sorghum. The drought-tolerant pigeonpea has a unique role in meeting food security needs of subsistence farmers in climatic risky regions of ESA (Snapp et al. 2003). With the regional breeding approach in place, the crop can now be grown in more targeted areas and breed for a wide range of uses.

1.3.2. Increased market demand for pigeonpea

Both local and export demand for pigeonpea exist in Africa, especially in ESA. Some studies indicate that a vibrant domestic, regional and export trade of dry grain and an emerging market for vegetable pigeonpea exist in ESA (Shiferaw et al. 2008a). ESA countries export about 200,000-ton grain annually to India. In ESA, Kenya and Malawi are the two biggest producers of pigeonpea. In Kenya, 45% of the crop is sold, while in Malawi, the share is 35% (Shiferaw et al. 2008b;

Abate and Orr 2012). During recent years, Tanzania and Mozambique have increased area under cultivation and contributing to large quantities of grain export. Although informally traded, cross-border trade of pigeonpea between ESA countries do exist, for instance, between the northern Tanzania and Kenya (Brink and Belay 2006). In addition, the large Indian and Afro-Caribbean communities in Europe and North America offer new potential markets that can be accessed through the application of improved processing technologies such as freezing (Jones et al. 2006).

1.3.3. Improved seed access and policy support

One of the key factors for stimulating technology uptake and increasing agricultural productivity in smallholder agriculture is access to quality seed of improved varieties (Shiferaw et al. 2008b).

Many countries in ESA have regulations that only permit the sale of certified seed (Abate and Orr 2012). Community-based seed production and marketing systems like quality declared seed, which is tested in Tanzania for dissemination of truthfully labelled seed of high quality, could be one strategy for easing the seed shortage problem, especially for open-pollinated cereals or self-pollinated legumes like pigeonpea (Abate et al. 2012). For an efficient seed system to operate, the public sector must play a bigger role in plant breeding and some aspects of quality control, while the private sector has better incentives in the area of seed multiplication, processing and distribution (Minot et al. 2007).

The ongoing seed policy reforms in the region have facilitated more participation of the private sector within pigeonpea seed systems. For instance, right now in Tanzania, there are more than 10 big companies/estates producing quality seeds and grain for sale excluding community-based organizations, NGOs, PMGs, farmer's groups and contract farmers (Rao et al. 2014). The move towards formation of strategic partnership with different stakeholders has accelerated the release of pigeonpea seeds as well as increasing the quantity of seeds produce in the region. Commercial seed companies are also expected to develop interest

in pigeonpea, due to ever growing demand for pigeonpea exports. About 200,000 tons of pigeonpea grain is exported annually by ESA countries to India.

1.3.4. Improved varieties and potential for hybrid pigeonpea

Breeding activities supported by ICRISAT over the years developed several region-specific genotypes through intensive genetic enhancement programme. In close collaboration with national programmes, 32 high-yielding varieties were released as follows; Malawi (7), Kenya (7), Tanzania (7), Mozambique (5), Uganda (2), Zambia (2), Ethiopia (1) and Sudan (1). Further, 10 varieties (4-Ethiopia, 2-Zambia, 4-Uganda) are being processed for release. Most of these varieties were developed from local germplasm with region-specific breeding priorities such as high grain yield, intercropping compatibility, photoperiod insensitivity, consumer-preferred grain quality, resistance/tolerance to Fusarium wilt, Helicoverpa pod borer and resilience to climate change. List of popular pigeonpea varieties released in ESA is given in Table 1.2.

Table 1.2. List of popular pigeonpea varieties released in ESA

Country	Variety	Year of release	Special varietal attributes
Kenya	KARI Mbaazi2 (ICEAP 00040)	1995	Long duration, large cream seed and Fusarium wilt resistant
	Katumani 60/8 (Kat 60/8)	1998	
	Karai (ICEAP 00936)	2011	
	Peacock (ICEAP 00850)	2011	Medium duration
Malawi	Sauma(ICP 9145)	1987	Long duration, fusarium wilt resistant
	Kachangu(ICEAP 00040)	2000	Long duration, large seeded, fusarium wilt resistant
	Mwaiwathualimi(ICEAP 00557)	2010	Medium duration
	Chitedze; pigeonpea 1 (ICEAP 01514/15)	2011	Medium duration and high pod load
Mozambique	ICEAP 00040	2011	Long duration
	ICEAP 00020	2011	Long duration
Tanzania	Komboa (ICPL 87091)	1999	Short duration (110–120 days)
	Mali (ICEAP 00040)	2002	Long duration (180–270 days)
	Tumia (ICEAP 00068)	2003	Medium duration (140–180 days)

Table 1.2: Continued

	Kiboko (ICEAP 00053)	2015	Long duration and erect plant type
	Karatu 1(ICEAP 00932)	2015	Long duration
	Ilonga 14-M1(ICEAP 00554)	2015	Medium duration
	Ilonga 14-M2 (ICEAP 00557)	2015	Medium duration
Uganda	Sepi I (Kat 60/8)	1999	Medium maturity
	Sepi II (ICPL 87091)	1999	Short duration, multiple cropping

A key to success factor in pigeonpea breeding in ESA was the adoption of the breeding strategy for the establishment of the regional approach taking into consideration the key factors such as adaptation, crop phenology, market preference and pathogen specificity (Silim et al. 1995; Silim and Omanga 2001; and Silim et al. 2006). Kenya transect considered as an open laboratory (Table 1.3) was used. It varied from 50 to 2500 m above sea level and where temperature decreases with increase in altitude. It was the basis for understanding the adaptation for developing and targeting varieties (Table 1.4). In addition, sources of resistance in the medium- and long-duration background were also identified. Efforts are under way to increase the adoption of these varieties in farmers' fields.

Table 1.3. Geographical positions and weather information of the location (study sites in Kenya)

Latitude/Altitude		Location									
(m)	Temp (°C)	Kabete		Katumani		Kiboko		Mtwapa		Muguga	
1° 15^0(1825)	Max	24.6[1]	22.1[2]								
	Min	12.9[1]	12.2[2]								
	Mean	18.7[1]	17.1[2]								
1° 35^0(1560)	Max			25.6[1]	23.6[2]						
	Min			14.4[1]	12.9[2]						
	Mean			20[1]	18.7[2]						
4° 25^0(900)	Max					29.4[1]	27.8[2]				
	Min					17.7[1]	15.5[2]				
	Mean					23.5[1]	21.6[2]				
4° 25^0( )	Max							31. 4[1]	28.9[2]		
	Min							23.2[1]	21.5[2]		
	Mean							27.3[1]	25.2[2]		
1° 15^0(2110)	Max									22[1]	1 9.4[2]
	Min									11.5[1]	10.2[2]
	Mean									16.8[1]	14.9[2]

KEY: Rainfall duration = [1]Short, [2]Long

Table 1.4. Days to flowering of the selected pigeonpea genotypes tested under natural day length at the selected study sites in Kenya

		Location				
Genotype	**Maturity**	**Kabete**	**Katumani**	**Kiboko**	**Mtwapa**	**Muguga**
ICEAP 00040	Long	149	178	227	300	156
T-7	Long	150	185	164	–	170
ICP 6927	Medium	123	105	121	119	160
ICP 7035	Medium	119	94	125	122	
ICPL 87091	Short	91	81	74	84	
ICPL 9001	Extra short	80	78	64	79	

ICRISAT in Asia has developed a number of hybrid varieties that have been released by NARS and commercial seed companies. The hybrid varieties have a 20–40% yield advantage over the open pollinated varieties (Shiferaw et al. 2008b). Hybrid varieties that are regional –specific, meeting consumer preference and adapted to cropping systems are the priority of ICRISAT-ESA. Efforts are underway to identify stable CMS lines that are adaptable to ESA, maintainers in local germplasm and heterotic parental combinations as hybrid vigour is associated with genetic diversity, crosses between the genetically diverse African and Asian gene pools could result in considerable yield improvement and create greater incentive for adoption of such varieties (Kimani 1991).

1.3.5. Availability of genomic resources for pigeonpea genetic enhancement and breeding

To meet the growing demand for pigeonpea in ESA, conventional breeding on its own will not be sufficient in developing superior genotypes. Pigeonpea genome has now been sequenced, availing more genomic resources for exploitation to speed up the ongoing conventional breeding activities (Bohra et al. 2011, Varshney et al. 2011, and Varshney et al. 2012).

Availability of DNA markers for pest and disease resistance will be of utmost importance, as it will be easier to conduct resistance breeding to achieve both

stability and productivity of the crop which is top priority in the genetic enhancement of this pulse in ESA (Crouch and Ortiz 2004).

1.4. Conclusions

This chapter has shown that pigeonpea breeding research in ESA has moved knowledge forward and has resulted in impacts on the ground over a very short period, moving the crop from an orphan crop to where both national governments and development partners are now paying attention to it. In addition, the review has shown that much needs still to be performed to unlock the opportunities that exist in this crop. This will require a multifaceted approach from science-based solutions, policies to market requirements.

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CHAPTER TWO

Phenotypic variability among F_2 individuals of pigeonpea derived from three genetic backgrounds

Abstract

Information on phenotypic variability of F_2 individuals is useful for genetic analysis and selection programs. The objective of this study was to assess the phenotypic variability and to identify promising genotypes among F_2 segregants of pigeonpea populations derived from three genetic backgrounds. Six parents including AL 201, ICPL 20325, ICP 8863, ICPL87119, ICP 5529 and ICP 7035 were selected and crossed using a bi-parental mating scheme. The six parents and families derived from the three genetic background (AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035) were field evaluated. Data on F_2 individuals were collected including days- to-50% flowering, plant height, number of primary branches, number of secondary branches, number of pods plant^{-1}, number of seed plant^{-1}, 100-seed weight and seed yield plant^{-1}. Significant phenotypic variation was observed in the medium maturing (ICP 8863 and ICPL 87119) and long maturing (ICP 5529 and ICP 7035) parents. Significant variation was exhibited for days to 50% flowering, number of pods plant^{-1}, number of seeds pod^{-1} and seed yield plant^{-1} among F_2 individuals derived from medium maturity parents, whereas individuals from late maturing parents showed significant variations in plant height, number of pods plant^{-1} and seed yield plant^{-1}. Transgressive segregations were recorded for all studied characters except seed yield. Transgression was more pronounced in the families of ICP 5529 × ICP 7035 and AL 201 × ICPL 20325. This study demonstrated the presence of considerable genetic variation among F_2 individuals derived from the three genetic groups. Identified transgressive segregants are useful genetic resources for further selection and breeding of pigeonpea.

Key words: genotype, phenotype, pigeonpea, segregation analysis, transgressive segregation

2.1.Introduction

Genetic variation and variability are crucial in biology (Hallgrímsson and Hall 2005) and plant breeding programs. Although the terms are used interchangeably in the scientific literature, variation and variability are not synonymous (Wang et al. 2014). Plant breeding is a three step process, wherein choice of appropriate mapping population that is critical for the success of quantitative trait loci (QTL) mapping project is made (Semagn et al. 2010), individuals with superior phenotypes are identified, and improved cultivars are developed following designed crosses and selections (Moose and Mumm 2008).

Mapping population can either be obtained from controlled crosses involving selected parent's (experimental approach) or from naturally occurring mating systems (without artificial control). Parents of a mapping population must have sufficient genetic variation for the traits of interest both at DNA and phenotypic level (Liu 1997). This is important for creation of segregating progenies with maximum genetic variability for further selection (Barrett and Kid-well 1998), and to introgress desirable genes from diverse germplasm into the available genetic base (Thompson et al. 1998). Selection of superior genotypes in any crop is undoubtedly proportional to the amount of genetic variability present in the population and the degree to which the traits are inherited (Udensi et al. 2011).

Once suitable parental lines have been selected, controlled crosses are undertaken to produce heterozygous F_1 individuals, which are then selfed to generate F_2 populations from which variable phenotypes and genotypes are scored (Falconer and Mackay 1996). Due to high level of segregation the F_2 population provides maximum genetic information when analyzed using co-dominant marker system which is more powerful for detecting quantitative trait. Furthermore, the F_2 population can also be used to estimate the degree of dominance for detected QTL. Data obtained from backcross (BC) populations using either co-dominant or dominant markers is less informative than that

obtained from F_2 populations because one, rather than two, recombinant gametes are sampled per plant (Semagn et al. 2006). Dominant markers supply as much information as co-dominant markers in recombinant inbred lines (RILs), near isogenic lines (NILs) and doubled haploids (DHs) (Burr et al. 1988). In practice, the population size used in preliminary genetic mapping studies varies from 50 to 250 individuals (Mohan et al. 1997) but a larger population size is needed for high resolution fine mapping. Although molecular breeding strategies, such as marker-assisted recurrent selection (MARS) and genomic selection, place greater focus on selections based on genotypic information, they still require phenotypic data (Jannick et al. 2010).

Phenotyping can be defined as the set of methodologies and protocols used to measure plant growth, architecture, and composition with a certain accuracy and precision at different scales of organization, from organs to canopies (Fiorani and Schurr 2013). Replication of individual accessions within a site is usually needed to increase precision in phenotypic measurements, by eliminating environmentally induced noise and measurement errors (Hall et al. 2010). Phenotypic characterization and evaluation of germplasm are pre-requisites for the utilization of the available diversity in any crop improvement programme.

Some studies of quantitative traits in segregating populations reported the presence of phenotypes that are extreme relative to those of either parental lines (deVicente and Tanksley 1993; Rieseberg and Ellstrand 1993; Cosse et al. 1995). This is referred to as transgression or transgressive segregation, the appearance of individuals in segregating populations that fall beyond their parental phenotypes (deVicente and Tanksley 1993).

Transgressive segregation is the result of additive gene action and reported due to fixation of dominant and recessive genes in separate individuals (Rick and Smith 1953; Singh 1996), and/or from complementary gene action (Vega and

Frey 1980). Complementary gene action has been a more popular general explanation for transgressive segregation in plant genetics (Grant 1975; Vega and Frey 1980).

Pigeonpea (*Cajanus cajan*) is an important pulse crop grown world-wide. It is predominantly grown by small-holders in marginal environments in sub-Saharan Africa and Asia. Breeding efforts for this crop are challenged by, low level of genetic diversity present in the primary gene pool and non-availability of marker-trait information for traits of interest.

Several studies have been conducted to determine the phenotypic and genotypic variability of different agronomic characters for this crop. For instance, Fakir et al. (1998), studied the phenotypic variability of selected yield –related characters and identified the number of pod plant-1, to be the main source of yield variation among studied characters. Sarsamkar et al. (2008), investigated the variation among the crosses consisting of parents and F_2 of pigeonpea and found the wide variability to be exhibited by number of pods plant-1, plant height and seed yield plant^{-1}.

Niranjana et al. (2014), studied the genetic diversity, genetic variability and correlation on various morphological traits, yield and yield related components of pigeonpea, and observed greatest variation in number of pods plant^{-1} at phenotypic and genotypic level.

Chaturved et al. (2013), studied variability in thirty genotypes of pigeon pea for grain yield and its attributing characters and recorded the highest Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) for number of pods per plant followed by seed yield per plant and number of cluster per plant indicating the presence of ample variation for these traits in the present material.

Vange and Egbe (2009), conducted a study to evaluate genetic variability among 29 new pigeonpea genotypes and a local variety for the yield and yield component

traits, and the results indicated the phenotypic coefficient of variability and genotypic coefficient of variability to be relatively high for number of pods per plant, dry pod weight, dry grain yield and number of primary branches.

Yerimani et al. (2013), studied genetic variability generated from the Gulyal white × Maruti cross in F_3 and F_4 generations and the results indicated the higher magnitude of variability to be recorded for 50 per cent flowering, number of secondary branches, number of seeds per pod, number of pod per plant, seeds yield per plant and seed yield (Kg/ha).

To initiate any selection program of pigeonpea and for genetic mapping, it is necessary to assess the phenotypic variability present in the early segregation generation after designed crosses for yield and its components. Therefore, the objective of this study was to assess the phenotypic variability and to identify promising genotypes among F_2 segregants of pigeonpea derived from three genetic backgrounds.

2.2. Materials and Methods

2.2.1. Study site

The study was conducted at the International Crops Research for the Semi-Arid Tropics (ICRISAT), Patancheru (18° 78° E), Andhra Pradesh India. Field experiments were conducted during the rainy seasons over three growing seasons (2012/2013, 2013/2014 and 2014/2015).

2.2.2. Parental materials, crosses and field management

Six diverse parents were used to generate three families (Table 2.1). The parents were AL 201, ICPL 20325, ICPL 87119, ICPS 8863, 7035 and 5529. The parents were selected on the basis of their genetic diversity and high yield potential based on prior yield data. Original seeds of all parents were obtained from ICRISAT seed bank. Characteristics features of the parents are described in Table 2.1.

Table 2.1. Some agro-morphological features of the parental genotypes used in the study.

Genotype	Features
AL 201	Selected from a cross of AL 16 × QLP 200. It was released in 1993 in Punjab. It has indeterminate growth habit. Matures in about 140 days, suitable for pigeonpea -wheat rotation. Average grain yield is about 1550 kg/ha with potential yield of 2500 kg/ha.
ICPL 20325	Extra early maturity, indeterminate plant height, good yielding.
ICP 8863	Erect, mid to late maturing, highly resistant to *Fusarium* wilt (FW) and susceptible to Sterility Mosaic Disease (SMD). It is an extensively grown variety in Northern Karnataka and Maharashtra region of India. Red seeded genotype.
ICPL 87119	Genome sequence available, leading variety in India which is resistant to FW and SMD.
ICP 5529	Medium maturity, indeterminate plant height, good yield showing more branching habit.
ICP 7035	Medium maturing, SMD resistant to both Patancheru and Bangalore races, large purple seed with high sugar content.

The six selected parents were crossed using a bi-parental mating scheme resulting in the following three mapping populations: AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035. The F_1 and F_2 generations were grown under field conditions at the research farm of ICRISAT, Patancheru during the rainy season of 2013 and 2014. The three mapping populations were field grown with inter-row spacing of 60 cm and intra-row spacing of 20 cm. True F_1s were selfed using the single seed decent method to generate the mapping populations in each genetic combination. A total of 250 genotypes from AL 201 × ICPL 20325, 233 from ICP 8863 × ICPL 87119 and 128 from ICP 5529 × ICP 7035 were selected for phenotyping.

Legume/cereal rotation is the common agronomic practice at ICRISAT experimental fields. The field was thoroughly prepared for planting to prevent disease/pathogen build up in the soil for the next crop. Rotary cutter was used to cut the stubble followed by shedder to incorporate into the soil. Disc arrow was then repeatedly used in different directions to ensure fine tilt. Leveler was used to level the field.

Sowing was done by hand in the shallow furrows opened at the top of the ridge with spacing of 60 cm between rows and 20 cm between plants. Two seeds were sown per hill and thinning was done later to one plant per hill. Each F_2 plant represent a genotype and replication was not required.

Diammonium Phosphate (DAP) fertilizer as a source of 18% N and Triple Superphosphate (TSP 46% P2O5) was applied at a basal dose of 100 kg/ha, while 1st and 2nd mechanical intercultural fertilizer was applied 30 days and 45 days after sowing. A pre-emergence herbicide was applied from a tank containing Fluchloralin 45% at 2.0 kg ha^{-1}, Prometry 50% at 1.5 kg ha^{-1} and Paraquat 0.25 at 3.0 kg ha^{-1}. The 1st and 2nd weeding were done 30-35 days after sowing (DAS) and 60 DAS, respectively. After sowing soil was uniformly irrigated to field capacity using perforated pipes (provided with check gates for the control of water flow) so that soil moisture was sufficient for seed germination and good crop establishment. Furrow method of irrigation was opted. Harvesting was done by hand.

2.3. Data collection and analysis

Data was collected on individual plants in which observations were recorded on days to 50% flowering (DTF), plant height (PHT, expressed in cm), number of primary branches per plant (PB), number of secondary branches per plant (SB), number of pods per plant (PD), number of seeds per pod (SP), 100 seed weight (gram per 100 seed), and seed yield per plant (SYLD) (gram per plant). Briefly, these data were collected as follows:

Days to flowering were recorded as number of days from planting to the date when 50% of the plants showed flowers. Plant height was measured as the height to the nearest centimeters of a stretched plant from ground level to the tip of the main stem at harvest. Number of primary branches were counted as number of branches (productive and unproductive) arising from the main stem and counted at harvest. Numbers of secondary branches were determined as the total number

of branches arising from primary branches. Number of pods were counted as the total number of matured pods obtained at harvest. Number of seeds per pod was determined as the average number of seeds of 10 sampled pods, 100-seed weight was determined as the weight to the nearest grams of one hundred clean whole dry seeds. Seed yield was the seed weight measured to the nearest grams per plant. With an exception of DTF, all other measurements were recorded at maturity.

Data collected were subjected to descriptive statistical analyses to calculate mean, standard deviation, standard error and coefficient of variation. The statistics were used to describe the phenotypic variability and draw various distribution curves using the SAS (Statistical Analysis System software (Littell 2006) to make inferences.

2.4. Results

2.4.1. Summary statistics of traits among parents and F_2 segregants

Genetic variation is an important pre-requisite in designing any breeding program. Genetic variation enables identification of individuals with suitable characters for selection. Statistical parameters for yield and yield-related characters were computed for the six parental lines; AL 201, ICPL 20325, ICP 8863, ICPL 87119, ICP 5529 and ICP 7035 and their families. Pairwise statistical tests indicated the presence of significance differences ($P<0.0.0$) among parents used in the crosses. Table 2.2 summarizes the statistical analysis of six parental genotypes and F_2 progenies in three crosses.

There were no significant differences observed between AL 201 and ICPL 20325 in any of the characters studied.

The significance differences among the two parents ICP 8863 and ICPL 87119 were obvious, with ICPL 87119 exhibiting superior performance than ICPL 8863 for the majority of the yield parameters with an exception of plant height. Implying

that these characters, DTF, NSB, NPP and NSP contributed to the observed higher seed yield in ICPL 87119 as compared to ICP 8863.

Parents ICP 7035 and ICP 5529 had significance differences for the observed characters. The ICP 7035 had more days to flowering, plant height, number of primary branches, number of seeds per pod and seed weight than ICP 5529.The NPP was the only character in which ICP5529 exhibited higher score than ICP 7035, however this did not have significant effect on the seed yield as ICP 7035 compensated this with its seed size that was more compared to those of ICP 5529.

2.4.2. Variation among F_2 progenies for yield and related traits

The F_2 individuals derived from the three cross combinations showed within and between cross variation for all studied characters (Table 2.2).

The family of AL 201 × ICPL 20325 recorded the highest coefficient of variation (13.3%) among the three crosses in plant height. The mean plant height of the cross AL 201 × ICPL 20325 was 95± 0.8 (Table 2.2). The family of ICP 8863 × ICPL 87119 had the highest coefficient of variation recorded in DTF (9.6%), with a mean of 93.59 ±0.59, followed by the number of seeds per pod (cv=15.0%) and a mean of 3.44 ± 0.03.

In the cross ICP5529 × ICPL 7035, the highest coefficient of variation was observed in the number of primary branches (34.2%) with a mean of 14.13 ± 0.43. The CV of the number of secondary branches was 7%, with a mean of 35.41 ± 1.28. The number of pods plant^{-1} had a cv of 77.4% and a mean of 125.65 ± 8.6. Hundred seed weight displayed a cv of 31.2% and a mean of 21.4 ± 0.59 while the cv for seed yield plant^{-1} was 65.2% with a mean of 50.52 ± 1.92.

Overall, this family recorded the highest phenotypic variation for the studied characters, as compared to the other two families. t.

Table 2.2. Summary statistics of the parental lines and their F_2 families for seven yield and related traits in three studied crosses of pigeonpea

	Traits							
Parent/Cross	**DTF**	**PHT**	**NPB**	**NSB**	**NPP**	**NSP**	**HSW**	**SYDP**
AL201	59 ±0.00	137±3.08	6.87± 0.27		98.67±9.08	3.78±0.05	8.01±0.14	18.23± 1.55
ICPL2035	59±0.00	134.3± 3.54	7.8 ±0.43		104.47±9.74	3.77±0.10	8.32 ±0.09	19.1±2.09
t-value and sig. test	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
AL201×ICPL20325								
Mean	50.7±0.3	95±0.8	7.1±0.1		63.5±1.9	3.6 ±0.0	7.8± 0.0	17.4 ±0.4
Range	43-61	65-163	2-14		7-170	2-8.2	5.9-12.3	5-40.8
STD	4.7	12.6	2.2		29.4	0.4	0.8	6.9
CV (%)	9.2	13.3	31.1		46.3	12.3	10.00	39.6
ICP8863	94±0.83	213.78±2.27	2.44±0.18	12.44±0.99	98.78±13.83	3.22±0.28	10.2±0.08	22.69±3.10
ICPL87119	102.33±4.66	207.2±5.98	3.00±0.32	33.2±2.08	455±68.59	4.00±0.0	11.84±0.29	120±17.16
t-value and sig. test	*	n.s.	n.s.	**	*	ns	**	**
ICP8863×ICPL87119								
Mean	93.59±0.59	192.9±0.75	2.55±0.05	13.04±0.28	157. 86±6.9	3.44±0.03	11.7±0.06	50.52±1.92
Range	53-110	165 -230	1-5	5-28	16- 748	1.9-4.2	8.6-14.1	9- 192
STD	8.97	11.38	0.77	4.29	105.38	0.52	0.94	29.33
CV (%)	9.6	5.9	30.1	32.9	66.8	15.0	8.0	58.1
ICP 5529	114.8±1.99	203±2.10	12.2±1.11	12.8±0.97	538.8±111.18	2.72±0.17	10.5±0.29	59.2±14.22
ICP 7035	122±2.00	240±5.00	13.2±0.12	10.75±1.35	396±146.00	3.75±0.65	23.5±0.5	258.5±48.5
t-value and sig. test	n.s.	*	n.s.	**	n.s.	n.s.	**	n.s.
ICP 5529 × ICP 7035								
Mean	123.5± 0.78	209.43±1.10	14.13±0.43	35.41±1.28	125.65± 8.6	3.38±0.04	21.4± 0.59	103.54± 5.97
Range	109 -148	179-240	5 -29	7- 69	21.8- 685	2.1 -5	7- 34.2	12.9 – 303.7
STD	8.83	12.47	4.83	14.52	97.2	0.5	6.68	67.53
CV (%)	7.1	6.0	34.2	41.0	77.4	14.8	31.2	65.2

KEY: CV =Coefficient of variation, STD=Standard Deviation; n.s = non-significant ***,** and ******Significant at 5% and 1% levels of probability, respectively.DTF=Days to 50% flowering, PHT =Plant height (cm), NPB=Number of primary branches, NSB=Number of secondary branches, NPP=Number of pods plant^{-1}, NSP=Number of seeds pod^{-1}, HSW=100-seed weight (g/100 seed), SYDP =Seed yield plant^{1} (grams/plant).

Distribution patterns of parents and F_2 progenies for yield and yield related traits

Response of parental lines AL 201 and ICPL 20325 and their crosses

Figure 2.1 summarizes the distribution of F_2 progenies with respect to their parental lines (AL 201 and ICPL 20325) for the four selected characters: days to 50% flowering, number of pods plant^{-1}, 100- grain weight and grain yield plant^{-1}

Days to 50% flowering

Parental lines (AL 201 and ICPL 20325) had no significance difference for the mean value of the days to 50% flowering (Figure 2.1). Progenies had DTF ranging from 43 to 61 days with a mean of 50.7 days, indicating that majority of the genotypes are early flowering. There were 234 F_2 progenies with mean DTF less than that of the two parents, varying from 43 to 58 days. There were seven individuals with mean DTF of 60 to 61 days, greater than the parents (Figure 2.1).

Plant height

The mean PHT of AL 201 and ICPL 20325 were 137±3.08 and 134.3± 3.54 cm respectively (Table 2.2). F_2 progenies had PHT ranging from 65 to 163 cm, with a mean of 95 cm. Only 2 F_2 individuals had PH at 135 and 163 cm which are greater than the mean of AL 201 (137.3 cm) or ICPL 20325 (134.3 cm). The mean PHT of the remaining 248 individuals varied from 65 to 130 cm. These values were less than the mean PHT of parents.

Number of primary branches

The mean number of branches for AL 201 was 6.87± 0.27, while ICPL 20325 had 7.8 ±0.43 (Table 2.2). Most genotypes had mean NPB varying from 2 to 14, with a mean of 7.1 branches. A total of 103 individuals displayed NPB from 2 to 6 less than AL 201 (6.8). There were 50 individuals displaying NPB values

falling between the mean values of two parents. A total of 97 individuals had values greater than the mean of ICPL 20325 (7.8).

Number of pods plant^{-1}

The mean number of pods plant $^{-1}$ for AL 201 and ICPL 20325 were 98.67±9.08 and 104.47±9.74, respectively (Figure 2.1). Progenies had number of pods per plant ranging from 7 to 170 days with a mean of 63.5 suggesting the presence of a wide genetic variation useful for selection of transgressive segregants. There were 213 individuals with mean number of pods plant^{-1} less than AL 201 (98.6). Eight individuals were recorded with mean NPP falling between values of AL 201 (98.6) and ICPL 20325 (104.5). Furthermore, 29 genotypes had greater than 104.4 pods per plant (Figure 2.1).

Number of seeds pod^{-1}

The two parents, AL 201 and ICPL 20325 had 3.78 ± 0.05 and 3.77 ±0.10 seeds per pod, respectively (Table 2.2). The F_2 individuals showed 2 to 8.2 seeds per pod with a mean of 3.6 seeds per pod. In this character, a total of 139 individuals had fewer seeds per pod than AL 201 (3.78 seeds per pod) and ICPL 20325 (3.76 seeds per pod). About 111 genotypes, had 3.8 to 4.3 mean seeds per pod greater than the two parents.

100-seed weight

A mean HSW for parents AL 201 and ICPL20325 were 8.01±0.14 and 8.32 ±0.09 g/100 seed, respectively (Figure 2.1). The 100-seed weight of F_2 individuals varied from 5.9 to 12.3 g/100 seed, with a mean of 7.8 g/100 seed. This implies that many genotypes had poor grain filling capacity and hence less seed weight. About 155 F_2 individuals had mean 100-seed weight less than AL 201 (8.01g/100 seed). A total of 35 individuals had 100-seed weight greater than AL 201 (8.01 g/100 seed), but less than ICPL 20325 (8.32 g/100 seed). The 100-seed weight ranged between 8.06 and 8.31g/100 seed.

Seed yield plant^{-1}

The mean number seed yield plant $^{-1}$ for AL 201 and ICPL 20325 were 18.23± 1.55 and 19.1±2.09 g/plant, respectively (Figure 2.1). For F_2 progenies, seed yield plant^{-1} ranged from 5 to 40.8, with the general mean of 17.4, suggesting that most of the genotypes have low seed yield. The distribution of F_2 progenies followed binomial distribution with values between 10 and 30. Individuals with values less than mean value of AL 201 (18.23) were 156 ranging from 5 to 18.1. Individuals with values greater than the mean value of ICPL 20325 (19.1) were 79 ranging from 19.4 to 40.8.

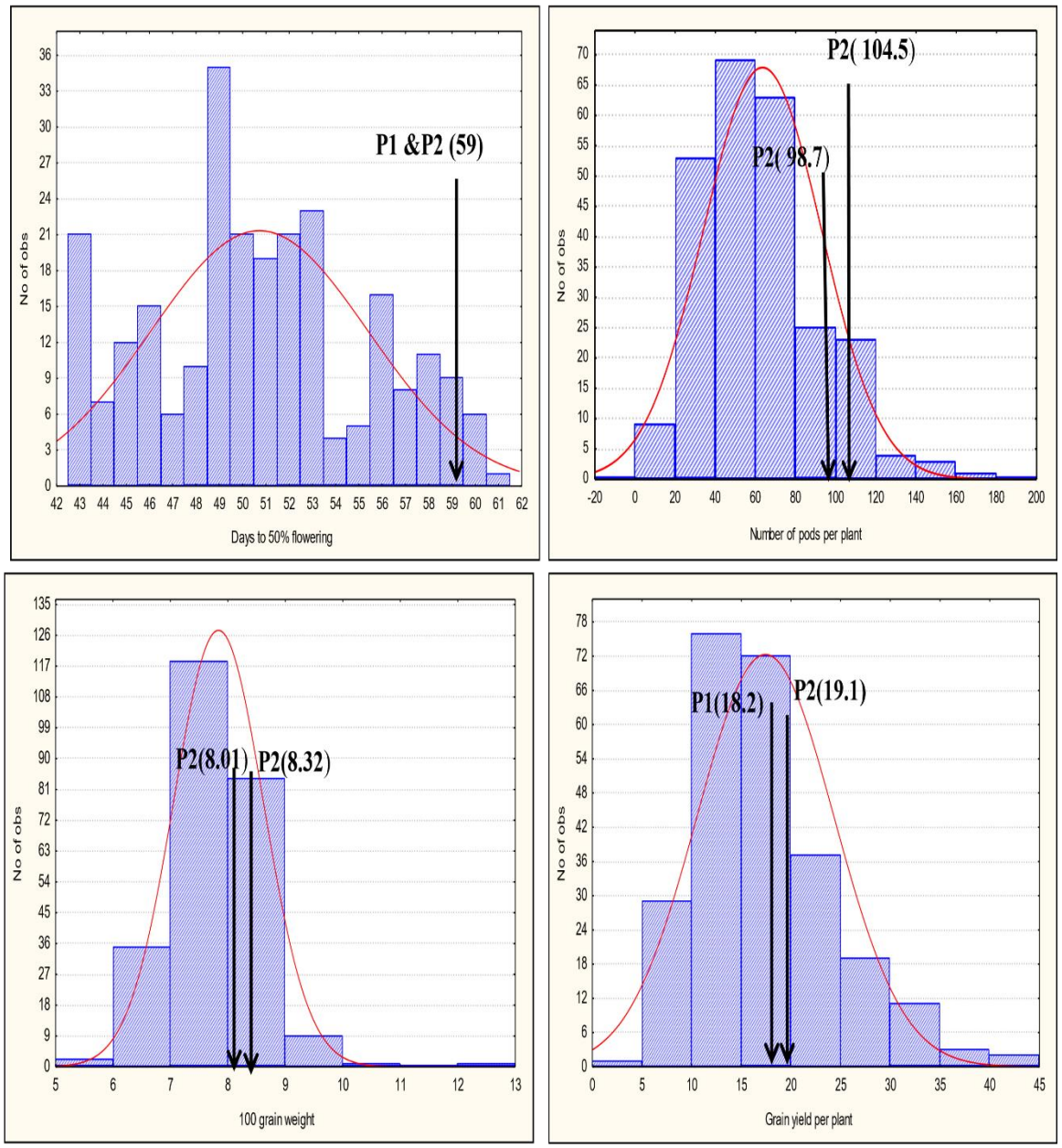

Figure 2.1: Distribution of F_2 progenies for days to 50% flowering, number of pods plant, 100 grain weight and grain yield plant in the F_2 population of the cross AL 201×ICPL 20325.

Response of ICP 8863, CPL 87119 and their crosses

Days to 50% flowering

ICP 8863 and ICPL 87119 had mean DTF of 94±0.83 and 102.33±4.66 days, respectively (Figure 2.2). The DTF of F_2 progenies ranged between 53 and 110 days with a mean of 93.59 days. This implies that most of the genotypes

are late flowering. A total of 132 F_2 progenies had less DTF (5 to 95 days) than ICP 8863 (96 days). A total of 87 progenies had DTF (97 to 110 days) falling between the mean values of 2 parents. There were no individuals that had DTF score greater than the mean value of ICPL 87119 (116 days) (Figure 2.2).

Plant height

The mean PHT of ICP8863 and ICPL 87119 were 213.78 ±2.27 and 207.2 ±5.98cm respectively (Table 2.2). The PHT of F_2 genotypes ranged from 165 to 230 cm with a mean of 192.9. A total of 209 had mean PHT less than ICP 8863 (213), and ICPL 87119 (207.2 cm). A total of 19 individuals had mean PH of 208 to 212 cm falling between the mean values of the two parents. There were five plants with PH varying from 215 to 230 cm which was greater than the two parents.

Number of primary branches

The mean number of primary branches for ICP 8863 and ICPL87119 were 2.44±0.18 and 3.00±0.32 respectively (Table 2.2). The F_2 individuals had mean branches ranging from 1 to 5 with a grand mean of 2.55 branches. A total of 125 individuals displayed mean NPB less than ICP 8863 which had 2.4 branches per plant. There were 26 individuals whose values (4 to 5) were greater than the mean of ICPL 87119 (3). The remaining 82 F_2 individuals had equal number of branches with that of ICPL 87119.

Number of secondary branches

ICP 8863 had a mean NSB of 12.44±0.99, while ICPL 87119 displayed a mean of 33.2± 2.08 (Table 2.2). F_2 individuals had mean branches of 13.04 branches. A total of 125 individuals had fewer branches (5 to 12) than ICP 8863 (12.4). The rest of the F_2 progenies (108 individuals) had branches varying from 13 to 28 with a mean of ICP 8863 (12.4) and ICPL 87119 (33.2).

Number of pods plant^{-1}

The mean number of pods per plant for the two parents; ICP 8863 and ICPL 87119 were 98.78±13.83 and 455±68.59 pods, respectively (Figure 2.2). F_2

individuals showed 16 to 748 pods plant^{-1} with a grand mean of 157 pods. Most genotypes had larger number of pods plant^{-1}. A total of 70 F_2 individuals were noted showing fewer pods (73-90 pods) than ICP 8863 (98.8). There were 158 individuals showing 100 to 440 pods per plant fallen between the mean values of the two parents. Only 5 individuals had mean number of 520 and 748 pods plant^{-1} greater than 455 pods which is the mean value of ICPL 87119 (Figure 2.2).

Number of seeds pod^{-1}

Number of seeds pod^{-1} for ICP 8863 and ICPL 87119 were 3.22±0.28 and 4.00±0.0 seeds respectively (Table 2.2). The range of F_2 individuals were between 1.9 and 4.2 seeds, with a general mean of 3.44 days. In this character, individuals were distributed as follows; 61 had values less than that of ICP 8863 (3.2), the range was from 1.9 to 3.1.Nine individuals had values equivalent to the mean value of ICP 8863 (3.2).A total of 112 individuals had values ranging from 3.3 to 3.9, falling between the mean averages of the two parents(3.22 and 4.00).Forty eighty individuals had value equal to the mean value of parent ICPL 87119 (4.00).The remaining three individuals had values greater than that of ICPL 87119,and their range was from 4.1 to 4.2 seeds.

100-Seed Weight

As for 100-seed weight, the mean values of ICP 8863 and ICPL 87119 were 10.2±0.08 and 11.84±0.29g/100 seed respectively (Figure 2.2). Genotypes were in a range of 8.6 to 14.1 g/100 seed and a general mean of 11.7 g/100 seed, suggesting that most of the genotypes had moderate seed weight. A total of 17 individuals had values below that of the mean value of (10.2). They were in a range of 8.6 to 10.2 g/100 seed. A total of 110 F_2 progenies had values found between the mean values of the two parents. These ranged from 10.3 to 11.8. Individuals with values greater than the mean value of ICPL 87119 (11.84) were 106.Their range was from 11.9 to 14.1 g/100 seed.

Seed yield plant^{-1}

In this character, the two parents; ICP 8863 and ICPL 87119 had mean scores of 22.69±3.10 and 120±17.16 g/ plant respectively (Figure 2.2). For F_2 progenies, the lowest and the highest values of seed yield plant $^{-1}$ were 9 and 192 grams per plant respectively, with the general average of 50.52 g/plant. This indicates that most of the genotypes had low seed yield. Individuals which had values that are less than that of ICP 8863 (22.7) were 29. The values ranges from 9.1 to 22.3. Whereas, the number of progenies with values found between the two mean values of the parents were 195.They ranged between 23.6 and 116.9. A total of 9 individuals had values greater than the mean value of ICPL 87119 (120). Their range was between 124 to192.3. Figure 2.2 shows the distribution of F_2 progenies in four selected characters; days to 50% flowering, number of pods plant^{-1}, 100 grain weight and grain yield plant^{-1} in ICP8863 × ICPL 87119.

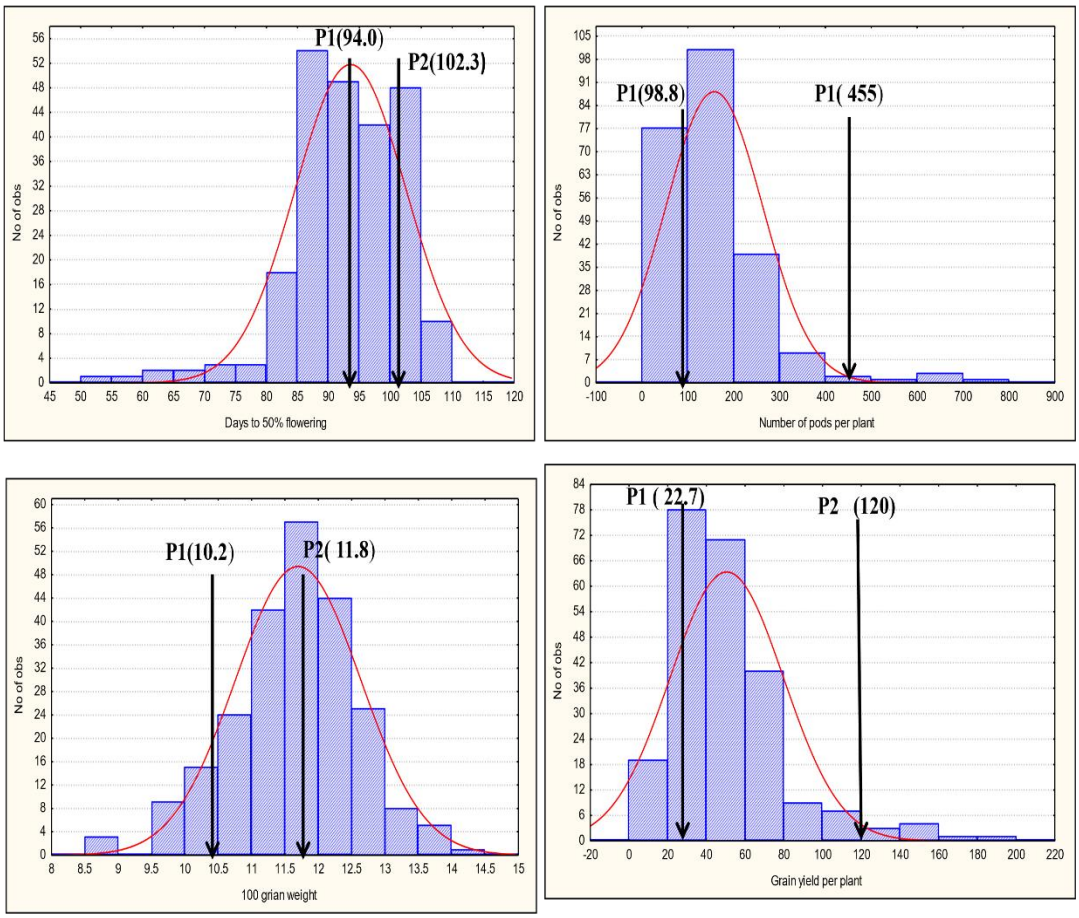

Figure 2.2: Distribution of F_2 progenies for days to 50% flowering, number of pods per plant, 100 grain weight and grain yield per plant in the F_2 population of the ICP8863 × ICPL 87119.

Parental lines ICP 5529 and ICP 7035 and their crosses

Days to 50 % flowering

The mean scores of days to 50 % flowering of the two parental lines; ICP5529 and ICP 7035 were 114.8±1.99 and 122±2.00 days (Figure 2.3). The range of the F_2 progenies were from 109 to 148 days, and a general mean of 123.5 days, suggesting that most of the genotypes had moderate values. Individuals with values less than the mean value of ICP 5529 (116) were 22, ranging from 109 to 115.The F_2 progenies which had values greater than the mean value of ICP 5529 (116), but less than that of ICP 7035(122), were 39. These ranged between 117 -121. A total of 58 individuals had values which were greater than

the mean value of ICP 7035(122), and their range was between 123 and 148 (Figure 2.3).

Plant height

For the plant height the mean scores of the parental lines ICP 5529 and ICP 7035 were 203±2.10 and 240±5.00 cm respectively (Table 2.2). F_2 progenies ranged from 179 to 240cm, having a general mean of 209.43cm. Most of the individuals (96) had values that were less than the mean value of ICP 5529 (220). The range was from 179 to 215.Individuals with values falling between the mean values of the two parents were 16 and their range was from 222-235.A total of 13 individuals had values which are equivalent to ICP 5529, whereas 3 individuals had values that is equivalent to that of ICP 7035 (240).

Number of primary branches

In this character, the ICP 5529 had a mean score of 12.2±1.11 branches, while ICP 7035 displayed 13.2±0.12 branches (Table 2.2). Number of primary branches for F_2 progenies ranged from 5 to 29 having a general mean of 14.1. Individuals who had number of primary branches less than that of ICP 5529 (12.2) were 50 and the range was from 5 to 12. A total of 10 individuals had a value of 13, which is falling between the mean values of the two parents (12.2 and 13.2). Most of the individuals had number of primary branches that are more than that of ICP7035 (13.2). Total number was 68 and the range was 14-29.

Number of secondary branches

Mean scores of the two parental lines; ICP 5529 and ICP 7035 were 12.8±0.97 and 10.75±1.35 branches (Table 2.2). F_2 genotypes in this character ranged from 7 to 69, and a general mean of 35.4. Two individuals had number of secondary branches that are less than that of ICP 7035 (10.75), and the range was from 7-9. Four individuals had values that are between the mean values of the two parents (10.75 and 12.8). These were in a range of 11-12. All remaining 122 individuals had values that are greater than that of the ICP 5529 (12.8), and their range was 13-69.

Number of pods plant^{-1}

The mean values of ICP 5529 and ICP 7035 were 538.8±111.18 and 396±146.00 pods respectively (Figure 2.3). Number of pods plant^{-1} for the F_2 progenies ranged from 21.8 to 685 pods, having a general range of 125.6 pods, suggesting that majority of the genotypes had less number of pods per plant^{-1}. Out of 128 F_2 individuals, only one had a value (685.0) which was greater than the mean value of ICP5529 (538.6). One individual had a value (524) that falls between the mean values of two parents. The remaining 126 F_2 individuals had number of pods plant^{-1} which are less than the mean value of the ICP 7035 (396). Their range was between 21.8 and 382.

Number of seeds pod^{-1}

Parental lines ICP 5529 and ICP 7035 had mean scores of 2.72±0.17 and 3.75±0.65 seeds for the number of seeds pod^{-1}(Table 2.2). The lowest and highest values of the F_2 individuals were 2.1 and 5 seeds respectively, having a general range of 3.38. In this character, most of the individuals (88), had values falling between the mean values of the two parents (2.72 and 3.75). The range was between 3.8 and 5. Individuals having a values less than the mean value of ICP 5529 (2.72) were 13, having a range of 2.1 to 2.7. A total of 27 individuals had values which are greater than the mean value of ICP 7035 (3.75). The range was between 3.8 and 5 (Table 2.2).

100-seed weight

For 100-seed weight, mean values of ICP 5529 and ICP 7035 were 10.5±0.29 and 23.5±0.5g/100 seed respectively (Figure 2.3). The range of F_2 progenies were from 7 to 43.2. The general mean was 21.45g/100 seed. This suggests that majority of the genotype had moderate seed weight. In this character, a total of 125 F_2 individuals had values that were greater than the mean value of ICP 5529(10.5), whereas three had values less than the same. The ranges were 10.8 to 34.2 and 7.7 to 9.7 respectively. A total of 64 individuals had values that were less than the mean value of ICP 7035 (23.5). The values ranged between 7.7 and 23.5.A total of 64 individuals had values that were

greater than the same. These were found in a range between 23.6 and 34.2(Figure 2.3).

Seed yield plant^{-1}

Parental lines ICP 5529 and ICP 7035 had mean score values of 59.2±14.22 and 258.5±48.55 g/plant respectively for the seed yield plant^{-1} (Figure 2.3). F_2 progenies ranged from 5 to 40.8, with the general mean of 17.4 g/ plant, suggesting that most of the genotypes have low seed yield. The distribution of F_2 progenies followed binomial distribution with values between 10 and 30. Individuals with values less than mean value of AL 201 (18.23) were 156 ranging from 5 to 18.1. Individuals with values greater than the mean value of ICPL 20325 (19.1) were 79 ranging from 19.4 to 40.8.

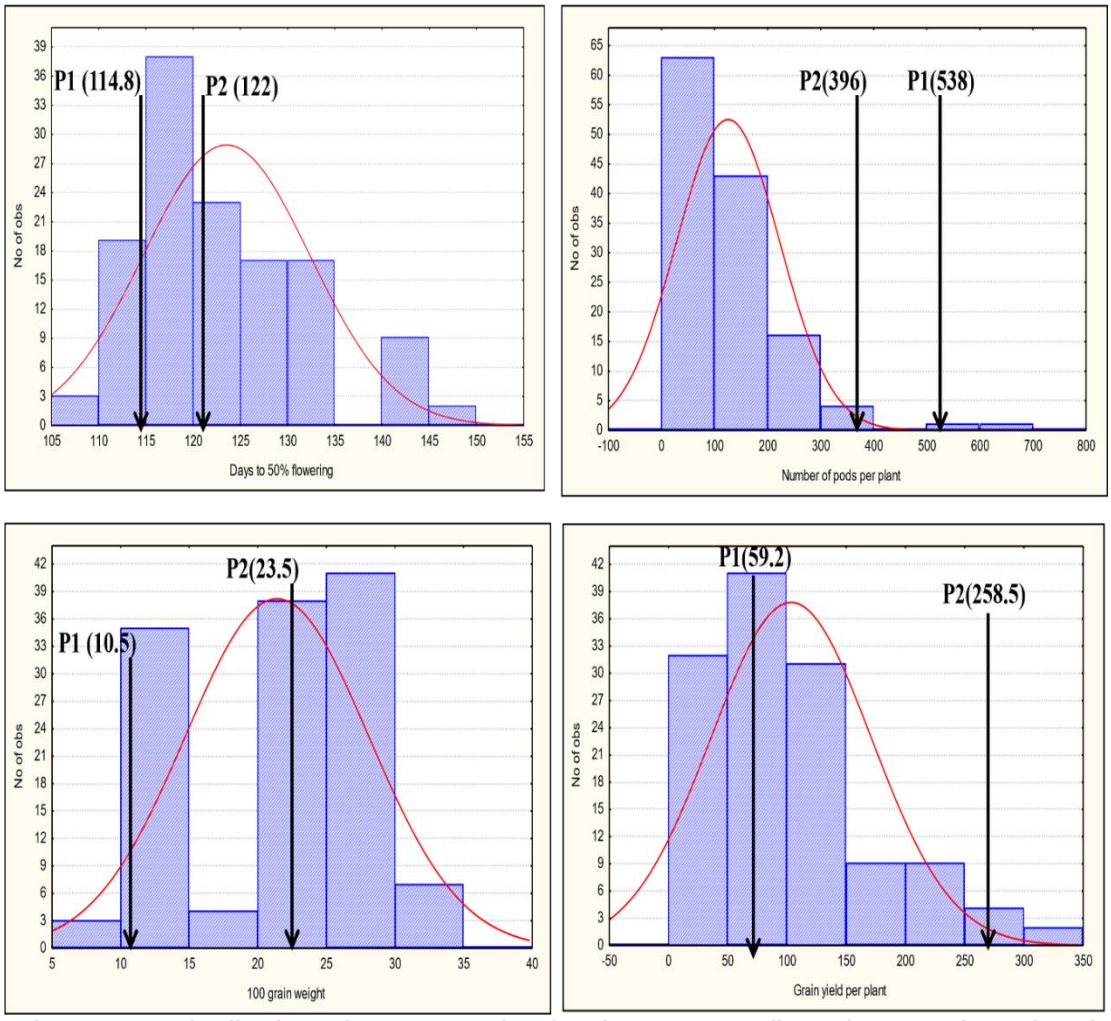

Figure 2.3: Distribution of F_2 progenies for days to 50% flowering, number of pods per plant, 100 grain weight and grain yield per plant in the F_2 population of the cross ICP5529 × ICP 7035.

2.5. Discussion

2.5.1. Phenotypic variability

The current study has indicated the variation existing between the parental lines as well as between crosses studied for the yield and yield –related characters. The parental germplasms used to generate the crosses, were selected based on the main pigeonpea maturity groups; early, medium and long duration.

ICP 5529 had superior scores in days to 50% flowering and highest number of pods plant^{-1} among all parental lines used in the current study. ICP 7035 had the highest score recorded for 100-seed weight, plant height, number of primary branches and seed yield plant^{-1}. ICP 7035 have faster dry matter accumulation rate attributed to its big seed size (Singh et al. 1991).

This indicates, that the two parental lines which belongs to late maturing group, possess attributes such as tall plant, higher number of branches plant^{-1}, more number of pods plant^{-1} and higher seed weight resulting in to higher seed yield plant^{-1}.

ICPL 87119 and ICP8863, which are medium duration accessions, recorded a relatively higher number of secondary branches plant^{-1} as well as the number of seed pod^{-1}.as compared to AL 201 and ICPL 20325 which are short duration accessions. Medium duration accessions possess attributes which also result into high seed yield plant^{-1} as it is the case with long duration accessions.

AL 201 and ICPL 20325 which are short duration accessions, had least scores in all the characters studied including seed yield plant^{-1}, as compared to parental lines belonging to medium and late maturity groups. In addition, these parents also didn't show any significant difference among them. The finding is in agreement with that of Upadhyaya et al. 2007, who studied the phenotypic diversity in the pigeonpea (*Cajanus cajan*) core collection and found diversity level of the quantitative traits to be increasing from extra early group to late maturity group indicating the association of these traits with late maturity. Increase in variation from the extra early group to the late maturity group for

above traits may be attributed to the high proportion of highly photoperiod sensitive indeterminate accessions in medium and late maturity groups (Remanandan et al. 1988).On the other hand, extra early and early maturing groups having maximum determinate type accessions, which are mostly the products of breeding programs and less sensitive to photoperiod and temperature has probably resulted in lower diversity (Saxena and Sharma 1990).

This study used F_2 individuals which normally are characterized by higher segregations. As expected, observations showed variations to be existing among studied characters as well as in segregation patterns of individuals within and among crosses under study. In the current study, the characters that showed high variation across all the studied crosses were; number of pods plant^{-1}, followed by seed yield plant^{-1}, number of secondary branches and number of primary branches. This suggests they are important traits for consideration in selection for breeding purposes. In the current study, the number of pods plant^{-1} had the highest phenotypic variation among all the crosses.

2.5.2. Distribution patterns and transgression of F_2 individuals

Understanding frequency distribution curves of characters is an important pre-requisite towards determination of distribution patterns of segregating individuals in a population. Variation of parental lines and F_2 progenies for the days to 50%, plant height, number of primary branches and number of secondary branches is shown in Figure 2.4, whereas Figure 2.5 summarizes the variability of parental genotypes and F_2 progenies for the number of pods plant^{-1}, number of seeds pod^{-1}, 100-seed weight and seed yield plant^{-1}.

The observed ranges of genotypes in AL 201 x ICPL 20325, indicates that majority of the genotypes are early flowering, have short stature, with less number of primary branches and pods. The cross also comprises genotypes with combination of low and higher number of seeds pod^{-1}, possessing less seed weight and are low yielding. Overall, this cross had the highest number of the transgressive segregants observed in DTF, PHT and NPP and NSP.

For the cross ICP 8863 × ICPL 87119, the pattern of distribution for most of the genotypes suggests they are late flowering, are of short stature had majority of individuals with less number of primary branches. In addition, F_2 progenies possess low to moderate number of secondary branches, having higher number of pods plant^{-1}and in all ranges from low, medium and high, with respect to the number of seeds pod^{-1}. Observations also suggests that majority of genotypes have seeds of moderate seed weight and seed yield. SYLD had the most observed transgressive segregants in this cross, compared to the remaining two.

Most of the genotypes in ICP 5529 × ICP 7035, had moderate values of days to 50% flowering, suggesting they belong to medium and late flowering. The observations, also suggests that progenies in this cross were having short to moderate stature, with higher number of primary and secondary branches. The cross also comprised genotypes possessing less number of seeds pod^{-1} and seeds of moderate weight. For seed yield, comparisons of the ranges and general means across the studied crosses indicates this cross to have the highest score among the three crosses, followed by ICP 8863 × ICPL 87119 and AL 201 × ICPL 20325 (Figure 2.5). The cross had majority of transgressive segregants observed in NPB, NSB and HSW.

The findings from this study is in agreement with that of Chauhan and Singh (1981), in which long duration accession produced higher mean seed yield than all other accessions. Late pigeonpea maturing varieties have comparatively more significant stem length, number of pods plant^{-1} and secondary branches plant^{-1} than varieties belonging to the other maturity groups.

Ranking studied crosses on the basis of phenotypic variation, on the present study indicates it was more in ICP 5529 × ICP 7035, followed by ICP 8863 × ICPL 87119, with AL 201 × ICPL 20325 having least variations on the studied characters. Early maturing accessions normally have determinate growth habit, whereas medium and long duration accessions are indeterminate. Generally, total dry matter and grain yield are relatively higher in the

indeterminate than in determinate group. Harvest index is relatively higher in the determinate group; however increased harvest index is not accompanied by increased total dry matter.

With an exception of seed yield plant^{-1} in which transgression was not observed, in the remaining seven characters examined, there was evidence for transgressive segregation based on the occurrence of significant numbers of extreme individuals in the F_2 generations. The occurrence of this transgression could be directly linked to the presence of complementary QTL alleles in the two parental species.

In this study, transgression was mostly observed in crosses AL201× ICPL 20325 and ICP 5529 × ICP 7035. In Cross ICP 8863 ×ICPL 87119, only two characters; plant height and days to 50 % flowering showed transgression. Parental phenotype similarity influences the occurrence of the transgression. Individuals formed by parental lines that have close phenotypic similarity are more likely to exhibit transgression. In other words, the more similar the phenotype of the parents, the greater the likelihood transgressive segregation will be observed in the F_2 (Rieseberg et al. 1999).

The genetic divergence of parental lines also influences the transgression, the more the genetic divergence of the parents, the higher the chances of transgression occurrence. This indicates that the phenotypes of parents used to generate crosses AL 201 × ICPL 20325 and ICP 5529 × ICP 7035 are closer in phenotypic similarity, but more genetically diverge as compared to the ones used in ICP 8863 × ICP 87119. Predominance of additive gene action among the loci controlling the traits in which segregation was observed, could also have contributed to the observed transgression. Observed transgression suggest the possibility of identifying positive and negative alleles in the superior parent and inferior parent respectively.

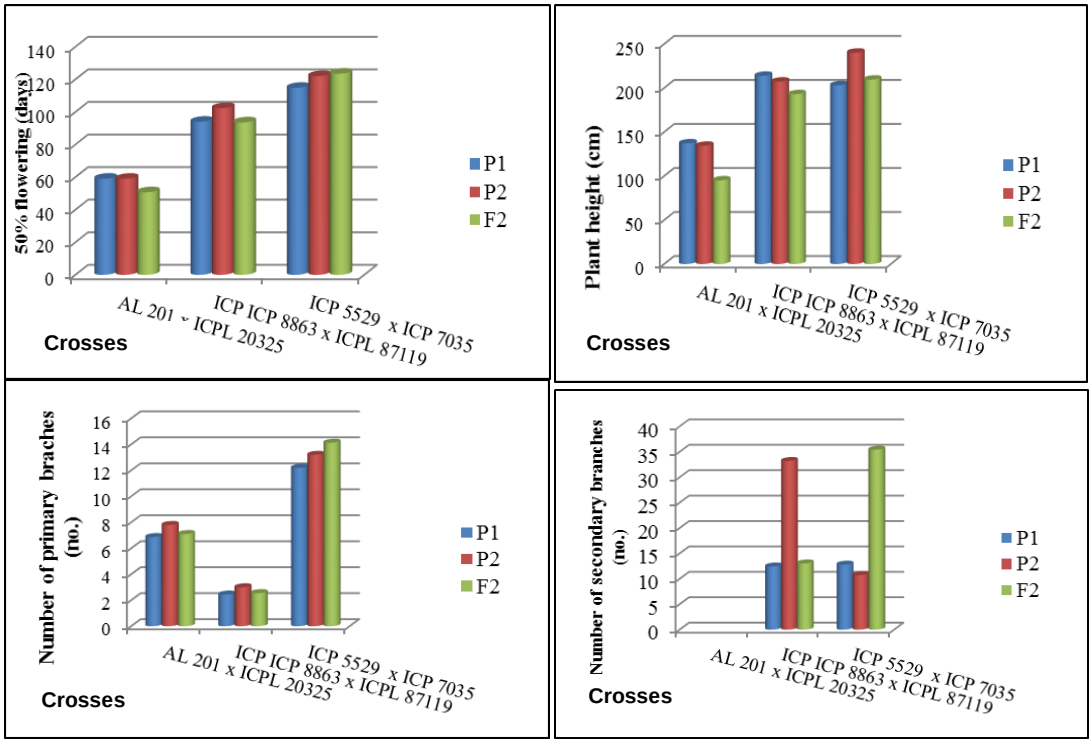

Figure 2.4: Variation in Days to 50% flowering, plant height, number of primary branches and number of secondary branches among parents and F_2 progenies in three populations

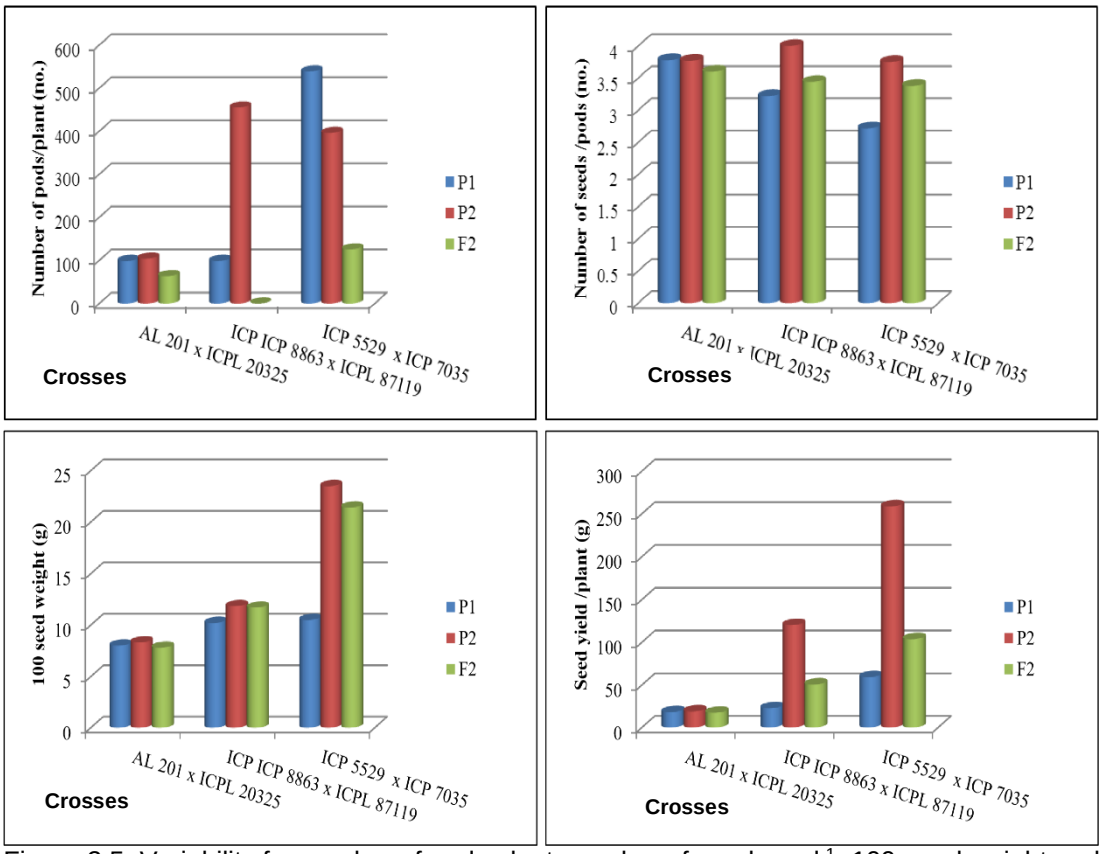

Figure 2.5: Variability for number of pods plant, number of seeds pod^{-1}, 100-seed weight and seed yield plant^{-1} among parents and F_2 progenies in three populations of pigeonpea

2.6. Conclusions

A study on the phenotypic characterization of six parental lines and three F_2 crosses of pigeonpea for eight yield and yield related traits has revealed the polymorphisms existing between parents and segregating individuals. Superior genotypes and characters exhibiting highest phenotypic variability have been identified for consideration on further breeding programme. In addition, transgression observed on the characters studied has provided useful information for crop improvement by identifying the potential source of genetic variation.

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CHAPTER THREE

Correlation and path-coefficient analyses of seed yield and related traits in newly developed pigeonpea populations

Abstract

Correlation analysis between yield and its contributing characters has been helpful as a basis for selection. Simple and path coefficient correlation analyses were conducted to determine association of seed yield and related traits using three newly developed populations of pigeonpea of the following crosses; AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035. Data were collected involving eight traits such as days to 50% flowering (DTF), plant height (PHT), number of primary branches (NPB), number of secondary branches (NSB), number of pods per plant (NPP), number of seeds per pod (NSP), hundred seed weight (HSW) and seed yield per plant (SYDP). The results indicated that NPP exhibited relatively the highest phenotypic correlation with SYDP across all mapping populations. Path-coefficient analyses were applied to pinpoint influential traits(s) for selection in segregating populations of pigeonpea. The results revealed that NPP had the highest path co-efficient value influencing SYDP across all families. In the family AL 201 × ICPL 20325, the NPP had indirect effect on the SYDP through PHT and HSW. In the families of ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035 selection for increased NSB and NSP had indirect effect on NPP. The high direct path value indicates that NPP tends to serve as a first order or principal selection criterion to improve SYDP among accessions. Results from simple correlation and path coefficient analyses suggest that PHT, NSP, HSW and NSB are the most important selection criteria for improving seed yield in the newly developed pigeonpea populations. Study also revealed that family ICP 5529 × ICP 7035 had the highest magnitude of correlation coefficient as well direct effect on the seed yield among the three studied families.

Key words: Correlation analysis, path-coefficient analysis, pigeonpea population, selection criteria

3.1. Introduction

Pigeonpea [*Cajanus cajan* (L) Millsp.] is a multi-use shrub legume of the tropics, sub-tropics and warmer regions of the world growing between 30°N and 35°S latitude. Unlike other grain legumes, pigeonpea production is concentrated in developing countries, particularly in south and south east Asia and eastern and southern African countries (Shuny et al. 2013).

Seed yield is an important economic trait in pigeonpea, and is controlled by several contributing characters (yield components) which are often highly correlated to each other. Yield components are under the influence of the genotype, environment and genotype x environment interaction (Rauf et al. 2004). Yield components do not only directly affect the yield, but also indirectly by influencing other components in negative or positive directions (Bidgoli et al. 2006). Therefore, knowledge on the nature and magnitude of trait association is relevant in a breeding population to identify the most influential trait(s) for selection (Sodavadia et al. 2009).

Correlation analysis can provide information that selection for one character results in progress for other positively correlated characters (Manggoel et al. 2012). It measures the mutual association between two variables, which aids in determining the most effective procedures for selection of superior genotypes (Udensi and Ikpeme 2012).

The path coefficient analysis is a standardized partial regression technique that measures the direct influence of one trait upon another and permits separation of a correlation coefficient into components of direct and indirect effects (Board et al. 1997). According to Cramer and Wehner (2000), a large path coefficient value indicates that the change will result in a proportional (or inversely proportional) change in another correlated trait, whereas a weak coefficient indicates that the change will have little effect on that trait. In addition to the direct effects, the indirect effects of yield components should be calculated (based on the correlations among yield components). The indirect effect for each yield component is calculated by multiplying the correlation between two components by the direct effect of the opposite yield component.

Correlation and path coefficient analyses studies have been conducted in pigeonpea involving various yield and yield components (Thanki and Sawargaonkar 2010; Nag and Sharma 2012; and Udensi and Ikpeme 2012).

A thorough knowledge of existing phenotypic variation and extent of association between various yield contributing characters is essential in a newly developed population for breeding high yielding genotypes in pigeonpea (Chaithanya et al. 2014). Therefore, the objective of this study was to apply simple correlation and path analyses and identify most useful yield and yield related components of newly developed pigeonpea mapping populations developed from three diverse genetic groups. The selected traits may serve as important landmarks for pigeonpea improvement using the new populations.

3.2. Materials and Methods

Plant materials

The study involved three mapping populations each comprising a total of 180 individuals. The six parents used to generate the three populations were AL 201, ICPL20325, ICP 8863, ICPL87119, ICP 7035 and ICP 5529. AL 201 was collected from Punjab Agricultural University (PAU) situated at 30.9028° N, 75.8086° E and altitude of 247 m above sea level (m.a.s.l.). All other accessions were obtained from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru. The parents belonged to three maturity groups: short maturity duration (AL 201 and ICPL 20325), medium duration (ICP 8863 and ICPL 87119) or long duration (ICP 7035 and ICP 5529).

3.2.1. Study site and experimental set up

The experiments were conducted in three consecutive growing years (2012-2013, 2013-2014 and 2014-2015) at the International Crops Research Institute for Semi-Arid Tropics, Patancheru (17.538 N^{o}, 78.278 E^{o}, 545 m.a.s.l.), Telangana, India.

Initial crosses were made involving six parents using a bi-parental mating system in the 2012-2013 cropping season and the F_1 seeds were self –

pollinated to obtain F_2 seeds in 2013-2014. The F_2 seeds were sown in the cropping season of 2014-2015.

Each experiment was laid out using an un-replicated augmented design. A total of six parental genotypes were used as comparative controls including AL 201, ICPL 20325, ICP 8863, ICPL 87119, ICP 5529 and ICP 7035. The check accessions were planted after every 15 respective F_2 genotypes. Each entry was established in a single row of 10-m length with inter-row spacing of 60 cm and intra-row spacing of 20 cm. All standard agronomic management practices were followed including basal fertilizer application, irrigation supplementation and pest and diseases control.

3.2.2. Data collection and analysis

Observations were recorded on eight quantitative traits: days to 50% flowering (DTF), plant height (PHT, expressed in cm), number of primary branches (NPB), number of secondary branches (NSB), number of pods plant^{-1} (NPP), number of seeds pod^{-1} (NSP), 100-seed weight (HSW, gram 100^{-1} seed) and seed yield per plant (SYDP, g plant^{-1}). The data recorded were subjected to correlation analysis to estimate phenotypic correlation coefficients between traits. Simple correlation and path coefficient values were computed according to the methods described by Dewey and Lu (1959) and Wright (1921). For the path analysis, seed yield per plant (SYDP) was used as response variable, while DTF, PHT, NPB, NSB, NPP, NSP and HSW were regarded as causal or independent variables.

3.3. Results.

Simple correlation analysis

Simple correlation coefficients showing pairwise associations between yield and related characters in three studied crosses are presented in Table 3.1.

The results show that, among the characters studied, the highest phenotypic correlation coefficient was observed between NPP with SYDP in the families of ICP 8863 × ICPL 87119 (r = 0.929), AL 201 × ICPL20325 (r =0.888), and ICP 5529 × ICP7035(r= 0.708) (Table 3.1).

A summary of the magnitude of the correlation coefficients and significant tests for each family are described below.

Family AL 201 x ICPL20325

Highly significant and positive correlations (P<0.01) were observed between SYDP with NPP (r=0.888). This was followed by correlation of SYDP with NPB (r=0.478), PHT (r=0.384), HSW (r=0.288), NSP (r=0.263) and DTF (r =0.245) in a decreasing order. Pairwise association of characters' revealed that PHT exhibited highly significant positive correlation (P<0.01) with DTF (r =0.254) (Table 3.1). Number of primary branches per plant showed highly significant (P<0.01) positive correlation with DTF (r=0.334) and PHT (r=0.492). Number of pods per plant exhibited significant and positive correlation (P<0.01) with NPB (r=0.523), PHT (r=0.353) and DTF (r=0.234).
The NSP exhibited highly significant positive correlations (P<0.01) with NPP (r=0.242). Generally, there were poor and non-significant associations between NSP with NPB, PHT and DTF (Table 3.1).

There were significant positive correlations (P< 0.05) between HSW with NSP (r=0.153) and PHT (r=0.134). This character also exhibited highly significant correlation (P<0.01) with NPP (r=0.154) and NPB (r=0.183).

Family ICP 8863 x ICPL 87119

A highly significant (P<0.01) phenotypic correlation was found between SYDP with NPP (r=0.943) only (Table 3.1). Further, significant positive correlation (P<0.05) was exhibited between SYDP with NSB (r=0.647), NSP (r=0.304), HSW (r=0.288), NPB (r=0.256), and PHT (r=0.243), in that order (Table 3.1). Number of primary branches had poor correlations with DTF and PHT. Number of secondary branches per plant exhibited highly significant and positive correlations (P<0.01) with NPB (r=0.26) and PHT (r=0.343). Highly significant and positive correlations (P< 0.01) were observed between NPP and NSB (r=0.652), NPB (r=0.314) and PHT (r=0.223). Significant and positive correlation was exhibited between NSP with NPP (P<0.01, r=0.314) and NSB

(P<0.01, r=0.225) (Table 3.1). Hundred seed weight exhibited highly significant and positive correlation (P < 0.05) with NPB (r =0.156). Generally, there were poor and non-significant associations between other agronomic traits with days to 50% flowering.

Family ICP 7035 x ICP 5529

Among the quantitative traits evaluated, NPP had the highest phenotypic correlation (P<0.01, r=0.707) with the SYDP (Table 3.1). Further, SYDP exhibited highly and significantly positive correlations (P<0.01) with NSB (r =0.664), HSW (r=0.607), and NPB (r= 0.457) (Table 3.1). Number of secondary branches per plant had significant and positive correlation with NPB (r = 0.691; P < 0.01) and PHT (r = 0.431; P < 0.01).

Significant and positive correlations (P<0.01) were observed between NPP with NSB (r = 0.751), PHT (r = 0.416), and NPB (r = 0.382) (Table 3.1). Significant and positive correlations (P < 0.01) were observed between NSP with NPP (r =0.242), and NPB (r=0.361). Highly significant and positive correlations (P <0.05) was observed between NSP with PHT (r=0.281) and NSB (r = 0.252). Hundred seed weight (HSW) was significantly and positively correlated (P < 0.01) with NPP (r=0531), NSB (r=0.492), NPB (r= 0.261) and PHT (r=0.126) in that order (Table 3.1). Days to 50% flowering had poor and non-significantly association with other characters.

Table 3.1. Pearson's correlations coefficients showing pair-wise correlation of eight agronomic characters in three populations of pigeonpea

AL 201 × ICPL 20325								
Traits	DTF	PHT	NPB	NSB	NPP	NSP	HSW	SYDP
DTF	1.00			n.a.				0.245**
PHT	0.254**	1.00		n.a.				0.384**
NPB	0.334**	0.492**	1.00	n.a.				0.478**
NPP	0.234**	0.353**	0.523**	n.a.	1.00			0.888**
NSP	0.024^{ns}	0.112^{ns}	0.125^{ns}	n.a.	0.242**	1.00		0.263**
HSW	0.072^{ns}	0.134*	0.183**	n.a.	0.154**	0.153*	1.00	0.288**
ICP 8863 × ICPL 87119								
Trait	DTF	PHT	NPB	NSB	NPP	NSP	HSW	SYDP
DTF	1.00							-0.001
PHT	-0.023^{ns}	1.00						0.243**
NPB	-0.046^{ns}	0.023^{ns}	1.00					0.256**
NSB	-0.042^{ns}	0.343**	0.26**	1.00				0.647**
NPP	-0.091^{ns}	0.223**	0.314**	0.652**	1.00			0.943**
NSP	-0.026^{ns}	0.135^{ns}	0.067^{ns}	0.225**	0.314**	1.00		0.304**
HSW	0.133^{ns}	0.082^{ns}	0.156*	0.041^{ns}	-0.02^{ns}	0NS	1.00	0.114
ICP 5529 × ICP 7035								
Trait	DTF	PHT	NPB	NSB	NPP	NSP	HSW	SYDP
DTF	1.00							-0.198
PHT	0.07^{ns}	1.00						0.333**
NPB	-0.323**	0.425**	1.00					0.457**
NSB	-0.316*	0.431**	0.691**	1.00				0.664**
NPP	-0.237*	0.416**	0.382**	0.751**	1.00			0.707**
NSP	-0.152^{ns}	0.281*	0.361**	0.252*	0.242**	1.00		0.349**
HSW	-0.235*	0.126^{ns}	0.261*	0.492**	0.531**	0.272**	1.00	0.607**

KEY: ns=non-significant. DTF=Days to 50% flowering, PHT =Plant height, NPB=Number of primary branches, NSB=Number of secondary branches, NPP=Number of pods plant^{-1}, NSP=Number of seeds pod^{-1}, HSW=100-seed weight. SYDP= Seed Yield per Plant.

n. a.= data not available

*Significant difference at the 0.05 probability level

** Significant difference at the 0.001 probability level.

3.3.2. Path coefficient analysis

Results on the path coefficient analysis for the three studied crosses with SYDP as the response variable and DTF, PHT, NPB, NSB, NPP, NSP, and HSW as independent variables are summarized in Table 3.2. Values of direct effects were <1, indicating that inflation due to multi-collinearity was relatively low.

In the cross AL 201 × ICPL 20325, relatively high direct path coefficients (0.826) and highly significant phenotypic correlation (r_p = 0.888, P<0.05) were

estimated between NPP with SYDP (Table 3.2). Path analysis indicated that selection for increased NPP would bring about simultaneous increase in PHT and HSW in a desirable direction (Table 3.2)

In the cross ICP 8863 × ICPL 87119, NPP also exhibited relatively the highest phenotypic correlation (r=0.929, P<0.01) and highest positive direct path coefficient (0.8765) on SYDP. The indirect effect via NSB and NSP were positive with 0.051 and 0.012, respectively.

Hundred seed weight had non-significant correlation with SYDP, but had the higher magnitude of direct effect on seed yield (0.113). The number of secondary branches also showed significant correlation (r=0.66) with SYDP and it followed HSW in term of its significant direct effect on seed yield (0.081). Thus, increased seed yield in this family can be achieved through simultaneous selection of genotypes that display higher number of pods, more seed weight and higher number of secondary branches.

In the family ICP 5529 × ICP 7035, NPP had the highest positive correlation (r=0.704) with SYDP, as well as the highest direct path coefficient value on SYDP (0.336). The indirect effect via NSB and NSP were positive with coefficients of 0.142 and 0.024, respectively. Hundred seed weight (0.277) and NSB (0.242) also had considerable direct effect on SYDP.

Table 3.2. Estimates of direct (boldfaced main diagonals) and alternate/indirect path coefficient values (off diagonals) of SYDP with seven related traits amongst F_2 pigeonpea genotypes of three crosses.

AL 201 × ICPL 20325								
Traits	DTF	PHT	NPB	NSB	NPP	NSP	HSW	SYDP
DTF	**0.029**	0.018	-0.003	n.a	0.193	0.001	0.008	0.245**
PHT	0.007	**0.071**	-0.001	n.a	0.288	0.004	0.015	0.384**
NPB	0.010	0.035	**-0.001**	n.a	0.412	0.003	0.020	0.478**
NPP	0.007	0.025	-0.001	n.a	**0.827**	0.009	0.022	0.888**
NSP	0.001	0.008	-0.001	n.a	0.202	**0.035**	0.016	0.260**
HSW	0.002	0.009	-0.001	n.a	0.158	0.005	**0.116**	0.288**
ICP 8863 × ICPL 87119								
Traits	DTF	PHT	NPB	NSB	NPP	NSP	HSW	SYDP
DTF	**0.040**	0.001	-0.001	-0.001	-0.058	-0.001	0.019	-0.001
PHT	-0.001	**0.042**	0.001	0.020	0.167	0.005	0.010	0.243**
NPB	-0.001	-0.002	**-0.025**	0.018	0.242	0.001	0.021	0.256**
NSB	0.001	0.010	-0.006	**0.080**	0.547	0.010	0.004	0.647**
NPP	0.003	0.008	0.007	0.051	**0.865**	0.012	-0.003	0.943**
NSP	0.001	0.004	-0.001	0.017	0.236	**0.044**	0.002	0.304**
HSW	-0.006	0.003	-0.004	0.003	-0.016	0.001	**0.133**	0.114
ICP 5529 × ICP 7035								
Traits	DTF	PHT	NPB	NSB	NPP	NSP	HSW	SYDP
DTF	**-0.103**	0.041	0.001	-0.033	-0.049	-0.009	-0.047	-0.198
PHT	-0.030	**0.143**	0.027	0.085	0.069	0.016	0.022	0.333**
NPB	-0.001	0.051	**0.075**	0.154	0.099	0.020	0.059	0.457**
NSB	0.014	0.050	0.048	**0.242**	0.197	0.021	0.092	0.664**
NPP	0.015	0.030	0.022	0.142	**0.336**	0.024	0.140	0.707**
NSP	0.008	0.019	0.013	0.043	0.066	**0.120**	0.081	0.349**
HSW	0.017	0.012	0.016	0.081	0.171	0.035	**0.277**	0.607**

KEY: DTF (Days to 50% flowering), PHT (Plant height in cm), NPB (Number of primary branches), NSB (Number of secondary branches), NPP (Number of pods plant^{-1}), NSP (Number of seeds pod^{-1}), HSW (100-seed weight in g/100 seeds), SYDP (Seed yield plant^{-1}(in g/plant). n a= not available. Residual factors=0.18905.
*Significant difference at the 0.05 probability level. ** Significant difference at the 0.001 probability level.

3.4. Discussion

Study of character association and path analysis helps the breeder in fixing the selection criteria for higher grain yield, so that selection will be effective in isolating the genotypes with desirable combination of characters (Vanisree et al. 2013). In the present study the number of pods plant^{-1} had the highest positive correlation and direct path coefficient on SYDP, among all other characters studied across all families. This association from the direct path value indicates that NPP tends to serve as a first order or principal selection

criterion to improve SYDP among accessions. Number of pods plant^{-1} has been reported to have maximum positive direct effect on SYDP in pigeonpea by several workers (Veeraswamy et al. 1973; Kingshlin and Subbaraman 1999; Singh 1999; and Padi 2003).

Number of pods produced by a crop plant is among the key yield components that significantly influence the seed yield production in grain legumes. It is a function of number of raceme/plant, number of flowers/plant and percentage floral abscission (Mostafa and Fakir 2008). Studies have shown that the pod is an important photosynthetic organ in re-fixing respired carbon within the pod wall that is then translocated to the developing seed (Ma et al. 2001; and Furbank et al. 2004).

In this study, SYDP had significantly and positively correlated with the NPB, NSB, HSW, NSP and PHT. Such correlations indicate the possibility of selection of genotypes with higher number of pods per plant, primary and secondary branches per plant and plant height for pigeonpea improvement. The significant positive interrelationship between SYDP and these traits have also been reported in pigeonpea (Brar 1993; Lal et al. 2002; and Sodavadiya et al. 2009).

In all the three populations studied, SYDP was positively and significantly associated with all the characters evaluated except with DTF in the population ICP 5529 × ICP 7035. This suggests that any positive increase in such traits will enhance SYDP. These findings are in agreement with the report by Thanki and Sawargaonkar (2010), who indicated that DTF to have negative and non-significant association with SYDP. A study by Kaveris et al. (2007), in green gram also revealed similar results. In this case, it could be suitable to select short duration lines for increasing other characters, including SYDP.

Partitioning of the correlation coefficients, into direct and indirect effects revealed that NPP exhibited the highest direct path coefficient on SYDP among all characters in all the studied crosses. High path coefficient value indicates that the change will result in a proportional (or inversely proportional) change in another correlated trait, whereas a strong correlation coefficient

indicates that the change will have marked effect on the second trait (Cramer and Wehner 2000).

Hundred seed weight is an important character for seed yield improvement in pigeonpea. In the current study, this character followed NPP in exhibiting high direct path coefficient affecting SYDP across all the three families. This implies NPP and HSW are the desired characters for selection in the breeding programmes to enhance SYDP in pigeonpea.

Except in the family ICP 8863 × ICPL 87119, increase in the two characters will contribute to increased SYDP in the studied populations. Increased HSW may be achieved by increasing the NSP, NPP and NSB.

Results of the current study agrees with the findings of (Yadvendra et al. 1981; Sodavadiya et al. 2009; Chandirakala and Subbaraman 2010; and Saroj et al. 2013), who reported 100-seed weight to be exhibiting high and positive direct effects on seed yield per plant^{-1}. Similar results have also been reported in chickpea (Padmavathi et al. 2013), and soybean (Khanghah and Sohani 1999; and Ball et al. 2001). In all these studies, the number of pods plant^{-1} and 100 seed weight had higher correlations as well as the highest positive direct effect on seed yield.

Except DTF which exhibited negative indirect effect on NPP, on the two populations; ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035, remaining characters such as PHT, NSB and NSP, exhibited indirect positive effects. The analysis indicated that selection for increased NPP would bring about simultaneous and favorable change towards increased plant height and hundred seed weight than selecting for PHT, NSB and NSP *per se*. A similar result was reported by Thanki and Sawargaonkar (2010). Overall, to increase SYDP, large number of pods per plant, tall and vigorously-branching genotypes are desirable for selection in all studied populations.

Ranking populations on the basis of the magnitudes expressed in their correlations and direct effects of the desired character on SYDP suggested that ICP 8863 × ICPL 87119 was the superior family, followed by ICP 5529 ×

ICP 7035 and AL 201 × ICPL 20325. The direct path co-efficiencies estimated for NPP with SYDP in all three crosses were > 0.7, which is considered to be a relatively higher value. Cramer and Wehner (2000), suggested a statistical test for the relative importance of path coefficient as 0.7 to 1.0 or - 0.7 to-1.0 (strong coefficients) and - 0.69 to 0.69 (weak coefficients).

3.5. Conclusions

Yield is an important polygenic trait that is a result of contribution of several interrelated characters. Knowledge on the degree of correlation and understanding of the relative direct and indirect effects of yield-related components is crucial in formulating the effective criteria in selecting desirable genotypes in early segregating populations. Through correlation and path coefficient analyses of eight yield and related characters of three F_2 pigeonpea populations, number of pods plant^{-1}, followed by 100 seed weight were identified as having the highest correlation and direct path coefficient on seed yield plant^{-1}. Ranking crosses on the basis of magnitudes of expression of correlation and direct effect on seed yield have shown that the families ICP 5529 × ICP 7035 to be superior among the three crosses. From the results of this study, it is concluded that effective selection for superior genotypes is possible considering number of pods plant^{-1}, 100-seed weight and number of secondary branches.

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CHAPTER FOUR

Prediction of gene action controlling yield and yield related traits in pigeonpea

Abstract

Seed yield is an important trait with quantitative inheritance. Understanding the inheritance of yield and related traits is a prerequisite in choosing a breeding strategy and methodology in crop improvement programs. Coefficients of skewness and kurtosis were used to determine gene action and to identify type of gene interaction for the yield and yield related traits in pigeonpea. The results indicated predominance of additive gene action affecting the studied characters conditioned by few to many genes. According to estimated coefficients of skewness and kurtosis of the characters tested, no gene interaction was observed for days to 50% flowering in the family of AL 201 × ICPL 20325 and for plant height in the crosses of ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035, and for the number of primary branches, secondary branches and 100-seed weight for family ICP 5529 × ICP 7035. The number of seeds pod^{-1} in the family AL 201 × ICPL 20325, and days to 50 % flowering, number of seed pod^{-1} and 100-seed weight in the family ICP 8863 × ICPL 87119, and 100-seed weight in ICP 5529 × ICP 7035 recorded negative skewness values indicating the presence of duplicate gene action. The remaining characters across all the tested families had positive skewness indicating the presence of complementary gene action. The result suggests that for the traits where complementary gene interactions was observed, targeted pure line selection can be made in the segregating generations for attaining faster genetic gain, whereas duplicate gene effect can be exploited by inter-mating the selected segregants and delay the selection of the traits for advanced generations.

Keywords: additive gene action, complementary gene action, duplicate gene action, kurtosis, pigeonpea, skewness

4.1. Introduction

Breeding procedures involve designed crossing of parents, selection from recombined parents, and fixation of superior genotypes for development and deployment of improved cultivars suited to the diverse needs of humans (Moose and Mumm 2008). The breeding method to be adopted for genetic improvement of any crop species depends mainly on its mating system and nature of gene action involved in the expression of quantitative traits, among others (Aziz et al. 2006). Gene action refers to the behavior or mode of expression of genes in a genome (Singh 1996). Knowledge of gene action and heritability involved in several quantitatively inherited traits helps to choose an appropriate breeding method (Lamkey and Edwards 1999; Amand and Wehner 2001; and Dias et al. 2004).

Depending upon the genetic variance, gene action is of three types; additive gene action, dominance gene action and epistatic gene action. Dominance and epistatic gene action are referred to as non-additive gene action (Singh 1996). The additive component describes the variance associated with the independent and additive contributions of alleles, while dominance describes the variance contributed by interactions between alleles at the same locus, and epistasis refers to the contribution of interactions between alleles at different loci (Wang et al. 2014).

Interaction between genes takes different forms such as duplicate gene interaction, complementary gene interaction or a more complex form of non-allelic interaction (Holland 2001). Duplicate gene interaction can occur when two or more loci serve the same function, whereas complementary gene interaction can result when two or more genes code for enzymes that function at different points on the same pathway, so that functional products from all genes in the set are needed to produce the final product (Holland 2001).

Complementary gene action has slower genetic gain with the mild selection and faster with intensive selection for that particular trait, whereas for duplicate epistatic gene action the gain is faster with mild selection and less rapid with

intense selection (Snape and Riggs 1975). Yield and its component characters have quantitative inheritance and exhibit either types of gene action (Saxena 2008).

Understanding the genetic architecture of complex traits is a major challenge in the post-genomic era (Yang et al. 2007), therefore, methods for identifying the nature of the gene interaction should be solicited to enhance genetic gain through breeding (Choo and Reinbergs 1982). Coefficients of skewness and kurtosis which are also referred to as third- and fourth-degree statistics are useful statistical tools for accurate determination of the presence or absence of gene interaction. Also these parameters help to identify the nature of gene interaction. Skewness describes the degree of departure of a distribution from symmetry and kurtosis is a measure of whether the data are heavy-tailed or light-tailed relative to a normal distribution (Jayaramachandran et al. 2010).

The coefficients of skewness and kurtosis corrects the deficiencies emanating from the use of first- and second-degree statistics. For instance, there is a major limitation for the diallel analysis when significant amount of additive × additive genetic effect is found limiting a further study of the nature of gene interaction (Choo and Reinbergs 1982). Graphical analysis (Mather and Jinks 1971) allows to detect complementary and duplicate gene action, but several assumptions should be met in order to interpret the results of this analysis (Choo and Reinbergs 1982). Furthermore, duplicate interaction is difficult to detect by a graphical analysis (Mather 1967).

Estimated values of skewness and kurtosis are useful statistical parameters to discern gene action. If no gene interaction is found, the kurtosis will be smaller than zero. The kurtosis larger than zero suggest the presence of gene interaction. If duplicate interaction occurs, the skewness will be smaller than zero. Skewness larger than zero shows presence of complementary gene interaction (Choo and Reinbergs 1982; Zhang and Xue 1997; and Yu et al. 1998).

In comparison to other economically important crops, limited effort has been made to understand the genetics of important quantitative traits in pigeonpea

(Yermani et al. 2013). Only few few studies have been conducted on this crop in which, both additive and non-additive gene effects have been reported to be influencing yield and related traits in pigeonpea (Saxena and Sharma 1990; and Pandey et al. 2014). Often pleiotropic gene effect, physiological changes, and the highly sensitive nature of pigeonpea due to environmental effects make difficult to interpret the inheritance of yield and associated traits (Byth et al. 1981).

Overall, there is limited study on gene action of seed yield and related traits in pigeonpea despite its economic importance. Underlying information on the genetics and inheritance of quantitative characters of this crop is necessary to develop populations for breeding and genetic analysis (Ajay et al. 2011). Therefore, the objective of present investigation was to determine the genetic control of eight yield and yield related traits involving a total of 460 F_2 pigeonpea progenies using three families of varied genetic backgrounds.

4.2. Materials and methods

Plant materials and crosses

The study used a total of 460 F_2 populations derived from bi-parent crosses of the following six parents: AL 201, ICPL 20325, ICP 8863, ICPL 87119, ICP 5529 and ICP 7035.

Study sites, field establishment and design

An experiment was conducted during the 2014/2015 rainy season at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (18° 78° E), Andhra Pradesh India.

Plant materials were established under a well-prepared field condition. Each genotype was sown by hand in shallow furrows opened at the top of the ridge with an inter-row spacing of 75 cm and intra-row spacing of 30 cm. Initially two seeds were sown per hill and later thinned to provide one plant per hill. Diammonium Phosphate (DAP) fertilizer was applied at a basal dose of 100 kg/ha (18% N and 46% P_2O_5). Urea (46% N-NH_2) was used as a top dressing fertilizer and was split-applied 30 days and 45 days after sowing. A pre-

emergent herbicide containing Fluchloralin 45% at 2.0 kgha^{-1}, Prometry 50% per cent at 1.5 kgha^{-1} and Paraquat 0.25 %at 3.0 kgha^{-1} were mixed and applied. After sowing the soil was uniformly irrigated to field capacity using perforated pipes (provided with check gates for the control of water flow) so that soil moisture was sufficient for seed germination and crop establishment. Experimental plots were irrigated using furrow irrigation.

4.2.1. Data collection and analysis

The following data were collected during the study: days to 50% flowering (DTF), plant height (PHT), number of primary branches (NPB), number of secondary branches (NSB), number of pods plant^{-1} (NPP), number of seeds pod^{-1} (NSP), 100-seed weight (HSW) and plant seed yield (SYDP). Description of data collection are presented in Chapter 2, Section 2.3. Genotypes AL 201 and ICPL 20325 have determinate growth habits. Consequently, data on secondary branches was not recorded in these genotypes and family AL 201 × ICPL 20325.

A total of 460 plants were available for data recording in the three families as follows: 180 plants from the cross of AL 201 × ICPL 20325, 180 (ICP8863 × ICPL 87119) and 100 (ICP 5529 × ICP 7035). From each parental genotype 20 plants were randomly selected and tagged for data collection.

Data collected was subjected to descriptive analysis to calculate the mean values of each trait. This was followed by calculation of the coefficients of skewness and kurtosis of phenotypic distribution in the F_2 population.

The parameters skewness and kurtosis were calculated with the following equations (Roy 2000):

$$Skewness = \frac{\sum_{i=1}^{N}(Yi-\overline{Y})^3}{(N-1)S^2}$$

$$Kurtosis = \frac{\sum_{i=1}^{N}(Yi-\overline{Y})^4}{(N-1)S^4}$$

Where: $Yi - \overline{Y}$ = deviation from the mean

S= Sample standard deviation

N= sample size

According to Roy (2000), a skewness value of < 0 imply additive gene action, while a skewness of > 0 shows complementary gene action. Likewise, negative kurtosis shows involvement of many genes influencing a trait, while a positive kurtosis shows control of a trait by few genes. Graphical distribution defining kurtosis are presented in Chapter 2, Section 2.4.3. Therefore, these reference values were used to deduce gene action in the studied populations.

Two samples independent t-test analyses were performed using Statistical Analysis Systems (SAS) program (SAS 2009), to determine significant differences among parental genotypes for the measured agronomic attributes.

4.3. Results

4.3.1. Significant test of differences among parents

A summary of an independent t-test with significant values comparing three pairs of parents is presented in Table 4.1. There was non-significant difference observed between parental genotypes AL 201 and ICPL 20325 for all the studied characters.

Highly significant differences (P<0.01) were detected between the parents ICP 8863 and ICPL 87119 for DTF (t=2.75), NSB (t= 10.29), NPP (t= -9.00), NSP (t=-2.79), HSW (t= -7.08), and SYDP (t=-7.45). The parental line ICPL 87119 had more number of secondary branches (33.2 branches) associated with increased number of pods per plant (245.0 pods) and higher number of seeds per pod (4.0 seeds), resulting in better seed yield per plant (120.0 g/plant) when compared to ICP 8863 (Table 4.2).

ICP 5529 and ICP 7035 were significantly different (P <0.05) for PHT (t= -6.82), NPP (t= -7.06) and SYDP (t= -14.51) (Table 4.1). The mean values of measured traits of the parental lines are summarized in Table 4.2. In general, ICP 7035 had higher plant height (240.0 cm) than ICP 5529 (203.0 cm). ICP 7035, popularly known as Kamica in India, has indeterminate growth habit (Rangaswamy et al. 2005). This variety has larger seeds (9–11 mm diameter) with purple seed coat and green cotyledons and suitable for consumption as

seed vegetable (Rangaswamy et al. 2005). These characteristics could have contributed to the observed higher seed weight (23.5 g/100 seeds) and higher seed yield (258.5 gm per plant) in line ICP 7035 than ICP 5529 (Table 4.2). However, ICP 7035 had significantly less number of pods per plant than ICP5529 (Table 4.1).

Table 4.1. Significant tests of differences among six pigeonpea parents for eight characters (n= 20; d.f. = 9)

	t-values and significant tests		
Character	AL 201 and ICPL 20325	ICP 8863 and ICPL 87119	ICP 5529 and ICP 7035
Days to 50% flowering (days)	0.06ns	0.78ns	-1.68ns
Plant height (cm)	0.64ns	1.03ns	-6.82*
Number of primary branches	-1.84ns	-1.54ns	-1.07ns
Number of secondary branches	n.a.	-10.29**	1.03ns
Number of pods plant^{-1}	-0.44ns	-9**	-7.06*
Number of seeds pod^{-1}	-0.44ns	-2.79	0.78ns
100-seed weight (gram)	0.12ns	-7.08**	-1.54ns
Seed yield plan t^{-1} (gram/plant)	-1.86ns	-7.45**	-14.51*

KEY: ns=non-significant; * and ** denote significant differences at 5% and 1% levels of probability, respectively. n. a. = data not available

4.3.2. Skewness and kurtosis of the F_2 populations

Gene action controlling the quantitative traits in the segregating generations was determined based on the frequency distribution of traits through third- and fourth-order statistics; skewness and kurtosis. Estimated coefficients of skewness and kurtosis for the studied characters in the three mapping populations are presented in Table 4.2.

In the family AL 201 × ICPL 20325, positive kurtosis was observed for PHT and HSW. The remaining characters had skewness and kurtosis values that were less than 1.0 (Table 4.2). The coefficients of skewness and kurtosis for the studied characters were as follows: 0.2 and -0.7 for DTF, 0.8 and 3 for PHT, 0.4 and 0.1 for NPB, 0.8 and 0.7 for NPP, -0.8 and 0.9 for NSP, 0.9 and

3.7 for HSW. SYDP had skewness and kurtosis values of 0.9 and 0.8, respectively (Table 4.2).

The family ICP 8863 × ICPL 87119, had positive skewness for seed yield plant^{-1} and number of pods plant^{-1}. Except for PHT, NPP and SYDP, all other traits in this family, had both skewness and kurtosis that were less than 1.0 (Table 4.2). The coefficients of skewness and kurtosis scored for studied characters in that order were -1.1 and 2.9 for DTF, 0.1 and -0.5 for PHT, 0.58 and 0.1 for NPB, 0.75 and 0.4 for NSB, 2.3 and 3.9 for NPP, -0.9 and 0.1 for NSP, -0.3 and 0.5 for HSW, and 1.7 and 3.1 for SYDP (Table 4.2.).

In ICP 5529 × ICP 7035, there was marked segregation of F_2 population except for the number of pods plant^{-1} and seed yield plant^{-1}. In this population the remaining traits had values of skewness and kurtosis that were less than 1.0. The values of skewness and kurtosis scored for studied characters respectively were 0.8 and 0.3 for DTF, 0.2 and -0.1 for PHT, 0.2 and -0.4 for NPB, 0.3 and -0.4 for NSB, 2.5 and 4.7 for NPP, 0.01 and 0.5 for NSP, -0.36 and -1.2 for HSW, and 1.1 and 0.6 for SYDP (Table 4.2).

Table 4.2. Mean values of parents and crosses and skewness and kurtosis of F_2 families for eight yield and yield-related traits of pigeonpea.

Genotype and parameters	Traits							
	DTF	PHT	NPB	NSB	NPP	NSP	SW	SYDP
AL 201	59.0	137.0	6.87	n.a	98.7	3.8	8.1	18.2
ICPL 2035	59.0	134.3	7.8	n.a	104.5	3.8	8.3	19.1
				AL 201 × ICPL 2035				
Mean	50.7	95	7.1	n.a	63.5	3.6	7.8	17.4
Skewness	0.2	0.8	0.4	n.a	0.8	-0.8	0.9	0.9
Kurtosis	-0.7	3.3	0.1	n.a	0.7	0.9	3.7	0.8
ICP 8863	94.0	213.8	2.4	12.4	98.8	3.2	10.2	22.7
ICPL 87119	102.3	207.2	3.00	33.2	245.0	4.00	11.8	120.0
				ICP 8863 × 87119				
Mean	93.6	192.9	2.55	13.04	157. 7	3.4	11.7	50.5
Skewness	-1.1	0.11	0.6	0.8	2.7	-0.9	-0.3	1.7
Kurtosis	2.9	-0.5	0.1	0.4	3.9	0.1	0.5	3.2
ICP 5529	114.8	203.0	12.2	12.8	538.8	2.72	10.5	59.2

Table: 4.2 Continued

ICP 7035	122	240.0	13.2	10.6	396.0	3.75	23.5	258.5
			ICP 5529 × ICP 7035					
Mean	123.5	209.4	14.2	35.4	125.6	3.4	21.4	03.5
Skewness	0.8	0.2	0.2	0.3	2.5	0.2	-0.4	1.2
Kurtosis	0.3	-0.2	-0.4	-0.4	4.6	0.5	-1.2	0.6

KEY: DTF=Days to 50 flowering, PHT =Plant height (cm), NPB=Number of primary branches, NSB=Number of secondary branches, NPP=Number of pods plant^{-1}, NSP =Number of seeds pod^{-1}, HSW=100-seed weight, SYDP =Seed yield plant^{-1}, n.a. data not available

4.3.3. Gene action

Based on scores of skewness and kurtosis, the gene action for each of the trait and estimated number of genes involved in each trait were calculated. Table 4.3 summarizes the results of the predicted gene action and interactions for each character in the three studied crosses. Additive gene action predominantly influenced the expression of studied characters across all the families (Table 4.3). Results indicated absence of gene interaction for DTF in the family AL 201 × ICPL 20325, for PHT in the families of ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035. Also gene interaction was not detected for NPB, NSB and HSW in the family ICP 5529 × ICP 7035 (Table 4.3). In these characters, the values of kurtosis were less than zero (Tables 4.2 and 4.3).

In AL 201 × ICPL 20325, all characters showed complementary epistasis gene action. Except in DTF where polygenes were involved in controlling this trait, the remaining characters were under the control of few genes (Table 4.3). Both duplicate and complementary gene actions were predominantly affecting some characters in ICP 8863 × 87119. Duplicate epistasis was responsible for DTF, NPP and NSP, whereas complementary epistasis was observed for NPB, NSB, SW and SYDP (Table 4.3). In this cross, except for PHT in which polygenic effect was observed, the remaining characters were under the control of few genes (Table 4.2).

For ICP 5529 × ICP 7035, complementary gene action was observed in all characters except for seed weight in which duplicate gene action was involved.

Characters in which polygenic gene action was observed included PHT, NPB, NSB, NPP, NSP and SYDP. However, DTF and HSW were under the control of few genes (Table 4.3).

Table 4.3. Summary of gene action for eight yield and yield related traits of three pigeonpea families.

Character	Families		
	AL201 × ICPL 20325	ICP 8863 × ICPL 87119	ICP 5529 × ICP7035
DTF	Additive, complementary, polygenic	Additive, duplicate, few genes	Additive, complementary, few genes
PHT	Additive, complementary, few genes	Additive, complementary, polygenic	Additive, complementary, polygenic
NPB	Additive, complementary, few genes	Additive , complementary, few genes	Additive ,complementary , polygenic
NSP	Additive, complementary, few genes	Additive, duplicate, few genes	Additive, complementary, few genes
HSW	Additive, complementary, few genes	Additive, complementary, few genes	Additive , duplicate, polygenic
SYDP	Additive, complementary, few genes	Additive, complementary, few genes	Additive, complementary, few genes

KEY: DTF=Days to 50% flowering, PHT =Plant height (cm), NPB=Number of primary branches, NSB=Number of secondary branches, NPP=Number of pods plant^{-1}, NSP=Number of seeds pod^{-1}, HSW=100-seed weight, SYDP=Seed yield per plant^{-1} n.a. =Data not available.

4.4. Discussion

The current study revealed the predominance of additive gene action controlling DTF, PHT, NPB, NSB, NPP, NSP, HSW and SYDP in populations of pigeonpea developed using three genetic background (Table 4.3). This was highly expected due to the mode of pollination of pigeonpea which is predominantly self-fertilizing. Additive gene action is mainly associated with homozygosity accumulated through continuous generation of self-fertilization (Singh 1996). Therefore, pure line or pedigree selection methods can be used to improve the genetic gain of these traits.

The estimated coefficients of kurtosis for DTF in the family AL 201 × ICPL 20325, PHT (ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035), NPB, NSB

and HSW (ICP 5529 × ICP 7035), were negative (Table 4.2). Coefficients of kurtosis helps in the prediction of the extent of genes involved in the control of quantitative traits. Traits which are under the control of few segregating genes exhibit positive coefficient of kurtosis. This is unlike traits that are under the control of many genes which will have negative kurtosis values (Roy 2000; Jayaramachandran et al. 2010).

In the current study, most of the traits in the three families had positive scores of kurtosis indicating the presence of few genes in controlling trait expression. These traits were DTF (in the families of ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035), PHT (AL 201 × ICPL 20325), NPB (AL 201 × ICPL 20325 and ICP 8863 × ICPL 87119), NSB (ICP 8863 × ICPL 87119), NPP (in all families), NSP (all families), HSW (AL 201 × ICPL 20325 and ICP 8863 × ICPL 87119) and SYDP (all families) (Table 4.2).

The magnitude of skewness helps to infer the type of gene action for a particular trait. Positive skewness indicates the presence of complementary epistatic gene action controlling a trait, whereas negative skewness indicates the presence of duplicate epistatic gene action (Roy 2000; and Jayaramachandran et al. 2010).

In this study, positive coefficients of skewness were recoded for DTF in the family AL 201 × ICPL 20325, NPB (AL 201 × ICPL 20325 and ICP 8863 × ICPL 87119), NSB (ICP 8863 × ICPL 87119), NPP (all families), NSP (ICP 5529 × ICP 7035), HSW (AL 201 × ICPL 87119) and SYDP in all families (Table 4.2). Presence of complementary gene action for most traits indicates that parents selected for crosses are diverse. If selected parents show complementary traits, then it is possible to realize enhanced genetic gain through selection in the ensuing breeding populations (Reynolds et al. (2009).

Varied coefficients of skewness were scored across all the studied families. In AL 201 × ICPL 20325, negative coefficient of skewness was observed only for NSP. In this family DTF, NSP and HSW, had positive skewness. In ICP 8863 × ICPL 87119, only HSW had negative skewness (Table 4.2). This indicates the preponderance of additive gene action and presence of duplicate epitasis

gene interaction among loci controlling these characters in the three families. Duplicate gene action is not easy to be fully exploited in a breeding programme (Kumar et al. 2009). It is therefore suggested that heterosis breeding may be used where large magnitude of non-fixable gene effects is observed. Findings of the present study is partially similar to the studies of Pandey (1972), Gupta et al. (1981), Sindhu and Sandhu (1981), Craufurd et al. (2001), Sreelakshmi et al (2011), and Kumawat et al. (2012) who reported the presence similar results in pigeonpea. However, the current findings contradict the results of Patel et al. (1990); Hooda et al. (2000); Perera et al. (2001); and Kumar et al. (2009).

The present study found that days to 50% flowering was under the influence of additive gene action. Except in the family AL 201 × ICPL 20325, where this character was found to be controlled by many genes, the other two families had few genes influencing the expression of DTF. The present findings are similar to the report of Gupta et al. (1981) who indicated predominance of additive gene action affecting days to flowering in pigeonpea.

A study by Craufurd et al. (2001), also reported duration from sowing to flowering to be controlled by two genes assorting independently and with predominantly additive quantitative effects. Contrarily, studies by Patel et al. (1990), Hooda et al. (2000), Perera et al. (2001), and Kumar et al. (2009) identified the significance of additive, dominance and epistatic gene effects in controlling days to flowering in pigeonpea.

In the present study plant height was found to be under the control of additive genes. Sharma (1981) reported this trait to be governed by both additive and dominance genes. The same author indicated that tallness was conditioned by dominant genes. Singh and Pandey (1974) reported additive gene action to be higher than non-additive gene action in governing plant height. Pandey et al. (2014) indicated additive gene action to be most important in the inheritance of the same trait.

The present study noted that additive gene action with complementary interaction were responsible in controlling the inheritance of the number of

branches per plant. Similar findings were reported by D'Cruz et al. (1971), who observed branching habit to be governed by three duplicate complementary factors. Marekar (1982), reported that close branching habit was controlled by one basic and two inhibitory complementary genes. The study of Kumawat et al. (2012), indicated the presence of positive additive effects due to QTL sqPB4.1 and qPB5 which were responsible for the control of the number of primary and secondary branches, respectively. Sreelakshmi et al (2011) reported additive gene action affecting primary and secondary branches. Unlike the above reports, D'Cruz and Deokar (1970) indicated that a single dominant gene controlled spreading habit, while erect types were under the control of homozygous recessive genes.

For the number of pods plant^{-1}, except in the family ICP 8863 × ICPL 87119, in which duplicate gene interaction was predominant, additive gene action with complementary gene interaction involving few genes were responsible for the expression of this trait. A study by Kumawat et al. (2012) indicated significant epistatic interaction effects of several QTLs affecting the number of pods plant^{-1} in which both complementary epistasis (additive x additive) and duplicate epistasis (additive x dominance) were expressed. Dahiya and Barar (1977) detected non-additive (over-dominance) gene action. Both additive and non-additive gene actions were detected by Venkateswarlu and Singh (1982), whereas Singh et al. (1983), reported additive gene action. Kumar et al (2009) indicated the presence of duplicatory epistatic type of gene action for the number of pods plant^{-1}.

The number of seeds pod^{-1} in the current study was under the influence of additive gene action with both duplicate and complementary gene interactions, involving few genes. This is contrary to the report of Venkateswarlu and Singh (1982), who detected both additive and non-additive gene action to be present in pigeonpea. Mohamed et al. (1985) reported dominance, additive x dominance and dominance x dominance gene interactions to be responsible for expression of this trait. Furthermore, Patel et al. (1990), noted additive,

dominant and epistatic genes affecting the inheritance of the numbers of pods plant^{-1} and seed yield plant^{-1}.

In the present study 100-seed weight was governed by additive genes in all the three families. Except in the family ICP 5529 × ICP 7035 in which duplicate and polygenic interaction was found, 100-seed weight in the remaining two families was under the control of complementary genes where few genes were responsible in the expression of this trait. Sharma et al. (1972) reported predominance of additive gene action for seed size. The authors suggested that genes controlling smaller seed size were dominant over larger seeds.

Other studies reported similar findings in pigeonpea (Gupta et al.1981; Sindhu and Sandhu 1981; and Mohamed et al. 1985). Conversely, studies by Dahiya and Barar (1977); Venkateshwarlu and Singh (1982); and Patel et al. (1987) indicated the presence of additive and non-additive (over-dominance) genes controlling seed size in pigeonpea.

Seed yield plant^{-1} in the present study was under the control of additive genes. Further, the study indicated the presence of complementary gene interaction involving few genes to be responsible for controlling this trait. Conversely, several co-workers (Singh and Pandey 1974; Dahiya and Barar 1977; Singh et al. 1983; and Sreelakshmi et .al 2011), reported non-additive gene action governing seed yield plant^{-1} in pigeonpea.

The current study was conducted on segregating F_2 populations. In this case, duplicate epistasis might restrict the expression and subsequent selection of a trait in early segregating generations (Jindal et al. 1993; and Amawate and Behl 1995). It is therefore recommended to exploit duplicate gene effects by intermating the selected segregants and delay the selection of the traits for advanced generations.

4.5. Conclusions

This study focused on prediction of gene action controlling yield and yield related traits in three families of pigeonpea using third- and fourth-degree statistics. The study revealed predominance of additive gene action controlling DTF, PHT, NPB, NSB, NPP, NSP, HSW and SYDP. Complementary and duplicate epistatic gene interactions were also present in conditioning the studied characters. Complementary gene interaction was noted influencing the expression of all studied traits in family AL 201 × ICPL 20325. Except DTF, NPP and NSP in which duplicate gene interaction was observed, complementary gene interaction was noted in all remaining traits in the family ICP 8863 × ICPL 87119, whereas in the family ICP 5529 × ICP 7035, only HSW was under the control of duplicate gene interaction. Additive genes are fixable; therefore, traits governed by such genes are expected to be effectively selected. Duplicate interaction indicates the presence of non-fixable genes, necessitating delayed selection involving advanced generations. For the characters observed to have large magnitude of non-fixable genes, heterosis breeding is recommended.

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CHAPTER 5

Quantitative trait loci mapping of yield and yield-related traits in pigeonpea

Abstract

Pigeonpea is an important multi-purpose crop widely grown in the tropics and sub-tropics. However, biotic and abiotic stresses significantly affect pigeonpea production and productivity. Genomic assisted breeding has the potential to enhance genetic gains in conventional pigeonpea breeding. The objective of this study was to identify quantitative trait loci (QTL) associated with eight yield and yield-related traits using 420 F_2 progenies developed from the following three diverse pigeonpea families: AL 201 × ICPL 20325, ICP 5529 × ICP 7035 and ICP 8863 × ICPL 87119. The following phenotypic data were collected: days-to-flowering (DTF), plant height (PHT), number of branches (NPB), number of secondary branches (NSB), number of pods per plant (NPP), number of seeds per pod (NSP), 100- seed weight (HSW) and seed yield per plant (SYDP). A total of 63 indel markers were used in AL 201 × ICPL 20325, and 51 and 56 simple sequence repeat (SSR) markers were used for ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035, respectively. Genotyping by sequencing (GBS) was used for genetic analysis and linkage analysis was performed using JoinMap version 4. Quantitative trait loci (QTL) analysis of the above yield and yield –related traits were performed using single marker analysis (SMA) employing composite interval (CIM) using stepwise regression linear model. A total of 42 QTL were detected in three families. In AL 201 × ICPL 20325, five QTL were identified for DTF, PHT, NPP and HSW on chromosomes 2, 3, 6 and 10. In ICP 5529 × ICP 7035, seven QTL were identified for DTF, PHT, NSB, NPP and HSW on chromosomes 2, 6 and 9, whereas in ICP 8863 × ICPL 87119, a total of 30 QTL were identified for DTF, PHT, NPB, NSB, NPP, NSP and SYDP on chromosomes 1, 2, 3, 4, 5, 6, 10 and 11. The number of QTL ranged from 1 for HSW to 16 for DTF, and the phenotypic value explained (PVE%) ranged between 10.35-16.27% in AL 201× ICPL 20325,10.44 -17.9 in ICP 5529 × ICP7035 and 10.71-89.12% in ICP 8863 × ICPL 87119. The detected QTL were co-localized within the same genomic regions indicating the presence of pleiotropic effect or linkage. Validation for the accuracy and consistency of the identified QTL in several, independent and diverse mapping populations is required for fine mapping and further use in marker-assisted selection programs.

Keywords: composite interval mapping, linkage maps, quantitative trait loci, pigeonpea

5.1. Introduction

Molecular markers and genetic maps are useful resources for genetic analysis or to undertake marker-assisted breeding in crop improvement programs (Yang et al. 2011). Genetic mapping involves several steps including DNA extraction from target species, identification of diagnostic molecular markers linked with desired genes, parental screening, genotyping, construction of linkage maps and linkage analysis (Semagn et al. 2006).

Molecular (DNA) markers are segments of DNA that can be detected through specific laboratory techniques (Datta et al. 2011). Genomic variation analysis is an essential component of plant genetics and crop improvement programs (Deschamps et al. 2012). DNA polymorphisms can be directly related to phenotypic differences or it may indicate genetic interrelationships between individuals in populations (Rafalski 2002).

Singe nucleotide polymorphism (SNP) markers have been increasingly used for QTL mapping studies (Jones et al. 2007). This is primarily, because SNPs are highly abundant in the genomes and, therefore, they can provide the highest map resolution compared to other marker systems (Jones et al. 2007).

Genotyping typically involves the generation of allele-specific products for SNPs of interest followed by their detection for genotype determination (Kim and Misra 2007). The two key components for genotyping germplasm are finding DNA sequence polymorphism and assaying the markers across a full set of test materials (Poland and Rife 2012). The strength of the sequence – based genotyping is the completion of the marker discovery and genotyping at the same time (Poland and Rife 2012).

Genotyping by sequencing (GBS) is among the popular genotyping platforms that are used nowadays. It is a robust genotyping method based on the sequencing of partial genome representations and has been developed for parallel high-throughput genotyping (Elshire et al. 2011). Generally, GBS utilizes one or more restriction enzymes (Poland et al. 2012) to digest the

genome into fragments that are then sequenced by parallel high-throughput methods. Genome complexity reduction, makes GBS to be easy, quick, extremely specific, highly reproducible, and enables reaching important regions of the genome that are inaccessible to sequence capture approaches. By choosing appropriate restriction enzymes, repetitive regions of genomes can be avoided and lower copy regions can be targeted with two to three-fold higher efficiency (Gore et al. 2007; Gore et al. 2009), which tremendously simplifies computationally challenging alignment problems in species with high levels of genetic diversity (Elshire et al. 2011).

Constructing a linkage map is, essentially, the finding of a linear arrangement of markers from recombination values (Stam 1993). A genetic linkage map is a representation of the genome that shows the relative position and distances between markers or genes along chromosomes. It does not show the physical distance between these markers but the genetic distance, defined as a function of the crossover frequency during meiosis (Foulongne-Oriol 2012). Distance between genes on chromosomes is usually expressed in centimorgans (cM) (Stam 1993). Genetic maps are constructed using different types and sizes of mapping populations, marker systems, statistical packages and procedures (Ferreira et al. 2006).

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an important food legume predominantly cultivated in the tropical and subtropical regions of Asia and Africa. It is a diploid (2n = 22), often cross-pollinated crop with a genome size of 858 Mbp (Greilhuber and Obermayer 1998). Intra- and inter-specific F_2 populations have been developed in pigeonpea for the purpose of constructing linkage maps for important agronomic traits such as *Fusarium* wilt resistance (Bohra et al. 2012), determinacy (Mir et al. 2014), plant type (Dhanasekar et al. 2010) and drought tolerance (Saxena et al. 2011).

During the last six decades, pigeonpea productivity has remained stagnant at around 780 kg/ha (Phazamala et al. 2015). The relatively low yields of the crop may be attributed to non-availability of improved cultivars, poor crop husbandry

and exposure to a number of biotic and abiotic stresses in pigeonpea growing regions (Varshney et al. 2012). Narrow genetic diversity in cultivated germplasm has further hampered the efficiency of conventional breeding as well as development and utilization of genomic tools (Varshney et al. 2010). Genomic assisted breeding has the potential to enhance genetic gains in conventional pigeonpea breeding.

Marker-assisted recurrent selection and genomic selection methods will now be feasible for pigeonpea breeding, and may be even further advanced by genotyping by sequencing that can be done with the help of the drafted genome sequence of the crop (Varshney et al. 2012). In pigeonpea, construction of linkage maps has been challenging. Nonetheless few linkage maps were constructed using different categories of markers, such as Diversity Array Technology (DArT) markers (Yang et al. 2011), simple sequence repeat (SSR) markers (Bohra et al. 2011; Gnanesh et al. 2011), Kompetitive Allele Specific PCR (KASP) assays (Saxena et al. 2012), and Golden Gate SNP assays (Kumawat et al. 2012).

Quantitative trait locus (QTL) analysis is a statistical method that links two types of information phenotypic data (trait measurements) and genotypic data (usually molecular markers) in an attempt to explain the genetic basis of variation in complex traits (Falconer and Mackay 1996; Kearsey 1998). There are several softwares that are useful in QTL analysis for gene detection and mapping. These includes MAPMAKER/QTL (Lincoln et al. 1993), QTL Cartographer (Basten et al. 1994), QGene (Nelson 1997), PLABQTL (Utz and Melchinger 1996) and MapQTL (Van Ooijen and Maliepaard 1996).

Different mapping studies have been conducted in pigeonpea for the major biotic and abiotic stresses, in which both QTLs and candidate genes have been reported for drought tolerance as well as for the major diseases such as *Fusarium* wilt and Sterility Mosaic Diseases (SMD). For instance, through pigeonpea genome analysis (Varshney et al. 2012), identified 111 proteins which were homologous to drought-responsive universal stress proteins. For *Fusarium* wilt, two random amplified polymorphic DNA (RAPD) markers were

reported by Kotresh et al. (2006), four sequence characterized amplified regions (SCAR) markers (Prasanthi et al. 2009), and six SSR markers (Singh et al. 2013). In the case of SMD, Gnanesh et al. (2011) identified six QTL explaining up to 24.72% phenotypic variation on linkage group (LG) 7 and LG 9. Further, other studies attempted to map genes controlling important agronomic traits in pigeonpea such as earliness and plant height (Kumawat et al. 2012), fertility restoration (Bohra et al. 2011), and determinacy (Mir et al. 2013, 2014).

Genomics-assisted breeding (GAB) is a useful breeding tool that enables breeders to select suitable parents for different crossing programs to achieve novel combinations leading to selection of elite breeding lines (Phazamala et al. 2015). However, inadequate genomic resources coupled with the narrow genetic base in cultivated gene pool caused serious impediment to applying GAB for pigeonpea improvement (Varshney et al. 2010). Therefore, there is need to explore and map candidate genes controlling economic traits in pigeonpea to accelerate conventional breeding and to enhance yield. The objective of this study was to identify QTL associated with eight yield and yield-related traits using 420 F_2 progenies developed from the following three diverse pigeonpea families: AL 201 × ICPL 20325, ICP 5529 × ICP 7035 and ICP 8863 × ICPL 87119.

5.2. Materials and methods

5.2.1. Development of mapping populations

Mapping populations derived from crosses involving three diverse parents were used in the current study. These were AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035. The F_1 and F_2 generations were grown under field conditions at the research farm of ICRISAT, Patancheru during 2013 and 2014. The three mapping populations were field grown with inter-row spacing of 60 cm and intra-row spacing of 20 cm. True F_1s were selfed in 2015 rain season using the single seed decent method to generate the mapping populations in each genetic combination. A total of 166 genotypes

from AL 201 × ICPL 20325, 131 from ICP 8863 × ICPL 87119 and 123 from ICP 5529 × ICP 7035 were selected for phenotyping.

5.2.2. Phenotyping of F_2 progenies

Phenotypic data was collected on individual plants in which observations were recorded on days to 50% flowering (DTF), plant height (PH, expressed in cm), number of primary branches per plant (NPB), number of secondary branches per plant (NSB), number of pods per plant (NPP), number of seeds per pod (NSP), 100 seed weight (gram per 100 seed), and seed yield per plant(SYDP) (gram per plant). Briefly, the data were collected as follows:

Days to flowering were recorded as number of days from planting to the date when 50% of the plants showed flowers. Plant height was measured as the height to the nearest centimeters of a stretched plant from ground level to the tip of the main stem at harvest. Number of primary branches were counted as number of branches (productive and unproductive) arising from the main stem and counted at harvest. Numbers of secondary branches were determined as the total number of branches arising from primary branches. Number of pods were counted as the total number of matured pods obtained at harvest. Number of seeds per pod was determined as the average number of seeds of 10 sampled pods. 100-seed weight was determined as the weight to the nearest grams of one hundred clean whole dry seeds Seed yield was the seed weight measured to the nearest grams per plant. With an exception of DTF, all other measurements were recorded at maturity.

5.2.3. DNA extraction

Seeds of the parents and F_2 progenies were sown in the field. Leaf samples were collected from parents and F_2 progenies. About DNA was extracted from 100 mg wet weight of a leaf sample collected from a three-week old plant. Extracted DNA was purified using NucleoSpin® 96 Plant II Core Kit protocol. The DNA was quantified and submitted for GBS analysis. The DNA from F_1 individuals and parents were isolated using the protocol suggested by Cuc et al. (2008).

5.2.4. Polymerase chain reactions

Polymerase chain reactions (PCRs) for amplification of SSR loci were performed in a 96-well micro titre plate (ABgene, Rockford, IL, USA) using thermal cycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The reaction volume consisted of 5µl containing 0.5 µl of 10 x PCR buffer (SibEnzyme, Novosibirsk, Russia), 1.0 µl of 15 mM $MgCl_2$, 0.25 µl of 2 mM dNTPs, 0.50 µl of 2 pmol/µl primer anchored with M13-tail (MWG-Biotech AG, Bangalore, India), 0.1 U of Taq polymerase (SibEnzyme, Novosibirsk, Russia) and 1.0 µl (5 ng/µl) of template DNA. A touch down PCR programme was used to amplify the DNA fragments: initial denaturation was for 5 min at 95 °C followed by five cycles of denaturation for 20 s at 94 °C, annealing for 20 s at 60 °C (the annealing temperature for each cycle being reduced by 1 °C per cycle) and extension for 30 s at 72°C. Subsequently, 35 cycles of denaturation at 94 °C for 20 s followed by annealing for 20 s at 56°C and extension for 30 s at 72°C and 20 min of final extension at 72°C. The PCR products were checked for amplification on 1.2 % agarose gel. Amplified products were separated on capillary electrophoresis using ABI 3730 (Applied Biosystems, Foster City, CA, USA).

5.2.5. SSR analysis

Markers polymorphic between the parental lines as identified in Bohra et al. (2011) were used for genotyping the respective mapping population. Indel primers were used for the identification of polymorphic markers in AL 201 × ICPL 20325, whereas SSR markers were used in ICP 8863 × ICPL 87119 and ICP5529 × ICP 7035.

5.2.6. Genotyping-by-sequencing

A GBS was used for SNP calling between the parents and genotyping the F_2s as described by Elshire et al. (2011). GBS libraries from the parental lines and F_{2s} were prepared using *Ape*KI endonuclease (recognition site: G/CWCG) and sequenced using the Illumina HiSeq 2000 platform (Illumina Inc, San Diego, CA, USA). Genomic DNA of selected mapping population and parental lines were subjected for restriction digestion using endonuclease *ApeK*I for 2 h at

75 °C. Adapters with unique multiple× sequence index (barcodes) were ligated to the sticky ends using ligase buffer with ATP and T4 ligase. Samples were incubated at 22 °C for 1 h and heated to 65 °C for 30 min to inactivate the T4 ligase. Aliquot of each sample (5 µl) was pooled (multiplexed) and purified to remove the excess adapters. DNA samples were eluted in a final volume of 50 µl. PCR was performed to increase the restriction fragments from each library using primers complementary to the corresponding adapters. The amplified pools constituting the “sequencing library,” were cleaned up and evaluated for fragment sizes using a DNA analyzer. Libraries without adapter dimers were subjected to sequencing.

5.2.7. Linkage mapping

The GBS data obtained were first de-multiplexed and SNPs were identified using GATK pipeline. Those SNPs with known and polymorphic alleles in parental genotypes (AL 201, ICP20325, ICP 8863, ICPL 87119, ICP 5529 and ICP 7035) were extracted and used for further processing. Initially, SNPs having more than 30% missing data were filtered out. Lines having more than 70% missing data were also removed from further analysis. Parent dependent sliding window based bin mapping approach was utilized to identify recombination breakpoints. The LD analysis clearly differentiates the SNPs on 11 pigeonpea pseudomolecules.

Genotype data were assembled for all segregating markers on all individuals from three mapping populations and linkage analysis was performed using Join Map version 4.1 using Regression mapping algorithm (Van Ooijen 2006). All markers were subjected to a Chi-squared test for fit to a 1:2:1 (A: H: B) ratio at 5% level of significance to identify markers with distorted segregation ratios. Logarithm of Odds (LOD) scores of 6 to 10 were examined, using the Kosambi map function, and a maximum distance of 40 cM was employed to determine the linkage between two markers. A LOD score of 10.0 was selected to develop the linkage maps. Recombination values were converted to genetic distances using the Kosambi mapping function (Kosambi 1994).

''Locus genotype frequency'' function was used to calculate the χ^2 values for all the markers. Map calculations were performed with parameters like LOD value ≥3.0, recombination frequency B0.40 and the χ^2 jump threshold for removal of loci = 5. Placement of markers into different linkage groups (LGs) was done with ''LOD groupings'' and ''Create group using the mapping tree'' commands. Mean χ^2 contributions or average contributions to the goodness of fit of each locus were also checked to determine the best fitting position for markers in genetic maps. The markers showing negative map distances or a large jump in mean v2 values were discarded. Final maps were drawn with the help of Map-Chart version 2.2 (Voorrips 2002).

5.2.8. QTL analysis for seed yield and yield –related traits

Genotyping and phenotyping data from three mapping populations; AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035) were analyzed employing composite interval (CIM) in the WinQTL Cartographer version 2.5 (Wang et al. 2007). CIM analysis was performed applying the standard model 6, with a genome scan interval (walk speed) of 1cM. The ''forward-backward stepwise regression'' was used to set number of marker co factor as background control. A window size of 10cM was used to block out signals within 10cM on either side of the flanking markers or QTL test site. Thresholds were determined by permutation tests using 1,000 permutations and a significance interval of 0.05.

MapChart program was used to displays charts of a series of linkage groups and imports linkage data from map files, produced by software for linkage analysis.

In summary, the steps that were used for QTL analysis in this study included, making crosses and generating mapping population**,** identifying markers that are polymorphic between the three pairs of parents, generating marker data**,** generating linkage maps of molecular markers**,** collecting phenotypic measurements of QTL yield and yield-related traits**,** and mapping QTLs (Association of QTL with marker).

5.3. Results

5.3.1. Parental screening for marker polymorphism

Out of 63 SSR markers screened for parental polymorphism between AL 201 and ICPL 20325, only 2 (2%) were found to be polymorphic.These were CciD 260 and 261. For two crosses, i.e., ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035, 51 and 56 SSR markers were used, in that order. Out of 51 SSR markers used for parental polymorphism between ICP 8863 and ICPL 87119, only 3 (2%) were found to be polymorphic.These markers were CcM 0095, 0126 and 0147. For polymorphism between ICP 5529 and ICP 7035, polymorphic SSR markers identified were CcM 0047, 0195 and 1001, which were 2% of the total numbers of markers used. All these markers were used for genotyping F_2 plants as follows; 166 plants in AL 201 × ICPL 20325, 123 plants in ICP 5529 × ICP 7035 and 131 plants in ICP 8863 × ICPL 87119.

On checking the genotyping data obtained for all polymorphic loci for segregation ratio, 72.42%, 81.34% and 90.35% marker loci were found in normal segregation (1A:2H:1B) in AL 201 × ICPL 20325, ICP 5529 × ICP 7035 and ICP 8863 × ICPL 87119 respectively.

5.3.2. Linkage map construction

The segregation data for the SSR loci showing the normal segregation in each of the above mapping population were used for constructing genetic maps for the respective mapping population by using MapChart. Three linkage maps were constructed in the present study (Table 5.1). The arrangements of the linkage groups as it appears in the constructed maps, followed the order of chromosomes, e.g., LG1 represents chromosome 1, LG 2 represents chromosome 2 etc.

5.3.2.1. Descriptions of three linkage maps constructed.

The linkage map of 1901 loci resolved into 11 linkage groups covering a total length of 1574.982 cM in AL 201 × ICPL 20325. An average distance between

the adjacent markers were 0.83 cM. Map lengths between linkage groups ranged from 35.606 cM in LG 5 to 285.358 cM in LG 2 (Table 5.1).

In the population of ICP 8863 × ICPL 87119, a total of 2132 loci were mapped with a total length of 1446.725cM (Table 5.1). The average marker interval was 1.47cM. LG 5 had the lowest map length (74.87cM) whereas LG11 had the highest map length of 224.27cM (Table 5.1).

In the population of ICP 5529 × ICP 7035 population, a total of 1831 loci were used to construct a map spanning 1733.16 cM, and, an average inter-marker distance of 0.94 cM. LG 5 had the least map length of (4.891cM), whereas LG11 had the highest map length of 470.903 cM (Table 5.1).

Table 5.1 Summary features of linkage maps constructed in three mapping populations

	Mapping populations		
Summary parameters	AL 201 × ICPL 20325	ICP 5529 × ICP 7035	ICP 8863 × ICPL 87119
Number of F_2 lines	166	123	131
Number of total scored markers	3486	2256	9403
Number of total mapped loci	1901	1831	2132
Total map length(cM)	1574.98	1733.16	1446.725
Range of map length(cM)	LG 5(35.61)-LG 2(285.36)	LG 5(4.89)-LG 11(470.9)	LG 5(74.87)-LG 11(224.27)
Inter-marker distance (cM)	0.83	0.94	1.47

5.3.3. QTL analysis

A total of 42 QTLs were identified for eight yield and yield-related traits across three mapping populations of pigeonpea using CIM in the current study. The population that had the highest number of QTL (30) was ICP 8863 × ICPL 87119, followed by ICP 5529 × ICP 7035 (7) and AL 201 × ICPL 20325 (5). When comparing the number of QTLs detected across 11 chromosomes in three populations, chromosome 2 had highest number (8), whereas

chromosomes 4 and 5 each had the lowest (1) QTL. The details of the QTL analysis for each population is presented below:

QTLs for yield and yield-related traits based on AL 201 × ICPL 20325 population

This family had the lowest number of QTLs detected amongst the three test populations (Table 5.2). A total of 5 QTLs were detected for DTF, PHT and NPP in this family. Of the 5 QTLs, three were contributed by ICPL 20325 and the remaining two contributed by AL 201. Table 5.2 summarizes the QTLs detected in this family. Distribution of the QTLs on the linkage map of AL 201 × ICPL 20325 is shown in Figure 5.1

One QTL, qDF6.1, was detected for DTF on the chromosome 6. It was situated on position 164.91cM with LOD value of 3.4. The percentage phenotypic variation explained by this QTL was 14.19%. The qDF6.1 showed negative additive effect indicating that the allele for increasing days to 50% flowering at this locus was contributed by ICPL 20325 (Table 5.2).

Two QTLs, qPH10.1 and qPH10.2, were detected for PHT on chromosome 10. Their positions were 13.61 and 53.2cM, with the LOD values of 3.6 and 2.8, respectively. The percentage phenotypic variations explained were 10.45 and 10.35%, respectively (Table 5.2). The two QTLs showed negative additive effect indicating that the allele for increasing plant height at this locus was contributed by ICPL 20325.Thus, AL 201 has a dwarfing allele at the two identified loci.

One QTL, qPD3.1, was detected for the number of pods plant^{-1} on chromosome 3 positioned at 142.81cM. The LOD and PVE of this QTL were 2.8 and 16.27%, respectively. The qPD3.1 showed positive additive effect indicating that AL 201 contribute allele that is responsible for increasing the number of pods in the locus. One QTL, qSW2.1, was detected for 100-seed weight on chromosome 3. This QTL was detected on position 56.01cM with LOD of 3.5. The phenotypic variation explained by this QTL was 11.73% (Table 5.2). This QTL showed positive additive effect, indicating that alleles for increasing number of seeds per pod were contributed by AL 201.

Table 5.2 Additive quantitative trait loci (QTLs) for yield and related traits detected in the AL 201 × ICPL 20325 population of 166 F_2 progenies by composite interval mapping (CIM) and single marker analysis (SMA).

Trait	QTL	Chromosome	Left marker	Right Marker	Chromosome position (cM)	[a]LOD	[b]PVE(%)	a[c]
DTF	qDF6.1	6	bin_6_14812515	bin_6_16686080	164.91	**3.4**	14.19	-2.01
PHT	qPH10.1	10	bin_10_9715332	bin_10_1018363	13.61	**3.6**	10.45	-3.57
	qPH10.2	10	bin_10_1415083	bin_10_1487802	53.21	2.8	10.35	-3.64
NPP	qPD3.1	3	bin_3_17446054	bin_3_17032102	142.81	2.8	16.27	12.3
HSW	qSW2.1	2	bin_2_24823105	bin_2_17156189	56.01	**3.5**	11.73	0.22

KEY: DTF=Days to 50% flowering, PHT = Plant height in cm, NPB = Number of primary branches, NSB = Number of secondary branches, NPP= Number of pods plant^{-1}, NSP = Number of seeds pod^{-1}, HSW =100-seed weight in g/100 seeds, SYDP = Seed yield plant^{-1}(in g/plant).

[a]LOD score that exceeds the threshold are shown with font bold script (QTLs below a LOD score of 3.0 were not included, except where QTLs were available only at LOD >2<3. cM=Centi Morgan

[b]PVE: Variance explained by the QTLs

[c]Additive effect: positive values of the additive effect indicate that alleles from AL 201 were in the direction of increasing the trait score.

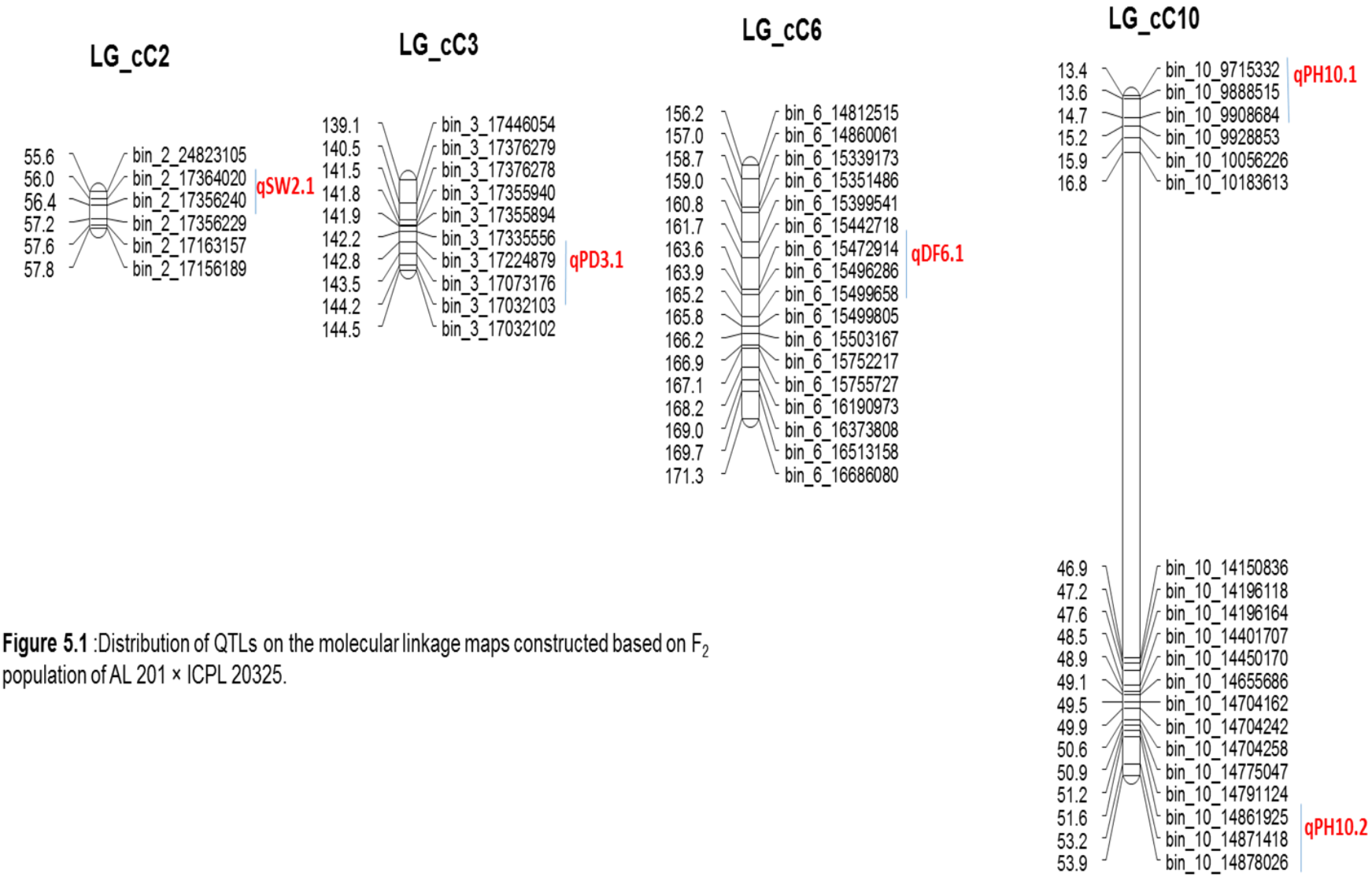

Figure 5.1 :Distribution of QTLs on the molecular linkage maps constructed based on F_2 population of AL 201 × ICPL 20325.

QTLs for yield and yield-related traits based on ICP 5529 × ICP 7035 population

A total of 7 QTLs were detected in this family. Of these QTLs, only one was contributed by ICP7035, whereas the remaining six were contributed by ICP5529. Summary of the QTL analysis for ICP 5529 × ICP 7035 is presented in Table 5.2, while distribution of the QTLs on the linkage map of ICP 5529 × ICP 7035 is shown in Figure 5.2.

Two QTL, qDF9.1 and qDF9.2, were identified for days to 50% flowering on chromosome 9 at 29.41 and 38.51cM, respectively. These QTL had LOD values of 5.4 and 3.8, in that order. The phenotypic variations explained by the two QTLs were 17.9 and 13.68%, respectively (Table 5.3). Both QTL showed positive additive effect indicating that allele for regulating days to 50% flowering was contributed by ICP 5529.

One QTL, qPH6.1, was detected for plant height on chromosome 6 (Table 5.3). It was located on position 89.71cM. The QTL had LOD score of 3.4 with PVE of 12.74%. The QTL exhibited a positive additive effect implying that ICP 5529 was responsible for increasing plant height, thus ICP 7035 has a dwarfing allele at qPH6.1 (Table 5.3). One QTL (qSB9.1), was detected for the number of secondary branches on chromosome 9 (91.81cM). The LOD and PVE values of this QTL were 3.6 and 10.85% respectively (Table 5.3). This QTLs showed a positive additive effects suggesting that ICP 5529 contributed allele for the number of secondary branches.

Two QTLs were detected for the number of pods plant^{-1} (Table 5.3). One QTL (qPD2.1), was detected on chromosome 2 (265.41cM), with LOD of 2.8 and PVE of 15.06% (Table 5.3). One QTL (qPD9.1 was detected on chromosome 9(66.71cM) with LOD of 3.4. The Phenotypic variation explained by this QTL was 11.3% (Table 5.3). The two QTLs exhibited contrasting additive effect, with qPD2.1 showing positive effect implying ICP5529 contributed to the increasing number of pods per plant, whereas qPD9.1 showed negative additive effect, indicating that allele for increasing the number of pods per plant at this locus was contributed by ICP 7035.

One QTL was detected for seed size on chromosome 2. The QTL (qSW2.2) was located on position 209.41cM with LOD of 2.9 and PVE of 10.44% (Table 5.3). It showed positive additive effect, indicating that alleles for the seed size were contributed by ICP 5529 in ICP 5529 × ICP 7035.

Table 5.3. Additive quantitative trait loci (QTLs) for yield and related traits detected in the ICP 5529 × ICP 7035 population of 123 F_2 progenies by composite interval mapping (CIM) and single marker analysis (SMA).

Trait	QTL	Chromosome	Left Marker	Right Marker	Chromosome position (cM)	[a]LOD	[b]PVE(%)	a[c]
DTF	qDF9.1	9	bin_9_7216021	bin_9_7130175	29.41	**5.4**	17.90	4.89
	qDF9.2	9	bin_9_5264669	bin_9_5235033	38.51	**3.8**	13.68	3.84
PHT	qPH6.1	6	bin_6_9816505	bin_6_11736735	89.71	**3.4**	12.74	4.73
NSB	qSB9.1	9	bin_9_2552282-	bin_9_873651	91.81	**3.6**	10.85	3.80
NPP	qPD2.1	2	bin_2_20119592-	bin_2_20797782	265.41	2.8	15.06	40.51
	qPD9.1	9	bin_9_4870443-	bin_9_3995906	66.71	**3.4**	11.30	-8.88
HSW	qSW2.2	2	bin_2_17046545-	bin_2_17611489	209.41	2.9	10.44	2.75

KEY: DTF =Days to 50% flowering, PHT =Plant height in cm, NPB = Number of primary branches, NSB = Number of secondary branches, NPP = Number of pods plant^{-1}, NSP = Number of seeds pod^{-1}, HSW= 100-seed weight in g/100 seeds, SYDP= Seed yield plant^{-1}(in g/plant.

[a]LOD score that exceeds the threshold are shown with font bold script (QTLs below a LOD score of 3.0 were not included, except where QTLs were available only at LOD >2<3. cM=Centi Morgan

[b]PVE: Variance explained by the QTLs

[c]Additive effect: positive values of the additive effect indicate that alleles from ICP5529 were in the direction of increasing the trait score.

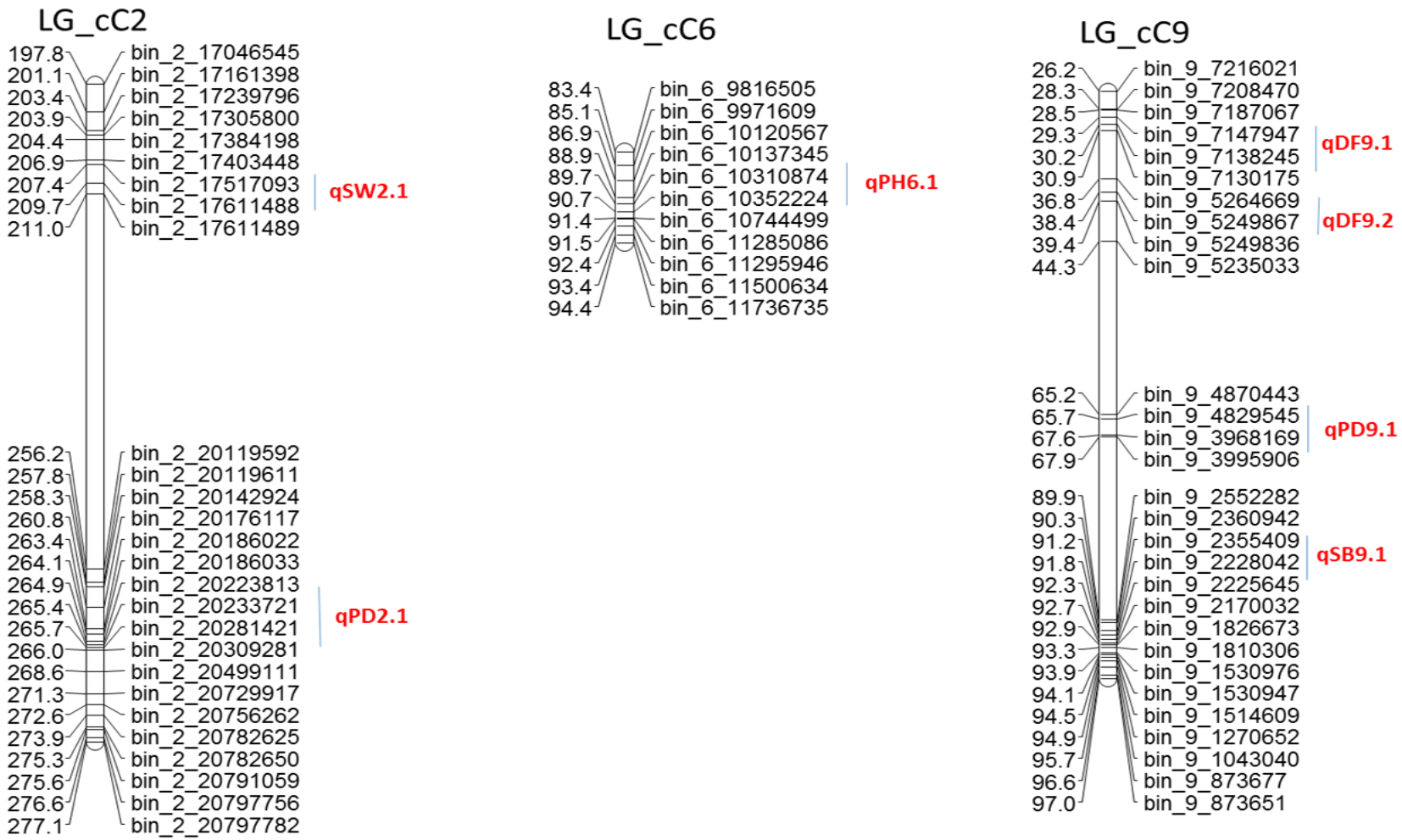

Figure 5.2 :Distribution of QTLs on the molecular linkage maps constructed based on F_2 population of ICP5529 × ICP 7035.

QTLs for yield and yield-related traits based on ICP 8863 × ICPL 87119 population

This family had the highest number of QTLs (30). Of the 30 QTLs,18 were contributed by ICP 8863, whereas ICPL87119 contributed 12 QTLs. Summary of the QTLs detected in ICP 8863 × ICPL 87119 are presented in Table 5.4 and distribution of the QTLs on the linkage map of ICP 5529 × ICP 7035 is shown in Figure 5.3.

Days to 50% flowering had the highest number of QTLs (16) detected amongst traits studied in this family. The number QTLs were 2, 1, 3, 1, 2, 3 and 4, positioned on chromosomes 1, 2, 3, 4, 6, 10 and 11 respectively. The detected QTL ranged in their LOD scores from 3.0 to 5.7, each explaining 11.8 -76.1% phenotypic variation (Table 5.4). All observed QTLs showed negative additive effect indicating that ICP8863 is responsible for contribution of alleles increasing days to 50% flowering at all loci controlling this trait.

One QTL, qPH2.1, was detected for plant height on chromosome 2 (78.21cM). The QTL had LOD score of 3.8 and PVE of 16.23%. The detected QTL showed a positive additive effect indicating that allele for increasing plant height was contributed by ICP 8863.

A total of five QTL (qPB 2.1, qPB 6.2, qPB 9.2, qPB 10.1 and qPB 11.1) were detected for the number of primary branches in this family. These QTLs were located on chromosomes 2, 6, 9, 10 and 11 (Table 5.4). The LOD of these QTLs ranged from 3.1 to 5.0 explaining 25.26 to 63.85% PVE. All five QTLs showed a positive additive effect indicating that allele for increasing the number of primary branches was contributed by ICP 8863.

One QTL (qPD1.1) was detected for the number of pods plant^{-1} on chromosome 1 (43.81cM). The LOD and PVE of this QTL were 4.2 and 10.71%, respectively (Table 5.4). The qPD1.1 showed positive additive effect showing that ICP8863 contribute allele that increases the number of pods per plant.

Only one QTL (qNS3.1) was detected for the number of seeds per pod in this family. The QTL was located on chromosome 3 (64.41cM). The detected QTL had LOD score of 2.6 and PVE of 36.69% (Table 5.4). The qNS3.1 showed positive additive effect indicating that ICP8863 contribute allele that increases the number of seed per pods.

Two QTLs (qSY1.1 and qSY11.1) were detected for seed yield plant^{-1} (Table 5.4). The qSY1.1 was located on the chromosome 1 (36.51cM) and had an LOD score of 3.2. The percentage phenotypic variation explained was 11.56%. The qSY11.1 was located on chromosome 11 (173.11) with LOD score and PVE of 2.9 and 52.86%, respectively. The qSY1.1 showed negative additive effect indicating that allele for increasing seed yield per plant at this locus was contributed by ICPL 87119. The qSY11.1 showed positive additive effect indicating that ICP 8863 was responsible for increasing seed yield per plant at this locus.

Table 5.4: Additive quantitative trait loci (QTLs) for yield and related traits detected in the ICP 8863 × ICPL 87119 population of 131 F_2 progenies by composite interval mapping (CIM) and single marker analysis (SMA).

Trait	QTL	Chromosome	Left Marker	Right marker	Chromosome position (cM)	[a]LOD	[b]PVE(%)	a[c]
DTF	qDF 1.1	1	bin1_9157099	bin1_7200644	50.21	**4.0**	34.44	-14.792
	qDF 1.2	1	bin1_14030807	bin1_1448640	43.81	**3.9**	17.14	20.674
	qDF 2.1	2	bin2_4297466	bin2_19392982	48.71	**3.1**	21.18	-6.8775
	qDF 3.1	3	bin3_1207862	bin3_4954564	68.01	**4.6**	34.47	-17.311
	qDF 3.2	3	bin3_10209623	bin3_7169460	48.61	**3.8**	20.81	-20.932
	qDF 3.3	3	bin3_24769020	bin3_27949764	99.71	**3.0**	18.61	-7.7634
	qDF 4.1	4	bin4_1914814	bin4_7430469	59.11	**5.7**	76.10	-12.524
	qDF 6.1	6	bin6_1722679	bin6_1268432	75.11	**4.2**	66.68	-11.113
	qDF 6.3	6	bin6_12699085	bin6_21827939	142.11	**4.0**	12.20	-17.451
	qDF10.1	10	bin10_1685243	bin10_1193309	57.81	**3.7**	51.47	-20.932
	qDF10.2	10	bin10_9493594	bin10_5774921	43.01	**3.1**	28.11	-17.156
	qDF10.3	10	bin10_4017384	bin10_1523546	50.21	**3.4**	22.44	-13.713
	qDF11.1	11	bin11_3694012-	bin11_3938723	132.61	**3.4**	60.23	-18.351
	qDF11.2	11	bin11_4136792	bin11_3885695	148.81	**3.5**	47.29	-19.314
	qDF11.3	11	bin11_1124941	bin11_6660445	143.41	**5.6**	45.08	-17.651
	qDF11.4	11	bin11_7081991	bin_449383099	154.81	**3.3**	11.80	-20.948
PHT	qPH2.1	2	bin2_35999980	bin2_27801880	78.21	**3.8**	16.23	7.5002

Table 5.4 continued

Trait	QTL	Chromosome	Left Marker	Right marker	Chromosome position (cM)	[a]LOD	[b]PVE (%)	a[c]
NPB	qPB 2.1	2	bin2_23127871	bin2_28049878	25.01	**3.1**	57.84	1.352
	qPB 6.2	6	bin6_16002188	bin6_4934	102.21	**3.7**	59.70	0.9571
	qPB 9.2	9	bin9_10383967	bin9_7150073	29.41	**5.0**	63.85	0.7812
	qPB10.1	10	bin10_3088979	bin10_1179518	91.41	**3.4**	38.03	0.5007
	qPB11.1	11	bin11_3080784	bin11_4511922	155.31	**3.4**	25.26	1.4128
NSB	qSB 2.1	2	bin2_32984051	bin2_1926109	63.51	**3.4**	89.12	7.437
	qSB 2.2	2	bin2_33644833	bin2_31156015	90.71	**3.3**	18.19	4.2416
	qSB 5.1	5	bin5_2573523	bin5_2848647	1.01	**3.5**	64.14	7.8219
	qSB 6.1	6	bin6_4936	bin6_11033681	112.01	**3.3**	24.33	7.8793
	qSB11.1	11	bin11_2589648	bin11_4405707	112.51	**3.1**	76.01	4.6353
NPP	qPD 1.1	1	bin1_17633179	bin1_12822551	43.81	**4.2**	10.71	91.831
NSP	qNS 3.1	3	bin3_2154488	bin3_28432102	64.41	2.6	36.69	-0.6663
SYLD	qSY 1.1	1	bin1_5674168-	bin1_11262983	36.51	**3.2**	11.56	-11.634
	qSY11.1	11	bin11_2064592	bin11_4558034	173.11	2.9	52.86	12.477

KEY: DTF =Days to 50% flowering, PHT =Plant height in cm, NPB = Number of primary branches, NSB= Number of secondary branches, NPP =Number of pods plant^{-1}, NSP = Number of seeds pod^{-1}, HSW =100-seed weight in g/100 seeds, SYDP =Seed yield plant^{-1}(in g/plant.

[a]LOD score that exceeds the threshold are shown with font bold script (QTLs below a LOD score of 3.0 were not included, except where QTLs were available only at LOD >2<3. cM=Centi Morgan

[b]PVE: Variance explained by the QTLs

[c]Additive effect: positive values of the additive effect indicate that alleles from ICP5529 were in the direction of increasing the trait score.

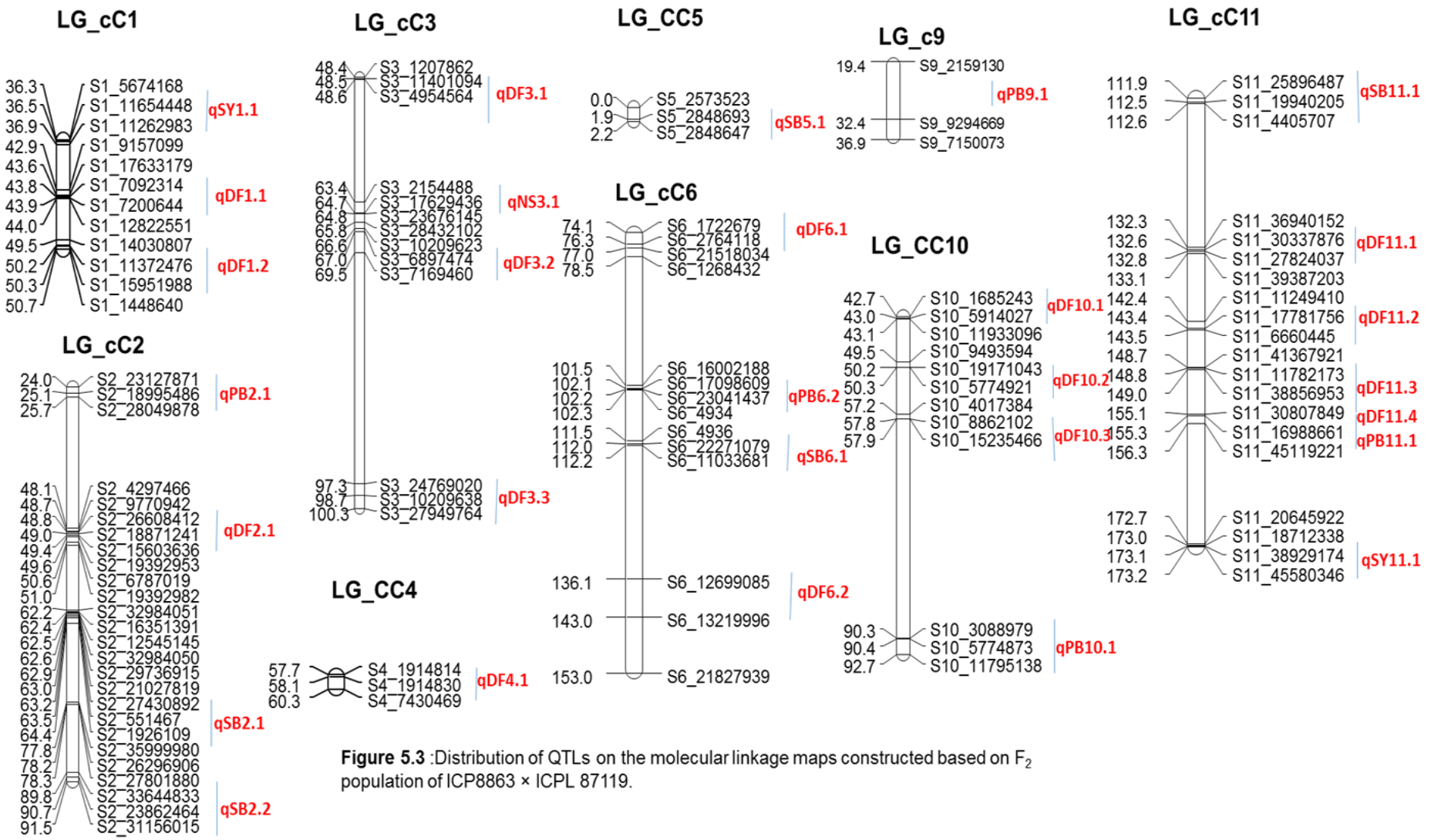

Figure 5.3 :Distribution of QTLs on the molecular linkage maps constructed based on F_2 population of ICP8863 × ICPL 87119.

5.4. Discussion

Mapping of quantitative trait loci associated with the yield and yield-related trait is an important step towards identification of important genomic regions associated with the key agronomic traits in crop species. In the present study a total of 42 QTLs associated with eight important yield components of pigeonpea were identified. On the linkage groups where the QTLs are located, one to four QTLs were detected per group, with days to 50% flowering in ICP 8863 × ICPL 87119 population having the highest number (4) of QTLs detected per linkage group (Table 5.4, Figure 5.3).

Three mapping populations were used in the present study. Linkage maps were constructed in the two mapping populations; AL 201× ICPL 20325 and ICP 5529 × ICP7035. For a third population; 8863 × ICPL 87119, marker data were provided, in which more QTLs (30) were detected. AL 201× ICPL 20325 had the least number of QTLs detected (5), whereas ICP 5529 × ICP7035 had 7 QTLs. This may be partly due to the high density of marker loci (2132) used in ICP 8863 × ICPL 87119 population, as compared to AL 201× ICPL 20325 (1901) and ICP 5529 × ICP7035 (1831). In addition, more divergent parents were used for developing ICP 8863 × ICPL 87119 population.

The proportion of phenotypic variation explained (PVE) by individual QTLs in the current study ranged from 10.35 -16.27% in AL 201× ICPL 20325, 10.44 -17.9 in ICP 5529 × ICP7035 and 10.71-89.12% in ICP 8863 × ICPL 87119. This is very high variation, and there could be chances of being overestimated due to smaller size of all three mapping populations used. AL 201× ICPL 20325 had the highest number of genotypes used (166), whereas ICP 5529 × ICP7035 had the lowest (123). A population size of at least 200 genotypes is needed to detect a QTL with an explained variance of 5% (Van Ooijen 1992). For enhanced accuracy and power of QTL detection, increased number of genotypes rather than the number of loci used for analysis are crucial (Herrmann et al .2006). On the other hand, the power of detecting a QTL remains virtually the same no matter a map with an average locus distance of 10cM or with an infinite number of loci is used (Darvasi

et al. 1993). All maps constructed in the present study had average-inter loci distance ranging from 0.83 to 1.47cM implying that they are dense maps ideal for QTL mapping.

The present study has added the QTLs information for three more traits; number of seeds per pod, 100-seed weight and seed yield per plant, which were not reported in the previous studies. Interestingly, the QTLs for 100-seed weight were detected in linkage group 2 in AL 201 x ICPL 20325 and ICP 5529 x ICP 7035.

The findings in the present study are similar to that reported by Kumawat et al. (2012) in terms of co-localization of the QTLs for yield and yield related traits in pigeonpea. For instance, the region between 29.41 – 91.81cM in linkage group 9 of the ICP5529 x ICP7035 population, five QTLs for days to 50% flowering, number of pods, plant height and secondary branches were located, with the QTLs for the later two separated by 2.1cM (Table 5.3).

Likewise, in linkage group 2 of the ICP8863 x ICPL87119, five QTLs for days to 50% flowering, plant height, number of primary branches and number of secondary were located in the region between 25.01cM - 90.71cM. This indicates the presence of pleiotropic effect or linkage.

The usefulness of the identified QTLs is made based on their LOD scores. If the LOD score is high (Positive LOD), it means that the traits are closely linked, and therefore usually inherited together. Ideally, a LOD score greater than 3 is desired.

In the present study, 42 may be categorized in to 4 groups, based on the range of their LOD scores., The QTLs that had LOD score ranging 5- 6 were 3, those with LOD 4-5 were 6, LOD ranging from 3-4 (28) and a total of 5 QTLs had QTLs ranging between 2-3.

However, QTLs detected in the present study requires further validation in different genetic background before they can be used in marker assisted selection (MAS). Therefore, there is need for QTL validation. Furthermore, unreplicated data of the F_2 population was used in the current study which may have resulted

into higher-overestimated PVE. The future plan is to advance the same mapping populations to $F_{2:3}$ populations to map the QTLs using available genotypic data. To be more confident, it will be feasible to use several diverse mapping populations for complete dissection of the yield and yield –related traits.

5.5. Conclusions

The present chapter aimed at identifying the genomic regions associated with days to 50% flowering, plant height, number of primary branches, number of secondary branches, number of pods plant^{-1}, number of seeds pod^{-1}, seed size, and seed yield plant^{-1} in three pigeonpea families of diverse genetic backgrounds using linkage mapping. Linkage map construction was followed by genetic analysis using Genotyping by Sequencing (GBS) platform. Quantitative Trait Linkage (QTL) analysis using single marker analysis (SMA) employing composite interval (CIM) detected a total of 42 QTLs with PVE ranging 10.35 -16.27%. The present work adds on to the QTLs information that have been reported so far on few studies related to QTL mapping for yield and yield-related traits in pigeonpea. The QTLs reported here on the key agronomic traits provide the basis for fine mapping studies. The detected QTLs and identified genomic regions will be useful for marker- assisted selection. However, detected QTLs requires further validation in several diverse, large and independent mapping populations.

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General overview of the thesis findings

Introduction and objectives of the study

Pigeonpea is an important multi-purpose crop grown in the semi-arid regions of the world including in Tanzania. The yield potential of this crop, however, has been significantly affected by various biotic and abiotic stresses. Further, low level of genetic polymorphism limited the progress of conventional breeding necessitating the use of complementary genomic tools to develop high yielding pigeonpea varieties.

Research findings in brief

Phenotypic variability among F_2 individuals of pigeonpea derived from three genetic backgrounds

Six parents including AL 201, ICPL 20325, ICP 8863, ICPL87119, ICP 5529 and ICP 7035 were selected and crossed using a bi-parental mating scheme. The six parents and families derived from the three genetic background (AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035) were field evaluated.

The main findings of the chapter two were as follows:

- Significant($P>0.05$) phenotypic variation was observed in the medium maturing (ICP 8863 and ICPL 87119) and long maturing (ICP 5529 and ICP 7035) parents.
- Significant variation was exhibited for days to 50% flowering, number of pods plant^{-1}, number of seeds pod^{-1} and seed yield plant^{-1} among F_2 individuals derived from medium maturity parents, whereas individuals from late maturing parents showed significant variations in plant height, number of pods plant^{-1} and seed yield plant^{-1}.
- Transgressive segregations were recorded for all studied characters. Transgression was more pronounced in the families of ICP 5529 × ICP 7035 and AL 201 × ICPL 20325.

- The study demonstrated the presence of considerable genetic variation among F_2 individuals derived from the three genetic groups.
- Transgressive segregants were selected for further selection and breeding of pigeonpea.

Promising genotypes were identified for all the characters, on the basis of their performance (scored value), which was greater as compared to the mean value of their parental lines.

Correlation and path-coefficient analyses of seed yield and related traits in newly developed pigeonpea populations

The correlation study was carried out using three mapping populations; AL 201 × ICPL 20325, ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035, each comprising a total of 180 individuals. The F_2 individuals were phenotyped for eight yield and yield-related traits. The data recorded were subjected to correlation and path-coefficient analyses. The main findings of this chapter were as follows:

- The highest phenotypic correlation with SYDP and the highest path co-efficient value influencing seed yield plant^{-1} across all families was exhibited by the number of pods per plant (NPP).
- NPP exhibited relatively the highest phenotypic correlation with SYDP across all mapping populations.
- Path-coefficient analysis revealed that NPP had the highest path co-efficient value influencing SYDP across all families.
- In the family AL 201 × ICPL 20325, the NPP had indirect effect on the SYDP through PHT and HSW.
- In the families of ICP 8863 × ICPL 87119 and ICP 5529 × ICP 7035 selection for increased NSB and NSP had indirect effect on NPP.
- The association using the direct path value indicates that NPP tends to serve as a first order or principal selection criterion to improve SYDP among accessions.

- Results from simple correlation and path coefficient analyses suggest that PHT, NSP, HSW and NSB are the most important selection criteria for improving seed yield in the newly developed pigeonpea populations.

Prediction of gene action controlling yield and yield related traits in pigeonpea

A total of 460 F_2 plants from three F_2 families; AL 201 × ICPL 20325, ICP8863 × ICPL 87119 and ICP 5529 × ICP 7035 were selected and phenotyped. Data collected were subjected to descriptive analysis to calculate the mean values of each trait, followed by calculation of the coefficients of skewness and kurtosis of phenotypic distribution in the F_2 population.

The main findings of this chapter were as follows:

- Additive gene action conditioned by few to many genes predominantly affected the studied characters.
- Complementary and duplicate gene actions were observed in the studied characters, with the former more pronounced in the studied population. For the traits where complementary gene interactions were observed, targeted pure line selection can be made in the segregating generations for attaining faster genetic gain, whereas duplicate gene effect can be exploited by inter-mating the selected segregants and delay the selection of the traits for advanced generations.

Quantitative trait loci mapping of yield and related –traits in pigeonpea

This study identified quantitative trait loci (QTL) associated with eight yield and yield-related traits using 420 F_2 progenies developed from three pigeonpea families: AL 201 × ICPL 20325, ICP 5529 × ICP 7035 and ICP 8863 × ICPL 87119. Data on important yield and yield-related parameters were collected. A total of 63 indel markers were used in AL 201 × ICPL 20325, and 51 and 56 simple sequence repeat (SSR) markers were used for ICP 8863 × ICPL 87119 and ICP 5529 × ICP

7035, respectively. Genotyping by sequencing (GBS) was used for genetic analysis, while linkage analysis was performed using JoinMap version 4. Quantitative trait loci analysis of the above yield and yield–related traits were performed using single marker analysis (SMA) employing composite interval (CIM) using stepwise regression linear model.

The main findings of this chapter were as follows:

- A total of 42 QTL were detected in three families. In AL 201 × ICPL 20325, five QTL were identified for DTF, PHT, NPP and HSW located on chromosomes 2, 3, 6 and 10. In ICP 5529 × ICP 7035, seven QTL were identified for DTF, PHT, NSB, NPP and HSW on chromosomes 2, 6 and 9, whereas in ICP 8863 × ICPL 87119, a total of 30 QTL were identified for DTF, PHT, NPB, NSB, NPP, NSP and SYDP on chromosomes 1, 2, 3, 4, 5, 6, 10 and 11.
- The number of QTL ranged from 1 for HSW to 16 for DTF, and the phenotypic value explained (PVE%) ranged between 10.35-16.27% in AL 201× ICPL 20325,10.44-17.9 in ICP 5529 × ICP7035 and 10.71-89.12% in ICP 8863 × ICPL 87119. The detected QTL were co-localized within the same genomic regions indicating the presence of pleiotropic effect or linkage.
- Validation for the accuracy and consistency of the identified QTL in several, independent and diverse mapping populations is required for fine mapping and further use in marker-assisted selection programs.

Overall, the present study; (i) selected transgressive segregants that are useful genetic resources for further breeding, (ii) determined the most influential traits in pigeonpea breeding to improve seed yield and yield components and (iii) developed mapping populations segregating for yield and yield-related traits including AL 201 × ICPL 20325, ICP 5529 × ICP 7035 and ICP 8863 × ICPL87119. This provided 42 putative QTLs and genomic regions associated with seed yield in pigeonpea which could enable marker-assisted breeding in the crop.

Future work and recommendations

The study involved F_2 generation which is segregating. Individuals in early generation such as F_2 population are heterogeneous, thus evaluation is made on individual plants and not on plot basis as it is the case with the advanced, homogeneous populations such as Recombinant Inbred Lines (RIL). There is a need of advancing the mapping populations to $F_{2:3}$ and to advanced generations so that the progenies can be replicated. The use of single plant data at F_2 generation is biased. In addition, the Phenotypic Value Explained(PVE) for some of the QTLs were too higher and there could be chances of overestimation, due to the nature of the populations used.

The QTLs have been detected using the mapping populations that had insufficient number of individuals to draw the conclusion. The future work is to increase the number of individuals so that the detected QTLs can be validated. The higher the number of individuals involved in QTL studies the more precise is the QTL detected from mapping studies. Multi location studies will further give indication on the consistence of the detected QTLs.

Recommendations

The choice of the appropriate parents for the development of a mapping population for QTL study is crucial. Mapping population of ICP 8863 × ICPL87119 had more number of QTLs detected as compared to the other two populations. This could be due to the diverse nature of the parents that were used to develop this population. The more the diverse the parents are, the higher the chances of detecting the variations and QTLs. It would be feasible to give more attention to this mapping population in future mapping studies.

Overall, the results obtained in this study can be a basis for the future QTL studies at advanced populations.