Identification and Characterization of Chickpea Genotypes for Early Flowering and High Seed Germination through Molecular Markers

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Research Article

Keywords: Chickpea, Cross-species transferability,Genetic diversity, ISSR markers

Posted Date: November 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1033758/v1

Abstract

Chickpea is the fourth most important legume crop contributing 3.1% to the total legume production and rich source of proteins, minerals and vitamins. Genetic diversity of wild and elite cultivar is quintessential for variety improvement. Determination of genetic diversity is more reliable and accurate, therefore, commonly used. In the present study, we analyzed the genetic diversity, population structure, cross-species transferability and allelic richness in 50 chickpea collection using 23 ISSR markers. The observed parameters such as allele number varied from 3 to 16,and polymorphic information content (PIC) varied from 0.15 to 0.4988, respectively. Further, range of allele size varied from 150 to 1600 bp, which shows the significance of ISSR markers for chickpea germplasm characterization. On the basis of ISSR marker genotypic data, dendrogram was constructed which divides these 50 chickpea in group I and II showing the reliability of ISSR markers. Among 50 chickpea, the accession P 74-1 is in group I and rest are in group II. Further we made mini-core collection of 15 diverse chickpea and sub-grouped them. Dendrogram, PCA, Dissimilarity matrix and Bayesian model based genetic clustering of 50 chickpea germplasms revealed that P 74-1 and P 1883are very diverse chickpea accession. Further selected 15 diverse chickpea screened for early flowering and high seed germination. Among 15 diversechickpea germplasms P 1857-1 and P 3971 has early flowering and high seed germination compared to P 1883 and other germplasm. Characterization of these diverse chickpea for early flowering and high seed germinationwould help in reducing crop duration and enhancing seed qualities. Utilization of these ISSRs markers in diversity analysis and population structure characterization of 50 chickpea germplasm suggests their wider efficacy for molecular breeding ofearly flowering and high seed germination.

Introduction

Among various leguminous crops, chickpea is the fourth most important legume contributing 3.1% to the total legume production (http://faostat.fao.org). Chickpea is the rich source of proteins, minerals and vitamins which makes them suitable for both food and feed. There are total 43 species that have been reported under the genus *Cicer* among which only one species i.e., *Cicer arietinum* L. is under cultivation that have economic importance (Sethyet. al., 2006a). The genome sequence data revealed the genome size (~750 Mbp) with 8 basic set of chromosome (Sethy et. al., 2006b). For any crop to be improved, the knowledge of genetic diversity of wild and elite cultivar is very important. Before 1980s when molecular markers were not known, genetic diversity and relationship were analyzed by biochemical markers. Further, the discovery of molecular markers has made the genetic diversity analysis easy.

Among various available marker systems, molecular markers are more reliable and accurate, therefore are very commonly used for genetic diversity analysis, phylogenetic studies and cultivar identification. Due to continuous selection breeding for crop improvement, genetic polymorphisms in the cultivated *Cicer arietinum* L. are very low, hence marker assisted selection (MAS) and marker assisted breeding (MAB) could play very potential role in the development of new varieties. Since past many years, genetic diversity analysis with different molecular markers in various crops has been done efficiently (Ahmad, 1999; Queen et. al., 2004; Schulman, 2007; Gill-Langaricaet. al., 2011; Noormohammadiet. al., 2013;

Bonmanet. al., 2015; Moggaet. al., 2018; Elshafeiet. al., 2019; Delfiniet. al., 2021). Due to several advantages of Inter Simple Sequence Repeat (ISSR) markers over other markers such as PCR based, no requirement of sequence information, distribution across the whole genome, require small quantity of template DNA and cost effective, ISSR markers are extensively used for genetic diversity analysis. The genotypic data of ISSR marker, pedigree analysis and various phenotypic data can assist in the selection of germplasm for further crop improvement through molecular breeding programs (Raoet. al., 2007).

In this study we analyzed the genetic diversity, structure, cross-species transferability and allelic richness in 50 chickpea collection using 23 ISSR markers. The observed parameters such as allele number varied from 3 to 16,and PIC varied from 0.15 to 0.4988, respectively. Further, range of allele size varied from 150 to 1600 bp which shows the significance of ISSR markers for chickpea germplasmscharacterization. On the basis of ISSR marker genotypic data dendrogram were constructed which divides these 50 chickpea in group I and II showing the reliability of ISSR markers. Among 50 chickpea, the accession P 74-1 is in group I and rest are in group II. Further we made mini-core collection of 15 diverse chickpea and sub grouped them. Dendrogram, PCA, Dissimilarity matrix and Bayesian model based genetic clustering of 50 chickpea germplasms revealed that P 74-1,P 1883, P 1260 very diverse chickpea accession.Additionally we have screened 50 chickpea germplasms for early flowering and high seed germination. Among 15 diverse chickpea germplasms P 1857-1 and P 3971 have early flowering and high seed germination compared to P 1883 and other germplasm. Characterization of these diverse chickpea would help in maintenance breeding, conservation and in future could be used to develop climate resilient elite cultivar of chickpea. The study of genetic diversity and population structure of 50 chickpea and development of 15 chickpea core collection will serve as important knowledge resources for future studies like GWAS, and mapping and introgression of early flowering and high seed germination in elite cultivar.

Material And Methods

Plant Material

Total fifty individuals of wild chickpea germplasm (Supplementary Table 1)were grown in the field of ICAR-IISS, Mau, India. Genomic DNA from freshly procured leaves of chickpeawas isolated by CTAB method with minor modification (Murray and Thompson, 1980). The wild chickpea germplasm having varying concentrations of secondary metabolite content and to overcome the inhibitory effect of secondary metabolite content during DNA extraction, the concentration of polyvinyl pyrrolidone K-30 and β-mercaptoethanol were standardized. Further, the quality of extracted DNA was examined over 0.8% agarose gel using lambda uncut marker (Fermentas, Lithuania) and quantified by NanoDrop 2000 (Thermo Scientific, USA).

Amplification validation and polymorphic potential evaluation

Fifty chickpea germplasm were genotyped by 23 inter simple sequence repeat (ISSR) markers (Supplementary Table- 1) and DNA fingerprint was developed (Table-1). For all the, PCR amplification efficiency with 23 ISSR markers were analyzed in 25µL reaction volume with 20 ng of DNA template. The PCR program initiated by pre-denaturation step at 94°C for 5 min, subsequent 35 cycles of denaturation at 94°C for 1 min, annealing at optimum temperature 50°C for 1 min, primary extension at 72°C for 2 min and the final extension was done at 72°C for 10 min in Thermo Cycler (Eppendorf). The amplified PCR products were separated on 1.5% agarose gel, and amplicon size were estimated based on 100 bp DNA ladder (Genedirex) as a reference.

Molecular data analysis

The amplification profiles with ISSR markers were scored based on their presence (1) or absence (0) across all individuals (SupplementaryTable-2). The amplified product were categorized as monomorphic, polymorphic and null alleles on the basis of same, different and absence of amplified PCR product across all the individuals. Cross-transferability of the ISSR markers and amplicon size were recorded for all the chickpea germplasms to construct UPGMA dendrogram and Principle Component Analysis (PCA) based on Jaccard's coefficient using DARwin6 (ver.) software (Perrier and Jacquemoud-Collet 2006). The polymorphic information content (PIC) values were calculated for each marker using online PIC calculator (https://www.liverpool.ac.uk/~kempsj/pic.html) (Table-1). Genetic structures of fifty individuals of chickpeawere inferred using Bayesian algorithm-based STRUCTURE software (ver. 2.3.3) (Pritchard et al., 2000; Falush et al., 2007). Further dissimilarity matrix (SupplementaryTable-3) was measured using DARwin6 software (Perrier and Jacquemoud-Collet 2006).

Phenotyping of minicore collection for early flowering and high seed germination

50% flowering data were taken day after showing between45 days to 65 days to select early, moderate and late flowering germplasms. For germination test, paper towel was moistened with distilled water and kept at 25 ºC and constant light. Further the days of 50% emergence under field condition was measured.

Result And Discussion

Genome of plants has several repeated DNA sequence such as Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSRs). In the past, when these sequence repeats were considered as'junk' DNA which have been used for plant genetic diversity analysis but with the time when sequencing technologies evolved, now it is well known that these sequence repeats are the major part of plant genome regulate gene expression and packing of genomes. ISSRs are usually repetitive DNA sequences mostly 1-6 bases (Litt*et al.,* 1989) which are PCR based, do not require prior sequence information, distributed throughout the genome, polymorphic and are cross transferable (Welsh *et al.,* 1990, Wang *et*

al., 1994). Chickpea (*Cicerarietinum*L.) belongs to the family leguminosae, is the fourth most important pulse crop of the world and India is among the
largest producer country (FAO, 2010).Chickpea seeds are nutrient rich due to the presence of balanced protein, carbohydrates, vitamins and minerals with the low levels of anti-nutritional factors (Wang *et. al.,* 2010; Santiago *et. al.,* 2010). Various biotic and abiotic stresses reduce the chickpea production across the globe, therefore, bringing of desirable traits in elite cultivar of chickpea from crossable donor cultivar is need of the hour (Singh *et. al.,* 2008). In this scenario, ISSRs markers could play potential role for comparative genomics study, traits identification, genetic purity, association studies and introgression of desirable traits in chickpea through marker-assisted breeding (Gautam*et. al.,* 2016; Jayaswall*et. al.,* 2019b; Jayaswall*et. al.,* 2019a).

Scoring and data analysis

Twenty five ISSR markers were selected for identification of polymorphism, cross-species transferability, genetic diversity and population structure analysis in 50 chickpea germplasms. Among twenty five only 23 ISSR markers were amplified at least in one of the individuals of chickpea. The amplification failure in some chickpeas could be due to not binding of primers in the genome. In present study, polymorphism was found to be higher than SSRs due to random binding of ISSR primers to the genome of diverse chickpea germplasms. The observed parameters like allele number varied from 3-16, allele size varied from 150 – 1600 bases and polymorphic information content (PIC) value ranges from 0.15 to 0.4988 (Table 1).

Table 1

List of primers and their amplification characteristics

S. No.	Locus name	Primer sequence	Annealing temperature (*T*a)°C	No. of alleles	PIC	Approximate size range (bp)
1	UBC 807	AGAGAGAGAGAGAGAGT	50	13	0.48	150-650
2	UBC 808	AGAGAGAGAGAGAGAGG	50	7	0.4669	320-1400
3	UBC 809	AGAGAGAGAGAGAGAGG	50	5	0.2522	300-1200
4	UBC 810	GAGAGAGAGAGAGAGAT	50	8	0.2952	300-1300
5	UBC 811	GAGAGAGAGAGAGAGAC	50	11	0.4721	250-1300
6	UBC 812	GAGAGAGAGAGAGAGAA	50	12	0.4916	250-1450
7	UBC813	CTCTCTCTCTCTCTCTT	50	3	0.3432	400-1200
8	UBC 815	CTCTCTCTCTCTCTCTG	50	6	0.4997	350-1200
9	UBC 817	CACACACACACACACAA	50	3	0.457	1000-1250
10	UBC 818	CACACACACACACACAG	50	3	0.1583	400-1200
11	UBC 819	GTGTGTGTGTGTGTGTA	50	8	0.499	300-1200
12	UBC 820	GTGTGTGTGTGTGTGTT	50	7	0.4953	400-1200
13	UBC 822	TCTCTCTCTCTCTCTCA	50	6	0.4002	300-1200
14	UBC 824	TCTCTCTCTCTCTCTCG	50	16	0.4947	250-1200
15	UBC 825	ACACACACACACACACT	50	6	0.3394	700-1250
16	UBC 826	ACACACACACACACACC	50	5	0.463	750-1500
17	UBC 827	ACACACACACACACACG	50	6	0.4772	600-1100
18	UBC 834	AGAGAGAGAGAGAGAGYT	50	9	0.4975	250-1300
19	UBC 835	AGAGAGAGAGAGAGAGYC	50	9	0.3761	250-1250
20	UBC 840	GAGAGAGAGAGAGAGAYT	50	8	0.4955	300-1600
21	UBC 841	GAGAGAGAGAGAGAGAYC	50	6	0.3506	300-800
22	UBC 842	GAGAGAGAGAGAGAGAYG	50	9	0.4988	200-1100
23	UBC 856	ACACACACACACACACYA	50	3	0.0768	500-750

Understanding of taxon and genetic relationship among 50 chickpea germplasms

In plant molecular breeding, the genetic variability is a primary requirement and further, population structure analysis in the selection of elite diverse germplasms (Chakraborty*et. al.,* 2016). The rationale of the genetic structure analysis is to understand the population homogeneousity and genetic variability. Population structure is required for mapping of agronomically important genes and dissection of important traits (Wei et al., 2006).Twenty three ISSRs markers were selected for screening of cross transferability and polymorphism studies in 50 chickpea germplasm. Based on the data from 23 ISSR markers (2 ISSRs were not amplified) dendrogram were constructed that divides 50 chickpea germplasms into two group (group I, & II). In group I, germplasm P 74-1 is highly diverse and remaining 49 chick pea fall in group II which have been further divided in different subgroups. Based on UPGMA dendrogram (Fig.1) we selected diverse 15 chickpea core collection (Table 2).Germplasms P 74-1, P 1883, and P 1260 belongs to I, IIB, IIBb, subgroup respectively(Fig.1). Further in Polymorphic Component analysis (PCA), they fall in cluster I (Fig.2). Additionally in structure analysis they belongs to cluster A (Fig.3). Further it was observed that dissimilarity of P 74-1, P 1883, P 1260 to germplasm P 4051 is 78%, 55%, 54% respectively (Table 2 and Supplementary table 3). Dendrogram, PCA , Dissimilarity matrix and Bayesian model based genetic clustering of 50 chickpea germplasms reveal that P 74-1, P 1883, and P 1260 are very diverse than chickpea germplasms P 4051.

Table 2

Panel of core collection of 15 chickpea germplasms

19. No.	Variety/accession number	Group in dendrogram	Group in PCA	Cluster based on population Structure	Dissimilarity from P 4051 in %
1	P 74-1	I	I	A	78
2	P 1883	IIB	I	A	55
3	P 1260	IIBb	I	A	54
4	P 556	IIBa2	II	A	46
5	P 625	IIBa1v	II	A	40
6	P 886	IIBa1v	II	A	40
7	P 55	IIBa1v	II	A	40
8	P 341	IIBa1v	II	A	40
9	P 1107	IIBa1w	I	A	39
10	P 1137	IIBa1w	I	A	39
11	P 1857	IIBa1x	IV	B	35
12	P 1217	IIBa1y	III	B	33
13	P 1548	IIBa1y	III	B	33
14	P 1781	IIBa1z	III	B	28
15	P 3971	IIBa1z	III	B	16

Further, P 556, P 625, P 886, P 55, P 341 belongs to IIBa2, IBa1v, IBa1v, IBa1v, and IBa1v group respectively. Germplasm P 556, P 625, P 886, P 55, and P 341 falls in cluster II(Fig.1). P 556, P 625, P 886, P 55, P 341 germplasm also belong to cluster A of Bayesian model based genetic clustering of 50 chickpea germplasms(Fig.2). AdditionallyP 556, P 625, P 886, P 55, P 341 has dissimilarity 46%, 40%, 40%, 40%, and 40%, respectively(Table 2 and Supplementary table 3)from chickpea germplasm P 4051. P 1107 and P 1137 which belongs to cluster IIBa1w of UPGMA dendrogram(Fig.1).Additionally P 1107 and P 1137 belong to group I of two dimensional distributions of 50 chickpea germplasms(Fig.2).Germplasms P 1107 and P 1137 belong to cluster A of Bayesian model based genetic clustering of 50 chickpea germplasms(Fig.3). P 1107 and P 1137 both have dissimilarity 39% from chickpea germplasm P 4051(Table 2 and Supplementary table 3).P 1857 falls in group IIBa1x of UPGMA dendrogram (Fig.1).and belongs to IV cluster of two dimensional distributions of 50 chickpea germplasms (Fig.2). It belongs to cluster B of Bayesian model based genetic clustering of 50 chickpea germplasms(Fig.3).Its dissimilarity is 35% from chickpea germplasm P 4051(Table 2 and Supplementary table 3). Germplasm P 1217, P 1548, P 1781, P 3971 belongs to group IIBa1w of UPGMA dendrogram(Fig.1).Germplasms P 1217, P 1548, P 1781, and P 3971 belongs to cluster III of two dimensional distributions of 50 chickpea germplasms(Fig.2).P 1217, P 1548, P 1781, and P 3971 germplasms fall in cluster B of Bayesian model based genetic clustering of 50 chickpea germplasms(Fig.3).All P 1217, P 1548, P 1781, P 3971 has dissimilarity 33%, 33%, 28% and 16% respectively from chickpea germplasm P 4051(Table 2 and Supplementary table 3). So dendrogram of 50 chickpea germplasms based on 23 inter simple sequence repeats (ISSR) markers (Fig.1), ISSR marker based principle component analysis (PCA) showing two dimensional distributions of 50 chickpea germplasms (Fig.2), Bayesian model based genetic clustering of 50 chickpea germplasms (Fig.3), triangle plot of 50 chickpea germplasms (Fig.4) reveals that all 15 core collection of chickpea are very diverse and could be used to molecular breeding by utilizing these ISSR markers.

Identification of early flowering and high seed germination lines

Present study was carried out on chickpea genotype to identify early flowering and high seed germination lines.In current study, 50 individuals were DNA fingerprinted and 15 diverse chickpea minicore collection developed. All selected 15 chickpea lines screened for early flowering and high seed germination.Among these 15 individuals, four were identified under early flowering and six were high seed germination lines.Among 15 diverse chickpea P 1857-1 and P 3971 have both early flowering and high seed germination trait compared to P 1883 and other germplasms. Among these 15 lines,P 1857-1 and P 3971 both showed strong correlation among themselves for early flowering and high seed germination compared to other 13 individual(fig-5b,6b)The chickpea correlation heatmap matrix(fig-5a,6a) of the early flowering and high seed germination linesfurther confirmed the DNAgenotype and heatmap data of the obtained for early flowering and high seed germination lines for the identification of early flowering and high seed germinationlines.

Therefore information about relatedness of various chickpea within and between group I, and II provides radiant prospect to bring various agronomically desirable traits such as of early flowering and high seed germinationfrom donor chick pea to elite cultivated chickpea through marker assisted selection and breeding. Characterization of these diverse chickpeas would help in maintenance breeding, conservation and could be used in future to develop climate resilient chickpea to assist in food security.

Conclusion

ISSRs in genome influence activity and function of other nuclear and organelle coding gene due to their repeat length. Unfortunately, ISSR marker resources for chickpea have not been well harnessed for genotypic improvement of chickpea. Therefore, a set of 23 ISSRs have been used to expedite molecular breeding of chickpea. High cross transferability and polymorphism of these ISSR markers further reveal their novelty. Utilization of these novel ISSRs markers in diversity analysis and population structure characterization of 50 chickpea germplasm suggests their wider efficacy in superior scale for molecular breeding studies in chickpea. The study of genetic diversity and population structure of 50 chickpea and development of 15 chickpea core collection will serve as important knowledge resources for future studies like GWAS, and mapping.Identified P 1857-1 and P 3971 early flowering and high seed germination would act as a breeding material for introgression of early flowering and high seed germination trait into elite cultivar.

Declarations

Author Contribution statement

DJ, and KJ Conceived and designed the study. GY, AK and JT performed the experiment. ANS, AK, RC, AK, SK and JK helped in the preparation of draft manuscript. SK and JK performed the critical revision of the article. SPJK edited the final draft. All authors approved the final version of the article.

Ethical approval

This article does not contain any studies with animals performed by any of the authors.

Compliance with Ethical Standards

No funding was received for the current research.

Acknowledgements

Financial assistance for research by Indian Council of Agricultural Research-Indian Institute of Seed Sciences, Mau is gratefully acknowledged. Further ICRISAT,Hyderabad is gratefully acknowledged for

providing chickpea germplasms.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary Table

Supplementary Table 3 is not available with this version.

Figures

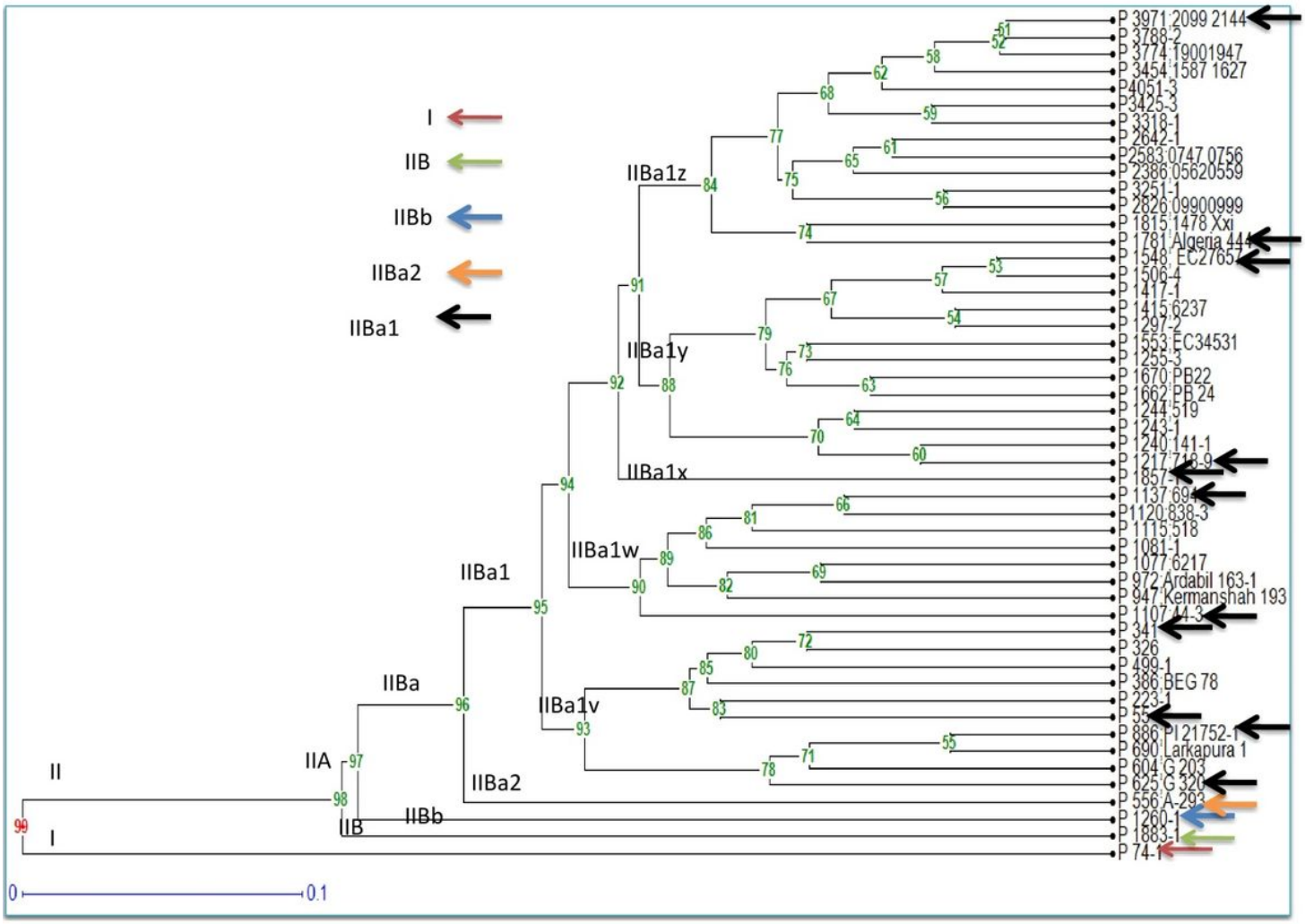

Figure 1

Dendrogram of 50 chickpea germplasms based on 23 inter simple sequence repeats (ISSR) markers

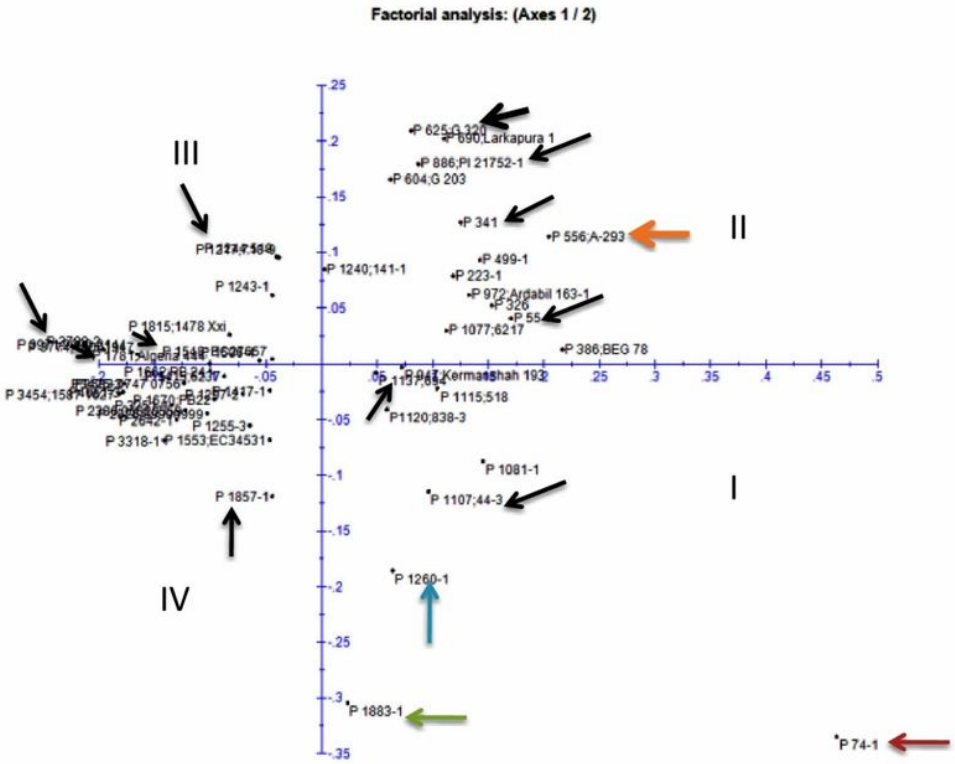

Figure 2

ISSRmarker based principle component analysis (PCA) showing two dimensional distributions of 50 chickpea germplasms

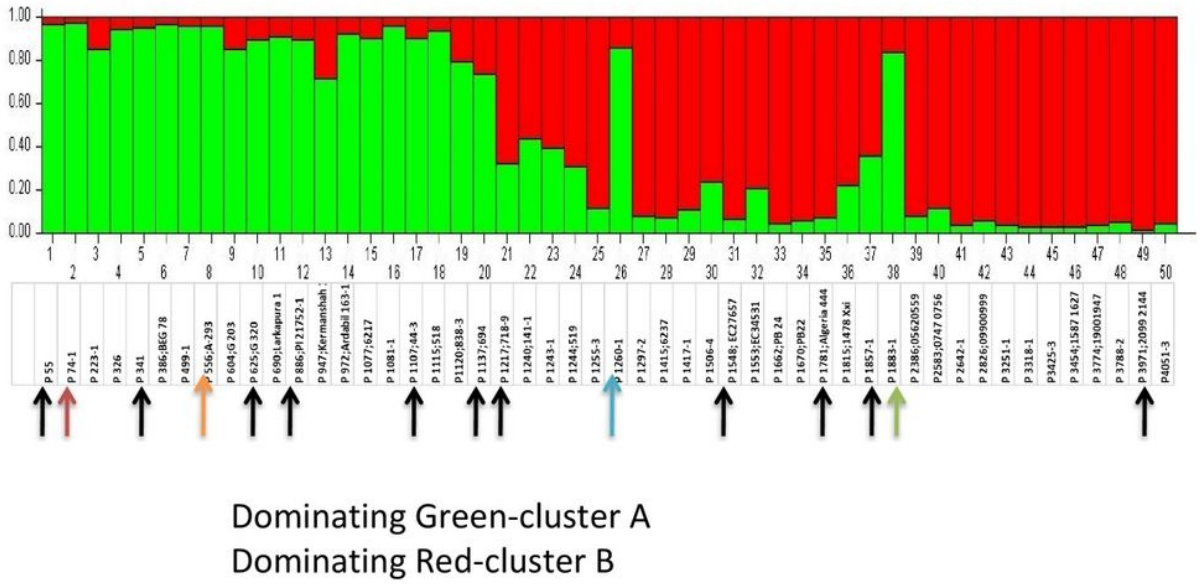

Figure 3

Bayesian model based genetic clustering of 50 chickpea germplasms

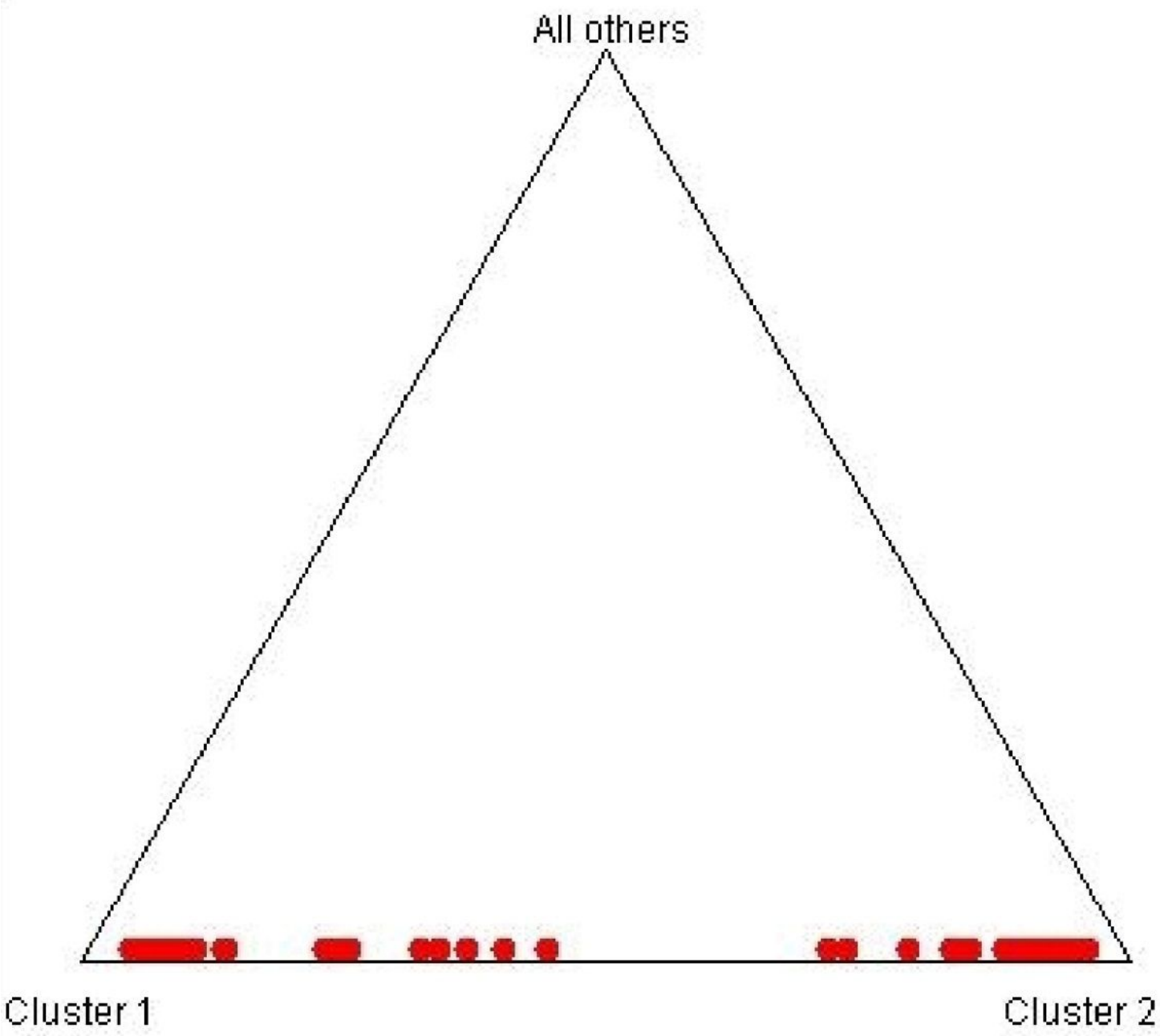

Figure 4

Trianle plot of 50 chickpea germplasms

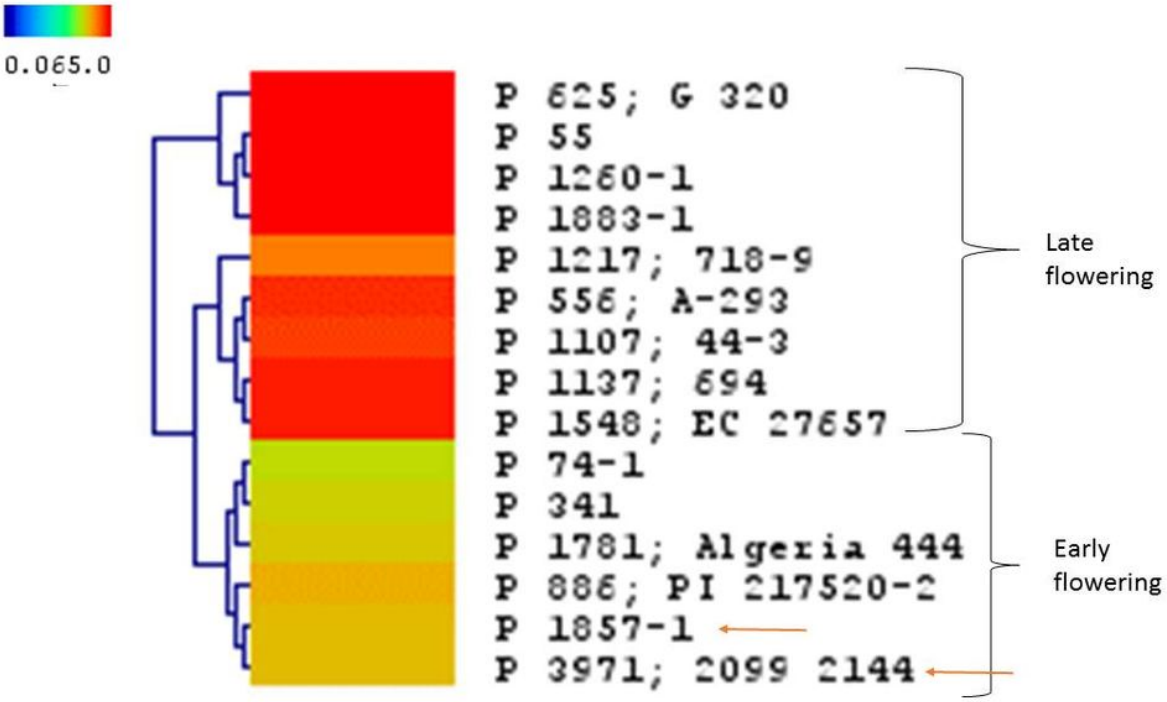

Figure 5

Heat map depicting early flowering profile of 15 chickpea germplasms

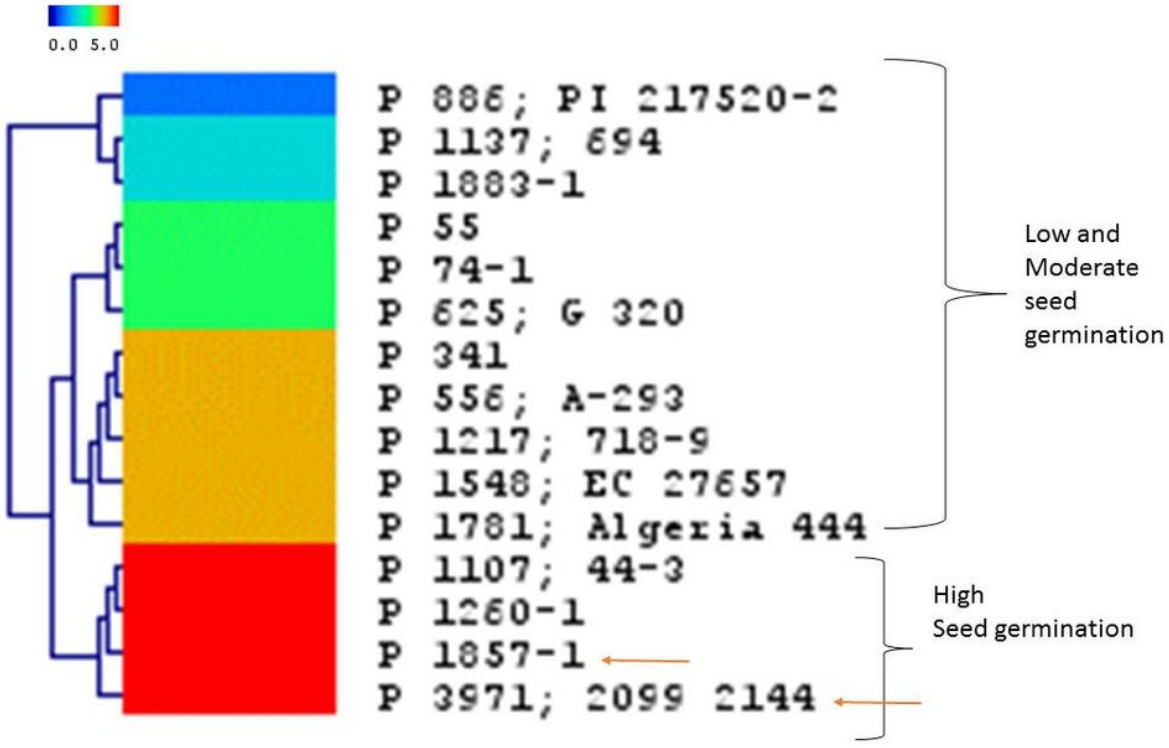

Figure 6

Heat map depicting high seed germination profile of 15 chickpea germplasm

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1.docx
- Supplementarytable2.docx