Genetic association of geographic populations and species of lac insects of the genus *Kerria* (Hemiptera: Coccoidea) with EST-microsatellite markers

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Abstract
Lac insects belonging to the genus Kerria are commercially exploited for lac which has diversified industrial applications. India is the global leader in lac production; much of commercial lac is derived from the Indian lac insect, Kerria lacca (Kerr). Molecular marker studies of Kerria species are limited to RAPD and ISSR. We evaluated genetic diversity among 27 Kerria geographic populations belonging to thirteen species of Kerria, using 31 microsatellite markers developed from transcriptome of the Indian lac insect. The number of alleles per locus ranged between 2 and 6 with a mean of 2.74, showing polymorphism of 66.7-100%. UPGMA dendrogram based on Jaccard's similarity coefficient grouped 27 Kerria accessions into three major clusters consisting of 2, 13 and 12 populations respectively and their similarity coefficient ranged between 0.61 and 0.95.

Based on similarity coefficients, the closest relationship was observed between Kerria pusana and Kerria pennyae and also between K. lacca and K. pennaye. K. chinensis appeared well separated from all other Kerria species studied. Geographically closer species were found more similar, in general. The present study reveals wide intra- and inter-specific genetic diversity among Kerria populations through EST-microsatellite markers and indicates the potential and usefulness of marker studies for lac insect improvement programmes.

Keywords: EST, Genetic diversity, *Kerria* spp, Lac, Simple Sequence Repeat, Scale insect.

Introduction

Genus *Kerria* (Hemiptera: Coccoidea: Kerriidae) commonly called lac insects holds the distinction of the only genus in the animal kingdom producing resin. Lac insects also secrete wax and produce dye. Lac insect products are preferred in the present era over synthetic products due to their unique physical, chemical properties and environmentally safe nature[1,2]. These products have diverse applications in food, pharmaceuticals, cosmetics, perfumes, varnishes, paints, polishes, adhesives, jewellery and textiles since ancient times[3-5].

Twenty-seven species of *Kerria* have been described so far[6]. Few species of *Kerria* are commercially exploited in India, China and some South East Asian countries. India being the global leader in lac production harbours more than 20 percent of lac insect biodiversity[7]. The most widely distributed species in India is the Indian lac insect, *Kerria lacca* (Kerr). Two infra subspecific forms of *K. lacca* which differ in duration of life cycle, host plant preference and quality of resin secreted have been reported from India[8]. *Rangeeni* infra subspecific form prefers *Butea monosperma* as host plant over *Schleichera oleosa*, whereas *kusmi* infra subspecific form prefers *S. oleosa* over *B. monosperma*. Other minor cultivated species in India include *Kerria sharda*[9] and *Kerria chinensis*[10].

Lac insects collected from different parts of the country are maintained by ICAR-Indian Institute of Natural Resins and Gums (IINRG), Ranchi, India. Based on the morphometrics, these lac insect lines were identified and grouped into 15 species[11]. The rapidly changing ecosystem, depletion of host trees and discontinuation of lac cultivation by lac growers in some regions are major reasons for the erosion of lac insect biodiversity[7]. Hence, there is an immediate and urgent need for conservation of lac insect genetic resources.

Molecular markers are one of the useful tools which aid in conservation of the unique genetic resources of lac insects. So far, genetic diversity of lac insect lines has been studied using only RAPD[12] and ISSR[1] markers. However, they are dominant markers and not specific for lac insects. Hence, there is a need to use lac insect specific markers for analysing the genetic diversity of lac insects. Polymerase chain reaction (PCR) based SSRs (Short sequence repeats) or microsatellites which are polymorphic, co-dominant, evenly distributed throughout the genome are markers of choice for population genetic analysis[13].

EST libraries could be considered as a valuable source of SSR markers for population genetic studies in insects and other animals[14]. EST-SSRs were markers of choice in population diversity studies in hemipteran insects such as flower thrips[15], bird cherry oat aphid[16], brown plant hopper[17] and rice plant hoppers[18] etc.

We have generated transcriptome of *K. lacca* adult female insects (https://www.ncbi.nlm.nih.gov/sra/SRX756856 [accn]) and designed numerous SSR primers from it. The primers developed from transcriptome data have been

utilized in this study for deciphering genetic diversity of 26 lac insect lines collected from different parts of India and one exotic line (from Thailand) including 13 species and 1 cross bred line.

Material and Methods

Sample Collection: Twenty-seven lac insect lines were studied (table 1) in the present investigation. These included populations of thirteen species of *Kerria* (*K. lacca*, *K. sharda*, *K. chinensis*, *Kerria pennyae*, *Kerria dubeyi*, *Kerria varshneyi*, *Kerria pusana*, *Kerria chamberlini*, *Kerria indicola*, *Kerria fici*, *Kerria ebrachiata*, *Kerria brancheata*, *Kerria thrissurensis*) from the principal lac growing states of India, geographical races, cross-bred lines, infra-subspecific forms namely *kusmi* and *rangeeni* and different coloured lac insects as described by Ahmad and co-workers[11]. The lac insect samples were obtained from the lac insect field gene bank of National Lac Insect Germplasm Centre (NATLIGEC), IINRG campus, Ranchi (23°19'51" N 85°22'18" E; Elevation ~2080 ft), India.

The lac insect populations were maintained on the lac host plant, *Flemingia macrophylla* grown in pots under protected conditions. The lac insect cultures were kept enclosed in synthetic mesh sleeves (60 mesh) to protect them from parasitoids and predators. The cultures were regularly sprayed with carbendazim (0.01%) to protect them from fungal infestation.

Preparation of Specimens and DNA Isolation: Mature female lac insects weighing up to 50 mg were kept in absolute ethanol for overnight at room temperature (20-25 ºC) to dissolve the resinous cover. The insect bodies were separated, cleaned and serially washed with 70% ethanol to get rid of protective resin, waxy secretions and other impurities. The insects were then stored in absolute ethanol in 1.5 ml micro centrifuge tubes at -20 ºC. Genomic DNA was isolated from lac insects adopting HiPurA™ insect DNA purification kit (HiMedia, MB529-50PR), with some minor modifications such as precipitating the DNA at -20 ºC for overnight and incubating the column at 65 ºC for 5 minutes before elution of DNA. The isolated DNA was suspended in 50-60 µl of elution buffer. The extracted DNA was quantified in Nanodrop 2000 spectrophotometer (Thermo Scientific) and was also checked on 1% agarose gel.

SSR Amplification: The software WebSat was used to find the SSRs in the transcriptome data of *K. lacca*[19]. Eighty-six primer pairs were designed from the transcriptome data. Primer3 was used to design primers flanking SSRs in the transcriptome sequences. The main design parameters were amplicon size (approximately 110–450 bp), TM (≥ 45 °C) and lack of secondary structures (self-dimers, primer dimers)[20]. The details of primers are given in table 2. The target regions of the lac insect genomic DNA were amplified in thermocycler (Sensoquest, Lab cycler). The amplification cocktail was prepared by mixing 0.5 µL of forward primer and 0.5 µL of reverse primer (10 pmol/µL concentration of each primer); 1.25 µL of PCR buffer; 0.1 µL of 25 mM dNTPs; 1.25 µL $MgCl_2$ (25 mM); 1.0 µL of DNA template (25 ng/µL); 0.15 µL of *Taq* DNA polymerase (Fermentas, 5 units/µL) and 7.0 µL of sterile distilled water to a final volume of 12.5 µL.

The annealing temperature for each primer pair was determined following the thermal program: an initial 5 minutes denaturation at 94 ºC, followed by 40 cycles of 1 minute at 94 ºC, 30 seconds at 50–58 ºC and 45 seconds at 72 ºC with a final extension at 72 ºC for 10 minutes. The PCR products were checked on 3% agarose gel along with 50 bp DNA ladder (Fermentas). The gel was stained by incorporating ethidium bromide (0.2 µg/ml) and documented using bioimaging system (Syngene). All the PCRs were repeated twice to ensure reproducibility.

Data Analysis: To analyze the genetic association among 27 *Kerria* genotypes assayed with 31 microsatellite markers, each band was considered as a single locus/allele. Data analysis was performed for evaluation of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products. It was used to generate similarity matrices on the basis of Jaccard's coefficients[21]. NTSYSpc 2.02i software package was used for dendrogram construction using unweighted pair group method with arithmetic means (UPGMA) according to Rohlf[22].

The estimation of genetic diversity parameters effective number of alleles (ne), percentage of polymorphic loci, observed (Ho) and expected heterozygosity (He), Shannon's information index of diversity (I), Nei's gene diversity (H) with deviations from the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium between loci were tested using POPGENE v. 1.31[23].

Results

Characterization of SSR loci: From the transcriptome data 494 di-nucleotide repeats, 909 tri repeats, 156 tetra repeats, 27 penta repeats and 6 hexa repeats were found. Out of 86 SSR markers developed from the transcriptome data, 31 produced polymorphic, satisfactory, clear and reproducible banding patterns for all the genotypes used herein and 55 did not produce either any or clear banding patterns. Out of these 31 markers, 14 produced more than expected size bands. Seven markers produce multiple banding patterns (table 2) and total of 85 amplification products were obtained across the genotypes studied. Among 31 selected primers on the basis of reported intra-*Kerria* polymorphism, 7 were found informative with polymorphism rate of 66.67%, primers MS 153, MS 123 and MS 122 revealed polymorphism rate of 75%, 80% and 83.3% respectively; the remaining 23 primers showed 100% polymorphism.

The number of alleles per locus ranged from 2 to 6 with mean number of alleles per locus as 2.74 and the size of amplified products ranged from 154 bp (for the marker MS

102) to 400 bp (for the marker MS 186). Private alleles were also observed in few genotypes. The species *K. chinensis* showed private alleles with the markers, MS109, MS113, MS123, MS149 which could be used for easily distinguishing it from other genotypes.

Besides this, the markers MS109 and MS110 revealed private alleles for LIK0017; MS108 for LIK0031; MS122 for LIK0060; MS156 for LIK0013. Few rare alleles have also been exhibited by lac insect accessions in this study; for instance, MS122 for LIK004 and LIK029; MS123 for LIK0020 and LIK0060. The functional products coded by these unigenes with SSS loci are also given (table 2).

Pair-wise comparison was performed among all the accessions. Jaccard's similarity coefficients as suggested by Nei[21] varied from 0.52 to 0.95 with a mean value of 0.735. The similarity coefficients for *K. sharda* varied from 0.62 to 0.84 with a mean value of 0.73 and for *K. chinensis*, similarity coefficients ranged from 0.52 to 0.82 with a mean value of 0.67 and for *K. lacca*, data varied from 0.52 to 0.95 with a mean value of 0.735. The similarity coefficients for *K. pennaye* varied from 0.59 to 0.94; for *K. ebrachiata* varied from 0.60 to 0.95; for *K. dubeyi* ranged between 0.62 and 0.85, for *K. thrissurensis* varied from 0.68 to 0.90, for *K. fici* ranged from 0.55 to 0.86, for the species *K. brancheata* ranged from 0.57 to 0.82, for *K. chamberlini* varied from 0.54 to 0.92, for *K. indicola* varied from 0.52 to 0.91, for *K. varshneyi* ranged between 0.63 and 0.91 and for *K. pusana* varied from 0.60 to 0.94 (table 4).

Interestingly, *rangeeni* and *kusmi* infra subspecific forms of *K. lacca* appear to be well separated from each other with JSC ranging between 0.55-0.91 for all the lines studied with mean and mode of 0.71 and 0.68 respectively. Comparisons of paired JSC showed a slightly positive skewed distribution (fig. 2). The least similarity was shown by *K. chinensis, K. indicola* and also by *K. lacca* and *K. pennyae* and the maximum similarity by *Kerria pusana* and *Kerria pennyae* and also between *K. lacca* and *K. pennaye*. Few comparisons were also made to understand the relations observed in JSC between different species used in the study (fig. 3).

Diversity among *Kerria* spp.: Effective number of alleles, Nei's genetic diversity, Shannon's information index, observed and expected heterozygosity were calculated for each locus (table 3). Observed and expected heterozygosity ranged from 0.02 (LIK0004, *K. ebrachiata*) to 0.49 (LIK0002, *K. lacca*) and from 0.01 (LIK0019, *K. lacca*) to 0.48 (LIK0005, *K. ebrachiata*) respectively. The effective number of alleles per locus ranged from 1.00 to 1.99 with an average of 1.39. Shannon's information index averaged 0.39 and showed the same trend as expected heterozygosity. Nei's genetic diversity showed a range of 0.08-0.69 with all 27 lac insect genotypes.

Genetic relationships among lac insect lines: The UPGMA dendrogram based on Jaccard's similarity coefficient revealed that 27 *Kerria* accessions were grouped into three major clusters consisting of 2, 13 and 12 genotypes, respectively (fig. 1) and similarity co-efficient ranged from 0.61-0.95. Cluster I consisted of two genotypes (LIK0031 and LIK0023); both *K. chinensis* lines. Cluster II, the largest group could be further sub-divided into two sub-clusters (IIA and IIB); IIA consisted of 1 *K. lacca* and 1 *K. indicola* (LIK0028 and LIK0029) accessions, both *rangeeni* lines from Jharkhand and Chattisgarh respectively; IIB consisted of 11 accessions (LIK0015, LIK0016, LIK0017, LIK0018, LIK0019, LIK0021, LIK0020, LIK0060, LIK0063, LIK0061, LIK0065), all *rangeeni* lac insect lines (from Gujarat, Maharashtra, Uttar Pradesh, Andhra Pradesh, West Bengal and Punjab) and one cross bred line developed at IINRG, Ranchi. This cluster consisted of different species including *K. lacca* lines (LIK0016, LIK0017, LIK0018, LIK0019, LIK0021), *K. chamberlini* lines (LIK0015, LIK0060 and LIK0061), *K. indicola* (LIK0020), *K. varshneyi* (LIK0063) and *K. pusana* (LIK0065).

Cluster III consisted of 12 accessions, which could be sub-divided into two sub-clusters (IIIA and IIIB); IIIA, the only cluster having *kusmi* lines consisted of 6 accessions (LIK0001, LIK0003, LIK0002, LIK0004, LIK0005, LIK0006) from Chhattisgarh, Jharkhand, Odisha and one *K. sharda* line from West Bengal. Out of these, LIK004 and LIK005 were *K. ebrachiata* lines, LIK001 was *K. pusana* line and LIK0003, LIK0002 were *K. lacca* lines. Cluster IIIB consisted of 6 accessions - *K. lacca*, *K. dubeyi*, *K. thrissurensis*, *K. fici*, *K. brancheata*, all *rangeeni* lines from Karnataka, Kerala, Rajasthan, Punjab and Jammu and Kashmir (LIK0008, LIK0010, LIK0011, LIK0013, LIK0014) and one *K. sharda* line from Odisha (LIK0007).

Discussion

Genetic diversity in genotypes of the economically important lac insects is pre-requisite to cope with environmental changes and was typically described using polymorphism by dominant markers such as RAPD[12] and ISSR[1]. Lac insects have limited genomic resources especially expressed sequence based (genic) markers to be used in genetic improvement programmes. The present paper employs a co-dominant EST-SSR marker to unravel the genetic diversity existing among lac insect genotypes. EST-SSR markers have been developed from the transcriptome data and used in this study for analyzing genetic diversity of different species of lac insects. Transferability or utility of EST-SSR markers among different *Kerria* species seems to be very high since the markers developed from *K. lacca* worked well with twelve other species of *Kerria*.

The present study aimed at evaluating genetic diversity among 27 *Kerria* genotypes using EST-SSR markers. Out of the 86 microsatellite markers, 31 produced polymorphic, satisfactory, clear and reproducible banding patterns for all the genotypes. The ratio of markers amplifying to the markers developed varied widely. In *D. virgifera*, 17 out of

38 markers amplified successfully[14]; in *Frankliniella occidentalis*, 18 out of 72 markers amplified successfully[15]. The number of alleles per locus ranged from 2 to 6 and mean number of alleles per locus was 2.74. The highest and lowest number of alleles were obtained for tri repeat markers (table 2).

Yang and co-workers[15] categorized 18 novel highly polymorphic EST-derived microsatellites marker in *F. occidentalis* for their population genetic studies and the number of alleles ranged from 2 to 15, with an average of 5.50 alleles per locus. It is known that a greater number of alleles and higher PIC value are expected for wild type genotypes.

Application of known polymorphic primers is helpful to get more information[24,25]. Results from this study indicate that the high level of intra specific polymorphism obtained is highly useful for primer selection in genetic diversity studies and also in breeding programmes involving *Kerria* spp. In the present investigation, tetra repeat markers showed 100% polymorphism whereas tri repeat markers showed polymorphism from 66.67% to 100% (table 2).

Both lines of *K. chinensis* form a distinct outgroup (I) from the rest of twelve Indian species of *Kerria* studied. This is similar to the results obtained from RAPD[12] and ISSR studies[1]. All the remaining twelve Indian species of *Kerria* appear to have descended from a common ancestor, forming a large clade comprising of two subgroups (II and III). The subgroup II consists of nine species of *Kerria;* This group contains only two populations of *K. lacca*: one *rangeeni* form from Rajasthan and one *rangeeni* population from Jharkhand.

The subgroup III contained the *kusmi* form of *K. lacca* which is adapted for the host *S. oleosa* along with *K. pusana*, *K. pennyae* and *K. sharda*, all of which prefer *S. oleosa* as their host. The subgroup III contains thirteen lines, which includes six lines of ubiquitous *K. lacca* and seven populations of four species. Since lac insects are immobile during most of its life, there is a limited chance for dispersion of the insects and mixing of genes between populations and as a result, higher similarity exists between populations of neighbouring geographical locations[1]. Though there is a tendency of geographically nearer populations/species to cluster together (Group II: LIK0028, LIK0029; LIK0015, LIK0016, LIK0017; LIK0060, LIK0061; Group III: LIK0001, LIK0003; LIK0002, LIK0004, LIK0005; LIK0013, LIK0014), there are several instances of geographically distant populations to be close to each other (Group II: LIK0018, LIK0019, LIK0021, LIK0020; Group III: LIK0007, LIK0008; LIK0010, LIK0011; LIK0063, LIK0065). This could be due to lac culture practices.

The lac insect population of different locations are cultured in the same location which could have led to mixing of gene pools. The dendrogram showed a very close similarity between LIK0004 and LIK0005, *rangeeni* lac insects from different regions of Jharkhand although one is crimson coloured insect and another is yellow coloured insect. Similarly, *rangeeni* lac insects from Gujarat (LIK0015 and LIK0016) shared a close genetic relationship.

Two populations of *K. pusana* from adjoining States, Chhattisgarh and Madhya Pradesh are distantly located in the dendrogram with a JSC of 0.66. Such genetic dissimilarity could be due to interbreeding between different populations due to natural processes or due to human interventions, as these two populations are from cultured ones for lac production. The JSC values of *K. chamberlini* of two populations from West Bengal, were 0.71 and 0.76 versus population of same species from Gujarat; the latter clustered with geographically proximal populations of *K. lacca*. All the three populations of *K. chamberlini* showed common ancestry (IIB).

Although *K. sharda* (LIK0006 and LIK0007) is described as a separate species and trivoltine in nature[9], it is grouped along with other *K. lacca* lines. Similar result was obtained in previous study of lac insects with ISSR markers as well[1]. Crossbreeding experiments using different geographic lac insect populations have shown absence of reproductive barriers among the Indian species of *Kerria* (Ramani and Sharma, unpublished). Only *K. chinensis* has shown reproductive isolation with *K. lacca*, with infertile F_1 progeny[26]. Occasionally cross breeding happens either in nature or in laboratory condition.

Cross breeding experiments have shown hybrid vigour resulting in 40-100% increase in lac production[27]. The trivoltine cross breed (LIK 0021) was developed in such an attempt at ICAR-IINRG, Ranchi. It is interesting to note that the dendrogram position of LIK0021, a cross bred line is relative to its parental lines, LIK0003 (*K. pennyae*) and LIK0007 (*K. sharda*).

The two infra subspecific forms *kusmi* and *rangeeni* do not separate into distinct clusters. In one instant, *kusmi* insects and *rangeeni* insects from neighbouring states clustered together in a sub cluster (III A). *Kusmi* insects are concentrated mainly in the eastern part of peninsular India and *rangeeni* insects are distributed throughout the country[12]. The *kusmi* and *rangeeni* forms also interbreed under laboratory conditions but the chances of crossbreeding could be rare under natural conditions due to differences in the life cycle creating asynchrony of the sexual maturity periods. Such breeding structure prevailing in *Kerria* probably accounts for the relationships among the different populations of *Kerria* in the dendrogram.

It is also reasonable to assume that these two forms could have been evolved from a common ancestor. The evolutionary/divergence history of the two forms can be studied in future using Ka/Ks values of some conserved genes.

A look at the similarity of population(s) of a given species of *Kerria* with other species reveals interesting patterns. The mean similarity (JSC) between different species varied from 0.57 to 0.89. Distribution of paired comparisons of JSC showed a slightly positive skewed distribution (fig. 2) due to genetic distance of *K. chinensis* from all other species examined. Fig. 3 depicts select instances to demonstrate the variations observed in similarity between different species investigated.

The mean similarity of given species with that of all remaining species, varied between 0.71 and 0.74 except *K. chinensis* and *K. thrisssurensis*; the values were 0.62 and 0.78 for the former and the latter respectively. *K. thrissurensis* showed a similarity of 0.69 with *K. chinensis*, which is highest for paired comparison of *K. chinensis* with other species. *K. lacca*, which is widely distributed and in India, showed a similarity of 0.70 to 0.78, except *K. chinensis*, with which it was 0.62.

Lac insects possess different body colour ranging from crimson, yellow, cream and albino (white). Wild type crimson insect possesses crimson body colour and produces yellow resin whereas yellow insect possesses yellow body colour and produces yellow resin. The yellow body colour gene is a simple recessive gene mutant[28]. In some natural populations both crimson and yellow insects coexist (LIK0010, LIK0014, LIK0028 and LIK0060). Although the dendrograms based on SSR markers does not differentiate between yellow and crimson colour insects, either a greater number of SSR markers or use of capillary electrophoresis or markers like SNPs (Single Nucleotide Polymorphisms) may be of great help in this regard.

Table 1
Details of the 27 lines of *Kerria* spp used in the study

S. N.	Code No	Species	Life cycle type	Colour form	Place of collection
1	LIK0001	*K. pusana*	*Kusmi* early maturing	Crimson	Korba, Chhattisgarh
2	LIK0002	*K. lacca*	*Kusmi* late maturing	Crimson	Ranchi, Jharkhand
3	LIK0003	*K. pennyae*	*Kusmi*	Yellow	Sundergarh, Odisha
4	LIK0004	*K. ebrachiata*	*Rangeeni*	Crimson	Palamau, Jharkhand
5	LIK0005	*K. ebrachiata*	*Rangeeni*	Yellow	Bokaro, Jharkhand
6	LIK0006	*K. sharda*	*Trivoltine*	Crimson	Medinipur, West Bengal
7	LIK0007	*K. sharda*	*Trivoltine*	Crimson	Sarat, Mayurbanj, Odisha
8	LIK0008	*K. dubeyi*	*Rangeeni*	Crimson	Bangalore, Karnataka
9	LIK0010	*K. thrissurensis*	*Rangeeni*	Crimson + Yellow	Thrissur, Kerala
10	LIK0011	*K. lacca*	*Rangeeni*	Yellow	Udaipur, Rajasthan
11	LIK0013	*K. fici*	*Rangeeni*	Crimson	Ludhiana, Punjab
12	LIK0014	*K. brancheata*	*Rangeeni*	Crimson + Yellow	Jammu, Jammu & Kashmir
13	LIK0015	*K. chamberlini*	*Rangeeni*	Crimson	Ambaji, Banaskantha, Gujarat
14	LIK0016	*K. lacca*	*Rangeeni*	Yellow	Alsipur, Chotaudepur, Gujarat
15	LIK0017	*K. lacca*	*Rangeeni*	Crimson	Ahmednagar, Maharashtra
16	LIK0018	*K. lacca*	*Rangeeni*	Yellow	Aurangabad, Maharashtra
17	LIK0019	*K. lacca*	*Rangeeni*	Crimson	Bhathat, Maharajganj, Uttar Pradesh
18	LIK0020	*K. indicola*	*Rangeeni*	Crimson	Echoda, Andhra Pradesh
19	LIK0021	*K. lacca*	Crossbred, Trivoltine	Yellow	Developed at IINRG, Ranchi, India
20	LIK0023	*K. chinensis*	*Rangeeni* type	Crimson	Thailand
21	LIK0028	*K. lacca*	*Rangeeni*	Crimson + Yellow	Bokaro, Jharkhand
22	LIK0029	*K. indicola*	*Rangeeni*	Yellow	Korba, Chhattisgarh
23	LIK0031	*K. chinensis*	*Rangeeni*	Crimson	Nongpoh, Meghalaya
24	LIK0060	*K. chamberlini*	*Rangeeni*	Crimson + Yellow	Purulia, West Bengal
25	LIK0061	*K. chamberlini*	*Rangeeni*	Crimson	Bankura, West Bengal
26	LIK0063	*K. varshneyi*	*Rangeeni*	Crimson	Patiala, Punjab
27	LIK0065	*K. pusana*	*Kusmi*	Crimson	Bankhedi, Madhya Pradesh

Except *K. sharda*, other species are bivoltine

Table 2
Primers, sequence information, annealing temperature, repeat motif, fragment size, number of alleles and polymorphism (%) of 31 SSRs used in this study

S. N.	Primers	Primer Seq F (5'-3') /R (3'-5')	Annealing temp. (^{0}C)	SSR motif	Fragment size	Number of alleles	Polymorphism (%)	Name of the transcript
1	MS102	F: TTGTACCTGTTTCTCGACCAAT R: ATGTTCAATCGTGGCTCATAAA	58	(ATA)14	154	5	100	SH3 domain-binding glutamic acid-rich protein
2	MS103	F: AATAGTTCTTCCGTAGCCGATG R: CAGATTTTGGCTCGACCTAGAC	58	(CAG)6	177	2	100	afadin
3	MS107	F: GCACTTCTTAATCCAAGAGGGA R: GCTGCGTGCCATAATACAAATA	50	(TTTA)10	195	2	100	Insulin-like growth factor 2 mRNA-binding protein
4	MS108	F: GTTCCCCAGCTATTGAAACAAG R: CGACGACAACAATGACAACTC	50	(ATC)8	204	2	100	WD repeat-containing protein 11
5	MS109	F: ACCATACGCCACAATTTCAGTA R: AGTTGCTTTTACAACGAGCACA	58	(AAT)6	206	3	100	protocadherin Fat 4-like
6	MS110	F:GTTCCCCAGCTATTGAAACAAG R: CGACGACAACAATGACAACTC	58	(ATC)9	207	2	100	hemicentin-1 isoform X1
7	MS112	F: AAATTAGTCAGAATCGTGCGGT R: CTGTTGGATTATTGGCACTTGA	58	(ACA)7	230	2	100	cuticular protein
8	MS113	F:AAAAGGTTTGCCTAAAAGGGAG R:GAAGAGCCTAATCCACGACAAT	56	(TAA)7	232	2	100	hypothetical protein
9	MS114	F: TAATACGAACGGACACACTTGC R:GTGACTGAGAGAAAATGCGAGA	58	(ACA)7	237	2	100	novel gene
10	MS118	F:AGTATAGGGAATGTTCTATTTTCCG R: TCCTTCATTTTCGTGTACCATC	58	(ATTT)8	245	2	100	novel gene
11	MS119	F: ATCAAGTGGTGGCTTCTGC R: TTTGTGGGTAAATGCTATGTGG	56	(TCG)9	246	2	100	lysine-specific demethylase 3B isoform X5

12	MS122	F: ATCCAACAGGAGATGTTTTCGT R: CCGATGCGGAATTAGAGAAAT	56	(TTC)7	258	6	83.3	reticulocyte-binding protein 2-like protein
13	MS123	F: TGTTCTGGGAATGACAGCAGTA R: TTTGAAGCATGGTCGTAACATC	56	(TGG)7	268	5	80.0	DNA repair protein RadA
14	MS124	F: CAACCTATCCGTCATCTCAACA R: GGATGCGTAATCGGGTTTATAG	56	(TAA)9	268	3	100	transport protein
15	MS125	F:AACTGAAAATGCAAATAGCCGT R: CATCGTCATCATCCAAATCATC	56	(GAC)7	269	2	100	transcription initiation factor IIA subunit 1 isoform 1
16	MS126	F: TAATTTTGGAAACGAAACATCG R:GCAGCAAAGGAAAAGAATTTAGT	53	(AAT)9	272	2	100	hypothetical protein
17	MS127	F: ACACAGGTACGCGAATTTAACA R: AAGCACTTCGATCAGTCGG	52	(ATA)20	272	3	100	ADP-ribosylation factor-like protein
18	MS128	F:CAAAGGCTCGTAGATACACAATTAG R: CCAAATCAACCACAGGATCA	55	(AAT)7	276	4	100	nuclear ribo-nucleoprotein U-like protein 1 isoform X2
19	MS132	F: CCTGTGAAACGTGCAAAACTTA R:AGCACTGATTGTGGTTGAAAAG	52	(AAT)17	283	3	66.67	ubiquitin carboxyl-terminal hydrolase
20	MS134	F:AAATACACCATCGAAAGATCGG R: GCGTAATTTGGCACTCGTTT	54	(ATT)16	290	3	100	short-chain dehydrogenase
21	MS136	F: CGAGCAGCAGAGTGTTCTCTTA R: CTTGATTTGCACGCAGTATCTC	56	(AGT)9	299	2	100	protein phosphatase
22	MS147	F: CGGGAGGAACGAGATAAAA R: ACGTAAAACTGAAAAGGCTCC	54	(TAAA)8	318	2	100	novel gene
23	MS149	F: AATGGTGAAGATGAGGCAAAC R: TGAGACGAATGTCAATTTCCG	55	(GAA)8	320	3	100	nuclear autoantigenic sperm protein
24	MS153	F: AATCGTTGTTATCCGACGTACC	55	(ATT)14	331	4	75.0	cAMP-specific 3',5'-cyclic

		R: AAATTCCTCCCGCACATTTA						phosphodiesterase
25	MS156	F: CCAGTTCGTTCAGCTTTCATTT R: TCTCCAAAATTATCCTCGCTTG	56	(GAT)9	335	2	100	uncharacterized protein
26	MS157	F:TAAGCGAGCAACGAGAAGTGTA R: GGAATGCTAATCGTCTATGGGT	54	(TAT)15	339	2	100	retinoid receptor
27	MS159	F: GGAATTTGTGCGAAACGTATTT R:TCAGAGAATGACTACAACGACCA	58	(TTC)7	351	2	100	novel gene
28	MS160	F: GCCGACAACATCGTTACACTTA R: CGCTGATCGAAATACATACGC	54	(ATTT)6	353	3	100	multiple PDZ domain protein
29	MS170	F:AACTTGACGAACCAAAATGAGC R:TAAAATGGAAAACAGCCACCAT	56	(TAT)18	365	3	66.67	novel gene
30	MS184	F:CCGTATGTATCGTAAAGGTATGGT R: CTTGTCCGTCAGCAGATGTAAT	58	(TTA)18	399	3	66.67	MAP kinase-interacting serine/threonine-protein kinase 1
31	MS186	F: GCGAAAATGTAGTTTGTGCC R: AGGCCATTCACCAGCATA	56	(TAA)13	400	2	100	myotubularin-related protein 4

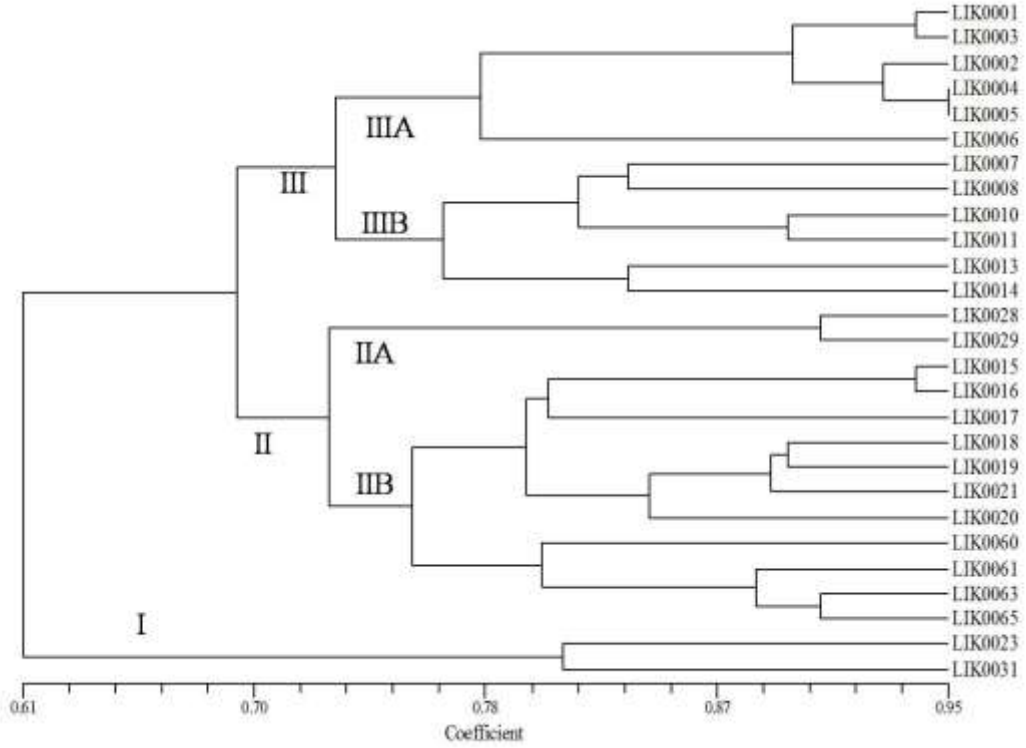


Fig. 1: UPGMA dendrogram of 27 germplasm of *Kerria* spp based on the 31 microsatellite primers

Table 3
Diversity parameters of the 27 accessions of *Kerria* spp. analyzed using SSR primers

S.N.	Code No.0	Ho	He	ne	h	i
1	LIK0001	0.23	0.14	1.36	0.26	0.43
2	LIK0002	0.49	0.38	1.99	0.49	0.69
3	LIK0003	0.48	0.44	1.95	0.48	0.68
4	LIK0004	0.02	0.02	1.03	0.03	0.08
5	LIK0005	0.48	0.48	1.92	0.48	0.67
6	LIK0006	0.07	0.03	1.06	0.06	0.14
7	LIK0007	0.34	0.31	1.51	0.33	0.52
8	LIK0008	0.32	0.29	1.47	0.32	0.50
9	LIK0010	0.45	0.38	1.85	0.46	0.65
10	LIK0011	0.23	0.21	1.29	0.22	0.39
11	LIK0013	0.05	0.05	1.05	0.05	0.11
12	LIK0014	0.16	0.15	1.19	0.16	0.30
13	LIK0015	0.14	0.13	1.16	0.14	0.27
14	LIK0016	0.49	0.38	1.99	0.49	0.69
15	LIK0017	0.13	0.07	1.13	0.12	0.24
16	LIK0018	0.12	0.11	1.00	0.11	0.23
17	LIK0019	0.03	0.01	1.03	0.03	0.08
18	LIK0020	0.18	0.17	1.21	0.17	0.32
19	LIK0021	0.22	0.21	1.27	0.21	0.37
20	LIK0023	0.21	0.20	1.26	0.20	0.36
21	LIK0028	0.49	0.40	1.95	0.48	0.68
22	LIK0029	0.31	0.23	1.43	0.30	0.47
23	LIK0031	0.09	0.08	1.09	0.08	0.18
24	LIK0060	0.06	0.05	1.06	0.05	0.13
25	LIK0061	0.20	0.15	1.26	0.21	0.36
26	LIK0063	0.16	0.15	1.19	0.16	0.30
27	LIK0065	0.49	0.40	1.99	0.49	0.69
Mean		**0.24**	**0.20**	**1.39**	**0.24**	**0.39**

*Ho= observed heterozygosity; *He= expected heterozygosity; * ne = Effective number of alleles; * h = Nei's (1973) gene diversity, * I = Shannon's Information index

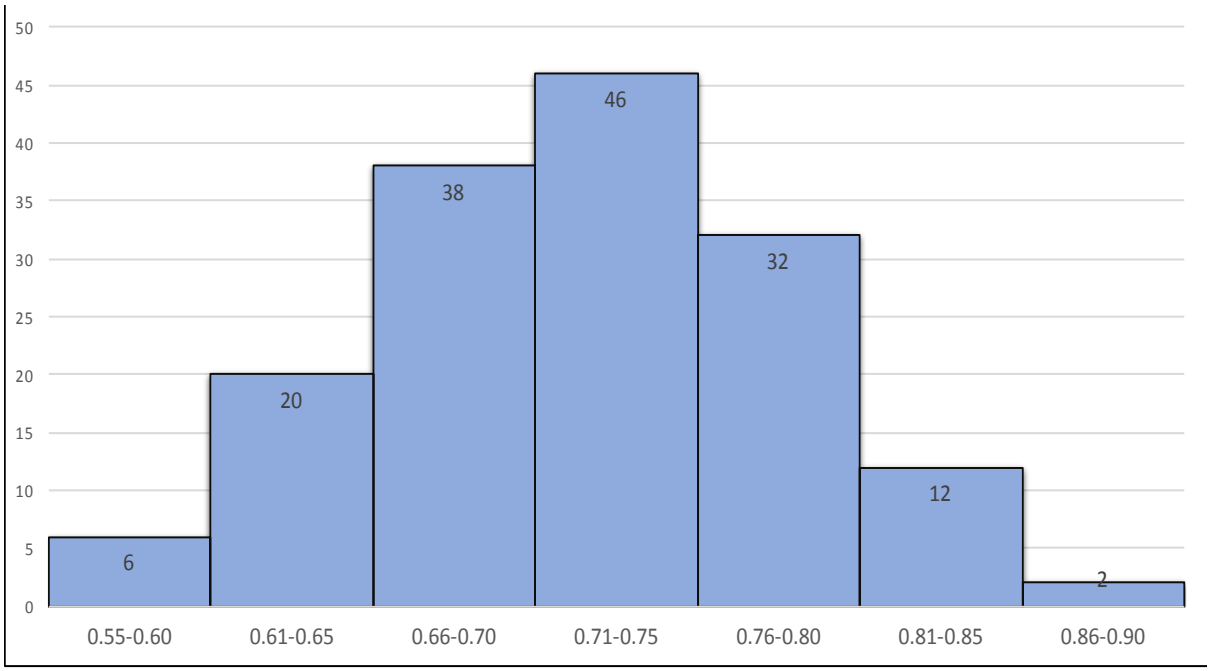
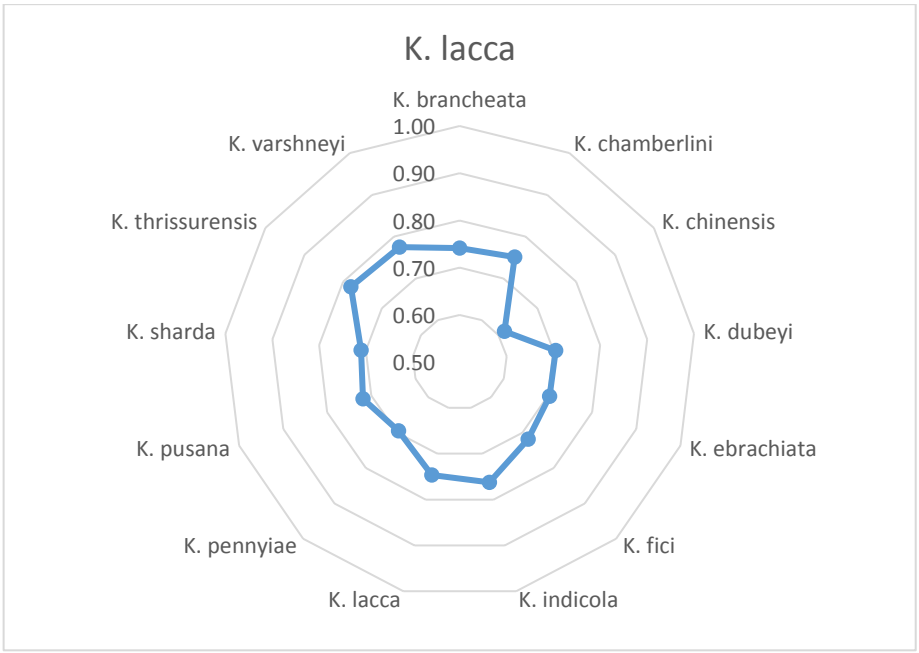


Fig. 2: Distribution of JSC values of paired comparisons of all the thirteen *Kerria* spp. studied

Table 4
Jaccard's coefficient of similarity from microsatellite data obtained for 27 lines of *Kerria*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**11**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**23**	**28**	**29**	**31**	**60**	**61**	**63**	**65**
1	**1.00**																										
2	0.91	**1.00**																									
3	0.94	0.94	**1.00**																								
4	0.86	0.91	0.90	**1.00**																							
5	0.86	0.91	0.87	0.95	**1.00**																						
6	0.77	0.79	0.80	0.75	0.77	**1.00**																					
7	0.77	0.77	0.76	0.77	0.79	0.77	**1.00**																				
8	0.68	0.70	0.71	0.72	0.70	0.72	0.84	**1.00**																			
10	0.78	0.78	0.82	0.76	0.74	0.78	0.83	0.85	**1.00**																		
11	0.75	0.77	0.78	0.77	0.72	0.77	0.82	0.79	0.90	**1.00**																	
13	0.68	0.72	0.71	0.79	0.75	0.68	0.75	0.75	0.78	0.86	**1.00**																
14	0.63	0.63	0.64	0.68	0.63	0.66	0.70	0.72	0.80	0.79	0.82	**1.00**															
15	0.70	0.68	0.71	0.66	0.63	0.66	0.68	0.70	0.80	0.75	0.72	0.82	**1.00**														
16	0.67	0.69	0.68	0.67	0.64	0.67	0.64	0.67	0.77	0.74	0.76	0.78	0.92	**1.00**													
17	0.61	0.63	0.62	0.68	0.70	0.66	0.66	0.63	0.71	0.68	0.70	0.75	0.79	0.83	**1.00**												
18	0.61	0.61	0.62	0.66	0.68	0.66	0.70	0.70	0.76	0.70	0.72	0.79	0.82	0.83	0.86	**1.00**											
19	0.67	0.64	0.66	0.67	0.67	0.71	0.74	0.71	0.82	0.76	0.69	0.78	0.83	0.82	0.80	0.90	**1.00**										
20	0.67	0.67	0.66	0.69	0.69	0.74	0.74	0.67	0.77	0.74	0.69	0.76	0.76	0.77	0.78	0.80	0.89	**1.00**									
21	0.68	0.68	0.67	0.66	0.70	0.70	0.75	0.70	0.78	0.70	0.63	0.70	0.79	0.80	0.77	0.89	0.90	0.85	**1.00**								
23	0.64	0.60	0.63	0.62	0.62	0.62	0.62	0.62	0.68	0.62	0.55	0.57	0.60	0.56	0.60	0.60	0.61	0.59	0.60	**1.00**							
28	0.60	0.55	0.59	0.60	0.62	0.64	0.69	0.74	0.72	0.74	0.67	0.71	0.67	0.66	0.69	0.76	0.75	0.70	0.71	0.56	**1.00**						
29	0.62	0.60	0.61	0.64	0.67	0.64	0.74	0.74	0.75	0.76	0.67	0.71	0.67	0.66	0.74	0.80	0.79	0.72	0.74	0.56	0.91	**1.00**					
31	0.60	0.64	0.63	0.64	0.64	0.64	0.62	0.62	0.70	0.64	0.64	0.62	0.67	0.68	0.69	0.67	0.61	0.61	0.64	0.82	0.52	0.52	**1.00**				
60	0.62	0.64	0.63	0.69	0.67	0.62	0.74	0.69	0.75	0.71	0.74	0.76	0.71	0.72	0.71	0.76	0.75	0.77	0.69	0.54	0.70	0.72	0.56	**1.00**			
61	0.69	0.71	0.70	0.74	0.71	0.67	0.76	0.74	0.82	0.80	0.76	0.71	0.76	0.77	0.74	0.78	0.77	0.75	0.76	0.61	0.77	0.79	0.66	0.86	**1.00**		
63	0.68	0.66	0.69	0.66	0.63	0.68	0.72	0.70	0.80	0.79	0.70	0.75	0.82	0.78	0.75	0.82	0.83	0.80	0.79	0.67	0.78	0.78	0.67	0.78	0.87	**1.00**	
65	0.66	0.68	0.67	0.68	0.66	0.68	0.68	0.68	0.76	0.75	0.70	0.70	0.72	0.76	0.72	0.79	0.78	0.76	0.77	0.64	0.76	0.76	0.71	0.78	0.90	0.91	**1.00**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K. ebrachiata
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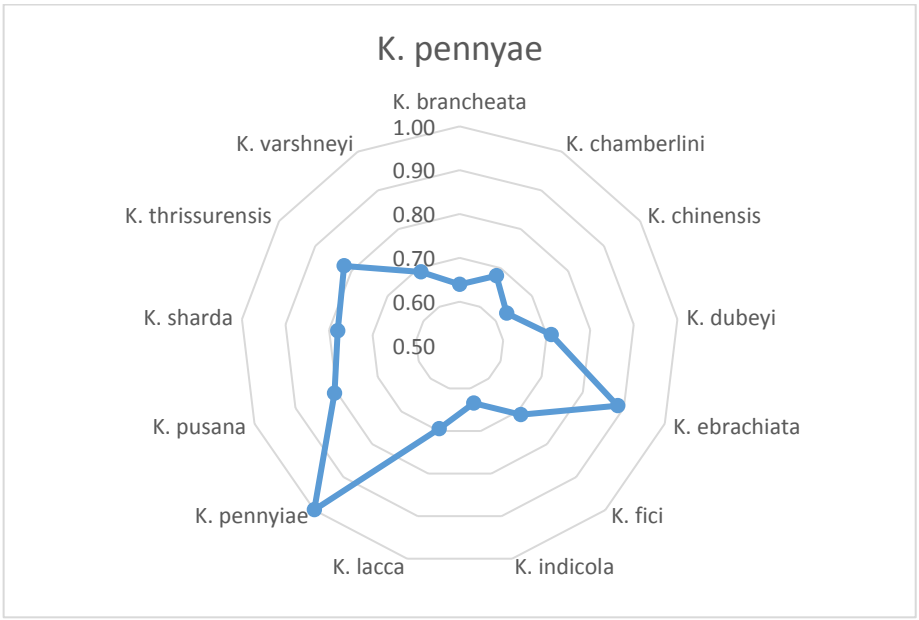
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K. chinensis
K. dubeyi
K. ebrachiata
K. fici
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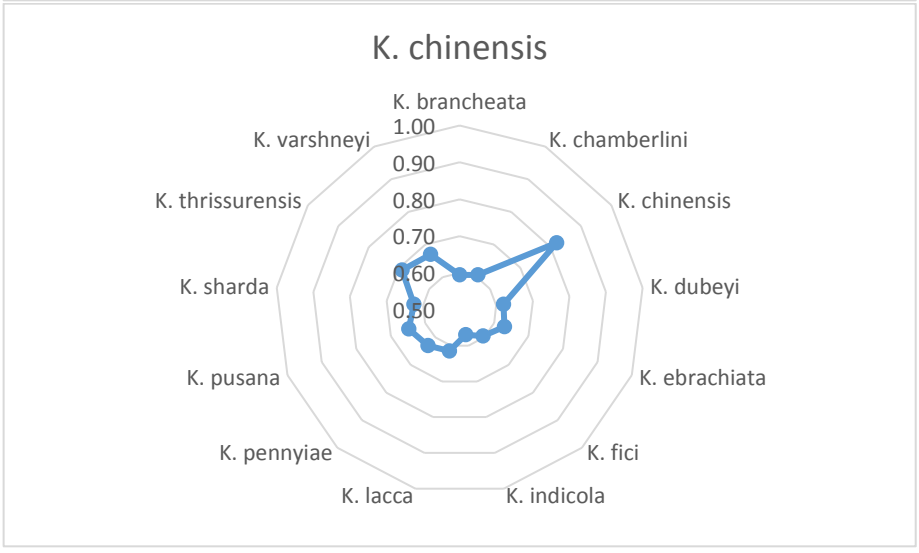
K. chinensis
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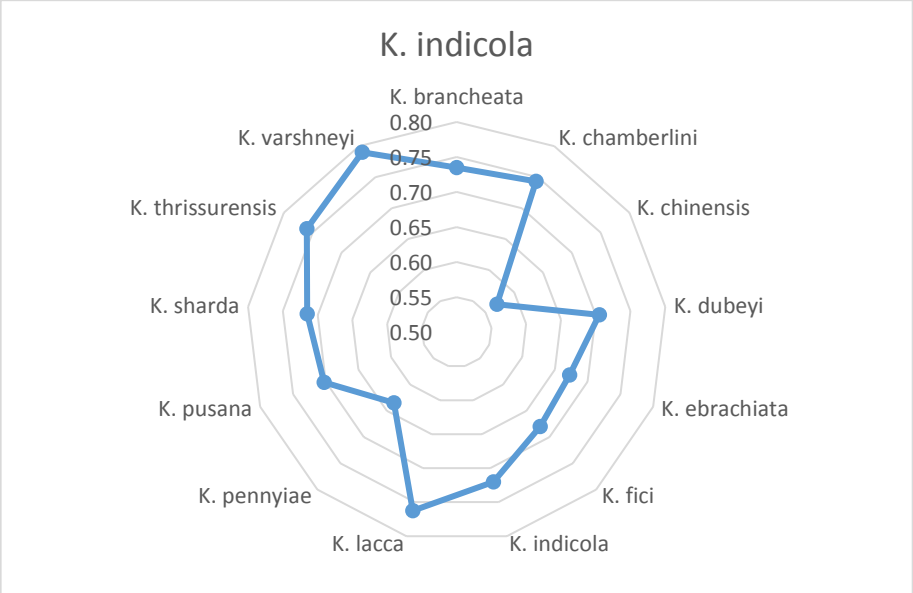

Fig. 3: Variation of similarity of select species of *Kerria* with other species based on paired comparison of similarity index

This study reveals a higher genetic diversity existing among *Kerria* populations, especially at infraspecific level. Private alleles specific for *K. chinensis* and some other accessions have been found which may help in developing fingerprinting marker to use in genetic resource conservation. The identified markers could be used in future lac crop improvement programmes to develop high lac resin yielding insects.

The conservation strategies either *in situ* or *ex situ* can also rely on these markers for better conservation and utilization of lac insect genetic resources available in the country. There is a future scope for using a greater number of markers and complementing with other markers to better understand the genetic relatedness among various populations and species of *Kerria*.

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