Unclasping potential chickpea resources for biofortification of the antioxidant enzyme Superoxide Dismutase

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Research Article

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Abstract

Context: The Superoxide Dismutase enzyme plays a very decisive role in governing abiotic and biotic stresses infused the hypothesis for the study.

Aims: The investigation was conducted to assess the diverseness and identify novel resources to be utilized in Superoxide Dismutase induced abiotic-biotic stress resistance breeding of chickpea.

Methods: The plants were grown in triplicates under recommended agronomic practices using PUSA 256 as check in a randomized block design. Fresh leaves were collected for estimation of enzyme superoxide dismutase and DNA extraction. Number of pods was recorded on 20 individual plants from middle of the row for each of the 12 genotypes. Employing 32 STMS markers together with morpho-biochemical data, Jaccard's similarity coefficients along with dendrograms were generated to compare and assess the diversity.

Key results: Amongst genotypes, the BGD-70 *vs* ICRISAT-3668 were identified as poorest *vs* best performers for superoxide dismutase activity. Out of 32 STMS primers, 80 alleles with 2.5 an average per loci were found. The marker TA-80 was identified as most polymorphic. The genotypes ICRISAT-3668 and SBD 377, distantly located on different molecular clusters, expressed higher SOD activity indicating genetic governance, probably by limited number of polygenes / OTLs and might be utilized as potential resources for abiotic-biotic stress resistance.

Conclusions: The genotypes ICRISAT-3668, SBD 377 and polymorphic marker TA-80 were identified as novel potential genetic resources.

Implications: The identified resources may be employed to widen the germplasm base, prepare maintainable catalogue, systematic blueprints and bifortification for future chickpea breeding strategies targeting abiotic-biotic stresses.

Introduction

Chickpea(*Cicer arietinum* L.), commonly known as Bengal gram or garbanzo beans belongs to Fabaceae family and on the basis of total production with 14.25million tons (MT), total harvested area of 13.72 million hectares (MHa), 1038.4 kg yield per hectare (Kg / Ha)(FAOSTAT 2019)andgrown over 40 countries representing all the continents, is the 2nd most important food legume globally. The 95% of the area, production and consumptions of chickpeas are represented by developing countries. During the span of last 30 years (1989-2019), the global chickpea area increased by 138.56%, yield by 143.29% and production by 198.53% (FAOSTAT 2019). Presently, it is cultivated in several countries with largest harvested area of 9.55 million hectares by India followed by Pakistan, Russian Federation, Turkey, Myanmar etc(FAOSTAT 2019). Currently, India represents as the largest producer of chickpeas accounting for around 69.76% of the global production followed by Turkey, Russian Federation, Myanmar and Pakistan are measured as the top five major world producers(FAOSTAT 2019).

India expects 11.99 MT of total chickpea production for the year 2020-21 as per recently released 4th advance estimates (GOISTAT 2021). However, In India,during 2019-20, it was cultivated in 10.17 M Ha acreage with 11.35 MT productions and 1116 Kg / Ha productivity (GOISTAT 2020).Rajasthan ranked 1st with the highest acreage of 2.46MHa followed by Maharashtra, Madhya Pradesh, Karnataka and Uttar Pradesh. The highest production of 2.66 MT was produced by Rajasthan followed byMaharashtra, Madhya Pradesh and Uttar Pradesh. The highest yield of 1574 Kg / Ha was produced by Gujarat followed by Telengana(1532 Kg / Ha), Uttar Pradesh (1371 Kg / Ha) and Madhya Pradesh (1288 Kg / Ha)(GOISTAT 2020).

In recent years, utilization of DNA-based markers is increasing owing to their phenotypic appearance, elevated polymorphism, alleviated development and varied applications in plant improvement. Chickpea mapping is acutely obstructed by amazing little genetic polymorphisms in cultivated genotypes and previously utilized molecular oligos such as isozymes, RFLP, RAPD, AFLP etc being unsuccessful to disclose intra-specific differentiations(Ghaffari*et al*.2014; Hajibarat *et al*.2015; Joshi and Reddy 2014). Amidst an array of DNA-based markers, Sequence Tagged Microsatellite Site (STMS) markers are often preferred in varied crop plants, inclusive of chickpea owing to their plethora, genomic compass, genetically co-dominant nature along with alleviated polymorphism (Bakshi et al. 2016; Bhardwaj et al. 2014; Harshavardhana et al. 2019; Katochet al. 2016; Kumar et al. 2017; Kumar

et al. 2020; Singh et al. 2011; Singh et al. 2013) and their distribution on various linkage groups or chromosomes (Bharadwajet al. 2011; Gaur et al. 2011; Verma et al. 2015). Chickpea specific STMS molecular markers were developed first timeby Huttel*et al.*1999 that divulged polymorphism up to prudent degree. After some time, legume family indicated that some of the species peculiar STMS were recognized as very efficient in divulging polymorphism also in additional species, it indicates once evolved in chickpea they can also be employed in other chickpea linked species (Choumane *et al.*2000). Thus, DNA marker analysis will help the identification and differentiation of landraces with different genetic make-up. The present study was conducted to ascertain the pattern and extent of molecular characterization, relatedness and potential utility of STMS markers in analyzing molecular polymorphism in chickpea.

Plants have developed an efficient antioxidant system that can protect plants from any disaster injury(Joseph and Jini 2011). The toxic effects of reactive oxygen species (ROS) are counteracted by enzymatic as well as non-enzymatic antioxidative system such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), ascorbic acid (AsA), tocopherol, glutathione and phenolic compounds, etc. Normally, each cellular compartment contains more than one enzymatic activity that detoxifies a particular ROS. The presence of these enzymes in almost all cellular compartments gives their clear crucial role in ROS detoxification for the survival of the plant (Ahmad *et al.*2013; Mittler 2002). SODs are ubiquitous metalloenzymes that constitute the first line of defense against ROS. SODs also constitute one of the major enzymatic components of detoxification of superoxide radicals generated in biological system by catalyzing its dismutation to H_2O_2 and finally to H_2O (Berwal and Ram 2018). SODs are metalloproteins working with Cu, Zn, Mn or Fe as cofactors and occur in the chloroplasts, mitochondria, cytosol, peroxisomes and the apoplast (Mittler 2002). Accordingly, three isoenzymes can be separated in plants having different structures and function as Cu/Zn-SOD, Mn-SOD and Fe- SOD (Alscher et al. 2002; Gill and Tuteja 2010). Mn-SOD is present in the mitochondria and peroxisomes, while Cu/Zn-SOD is mainly cytosolic, mitochondrial and plastidic. Similarly, Fe-SOD seems to be frequent in the chloroplasts, cytosol, mitochondria and peroxisomes (Arora et al. 2002; Giannopolitis and Ries 1977; Inze´ and Van Montagu 1995).

Materials And Methods

B.1. Experimental plots - The experimental research and field studies on chickpea were carried out during 2019-20 in the experimental plot “New Area A-Block” allocated to the Division of Genetics, IARI, New Delhi. This area is located between latitude 28.61^{o}N and longitude 77.23^{o}E and found at an altitude of 225 m above mean sea level. The topography of the experimental plot was uniform. The soil was sandy loam with physical and nutritional compositions as follows: The pH of soil was mild alkaline (7.5-8.5) with low EC (0.4-0.6 dS / m), low organic content (<0.5%), low nitrogen (<280kg/ha), high phosphorous (25-50kg/ha) and high potassium (>280kg/ha), medium sulphur (10-20mg/kg), adequate zinc (1-5mg/kg), deficient iron (5.8- 10 mg/kg) adequate manganese (10-25mg/kg) and adequate copper (0.5-10mg/kg) respectively.

B.2. Plant Genetic Material - 12 Chickpea genotypes (Table 1) were selected for the morpho-biochemical and molecular characterization, based on visual observations recorded for biotic and abiotic stresses over a large pool of germplasm including core germplasm obtained from gene bank (ICRISAT 2021) and maintained at Division of Genetics, Indian Agricultural Research Institute, New Delhi, India. Healthy seeds of each genotype were sown during 2019-20in the experimental field following a randomized block design with a set of three replications under all suitable agronomic practices using PUSA 256 as check. The mean daily minimum and maximum temperatures were 25^{o}C and 37.8^{o}C, respectively. The total annual rainfall during the season ranged from 0.2 mm to 66 mm with a mean of about 1.643 mm. The chickpea 20 individual plants from middle of each row for each genotype were used for collection of fresh leaves to estimating enzyme SOD and DNA extraction together with data recording for number of pods per plant.

Table 1
List of elite genotypes/ germplasms of Chickpea (*Cicer arietinum* L.)used as experimental material

S.N.	Genotype	Details	Origin / Source
1.	ICRISAT-3155 (ICC 3155)	Semi erect, white flower, maturity (104 days), Beige seed colour, Owl's head seed shape, smooth seed surface, seed yield (583 Kg / Ha), 100 seed weight (16 g), Protein (15.2%), traditional cultivar/Landrace, DOI: 10.18730/MDAWQ .	Shahr-e Pir, Fars, Iran
2.	BGD-70	Kabuli, developed from a cross between PUSA-256 x ICC-32, erect, very bold, low yielder, large seeded.	ICAR- Indian Agricultural Research Institute, New Delhi, India
3.	BGD-1004	Kabuli, low yielding, suitable for late planting.	ICAR- Indian Agricultural Research Institute, New Delhi, India
4.	Vijay (Phule G-81-1-1)	Desi cultivar, released in 1994, resistant to wilt, tolerant to terminal moisture stress, maturity (105-110 days), seed yield (19-21 q/ha) and suitable for Central Zone(MP, Maharashtra, Gujarat).	MPKV, Rahuri, Maharashtra, India
5.	PUSA-362 (BG-362)	Desi cultivar, developed through hybridization (BG-303 x P-179), released in 1995, tall, maturity (145-150 days), wilt resistant, drought tolerant, bold seeded, high seed yield (23-24 q/ha) and suitable for north-west plain zone.	ICAR- Indian Agricultural Research Institute, New Delhi, India
6.	ICRISAT-3673 (ICC 3673)	Desi, resistant to wilt &aschochyta blight, semi spreading, pink flower, maturity (115 days), black seed colour, angular seed shape, rough seed surface, seed yield (1200 Kg / Ha), 100 seed weight (10 g), Protein (17.4%), traditional cultivar/Landrace, DOI: 10.18730/MDV2Q .	Neyshabur, Khorasan, Iran
7.	PUSA-256 (BG-256)	Desi cultivar, wide adaptability, developed in 1984 through hybridization [(JG-62 x 8503 / 27)-1 x (L-550 x H-208], medium tall, tolerant to wilt and ascochyta blight, drought tolerant, salt sensitive, semi spreading to erect, leaves broad and suitable for North-East and North-West Plain zone, seeds light brown attractive, bold (22g / 100 seeds), high seed yield (18-20 q/ ha).	ICAR- Indian Agricultural Research Institute, New Delhi, India
8.	CSG-9505	High yielding, salt sensitive.	ICAR-Central Soil Salinity Research Institute, Karnal, India
9.	ICRISAT-3668 (ICC 3668)	Desi, semi spreading, pink flower, maturity (116 days), black seed colour, angular seed shape, rough seed surface, seed yield (1200 Kg / Ha), 100 seed weight (10 g), Protein (18.1%),traditional cultivar/Landrace, DOI: 10.18730/MDTXJ.	Neyshabur, Khorasan, Iran
10.	SBD-377	Desi breeding line derived from *Cicer arietinum* x *C reticulatum* cross, protein (26.66%), medium plant height, long internode length, erect growth habit, dark green leaf colour, simple pinnate leaf type, very large leaf size, pink flower colour, large flower size, brown seed colour, extra bold seed size (42.99g / 100 seed), early maturity, specific adaptability and drought susceptible	ICAR- Indian Agricultural Research Institute, New Delhi, India
11.	BGD-72 (Dharwad Pragati)	Desi cultivar, released in 1999, wide adaptability, medium maturity (115-120 days), resistant to wilt & root rot, drought toerant, bold seeded, high seed yield (25-30 q/ha) and suitable for CZ (MP, Maharashtra, Gujarat).	ICAR- Indian Agricultural Research Institute, New Delhi, India

S.N.	Genotype	Details	Origin / Source
12.	GPF-2 (GF-89-36)	Desi, Yellowish Brown, released in 1995, maturity (152 days), resistant to wilt & tolerant to ascochyta blight, seed yield (21-23 q/ha) and suitable for NWPZ (Punjab, Haryana, Delhi, North Rajasthan & West U.P).	PAU, Ludhiana, India

B.2.1. Morpho-biochemical Traits

B.2.1.1. Numbers of pods / plant - Total number of pods per plant were counted at the time of harvesting.

B.2.1.2. Superoxide dismutase estimation

B.2.1.2.1. Preparation of enzyme extract - For enzyme SOD extraction, fresh leaves of each chickpea genotypes were ground in liquid nitrogen with 10 ml extraction buffer (0.1M phosphate buffer, pH 7.5 containing 0.5 mM EDTA). Brie was passed through four layers of cheese cloth and filtrate was centrifuged for 20 minutes at 15000 rpm and the supernatant was used for estimation of activity of the SOD enzyme.

B.2.1.2.2. Enzyme Assay -SOD activity was estimated by recording the decrease in optical density of formazone made by superoxide radicle and nitro blue tetra-zolium dye by the enzyme. Reaction was started by adding 0.1 ml of 2 µM riboflavin and placing tubes under two 15W fluorescent lamps for 15 minutes. A complete reaction mixture without enzyme, which gave the maximal colour, served as control. Switching off the light and putting the tubes into dark stopped the reaction. A non-irradiated complete reaction mixture served as blank.

Separate controls (lacking enzymes) were used for total SOD and inhibition studies. The absorbance was recorded at 560 nm and 1 unit of enzyme activity was taken as that amount of enzyme, which reduces the absorbance reading to 50% in comparison with tubes lacking enzymes.

SOD = [(Control OD - sample OD) / (Control OD / 2)] x [{(ml extraction buffer) / (fresh weight in g × ml enzyme)} / time in minutes]

B.2.2. Statistical data analysis for morpho-biochemical traits -SPSS version 7.5 software used for all analysis of the morpho-biochemical traits evaluations stated as means of three repetitions. The outcomes were scrutinized by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests to compare means significance at $p<0.05$.

B.2.3. DNA isolation, amplification and detection of microsatellite alleles - Total genomic DNA of each genotype (individually) was extracted from fresh leaves of chickpea employing quality CTAB protocol narratedby Doyle and Doyle (1990) with slight alterations. The amounts together with standard of DNA were determined by biophotometeric analysis. A total of 32 STMS markers were used in this study to assess the diversity and relationships in chickpea. PCR was performed in a total reaction volume of 10 µl containing 1 U of Taq DNA polymerase (Bangalore Genei), 0.25 mM of each STMS primer (both forward and reverse), 250 µM dNTPs, 1.5 mM $MgCl_2$, and 1X PCR buffer (10 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl), 50 ng of template DNA, using a BIORAD Thermo Cycler. All amplifications had an initial denaturation of DNA for 2 min at 95^oC following 35 cycles comprising of denaturation for 20 sec at 95^oC, annealing for 50 sec at 55-59^oC, extension for 50 sec at 72^oC. The final extension was performed for 7 minutes at 72^oC and amplification products were analyzed using 3% Metaphor agarose (Resophor) gels stained with ethidium bromide. A 100 bp DNA ladder was run alongside the amplified products to determine the approximate size of the amplification products / bands.

The CCD camera (Sony XC-75 CE) attached to a gel documentation system with the Quantity One software (BIORAD) was used for photography of the gels. Each of the gel sections were scored done manually. The alleles were numbered as 'a_1', 'a_2' etc. sequentially from the largest to the smallest size bands. The bands were designated as '1' for presence of a band and '0' for absence of a band in the data matrix. The polymorphic information content (PIC) for each STMS marker, was ascertained followed by standard procedure (Senior et al. 1998). PIC is an estimate of allele variation at a locus and comparable to $1-\Sigma (P^2ij)$, where P_{ij} is the frequency of the j^{th} allele for i^{th} locus summated including all alleles in the locus. The 0-1 data matrix was further

used to calculate genetic similarity between genotypes following Jaccard'scoefficient (Jaccard 1908) using NTSYS software (Rohlf 2000). UPGMA (Sneath and Sokal 1973) on the similarity matrix was performed to identify genetic variation patterns among the chickpea genotypes using NTSYS pc version 2.11s.

B.2.4. Clusters analysis for measurement of distances - Software NTSYS-PC version 2.11s (Rohlf 2000) was employed to categorize genotypes into discrete conglomerations followed by dendrograms constructions using the UPGMA method (Sneath and Sokal 1973).

Results

C.1. Morph -biochemical traits analysis

C.1.1. Characterization for the enzyme SOD and yield trait number of pods - 12 Genotypes of chickpea used in present studies showed a wide variation for the enzyme SOD activity and yield trait number of pods (Supplementary Table 1). Among the chickpea genotypes, the highest and lowest values of superoxide dismutase were obtained in the genotypes ICRISAT-3668 (5.580±0.608) and BGD-70 (0.750±0.115) respectively with a mean value of 3.743 per plant / genotype. The minimum and maximum number of pods per plant were recorded in the genotypes SBD 377 (25.100±1.528 pods / plant) and BGD 72 (80.050±1.528 pods / plant), respectively with a mean value of 48.93 pods / plant / genotype.

C.1.2. Analysis of variance - The analysis of variance (Table 2) revealed significant variability @ 0.1% probability level amongst the genotypes for the morpho-biochemical traits SOD activity and number of pods per plant.

Table 2
Analysis of Variance

Traits →		**No of Pods / Plant**			**SOD Activity (%)**		
Source ↓	DF ↓	Sum of squares	Mean sum of squares	Calculated F	Sum of squares	Mean sum of squares	Calculated F
Replications	2	6.305	3.152	0.623	1.932	0.966	0.549
Genotypes	11	7683.976	698.543	138.019***	89.244	8.113	4.611***
Error	22	111.347	5.061	-	38.707	1.759	-
Total	35	-	-	-	-	-	-
*** indicates statistically significant at 0.01% probability level.							

C.1.3. Similarity Vs Dissimilarity Analysis - The integrated data for the enzyme SOD activity and yield trait number of pods was utilized for estimating pair wise genetic similarities among 12 genotypes of chickpea using Jaccard's coefficient method (Table 3). The genetic similarity matrix was used for construction of dendrogram (Fig. 1), showing the relationships among chickpea genotypes, following the UPGMA clustering method using computer software NTSYS pc version 2.11s. The generated dendrogram categorized the 12 chickpea genotypes into 4 diversified groups i.e. cluster I, II, III and IV containing 5, 4, 2 and 1 genotypes, respectively. BGD 1004 *vs* CSG 9505 and ICRISAT-3668 *vs* Vijay revealed remarkable proximity with similarity (0.991) followed by Pusa 256 *vs* GPF-2 and ICRISAT 3688 *vs* GPF-2 with uniform similarity values (0.981) and Pusa 256 *vs* CSG 9505 (0.931) respectively.

Table 3
Jaccard's genetic similarity (GS) matrix based on morpho-biochemical and molecular data

Morphological→ Molecular ↓	1	2	3	4	5	6	7	8	9	10	11	12
1	-	0.645	0.488	0.753	0.132	**0.101**	0.127	0.144	0.160	0.197	0.258	0.221
2	0.798	-	0.379	0.418	0.738	0.115	0.147	0.119	0.140	0.216	0.206	0.222
3	0.738	0.774	-	0.267	0.864	0.784	**0.110**	**0.991**	0.118	0.180	0.210	0.192
4	0.679	0.690	**0.845**	-	0.621	0.801	0.111	0.785	**0.991**	0.178	0.183	0.182
5	0.738	0.726	0.643	0.655	-	0.130	0.158	0.838	**0.107**	0.216	0.133	0.205
6	0.714	0.798	0.738	0.702	0.643	-	0.315	0.771	0.809	**0.101**	0.215	0.120
7	**0.583**	**0.619**	**0.607**	0.667	0.679	0.679	-	**0.931**	0.892	0.708	0.230	**0.981**
8	0.643	0.655	0.714	0.726	**0.619**	**0.609**	0.798	-	0.224	0.136	0.138	0.119
9	**0.821**	**0.810**	0.750	0.690	0.774	0.774	0.667	0.655	-	0.121	0.141	**0.981**
10	0.702	0.738	**0.845**	0.762	**0.607**	0.774	0.619	0.750	0.738	-	0.256	0.568
11	0.762	0.798	0.762	0.750	0.667	0.786	0.631	0.690	0.772	**0.821**	-	0.215
12	0.762	0.772	0.650	0.679	0.690	0.714	**0.607**	0.619	0.726	0.679	0.667	-

The largest distances were observed between the genotypes ICRISAT-3673 *vs* ICRISAT-3155 and ICRISAT-3673 *vs* SBD 377 with dissimilarity values (0.899) followed by ICRISAT-3668 *vs* Pusa 362 (0.893) and Pusa 256 *vs* BGD-1004 (0.890) respectively.

C.2. Molecular Characterization

C.2.1. Number of alleles and molecular polymorphism - In the present study, a total of 32 STMS loci were investigated, across various bin locations on various linkage groups (Supplementary Table 2). The 26 STMS loci in the scrutinized genetic material were obtained to be highly polymorphic and data generated only from loci were utilized for further statistical analysis. However, 6 markers namely CaSTMS 14, TA 13, TA 125, TA 96, TA 106 and TAA 58 were found to be non polymorphic. The 32 STMS loci together generated total 80 alleles with an average 2.5 per locus. The maximum number of alleles were noticed for CaSTMS 28 (6 alleles) followed by TA 2 (5 alleles), CaSTMS 22 (4 alleles), TA 45 (4 alleles), TA 80 (4 alleles), TR 58 (4 alleles), and lowest (single) alleles for CaSTMS 14, TA 13, TA 96, TA 106, TA 125 and TAA 58. Investigation on the distribution of the STMS allele covering the genotypes disclosed that maximum alleles were divided by *C. arietinum*. Supplementary Table 2provides the summated data on the allele number, allele frequencies along with allele dispersal in various genotypes. Considering the homozygosity of the populations, occurrence of more than 2 bands in some of the germplasm lines / varieties for different STMS loci was surprising.

The PIC values were calculated for various analyzed STMS loci on the basis of allele frequencies (Supplementary Table 2). The PIC values ranged from 0 to 0.705. CaSTMS 14, TA 13, TA 125, TA 96, TA 106 and TAA 58 showed the lowest PIC value with zero, while TA 80 showed maximum PIC (0.705) because of the well-distributed 4 alleles among the genotypes of *Cicer arietinum.* Out of 32 utilized markers, 20 markers gave higher PIC value as compared to average value (0.402) covering all the loci.

Estimation of size range of alleles were approximately estimated, as the resolution power of agarose gel is relatively less, as compared to that of polyacrylamide gel, which can resolve nucleotide difference of even one base pair. Specific STMS markers contained high differential capability for demarcating C. arietinum germplasm lines as the present study demonstrated that out of 80 STMS alleles, only 12 STMS alleles were found to be unique or rare; unique or rare allele is one with a frequency less than or equal to 0.10. The present findings also indicated instances where the STMS profiles for some of the genotypes displayed deviation from the expected pattern. Chickpea germplasm / varieties are assumed to be highly homozygous and thereby should

reveal only a single band (allele) per locus for a large majority of them if not all. However, double bands could clearly be seen in many of the lines.

C.2.2. Molecular Similitude Vs Dissimilitude Inspection - The STMS data was employed for inspecting pair wise genetic similarities among various genotypes using Jaccard's method (Table 3). The genetic similarity matrix was further explored following UPGMA clustering algorithm employing NTSYS pc version 2.11 software programme. The dendrogram extracted from the investigation as portrayed in Fig. 2 exhibited that the cluster I, II and III comprised of 5, 5 and 2 genotypes.

The molecular dendrogram together with pair wise genetic similarity index values indicated in parenthesis showed that the genotypes BGD 1004 *vs* Vijay (0.845), BGD 1004 *vs* SBD 377 (0.845), ICRISAT-3155 *vs* ICRISAT-3668 (0.821), SBD 377 *vs* BGD 72 (0.821) and BGD 70 *vs* ICRISAT-3668 (0.810) were very close to each other in their genotypic composition. The largest distances were observed between ICRISAT-3155 *vs*Pusa 256 (0.417) followed by BG-1004 *vs*Pusa 256 (0.393), Pusa 362 *vs* SBD 377 (0.393), Pusa 256 *vs* GPF-2 (0.393) and ICRISAT-3673 vsCSG 9505 (0.391) with their respective dissimilarity index values.

A comparative perusal of phenotypic and molecular cluster analysis dendrograms apparently does not show any co linearity. However, a meticulous comparative perusal revealed that the largest distances were observed between ICRISAT-3155 *vs* Pusa 256 (0.873, 0.417) followed by BGD-1004 *vs* Pusa 256 (0.890, 0.393), Pusa 362 *vs* SBD 377 (0.784, 0.393), Pusa256 *vs* GPF-2 (0.019, 0.393) and ICRISAT-3673 *vs* CSG 9505 (0.229, 0.391), ICRISAT-3673 *vs* ICRISAT-3155 (0.899, 0.286), ICRISAT-3673 *vs* SBD 377 (0.899, 0.226) and ICRISAT-3668 *vs* Pusa 362 (0.893, 0.226) with their respective phenotypic and molecular dissimilarity index values.

Discussion

The investigation targeted for dissecting the chickpea genetic variability utilizing morpho-biochemical traits (SOD activity, number of pods) and STMS markers for determining the potential utility of these genotypes and molecular markers. Molecular markers are employed for variable purposes including construction of linkage maps, assessing genetic relationships and identification of crop cultivars. Microsatellite genotypic data generated from an array of loci have prospective for furnishing distinct allelic profiles or DNA diagnostics for displaying genotypic signatures. Because of compartmentation of different isoenzymes, SOD plays more effective role in stress resistance mechanism. Increased activity of SOD is often correlated with increased tolerance of plant against environmental stresses. It was suggested that SOD can be used as indirect selection criterion for screening stress resistant plant materials. Over production of SOD has been reported to result in enhanced oxidative stress tolerance in plants(Shukla and Verma 2019).

Expression profiling of chickpea in response to biotic and abiotic stress has been reported (Mantri *et al.*2007), only based on customized microarray chip based on probe homologs. Metabolic pathways involved in cell wall synthesis, energy production, nitrogen metabolism, defense mechanism, regulation of transcription and signal transduction the key processes that were modulated by heavy metal stress also provide stress tolerance in chickpea. Many biotic stress associated genes were also reported to be differentially expressed during the exposure. The microarray data are available in the public domain and can be accessed to perform transcription factor based study. Insights from the transcriptome level expression profiling provide clues for a future molecular breeding approach to developing stress tolerant chickpea varieties (Yadav and Mani, 2018).

The diversity analysis studies on 12 chickpea genotypes for the morpho-biochemical traits number of pods and SOD activity revealed that the close uniform similarity (0.991) values amongst BGD 1004 *vs* CSG 9505, ICRISAT-3668 *vs* Vijay followed by Pusa 256 *vs* GPF-2, ICRISAT 3688 *vs* GPF-2 with uniform similarity values (0.981) could be due to ancestral association. The largest distances observed between the genotypes ICRISAT-3673 *vs* ICRISAT-3155, ICRISAT-3673 *vs* SBD 377 with dissimilarity (0.899) followed by ICRISAT-3668 *vs* Pusa 362 with dissimilarity (0.893) and Pusa 256 *vs* BGD-1004 with dissimilarity (0.890) values respectively, could be due to lack of ancestral association.

Molecular dissimilarity examination, employing 32 STMS markers generated 80 alleles along with an average 2.5 alleles per loci, indicated the presence of remarkable polymorphism for the examined microsatellite loci and disclosed a moderate level of genetic variability in the chickpea genotypes, which is also supported by several others (Ghaffari et al. 2014; Khan et al. 2010).

The PIC values ranging from 0 to 0.705 for the markers disclosed enough heterogeneity amongst genotypes and potential utility of already mapped marker TA 80 on the linkage group-6 (Gaur et al. 2011). Cluster formations using morpho-biochemical traits together with STMS markers segregated all chickpea genotypes into four and three perceptible groups, respectively. The occurrence of heterozygosity in pure lines revealed through STMS analysis could possibly draw the attention of the chickpea breeders for effective maintenance breeding. Thus, molecular characterization can give very useful information to chickpea breeder (Stephens and Lombardi 2014).

Results from the present investigation disclosed potential utility of STMS in characterizing chickpea germplasm and is supported by several others (Flandez-galvez *et al.* 2003; Singh et al. 2012; Singh et al. 2012; Soi et al., 2014). The reasonably high rate of polymorphism shown by the marker TA 80 with four alleles indicated the potentiality of this marker for further employability. The occurrence of rare alleles or specific STMS alleles gives an enormous chance for producing exhaustive fingerprint database. The noticed “null” alleles could be attained owing to mutations in the primer binding site causing to non-amplification. The PIC value is determined by the incidents of variants per locus along with relative dispersal of the alleles. The one to six extents of alleles per locus were found for example, CaSTMS 14 with single alleles with ‘0’ PIC value compared to TA 45 with four alleles with higher PIC value (0.680) disclosed the significance of dispensation of alleles over the genome (Supplementary Table 2). The molecular clustering along with pair wise genetic similarity index values as indicated in parenthesis revealed significant close association between the genotypes BGD 1004 *vs* Vijay (0.845), BGD 1004 *vs* SBD 377 (0.845), ICRISAT-3155 *vs* ICRISAT-3668 (0.821), SBD 377 *vs* BGD 72 (0.821) and distant relations between the genotypes ICRISAT-3155 *vs* Pusa 256 (0.417), BG-1004 *vs* Pusa 256 (0.393), Pusa 362 *vs* SBD 377 (0.393), Pusa 256 *vs* GPF-2 (0.393) and ICRISAT-3673 *vs* CSG 9505 (0.391) based on the dissimilarity index values. The observed molecular findings did not correlate with the phenotypic findings indicating control of expression of genotypes by environment and polygenes at large scale. The phenotypic diversity alone would not be a conspicuous barometer of genetic distinctness and large phenotypic dissimilarity amongst the genotypes deduced from the same source may be expected due to segregation (Nagahavi et al. 2013; Varshney and Thudi 2014).

However, a comparative meticulous perusal of morpho-biochemical and molecular data analysis revealed that the genotypes ICRISAT-3668, CSG 9505, SBD 377 and BGD 72 expressed the higher SOD activity with the largest distances between ICRISAT-3668 *vs* CSG 9505 (0.776, 0.345), ICRISAT-3668 *vs* SBD 377 (0.879, 0.262), ICRISAT-3668 *vs* BGD 72 (0.859, 0.228), CSG 9505 *vs* SBD 377 (0.864, 0.250), CSG 9505 *vs* BGD 72 (0.862, 0.310), SBD 377 *vs* BGD 72 (0.744, 0.179) with their phenotypic and molecular index values indicating genetic governance, probably by limited number of polygenes / OTLs along with environmental variations.

Thus, the genotype ICRISAT-3668 located on molecular cluster II and the molecular marker TA-80 mapped on LG-6 need further investigation and validation to be utilized as potential resources for the SOD activity in chickpea. The results included identification of SSR marker TA-80 and high SOD content chickpea genotype ICRISAT-3668. The identified resources will allow exploration for their utilization as potential marker / donor resources for the biofortification of SOD trait specific development of mapping populations, construction of genetic maps, marker trait associations, localization of genes / QTLs in future marker assisted selection (MAS) and abiotic-biotic stresses resistance breeding programmes.

Declarations

Author’s contribution

RK conceptualized and supervised the experiment. AS, RKS, PS and RY executed field and laboratory investigations. NS, VS and NY analyzed the data and interpreted the results. RK and RA contributed to the original writing, reviewing and editing of the manuscript. All authors read and approved the manuscript.

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Conflict of interest: The authors declare no conflicts of interest.

Ethical approval: Not applicable.

Data Availability Statement: Meta data are available as supplementary materials as electronic files.

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Figures

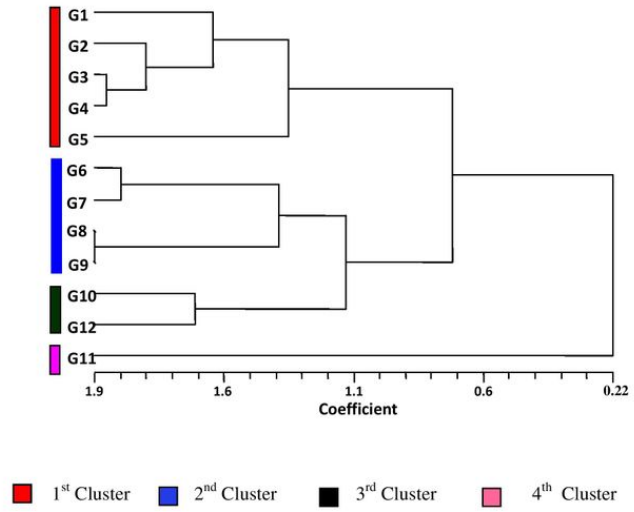

Fig. 1 - Amrendra Pratap Singh, Chickpea resources for the antioxidant enzyme Superoxide

Figure 1

Dendrogram depicting genetic relationship among selected genotypes of Chickpeas based on the enzyme SOD activity and yield trait number of pods data

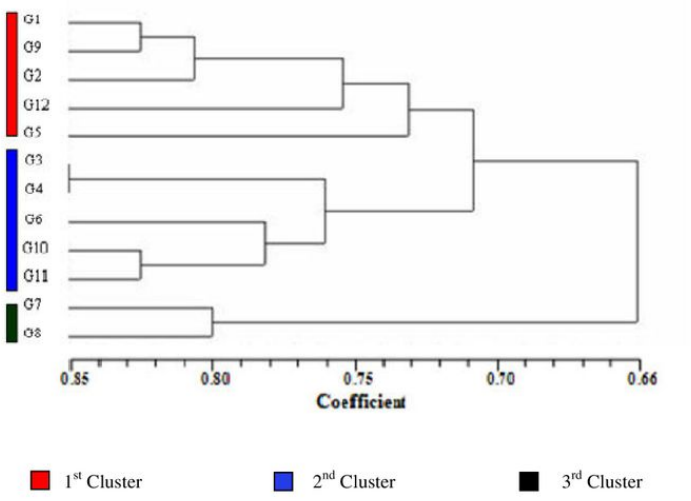

Fig. 2 - Amrendra Pratap Singh, Chickpea resources for the antioxidant enzyme Superoxide

Figure 2

Dendrogram depicting genetic relationship among selected genotypes of Chickpeas based on the STMS data through UPGMA cluster analysis

Supplementary Files

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- SupplementaryMaterials.pdf