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Genetic diversity analysis of selected Onion (*Allium cepa* L.) germplasm using specific RAPD and ISSR polymorphism markers

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A B S T R A C T

The rationale of the present study was to group nine cultivars of onion (*Allium cepa* L.), to determine their important traits and decipher their genetic variation. Methodology includes morphological observation, biochemical determination, genetic similarity and diversity using molecular markers of RAPD and ISSR in nine onion cultivars. In PCA studies, it was clearly observed that the germination percentage, root length, seedling length and plumule length were contributing most to the separation among the cultivars. Genetic diversity studies revealed that among 18 RAPD's 10 primers displayed specificity and produced a total of 52 amplified polymorphic fragments. In ISSR analysis, among 10 ISSR's 6 primers generated 28 variable polymorphic band patterns. The 16 primers of RAPD and ISSR discriminated nine cultivars into two groups by the method of UPGMA. Single cultivar Agrifound rose specified in group-I rest of all separated to another group. We conclude that phenotypic, genetic and biochemical variability of studied onion cultivars could boost the onion breeding programs. The cultivars exhibited statistically significant variability, which gives an opportunity to use them for varietal development through breeding programs. The key findings of the current study would be an insight for breeding strategies in onion cultivars

1. Introduction

Onion (*Allium cepa* L.) belongs to the family Amaryllidaceae and the genus *Allium*, is one of the largest genus consisting of 750 species (Fritsch and Friesen, 2002). Chromosome number (2n) = 16 with largest genome size of ~15290 Mbps/1C (King et al., 1998). This is the principal horticultural crop after tomatoes. Onion production in world has increased as a minimum of 25% during the past 10 years. In Worldwide ranking system, India occupied second rank in cultivation and production of onion. Onion is considered as a highly export oriented crop due to valuable foreign exchanges. Onions have been reported to possess wide range of benefits for mankind such as antibiotic effects, anticough and cold activity, cardiovascular helper, induction of appetite etc (Griffith et al., 2002). The knowledge of onion genetic diversity and resources is inadequate primarily owing to a scantiness of selective markers, germplasm resources, out breeding and biennial habit. Crop diversity is one of the most essentially important resources for human life. The conservation and sustainable use of plant genetic resources are essential to the development of agricultural production hence the knowledge of crop genetic diversity among cultivated crops is very important in germplasm collection and breeding. Genetic diversity assessment within the plant populations applied using various techniques such as morphological, biochemical and molecular markers. Morphological markers are those visually assessable traits, influenced by the environment, narrow diversity and variable expressivity. Biochemical markers are based on gene product and molecular markers relying on a DNA assay.

Molecular markers have provided evidence as invaluable tools in the evaluation and characterization of genetic diversity within and between species. However few marker systems viz. SSR; (Fischer and Bachmann, 2000) and RAPD; are simple, quick, economical and easily automated (Jones et al., 1997). Co-dominant simple sequence repeat (SSR) markers were effectively demonstrated to distinguish genetic diversity and relationships in onion germplasms. The knowledge acquired in this analysis may play a fundamental role in the application of molecular markers in onion improvement programs.

Classification of germplasm is established with multivariate statistical algorithms for analyzing the genetic relationships among the cultivars. According to Mohammadi and Prasanna (2003), the multivariate analytical technique simultaneously analyses multiple parameters of each individual under investigation of morphological and biochemical data. During the analysis of genetic diversity in onion correlation, principal component analysis (PCA) is calculated with Eigen values which defines the amount of total variation displayed on the axes (PC_1 and PC_2). and cluster analysis based on algorithms of un-weighted paired group method using arithmetic average (UPGMA). Currently there are many molecular markers routinely used to evaluate genetic diversity in plants. Improvement in yield and quality is normally achieved by desirable characters of the genome which is elevated through molecular markers (Rashid et al., 2013).

Currently, various techniques has been developed for identification of enormous varieties of vegetables through molecular markers mainly in onion, garlic and related species (Fischer and Bachmann, 2000), tomato, potato (Ashkenazi et al., 2001). In the current study, we have chosen the RAPD (Randomly Amplified Polymorphic DNA) and ISSR (Inter Sequence Simple Repeats) markers for genetic diversity studies in onion. Regardless of their huge economic significance, the genetic resources of the Indian onion varieties stay uncharacterized at the molecular genetic diversity. Hence, owing to the widespread impact of genetic variations in onion varieties on varied soil ecosystems, there is a great surge in recent years to understand and gain insight into factors that put in to the genetic diversity by using the molecular markers. The origin of few selected local onion cultivars are yet unknown, therefore we included these cultivars in the present study, which could significantly contribute to the process of crossbreeding. Therefore objectives of this study were to estimate the genetic variations in nine different onion cultivars for the first time and to evaluate the implications of the study for future breeding and genebank management programs.

2. Materials and methods

2.1. Plant material and growth conditions

Nine cultivars of onion viz. AF: Agrifound rose, BL: Bellary, PR: Prema-178, NR: Nasik red, AK: Arka kirthiman, AL: Arka lalima, GLR: Garvan light red, LR: Light red, RD: Red diamond were collected from local farmers with reference to the YSR Horticultural University, Kadapa and National Horticulture Research and Development Federation (NHRDF) Institute, Kurnool. A field experiment was conducted at green house conditions, Yogi Vemana University, Kadapa, India located at latitude 14.47° N longitude 78.92° E. To evaluate the responses of onion cultivars morphological observations were recorded up to 10 days. At the end of 10th day biochemical and molecular screening tests were carried out.

2.2. Morphological screening of Onion cultivars

The seeds were considered germinated when the tip of the radicles are grown free from the seed coat. The percentage of germination was calculated as followed by Wiese and Winning equation: GP-Ni/N × 100 (Where Ni: No. of germinated seed till ith day, N: Total no. of seeds) (Sai Sudha and Riazunnisa, 2015). Germination race with Maguire equation: GR - N1 / D1 + N2 / D2 + … + Ni / Di (Where, GR: Germination Race, Ni: No. of germinated seeds in every numeration, Di: No. of days till nth Numeration. N: No. of numeration times (Keshavarzi et al., 2011). Seed vigor was calculated as per Abdul-baki and Anderson equation: SV- {GP × (RL + PL) mm} ÷100 (Where, SV: Seed Vigor, RL: Radicle Length, PL: Plumule Length). Fresh weight, dry weight and the lengths of seedlings were measured for morphological screening.

2.3. Biochemical screening of Onion cultivars

The quantitative measurements of following biochemicals were done by using an UV–Vis spectrophotometer (Thermo Scientific Evolution-210). Protein concentrations of the extracts were determined by Lowry method (Lowry et al., 1951), Proline content by Bates et al. (1973), Total soluble sugars by DuBois et al. (1956), Alkaloids by Harborne (1973), Total flavonoids by Chang et al. (2002). Tannins by Van-Burden and Robinson (1981). Total chlorophylls by Arnon (1949), Total phenolic content by Sun et al., Relative water content by Yamasaki and Dillenburg (1999). Antioxidant studies were carried out with DPPH and FRAC assays; the radicle scavenging activity with DPPH was measured in nine cultivars of onion using Brand-Williams et al. (1995). Ferric reducing antioxidant capacity of the onion extracts were done according to the proposed method of Benzie and Strain (1999).

2.4. Molecular screening

2.4.1. DNA extraction and quantification by modified CTAB method

Approximately 100 mg of 10 days old onion seedlings were taken in 2 mL test tube and placed in liquid nitrogen for 10 min and then ground to fine powder. DNA Extraction procedure was followed by Doyle (1990). Quantification of the DNA was done by spectroscopic measurement at 260 nm. An aliquot of the DNA sample was diluted in TE buffer in a ratio of 1:1000 in a 1 mL cuvette (50 μg/mL). Optical density was determined at 260 and 280 nm against blank with TE buffer. The ratio of A_{260} to A_{280} was calculated to check the purity of DNA. DNA samples for sample analysis were diluted to 7.5 ng/μL for RAPD and ISSR marker studies.

2.4.2. RAPD and ISSR Amplifications using PCR

The isolated and quantified total genomic DNA amplified with 18 RAPD (decamer) primers and 10 ISSR primers. The amplifications were performed individually for both RAPD and ISSR primers. The reaction volume is composed with 20 μL of 10 X PCR buffers (Tris pH 9.0, 10 mM KCl 50 mM, $MgCl_2$ 1.5 mM, dNTP's 5 mM, 0.2 U (Taq DNA polymerase) (Genei, India), 5 μM RAPD, 30 ng DNA and final make up to ddH$_2$O. For ISSR amplifications 5 mM ISSR's primers were added to the above reaction mixture. Polymerase chain reactions of each primer (RAPD and ISSR's) was performed in Thermocycler (Eppendorf, Germany), with initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min; primer annealing at 36 °C for 1 min followed by extension at 72 °C for 1 min and final extension at 72 °C for 6–8 min. The amplified products were separated by gel electrophoreses in agarose (1.5%) in 1X TBE buffer. Then after gels were stained with EthBr and documented by gel documentation system (SynGene, UK).

2.5. Correlation, PCA and Cluster analysis

Before achieving cluster analysis, the means of quantitative characters of morphological and biochemical data were standardized in order to comprehend the phylogenetic relationship between nine different onion cultivars. A cluster analysis was applied on the basis of Unweighted Pair Group Method with Arithmetic Average (UPGMA), by employing NTSY/Spc software version 2.02. Jaccard's coefficient of similarity tool was employed to determine the pair wise similarity matrices among the samples. Jmp software was used for PCA (principal component analysis) and two way cluster analysis respectively.

2.6. Molecular marker analysis

Reproducible amplicons obtained after PCR amplification of RAPD and ISSR's were scored '1' as present or '0' as absent, each of phenotypes were assembled and used in statistical analysis. The fragments were taken in consideration when the bands were reproducible, the Polymorphic Information Content (PIC) was calculated by the following

Table 1
List of onion cultivars, season and place where the cultivars are grown.

S. No.	Cultivar	Season	Place
1.	Agrifound rose	Kharif Late Kharif Rabi	Andhra Pradesh Karnataka
2.	Bellary	Kharif	Andhra Pradesh
3.	Prema − 178	Early Kharif	Andhra Pradesh
4.	Nasik red	Kharif and Rabi	Andhra Pradesh Maharashtra
5.	Arka kirthiman	Kharif and Rabi	Andhra Pradesh Karnataka
6.	Arka lalima	Kharif and Rabi	Andhra Pradesh Karnataka
7.	Garvan light red	Kharif Late Kharif	Andhra Pradesh Madhya Pradesh Gujarat Maharashtra Karnataka
8.	Light red	Kharif	Andhra Pradesh Maharashtra Gujarat
9.	Red diamond	Kharif and Rabi	Andhra Pradesh Karnataka Madhya Pradesh Uttar Pradesh Himachal Pradesh Gujarat Rajasthan Punjab Delhi Odisha

formula: PIC = 2f (1-f) where frequency of polymorphic bands occurrence with different primers. Pair wise similarity matrices were created by Jaccard's coefficient of similarity tool and dendrogram was constructed by using the UPGMA by using NTSYS-pc 2.02 software (Rohlf and Slice, 1993) to reveal the phenotypic representations of genetic relationships with similarity coefficients.

2.7. Experimental design and statistical analysis

Each experiment was designed with minimum three replicates and randomized design. The data presented are the average values (± SE) of results from three experiments conducted on different days. The data were subjected to further statistical analysis by one way ANOVA (Holm-Sidak method) using Sigma Plot Version 13.0.

3. Results

In the present study, as a preliminary screening of nine onion cultivars (Table 1), morphological, biochemical and molecular screening experiments were performed. To access the phenotypic variability of genotypes first morphological experiments were conducted at field level, the results of the experiments were depicted statistically significant to higher germination percentage (GP) (91%), germination rate (GR) (10.88), seed vigor (SV) (9.74) in Red diamond, least was represented by Nasik red GP (37%), GR (4.14) and SV (4.25) (Fig. 1) which was also significant, but the length of seedling was more in Nasik red i.e. 11.24 mm. Arka kirthiman recorded highest plumule length (PL) (8.73 mm), least in Bellary (5.92 mm), however a greater radicle length (RL) was recorded in Agrifound rose (5.97 mm) and least in Prema-178 cultivar (3.18 mm). Fresh weight (FW) and dry weights (DW) were measured in milligrams (mg), high FW was observed in Garvan light red with 876.5 mg and least in Agrifound rose with 575.2 mg. The same pattern was observed in DW 32 mg in Garvan light red, 16.8 mg in Agrifound rose cultivars respectively (Fig. 2).

Biochemical study was carried out among the nine cultivars of onion

seedlings to investigate the quantitative analysis of important phytochemicals. All the studied seedlings of onion contain a significant amount of protein, proline, sugars, alkaloids, phenols, flavonoids, tannins, chlorophyll, RWC and antioxidants. Among the nine different onion seedlings Prema-178 exhibited high protein levels with 1130.23 µg g^{-1} FW, whereas Red diamond exhibited least protein levels with 683.72 µg g^{-1} FW. While in case of proline accumulation study, comparatively greater result was observed in Bellary (464.04 µg g^{-1} FW) and least in Nasik red (186.97 µg g^{-1} FW) correspondingly. The sugar determination revealed that the Agrifound rose demonstrated maximum content with 158.77 µg g^{-1} FW and minimum in Red diamond with 28.72 µg g^{-1} FW. The results of quantitative of alkaloids levels revealed that Bellary exhibited maximum alkaloid content (1.58 mg g^{-1} DW) which was trailed by Agrifound rose (1.46 mg g^{-1} DW) and Light red cultivars (1.32 mg g^{-1} DW) and the minimum was exhibited by Arka lalima i.e. 0.13 mg g^{-1} DW respectively. However in case of phenols content, the greater levels were observed in Bellary with 152.61 mg g^{-1} DW and lower in Prema-178 with 62.39 mg g^{-1} DW (Table S1).

Similarly, flavonoids quantification revealed highest in Arka lalima with 92.4 mg g^{-1} DW and lowest in Gavran light red (89.6 mg g^{-1} DW). While tannin levels were maximum in Gavran light red (9.014 mg g^{-1} DW) and minimum in Agrifound red (0.22 mg g^{-1} DW). In onion seedlings a significant amount of chlorophyll pigments were observed, maximum amount in Arka lalima with 0.568 g^{-1} FW followed by minimum in Agrifound rose cultivar with 0.354 g^{-1} FW. But in relative water content parameter highest percentage in Light red (91.09%) and lowest in Nasik red (71.96%) and likewise good quantity of total antioxidant capacity with DPPH and FRAC assays, a significant difference observed in all cultivars, highest DPPH activity in Red diamond (99.78%) and lowest in Nasik red (69%), FRAC levels were maximum in Prema-178 (34.44%) and minimum in Arka kirthiman (30.18%) with respect to scavenging activity.

PCA is a most authoritative tool in germplasm collection for having complete knowledge on the whole collection, it makes probable to categorize the most suitable cultivar among all. PCA can be used to calculate the morphological and biochemical distances of the cultivars used. During this case study, PCA was carried out with nine different cultivars of onion. PCA generated first 3 Eigen values of 6.95, 4.89 and 2.61 accounted 68.87 cumulative percentages. The first is 33.13 and second 23.29 Eigen gave 56.43 percentages (Table 2). PCA was conducted for the quantitative measurements of morphological and biochemical parameters confirmed that 3 of the components explicate the broad variability. The remaining components elucidate a small proportion of the variability. In view of these, a scattered plot was developed on account of principal components of axis- I and II (PC-I on X-axis and PC-II on Y-axis). Interestingly the analysis illustrated that nine cultivars are scattered in to different clades corroborating their clustering pattern and their differentiation within the cultivars of onion (Fig. 3). Among the nine cultivars, single cultivar (Light red) distributed to negative side of component I and II, rest of cultivars to positive side. Biplot based representation of PCA is a two dimensional approach for combining the allied characters. Here biplot confirmed that distribution of quantitative characters studied with morphological and biochemical parameters of PCA. This was plotted on X and Y-axis with component I (33.1%) vs. component II (23.3%). 21 quantitative characters used for principal component analysis, during biplot analysis 14% of 5 quantitative characters such as protein, FRAC, RWC, sugars and root length distributed to negative sides of component I and II. Remaining 76% of 16 quantitative characters to positive. The phenotypic dendrogram generated by UPGMA cluster analysis separated nine cultivars into four groups of clusters; they are I, II, III and IV clusters (Fig. 4) namely cluster-I with Agrifound rose (single cultivar), cluster-II with Bellary, Light red, Prema-178 (three cultivars), cluster-III with Nasik red and Garvan light red (two cultivars) and cluster-IV with Arka kirthiman, Arka lalima and Red diamond respectively.
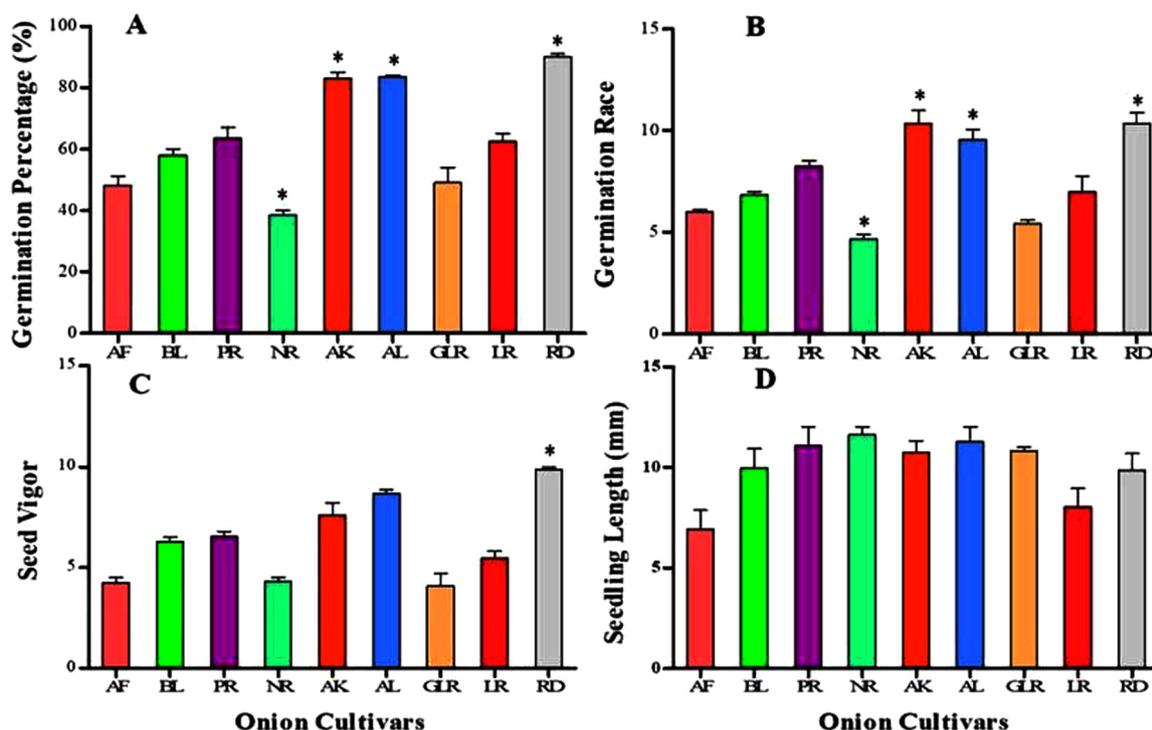
Fig. 1. Morphological parameters of onion cultivars with field experiments, A) Germination percentage (%), B) Germination race, C) Seed vigor and D) Seedling length. (AF: Agrifound rose, BL: Bellary, PR: Prema-178, NR: Nasik red, AK: Arka kirthiman,.
AL: Arka lalima, GLR: Garvan light red, LR: Light red, RD: Red diamond). Each bar is represented as mean average ± standard deviation of three replicates performed randomly at different time periods. Asterisks indicate that the differences (p < 0.05) between the different cultivars are statistically significant as determined by one way ANOVA (Holm-Sidak method).
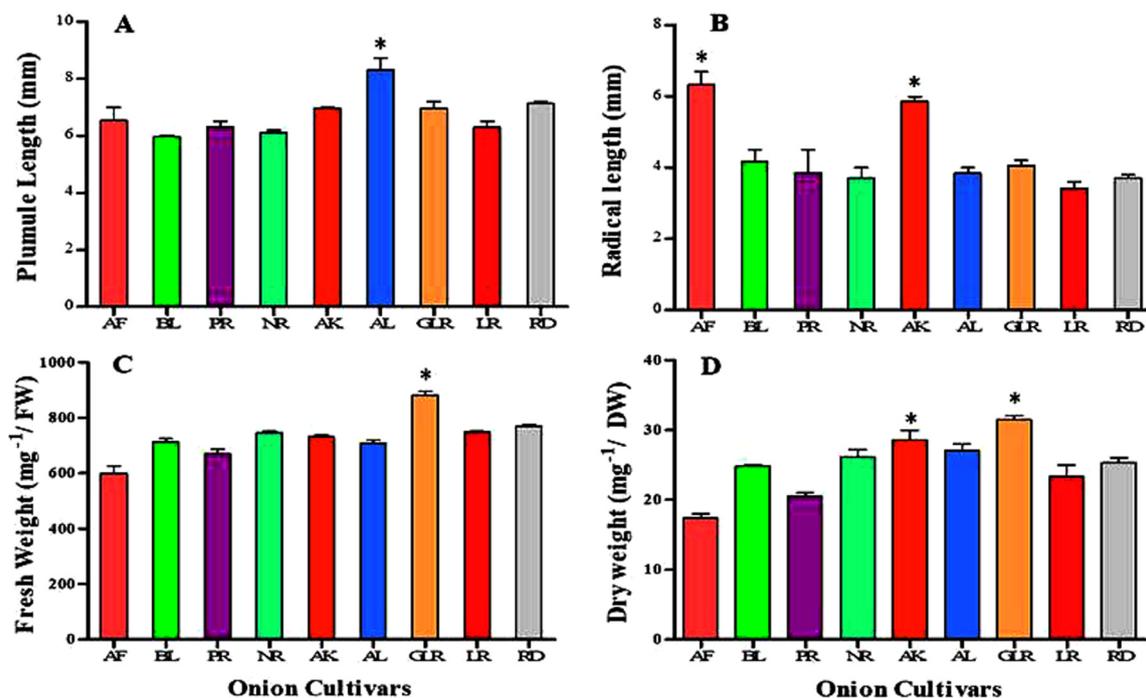

Fig. 2. Morphological measurements of onion cultivars A) Plumule length, B) Radical length, C) Fresh weight and D) Dry weight. (AF: Agrifound rose, BL: Bellary, PR: Prema-178, NR: Nasik red, AK: Arka kirthiman, AL: Arka lalima, GLR: Garvan light red, LR: Light red, RD: Red diamond). Each bar is represented as mean average ± standard deviation of three replicates performed randomly at different time periods. Asterisks indicate that the differences (p < 0.05) between the different cultivars are statistically significant as determined by one way ANOVA (Holm-Sidak method).

Table 2
Eigenvalues, percent and cumulative percent of total variance generated by principal component analysis of morphological and biochemical traits in onion cultivars.

| Number	Eigen value	Percent		Cum Percent	chi-square	F	Prob> ChiSq
1	6.9592	33.139		33.139	.	209.358	.
2	4.8913	23.292		56.431	.	201.494	.
3	2.6157	12.456		68.887	2.589	189.668	1.0000
4	2.1223	10.106		78.993	15.777	174.328	1.0000
5	1.6803	8.002		86.995	34.308	158.672	1.0000
6	1.3869	6.604		93.599	60.029	143.205	1.0000
7	0.8916	4.246		97.845	99.951	128.016	0.9685
8	0.4526	2.155		100.000	165.387	113.077	0.0010*

In molecular marker analysis, PCR amplicons with high reproducible visual bands were scored for both RAPD and ISSR marker analysis. To elevate genetic relationships among the cultivars of onion can thus assist onion breeders to predict sterile onion line. Strikingly the 18 RAPDs primers and 10 primers produced highly polymorphic reproducible banding pattern in *Allium cepa* L. genotypes. The 10-mer (decamer) primers were able to generate a minimum of 2 amplicons to maximum of 10 amplicons with an average of *PIC* 0.07–0.5 across the genotypes studied. A total of 6 ISSR primers, out of 10 primers were studied based on their polymorphic banding pattern (Table 3). These six 15–18 bp primers were able to amplify a minimum of 2–5 amplicons across the genotypes with a total of 17 amplicons of varying size and *PIC* value ranged between 0.06 and 0.26. Molecular analysis for informative primer pairs among nine onion cultivars scored through presence or absence of polymorphic DNA bands. Genetic matrix, consisting 77 informative alleles (Figs. 5 and 6) were subjected to cluster analysis to assess the diversity using NTSYS-pc program. Dendrogram of nine cultivars was constructed using Jaccard's genetic DICE similarity coefficients (Table S2, details in supplementary data). Notably, out of 18 RAPDs 10 evidenced reproducible amplicons, out of 10 ISSRs, 6 depicted reproducible amplicons. The polymorphic information content (*PIC*) was highest for the RAPD primer OPA 04 (0.43) and lowest for OPG 14 (0.08), in case of ISSRs highest was in 866 (0.35) and lowest in 880 (0.13) (Table 2).

A dendrogram generated by Unweighted Pair Group Method with Arithmetic Average (UPGMA method) cluster analysis nine cultivars were separable into two major clusters. Cluster-I Agrifound rose (single cultivar). Cluster-II with rest of eight cultivars, of which further fall into Sub-clusters, Sub cluster-IIa, six cultivars, Bellary, Arka kirthiman, Light red, Arka lalima, Gavran light red and Red diamond. Sub-cluster IIb, two cultivars Prema-178 and Nasik red. The present analysis is
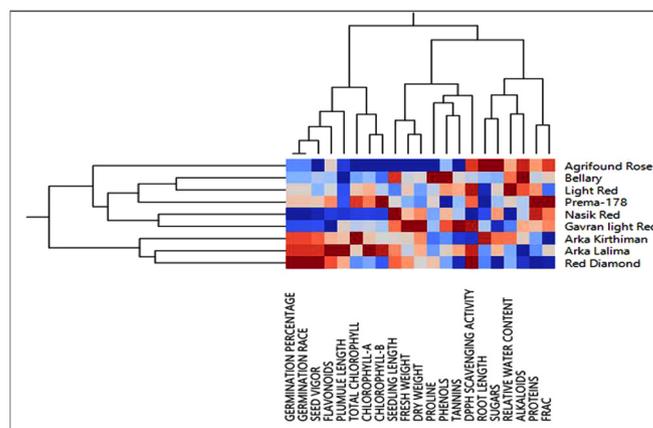
Fig. 4. Quantitative dendrogram generated by UPGMA clustering method with morphological and biochemical parameters of onion cultivars.
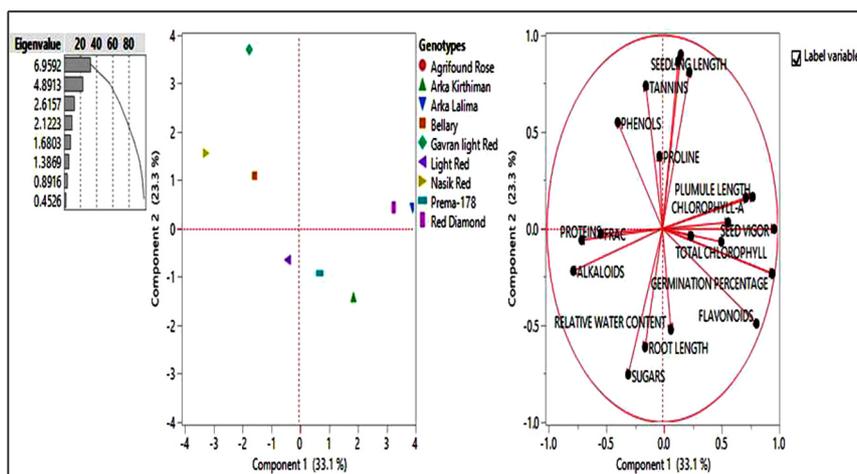
Fig. 3. Principal component analysis applied on morphological and biochemical characteristics of nine onion cultivars.

Table 3
Polymorphic information content (*PIC*) of *Allium cepa*. L cultivars using RAPD and ISSR primers.

Primer name	Sequence (5′-3′)	Annealing Temp*(°C)	No. of amplicons	No. of polymorphic bands	*PIC*
RAPD					
OPA-4	AATCGGGCTG	36	6	6	0.43
OPAL-20	AGGAGTCGGA	36	5	5	0.36
OPA-12	TCGGCGATAG	36	6	6	0.39
OPA-14	TCTGTGCTGG	36	5	5	0.22
OPAB-6	GTGGCTTGGA	36	5	5	0.49
OPB-5	TGCGCCCTTC	36	4	4	0.35
OPM-2	ACAACGCCTC	36	5	5	0.40
OPN-4	GACCGACCCA	36	6	6	0.40
OPP-7	GTCCATGCCA	36	3	3	0.25
OPG-14	GGATGAGACC	36	7	7	0.08
ISSR					
822	TCTCTCTCTCTCTCA	49	2	2	0.27
842	GAGAGAGAGAGAGAYG	49	5	5	028
881	GGGTGGGGTGGGGTG	49	3	3	0.35
880	GGAGAGGAGAGGAGA	49	9	9	0.24
866	CTCCTCCTCCTCCTCCTC	49	3	3	0.13
824	TCTCTCTCTCTCTCG	49	6	6	0.40
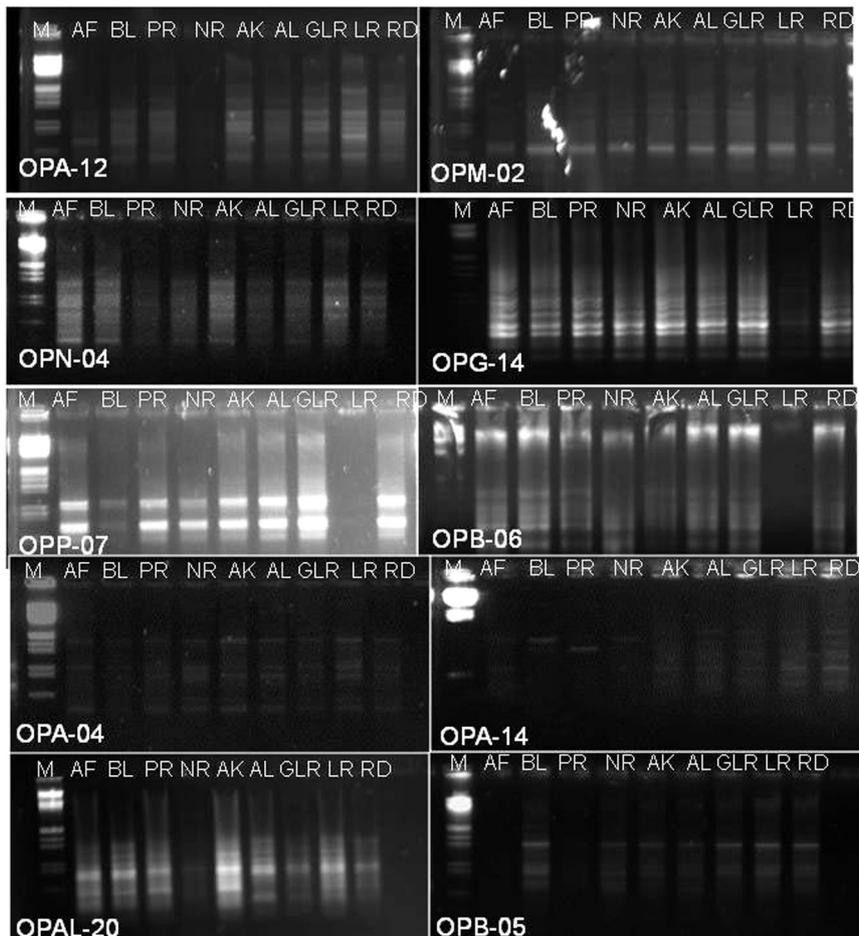
Fig. 5. Allelic pattern of RAPD marker studies in nine cultivars of onion (M: Marker (1 Kb, λ Hind - III) AF: Agrifound Rose BL: Bellary PR: Prema-178 NR: Nasik Red AK: Arka Kirthiman AL: Arka Lalima GLR: Garvan Light Red LR:Light Red RD: Red Diamond).
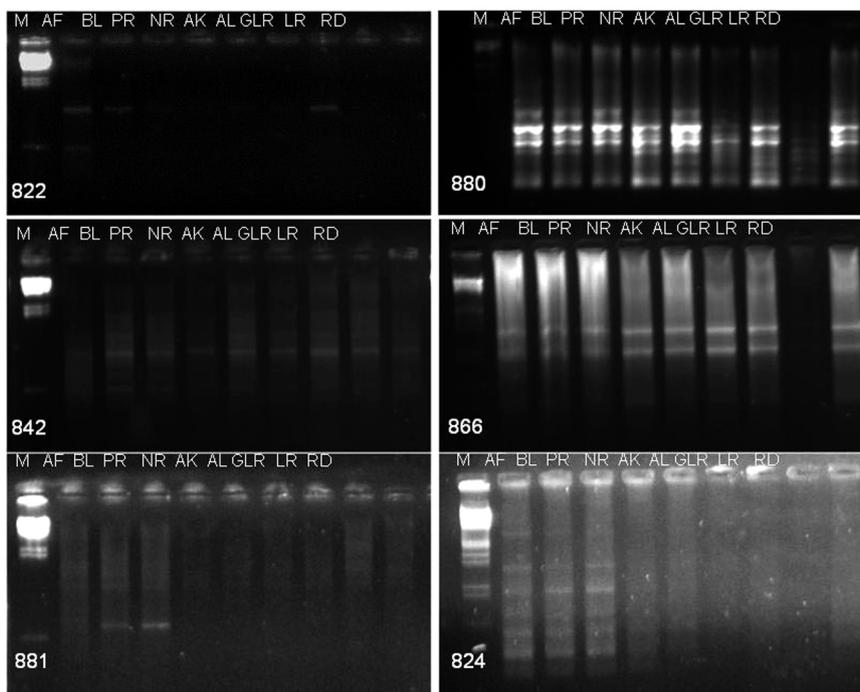
Fig. 6. Allelic pattern of ISSR marker studies in nine cultivars of onion (M: Marker (1 Kb, λ Hind - III) AF: Agrifound Rose BL: Bellary PR: Prema − 178 NR: Nasik Red AK: Arka Kirthiman AL: Arka Lalima GLR: Garvan Light Red LR: Light Red RD: Red Diamond).
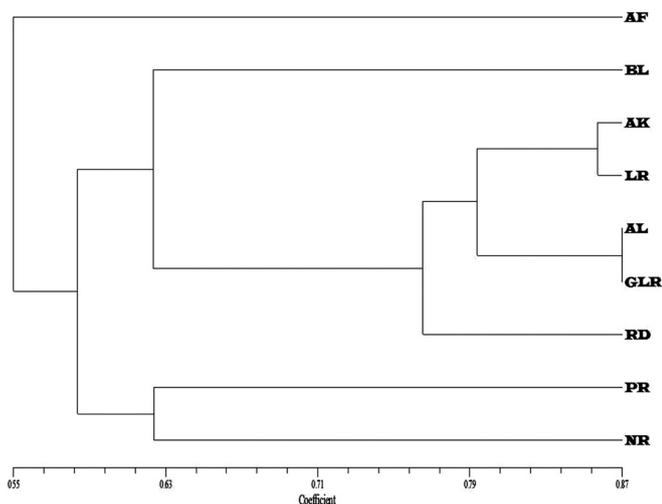
Fig. 7. Dendrogram obtained from nine cultivars of onion with UPGMA based on Jaccard's coefficient using RAPD and ISSR primers. (AF: Agrifound rose, BL: Bellary, PR: Prema − 178, NR: Nasik red, AK: Arka kirthiman, AL: Arka lalima, GLR: Garvan light red, LR: Light red, RD: Red diamond).

based on 10 RAPD and 6 ISSR markers which revealed significant genetic diversity among the onion cultivars studied. The similarity values in terms of genetic distance ranged from 0.55 to 0.87 (Fig. 7). Based on the aforesaid results, it is clearly evident that each cultivar is different from other cultivars and may possess novel genetic components.

4. Discussion

Plant breeding rests on the availability of genetic diversity, it refers to the differences in genome ranging from rearrangement of single base pair to entire chromosome (Hamrick and Godt, 1996). These variations in the genome interactions with environment, reveals conspicuous changes in diversity of plants (Huang and Han, 2014). It is notable that

genetic diversity is a fundamental strategy for developing improved cultivars or genotypes to maintain and enhance the productivity, stability and sustainability of agriculture crops. Interestingly, several breeding programs have been previously employed in the development of improved cultivars suitable for diverse agro climatic conditions (Moose and Mumm, 2008). Furthermore it is aimed at studying morphological, biochemical and molecular marker approach for better diverse patterns for improved agriculture in onion. Crop diversity is one of the most fundamentally important resources for human life on earth. The conservation and sustainable use of plant genetic resources are essential to the sustainable development of agriculture (Tilman et al., 2002). Hence the knowledge of crop genetic diversity in the cultivated crops is very essential in germplasm collection and breeding.

In the current study, onion diversity marker assessment approach applied at morphological, biochemical and molecular levels. Commonly, morphological markers are visually assessable traits, influenced by the environment. Our results revealed several morphologically variable changes with respect to percentage of germination, race, vigor and lengths of the seedlings or germinated among onion cultivars (Figs. 1 and 2). The morphological results in onion seedling revealed that variations in quantitative germination characteristics were compared among the nine cultivars, confirming their importance in selecting the cultivar (Aryakia et al., 2016). However morphological parameters have been used in many studies to specify species, families and cultivars. Strikingly the studied onion cultivars exhibited variations and highlighted wide range of phenotypic variation present in the collected germplasm. The main advantages of these markers are simple and inexpensive. Nonetheless the classical approaches for the identification of *Allium cepa* L. cultivars with morphological traits are exceptionally difficult and their evaluation is subjective. Therefore it is obligatory to identify and characterize selective candidate for achieving crop improvement in onion (Misra and Gupta, 2005).

Biochemical study of secondary metabolites in onion cultivars are potential sources of important phytochemicals for plant growth and development. Proline and sugar are involved in osmotic adjustment and acts as osmoprotectants, free radical scavenger and antioxidant activity (Sairam and Aruna, 2004). Flavonoids are found in dietary noted to

play effective roles in preventing cancer, traditionally which is an antimicrobial agent (Singh et al., 2016). Currently using principal component analysis (PCA) morphological, biochemical and molecular evidence is in practice to prove hybridization among different plants (Zimisuhara et al., 2015). During the correlation analysis of genetic diversity in onion, principal component analysis (PCA), is calculated with Eigen values as the amount of total variation displayed on the axes (PC_1 and PC_2). The PC_1 (first principal component) defines most of the variability achieved from the original data, whereas in PC_2 most of the variability is not summarized by PC_1 and uncorrelated with PC_1. PC (principal component) is expressed as the Eigen value divided by the sum of Eigen values and cluster analysis based on algorithms of unweighted paired group method using arithmetic average UPGMA (Table 1). In our studies, no parallel relationship was found with diversity and PCA analysis between the morphological, biochemical studies in onion cultivars because differences in their origin or may be the factors affected while germination and their growth (Fig. 3).

Molecular markers relying on DNA assay. During last decades, use of molecular markers revealing polymorphism at DNA level, has been playing a vital role in improvement of vegetables and the most use of this markers is germplasm characterization (El-Esawi, 2015). Diversity analysis is routinely carried out using molecular marker technologies. The desirable properties of molecular markers are high polymorphism, co-dominant, frequent distribution and even distribution throughout the genome (Madhumati, 2014). Genetic diversity assessment is a prerequisite using molecular markers for understanding genome structure, characterization and maintenance of genetic variation in germplasm to plant breeding strategies for crop improvement (Buckler and Thornsberry, 2002). Several sets of SSR markers have been previously used to detect genetic diversity in onion germplasm (Jakse et al., 2005; Chakraborty et al., 2015; Simó et al., 2014), but no studies including onion genotypes from South India, have been recorded so far.

In our work, the cultivars of *Allium cepa* L. formed two groups with RAPD and ISSRs markers, results depicted that RAPD and ISSRs were more efficient in expressing polymorphism at DNA level in selected onion cultivars (Table 2), the fact explained that arbitrary regions amplified by RAPD, while ISSRs targeted at specific genome sequence. Different onion breeding programs utilize RAPD technique to identify an onion line or cultivar. RAPDs have been applied with many other crops species. Sangeeta et al. (2006) applied 90 RAPD primers grouped 24 onion cultivars into northern and southern regions of India. Ten varieties of onion *(Allium cepa* L.) were analyzed, Bermis and India-2 were more dissimilar and Faridpuri and Bhati were the most genetically similar (Maniruzzaman et al., 2010). 32 germplasm resources of onion analyzed with ISSR markers and germplasms were divided into five groups (Chinnappareddy et al., 2013). Present analysis with RAPD and ISSR data were used as support for morphological studies that it could be possible to determine the extent of diversity and relation within and between the *Allium cepa* L. species (Fig. 5). However, the precise assessment of genetic diversity through molecular markers depends on the type of DNA polymorphism with length vs. sequence variation detected and the proportion of genome covered by such a marker system. The reports of Mohanty and Prusti (2002) give support for our obtained results. Evidence from Shanmugam and Rangasamy (1982) said that even though from same origin distributed in different clusters is a broad genetic base of genotypes belongs to same origin. To overcome the problem associated with phenotypic based parental selection, DNA based markers were previously proven to be extremely useful and of all marker kinds.

5. Conclusion

RAPD and ISSR markers were used to assess the genetic diversity of onion cultivars from Kadapa. The obtained results confirmed the efficiency of RAPD and SSR markers. The result of the current research is a clear understanding of the genotypic relationships of nine selected varieties of onion. Statistically mutivariant statistical tools dispense mode of evaluating diversity. Furthermore, it can be concluded from our findings that the molecular analysis of RAPD and ISSR markers were extremely powerful for studying the genetic relationship among nine cultivars of onion, it was observed with UPGMA dendrogram where Agrifound rose is in a group is alone and all other cultivars in another group. Interestingly, Agrifound rose cultivar is deemed unique in morphological and biochemical characteristics as revealed in quantitative dendrogram clustering. The molecular genetic analysis performed in this study endows with worthful information to researchers for prospective conservation studies novel genetic approaches on the specificity of local onion varieties.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2018.11.007.

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