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Utility of Simple Sequence Repeat (SSR) Markers to Realize Worth of Germplasm in Genus *Allium*

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Allium germplasm harbour novel genes which could be utilized in breeding programme for developing elite cultivars. Availability of wide genetic variability and its comprehensive knowledge is an important step towards the development of improved varieties in different cultivated alliums. Earlier, *Allium* germplasm characterized based on morphological markers but they are affected by environment and plant development stages which leads to biased estimates of variability. In various crop plants different molecular markers are utilized for diversity studies due to their unbiased nature and neutrality with no environmental effect. Also in genus *Allium* various marker systems such as isozymes, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) were used but they show number of disadvantages. To overcome their drawbacks, Simple Sequence Repeat (SSR) markers were developed in *Allium cepa*, *A. fistulosum* and *A. sativum* which are being utilized for DNA fingerprinting, genetic diversity analysis and cross amplification studies for better understanding of *Allium* germplasm. In future it will be desirable to use microsatellite markers for developing high density linkage map to follow marker assisted selection. Further understanding of population structure would be beneficial to exploit natural variation by using association mapping.

Key Words: *Allium*, Germplasm, EST, SSR, Transferability, Variation

Introduction

Allium is a species rich monocot genus as it includes about 780 species (Friesen *et al*., 2006) which are utilized as vegetables, condiments, medicinal and ornamental plants (Negi, 2006). Among alliums, bulb onion and garlic are cultivated worldwide while other species of economic importance such as bunching onion, shallot, leek and chives are grown in few counties. In addition to this, semi-domesticated and wild economic species are grown in a few pockets of Indian Himalayan region by local tribes (Negi, 2006; Pandey *et al*., 2008; Khosa *et al*., 2014).

Genetic resources enable the breeders to develop productive combinations of traits in elite cultivars to fulfill the needs in various agro-systems. Evaluation of germplasm is a prerequisite for utilization and the detailed evaluation determines the potentiality of an accession for specific purpose in crop improvement. Germplasm characterization and estimation of genetic diversity in plants on the basis of morphological characteristics is not much reliable. But, with DNA-based markers it is more desirable due to precise identification and quantitative estimation of genetic diversity (Glaszmann *et al*., 2010). However, development of reliable molecular markers is a challenging task in genus *Allium* due to its huge nuclear genome among monocots as onion genome (16,415 Mbp per 1C), which is nearly 36 times larger than rice and 6 times bigger than maize (Arumuganathan and Earle, 1991). Although, RAPDs were developed and used for diversity analysis (Bradeen and Havey, 1995; Ennequin *et al*., 1997; Tanikawa *et al*., 2002), but counter repeatability problem (Eicht *et al*., 1992) has made them less attractive to the researchers. Use of RFLPs and AFLPs has also been reported (Bark and Havey, 1995; Lampasona *et al*., 2010), but RFLPs cannot be employed for studies in routine due to their high cost, labour intensive procedure and low polymorphism, while dominant inheritance of AFLPs makes it less attractive to plant biologists (McCallum *et al*., 2008; Lampasona *et al*., 2010).

To overcome these problems, SSR markers are ideal due to their co-dominant inheritance, high hyper variability, wide genomic distribution, high reproducibility, multi-allelic nature, chromosome specific locations and ease to score (Varshney *et al*., 2005). Now-a- days due to the advances in next generation sequencing

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**Author for Correspondence: E-mail: jiffenvir.pau@gmail.com* technologies a wealth of genomic and transcriptome data was developed in various crop plants including genus *Allium* which were explored using different bioinformatic tools (Table 1) to find out SSR markers (Varshney *et al*., 2005; Baldwin *et al*., 2012; Sun *et al*., 2012; Duagnjit *et al*., 2013). As a result, in genus *Allium*, SSR markers have been developed in bulb onion (Fischer and Bachmann, 2000; Jakse *et al*., 2005; McCallum *et al*., 2008; Baldwin *et al*., 2012), bunching onion (Wako *et al*., 2002; Song *et al*., 2004) and garlic (Ma *et al*., 2009; Khar, 2012) which are utilized in different studies for realizing the worth of *Allium* germplasm. In the present review, the impact of utilizing SSRs in genus *Allium* for dissecting information about genetic resources is discussed.

Bulb Onion

Allium cepa is a highly valuable crop which is reported to have originated in central Asian region. Most of cultivars developed through selection and precise estimation of genetic variability using improved techniques is pre-requisite for efficient breeding (Brewester, 2008). In this context, sequence tagged microsatellites were developed from a genomic library enriched for microsatellite markers to determine genetic relationships between onion accessions. The eighty three bulb onion accessions were analyzed for diversity analysis where germplasm was partly grouped according to their geographical origins (Fischer and Bachmann, 2000). But, these markers had not proven reliable in various genetic studies due to their complex requirements and low transferability (McCallum *et al*., 2008). To overcome these barriers, Expressed Sequence Tags (ESTs) were generated and microsatellite markers were found in EST libraries during sequence analyses (Khul *et al.*, 2004). To test their utility in germplasm analysis, 35 elite populations from specific companies or breeding programmes were analyzed using EST- SSR markers and it was observed that different populations could be distinguished by them. Germplasm was found to be closely related with each other and grouping was according to their geographical origins (Jakse *et al.*, 2005). Later, more diverse germplasm was surveyed to estimate variation using 56 EST-SSR markers and four genomic SSR markers (McCallum *et al.*, 2008). Populations grown in long day, short day and Indian region clustered apart from each other (Fig. 1). They also suggested that resequencing of EST markers can readily provide SNP markers for purity testing of inbreds and other application in *Allium* genetics.

Table 1. Different bioinformatic softwares available to discover SSR markers

S. No.	Software Name	Reference
1.	Tandem Repeat Finder (TRF)	Benson *et al.* (1999)
2.	Tandem Repeat Occurrence Locator (TROLL)	Castelo *et al.* (2002)
3.	SSR Identification Tool (SSRIT)	Kantety *et al.* (2002)
4.	SSR Finder	Gao *et al.* (2003)
5.	MIcroSAtellite (MISA)	Theil *et al.* (2003)
6.	Build SSR	Rungis *et al.* (2004)
7.	SAT	Dereeper *et al.* (2007)
8.	SSR Locator	Maia *et al.* (2008)
9.	Rapid Identification of SSRs and Analysis (RISA)	Kim *et al.* (2012)
10.	GMATo	Wang *et al.* (2013)

Different diversity studies have reported uniqueness of Indian bulb onion germplasm than exotic germplasm but very few accessions/extant varieties were utilized (Mahajan *et al*., 2009). Later, greater number of accessions were used to assess the diversity of tropical Indian onion (Khar *et al*., 2011). It was reported that indigenous Indian short day onion formed separate cluster from the exotic short day and long day onions (Fig. 2). Also, Indian and North American germplasm are quite different from each other and central Asian revealed close relationship with Indian material (McCallum *et al*., 2008). It suggests that Indian cultivars and land races might provide novel germplasm sources to broaden bulb onion breeding base.

There is one concern with EST-SSR markers as they detect less number of amplicons while analyzing bulb onion germplasm. Also, onion has large genome size and low gene diversity because of which only a small region of onion genome has been explored. A new set of genomic SSR markers were developed that exhibited high allelic diversity during germplasm analysis. Most of the germplasm clustered according to their geographical origins but Algerian samples appeared to cluster across three groups and this may indicate multiple introductions into the country or independent selections (Baldwin *et al.*, 2012). In future, SSR markers will be useful to determine the levels of inbreeding and population structure for association mapping.

Bunching Onion

It is believed that bunching onion originated in North West China and is also being grown on a large scale in

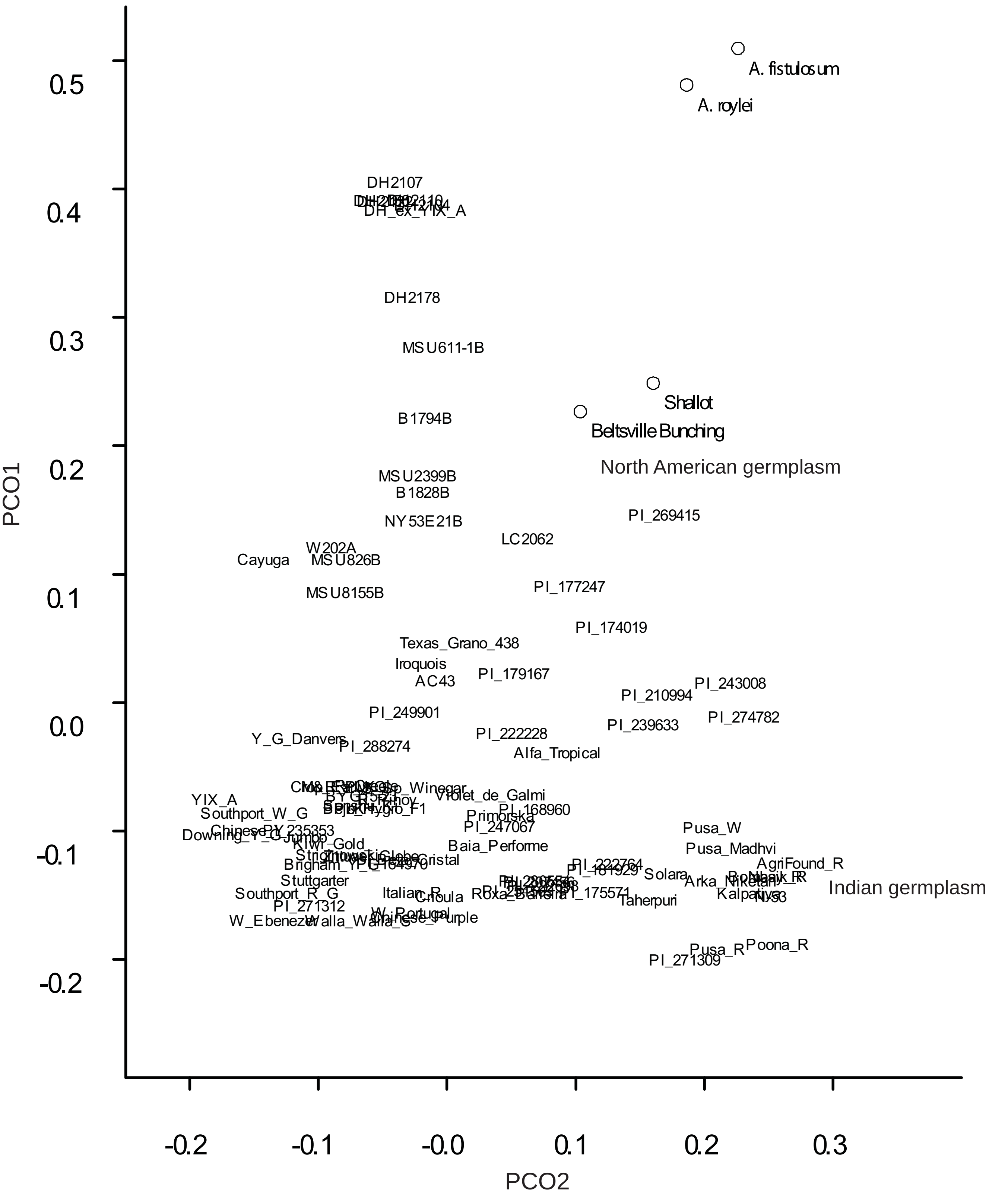

Fig.1. Principal co-ordinate analysis of Jaccard similarity matrix in bulb onion based on SSR markers (taken from McCallum *et al.*, 2008)

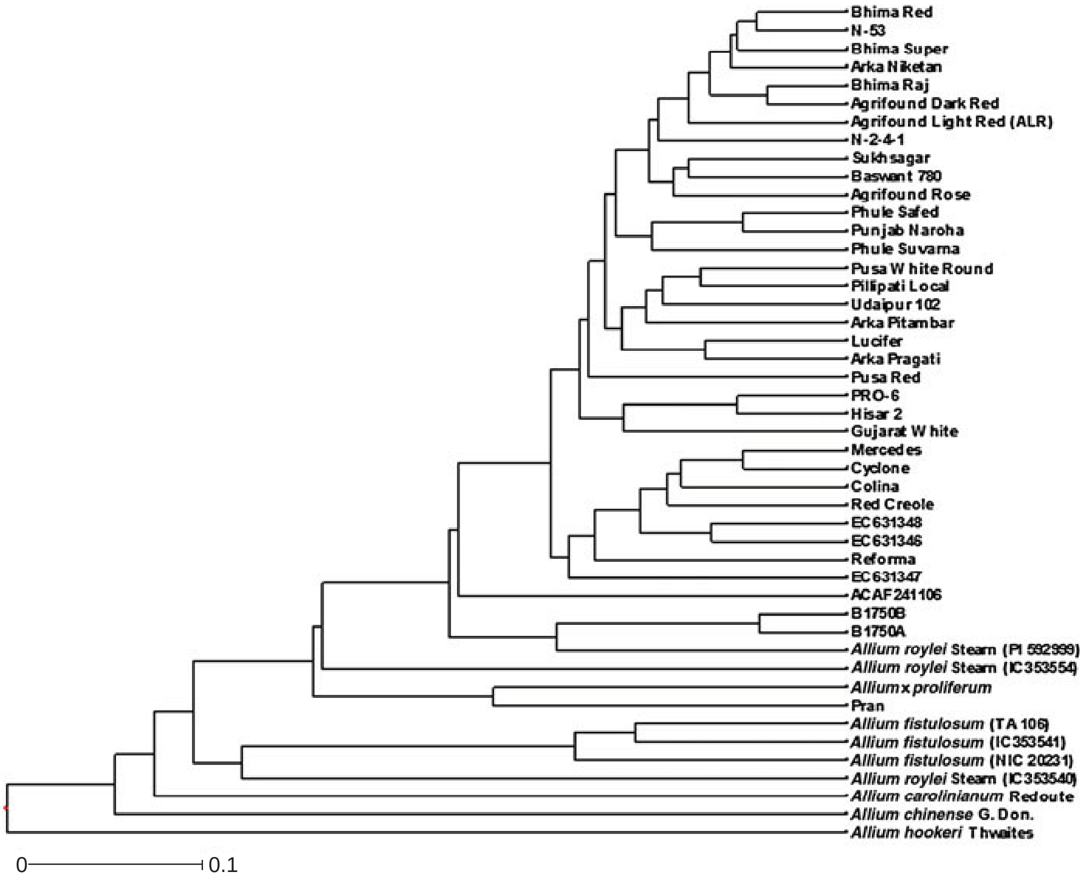

Fig.2. Clustering of bulb onion and allied species based on Jaccard dissimilarity index (Khar et al., 2011)

Japan and Korea (Brewester, 2008). In bunching onion, first set of SSR markers were developed from a genomic library and used for linkage map construction (Wako *et al*., 2002; Song *et al*., 2004; Tsukazaki *et al*., 2007; 2008). SSR markers were utilized to determine genetic uniformity in bunching onion hybrids and very low were detected at different polymorphic loci suggesting high level of heterozygosity (Tsukazaki *et al*., 2006). According to these results, SSR tagged breeding scheme was proposed in bunching onion and utilized to realize the utility of this scheme (Tsukazaki *et al*., 2009).

Thirty bunching onion cultivars were classified using SSR markers and it was consistent with the previously reported results based up on morphological characters. Average number of amplicons per primer in this study was high (10.6) in comparison to previous studies (Song *et al*., 2004) which indicates that high degree of genetic variability occurs in bunching onion germplasm (Tsukazaki *et al*., 2010). In bunching onion germplasm, SSR markers were more polymorphic than Sequence Related Amplified Polymorphism (SRAP) on the basis of an average number of polymorphic alleles (Li *et al*., 2008). But, the information given by SRAP was more consistent with morphological variability than that of SSR markers (Li *et al*., 2008). Bunching onion accessions collected from different regions of India found to be distinctive from exotic accessions (Khosa *et al*., 2013). In future, estimation of genetic variability in bunching onion germplasm of wide geographical origins must be investigated to detect some novel alleles.

Garlic

It is an asexually propagated *Allium*, which originated in central Asia is cultivated throughout the world (Brewester, 2008). The development of garlic cultivars has been limited to selection which hugely depends on the extent of variability available (Lampasona *et al*., 2010). SSR markers were also developed in garlic to study genetic diversity for planning efficient strategies for germplasm conservation (Ma *et al*., 2009; Cunha *et al*., 2012). These studies indicated that SSR markers act as useful tool for characterization of garlic germplasm. In this context, Acharya and Simon (2010) used 20 SSR markers on 48 accessions collected from different countries and got sufficient divergence in the germplasm. The Tunisian and French germplasm was characterized using ISSR markers where factor analysis of distances' table (AFTD) did not classify accessions on the basis of geographical origin or morpho-physiological characters, particularly bolting ability, but confirmed the appurtenance of analyzed accessions to s*ativum* botanical subspecies (Jabbes *et al.,* 2011). A core collection was developed, consisting of 95 accessions using heuristic approach which were the representative sample of whole germplasm. The degree of variability within accession (84.4%) was more than between the accessions (15.6%). There were four groups with - F_{ST} value of 0.1560, indicating a moderate differentiation among the groups (Zhao *et al.*, 2011). In China, considerable variability was observed in the garlic collections as 40 accessions were clustered into three groups (Xia *et al.*, 2012).

The garlic accessions collected from four countries were grouped into four clusters according to their geographical origin with SSR markers. It suggests that local selection pressure and differences in adaptability of garlic germplasm in particular regions is important cause of variability (Jo *et al*., 2012) Average number of alleles, observed heterozygosity, expected heterozygosity, Hardy Weinberg equilibrium, Shannon index and polymorphic information content values were 4.4, 0.468, 0.576, 1.073 and 0.518, respectively. Recently, EST-SSR markers were also developed in garlic and used for genetic diversity analysis (Ipek *et al.*, 2012; Khar, 2012). Indian garlic germplasm has considerable amount of variability and their clustering was independent of the geographical origin (Fig. 3) but based on the basis of vernalization requirement for bulb formation in garlic (Khar, 2012).

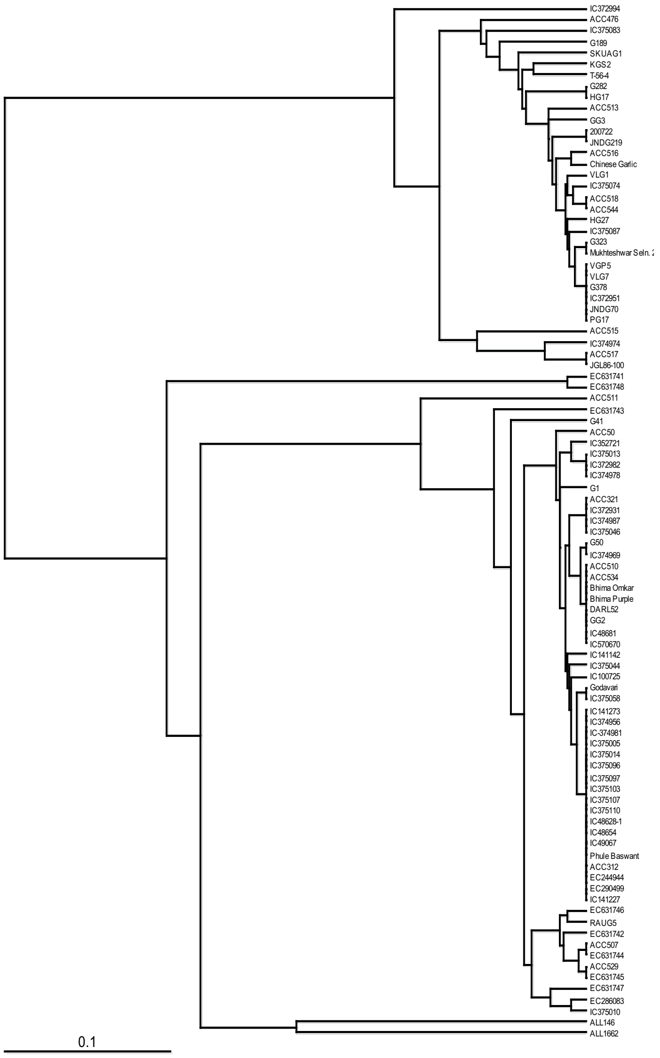

Fig. 3. The dendrogram based on Nei's genetic distance between 93 garlic accessions (Khar, 2012)

SSR Transferability

Availability of only limited number of SSR markers in *Allium* species is one of the major concerns for *Allium* research community. However, SSR transferability to related taxa is very useful approach as it was employed in various crops, which lacks sufficient sequence information (Varshney *et al.*, 2005).

Initially genomic SSR markers were screened for transferability studies but they exhibited low transferability in different *Allium* species (Fischer and Bachmann, 2000; Tsukazaki *et al.*, 2008; Araki *et al.*, 2010). It implies that markers developed from conserved coding regions would be more informative in transferability studies (Varshney *et al.*, 2005). In this context, bulb onion derived EST-SSR markers were employed for cross amplification in bunching onion and show high (75.10%) degree of transferability than genomic SSR (43.30%) markers (Tsukazaki *et al.*, 2008). Later, bulb onion derived SSR markers were used to estimate degree of transferability in wide range of *Allium* species and high transferability (100%, 87.17%, 25-91.70%) was observed in different studies (Khar *et al.*, 2011; Khosa *et al.*, 2013; Mallor *et al.*, 2014). The garlic derived SSR markers were tested for transferability in five related *Allium* species where highest transferability was observed in *A. porum* (73.00%) followed by A. *fistulosum* (48.00%) and *A. altaicum* (47.60%) which implies genome conservation among alliums (Lee *et al.*, 2011). Overall these findings suggest that in near future, SSR markers developed in bulb onion, bunching onion and garlic can be used in other *Allium* species for better understanding of *Allium* germplasm and their utilization.

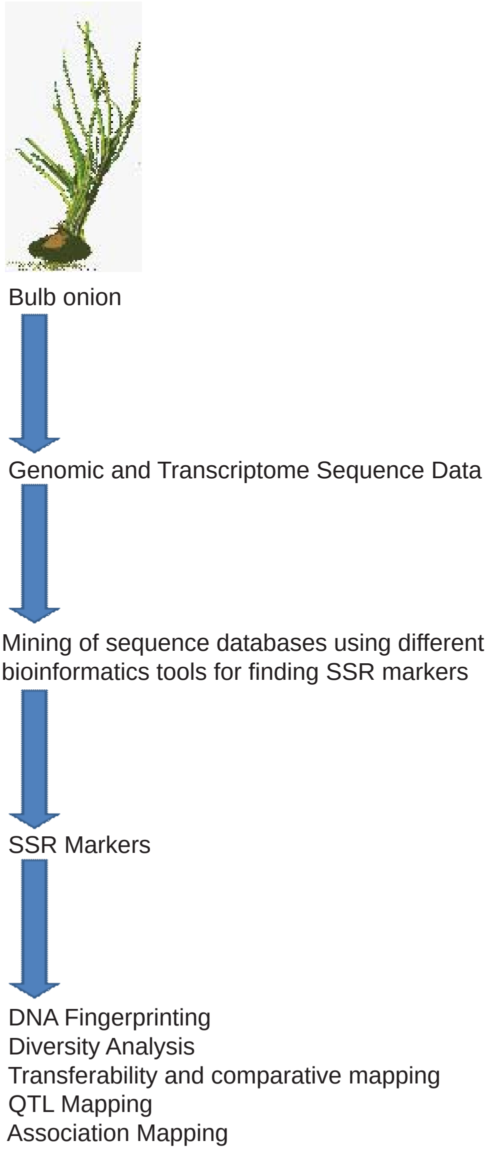

Fig. 4. A schematic flow chart for the development of SSR markers in bulb onion and their utilization

Conclusion

Utilization of SSR markers in genus *Allium* improves our understanding about genetic diversity and nature of *Allium* germplasm. This information would be useful to develop strategies for effective conservation and exploitation of *Allium* germplasm resources in improvement programs. Use of SSR markers to develop high density linkage map will facilitate the marker-assisted selection for useful traits in different *Allium* species. These markers could be utilized to study population structure which is pre-requisite to follow association mapping. Association mapping studies will be useful to find out the genomic regions associated with variability for various traits. Development of SSR-based interspecific linkage map will help us in revealing the synteny in various species. Establishment of syntenic associations will assist in the mining of orthologous genes and flow of genetic information between cultivated *Alliums* and their wild relatives for better understanding of *Allium* genomics. As transcriptome libraries were developed in garlic and bulb onion (Sun *et al.*, 2012; Duangjit *et al.*, 2013), it will be desirable to develop more EST-SSR markers to discover functional diversity in *Allium* germplasm.

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