Genome sequencing survey and identification of SSR of *Lycium ruthenicum*

Defang Zhang (defangstart2011@163.com)

Research

Keywords: Lycium ruthenicum, genome size, heterogeneity percentage, repetitive sequence

Posted Date: April 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23380/v1

Abstract

Background

Lycium ruthenicum had high economic and ecological role in western China due to the high content of active substances and tolerance to drought and salinity stress. But its genomic information was lack, which seriously affected the next breeding and forestation. **We** surveyed the genomic size and developed SSRs of *L. ruthenicum* based on the next generation sequencing technology to lay a theoretical foundation for next genomic research in this study.

Results

Totally 451,721,828 bp raw data were generated, 4,596,439 scaffolds were obtained after assembly. The estimated genome size of *L. ruthenicum* was 3,249.33 Mb, the heterozygosity rate was 1.13%, and repeat rate was 73.13%. Totally 958,619 SSRs were identified. The average SSRs density were 163.95 SSRs/Mb, the dinucleotide repeat motif accounted for larger proportion in all motifs, the AT/AT, AC/GT and AG/CT are dominant repeat motifs in *L. ruthenicum* genome.

Conclusion

These results could lay a foundation for next genome sequencing. And SSR data could alarge the molecular resources for *L. ruthenicum* and relatives, such as genetic mapping, QTL and population genetic study.

Background

Lycium ruthenicum Murrray (Solanaceae), mainly distributed in Qinghai Provinces, Ningxia and Xinjiang autonomous regions in China (Chinese flora,2006). *L. ruthenicum* has been cultivated for hundred years for economically and ecologically application. The Chinese medical classic “Jing Zhu Ben Cao” has recorded it for the treatment of heart disease, abnormal menstruation and menopause, and health care (Jia and Li 2005). Modern medicine research has recorded the abundant polysaccharides (Liu et al. 2013; Lv et al. 2013), anthocyanin (Zheng et al. 2011) and other active substances in fruit (Yabin et al. 2015).

L. ruthenicum has strong environmental adaptability, especially drought and salinity tolerance. *L. ruthenicum* was mainly growing in harsh environment, such as desert, highly saline and drought habitats (Dong et al. 2008; He et al. 2015). According to Geng (2012), even the soil water content dropped to 5%, it can be reached the normal metabolism stress point of *L. ruthenicum*. Previous research showed that appropriate salt stress was beneficial to the seed germination of *L. ruthenicum* (Chen et al. 2010; Wang et al. 2014). Based on the drought-resistance and saline-alkali resistance, *L. ruthenicum* has been widely planted in saline-alkali land improvement, water and soil conservation, desert management and so on

(Jalali et al. 2012; Peng et al. 2013). The cultivation of *L. ruthenicum* was asexual propagation by wild seedlings and lack of good cultivars. And because of its important medicinal value, the wild resources have been endangered due to overexploitation (Liu et al. 2012).

With the development of genomic technology and bio-informational analysis, many genomic researches about the Solanaceae plant have been reported, such as tomato (Anthony et al. 2014; The Tomato Genome Consortium, 2012), potato (Potato Genome Sequencing Consortium 2011), pepper (Cheng et al. 2014; Seungill et al. 2014), eggplant (Hideki et al. 2014), nicotiana (Bombarely et al. 2012; Nicolas et al. 2013; Sierro et al. 2014) and petunia (Aureliano et al. 2016). These genomic researches had laid foundation for genetic and genomic study, and accelerated breeding research. But few studies have been reported on *L. ruthenicum*, the genetic information has lack. The lack on genomics, genetics and cell biology of *L. ruthenicum* has restricted the cultivation and improvement in disease-resistant and new varieties of *L. ruthenicum*.

Considering its importance role in economy and ecology, it is urgent to reveal the potential genetic background in the synthesis of active components, as well as the genetic mechanism related to its drought and salinity resistance. Based on the genetic information, we can clarify the synthesis pathway and regulatory mechanism of its specific active components, so as to lay the foundation for targeted genetic improvement by means of molecular biology.

In this study, we surveyed the genome size and assessed the genomic characteristics of *L. ruthenicum*, such as heterozygosity and repeat sequence information based on the next generation sequencing technology. The aim of this study was to provide evidence and consult to a completely genome sequencing and assemble program for *L. ruthenicum*.

Results

A total of 451,721,828 bp raw data were generated from the Illumina HiSeq 2000 sequencing platform. The values of Q20, Q30 were 95.25% and 89.12% respectively, and the GC content was 41.66% (Table 1).

Table 1
Sequencing result of *L. ruthenicum*

Raw paired reads	Raw base(bp)	Effective rate (%)	Error rate (%)	Q20(%)	Q30(%)	GC content (%)
451,721,828	135,516,548,400	99.77	0.03	95.25	89.12	41.66

The peak of the depth distributed at 33,the estimated genome size of *L. ruthenicum* was 3,249.33 Mb (Table 2). The heterozygosity rate was 1.13%，repeat rate was 73.13%. About 10,000 high quality reads were randomly selected(5000 read 1 and 5000 read 2)，and mapped to the nucleotide database of NCBI based on the Blast program, the sample was considered free from potential contamination in the case of

homologous alignment. The Blast program found *Solanum lycopersicum* (0.71) and *Nicotiana tabacum* (0.33) were the top two homologue species with *L. ruthenicum*.

Table 2
Result of Genome feature

Kmer	Depth	N kmer	Genome size (M)	Revised Genome size (M)	Heterozygous rate (%)	Repeat rate (%)
17	33	108,822,300,737	3297.65	3,249.33	1.13	73.13

After assemble, a total of 5,257,494 contigs were obtained, with N 50 was 1,145 bp and N 90 was 150 bp. And 4,596,439 scaffolds were assembled; N 50 and N 90 were 1,693 bp and 150 bp respectively. All of assemble data showed in Table 3. The GC contents of assembled scaffolds were statistically and analyzed.

Table 3
Information of the assembled genome sequences

title	Total length	Total number	Max length	N50 length	N90 length
contig	2,484,092,388	5,257,494	1,233,094	1,145	150
scaffold	2,553,498,919	4,596,439	2,196,943	1,693	150

Totally 958,619 SSRs were identified from 4,596,439 examined scaffolds. Among these examined scaffolds, 502,615 sequences containing SSRs. The average SSRs density was 163.95 SSRs/Mb. The length of microsatellites ranged from 10 bp to 116 bp, most length was concentrated in the 10–25 bp, account for 96.57% (Fig. 1).

In mono nucleotide repeat motif, the length of microsatellites ranges from 10 bp to 116 bp, most length were concentrated 10–20 bp range, account for 94.29% in all SSRs. The length of dinucleotide repeat motif ranged from 12 bp to 70 bp, the range length of 12–25 bp accounted for 97.53%. And the length of trinucleotide repeat motif ranged from 15 bp to 54 bp (accounted for 99.23%), the length of teinucleotide repeat motif ranged from 20 bp to 47 bp (account for 99.77%), the length of penucleotide repeat motif ranged from 25 bp to 44 bp (account for 99.66%), and the length of hexanucleotide repeat motif ranged from 30 bp to 42 bp. Length distribution of all repeat tended to decrease as the length increases.

Among the identified purified SSRs, the number of mono nucleotides was 490,016 account for 57.74%, followed by dinucleotide (244,765, 28.84%), trinucleotide (87,734, 10.34%), tetranucleotide (15,187, 1.79%), pentanucleotide (6,271, 0.74%) and hexanucleotide (4,684, 0.55%) (Table 4).

Table 4
Statistics of the detected SSRs

Repeat type		Number	Percentage (%)	Density number·Mb^{-1})	SSR type	Max. number of repeats
compound type		109,962	11.47%	4.3063	109,962	
Purified type	Mononucleotide	490,016	51.12%	19.1900	2	116
	Dinucleotide	244,765	25.53%	95.8548	4	64
	Trinucleotide	87,734	9.15%	34.3584	10	44
	Tetranucleotide	15,187	1.58%	5.94758	32	32
	Pentanucleotide	6,271	0.65%	2.45588	93	24
	Hexanucleotide	4,684	0.49%	1.83438	267	17
Total		958,619	100	163.9473		

In dinucleotide repeat motif, the repeat type of AT/AT (134,917; 55.12%) was the dominant, followed by AC/GT (62,674; 25.61%), AG/CT (45,370; 18.54%) and CG/CG (1,804; 0.74%) repeat units. In the trinucleotide repeats, AAG/CTT (23,723; 27.04%), AAC/GTT (21,826; 24.88%) and AAT/ATT (14,809; 16.88%) were the top three dominant repeat units. In the tetranucleotide, AAAG/CTTT (38.12%), ACAT/ATGT (13.93%) and AAAT/ATTT (10.50%) were the top three repeat untis. Overall, the AT-rich tetranucleotide repeat units account for a great part of the tetranucleotide repeats, the others occurred at a very low frequency, especially the CCGG/CCGG was found only once. The ATATC/ATATG (23.74%), AAAAG/CTTTT (18.31%), AATTC/AATTG (13.87%) and AAGAG/CTCTT (11.86%) were the top four repeat motifs in the, pentanucleotide repeats motifs. There were no obvious distributed characters were found.

Discussion

Genome size greatly varied among angiosperms, from 130 Mb (*Arabidopsis thaliana*) to 127 Gb (*Fritillaria assyriaca*) (Bennett and Smith 1976; Bennett et al. 1982), it was related with evolutionary processing, such as chromosome polyploidy and rearrangement (Soltis et al. 2003).

In this study, the estimated genome size of *L. ruthenicum* was 3,297. 65 Mb, which was nearly similar to the genome size of pepper (~ 3.48 Gb) (Cheng et al. 2014; Seungill et al. 2014) and nicotiana (~ 3.1 Gb) in Solanaceae family (Bombarely et al. 2012; Nicolas et al. 2013; Sierro et al. 2014). But it was about three times of tomato (~ 0.95 Gb) (Anthony et al. 2014; The Tomato Genome Consortium,2012), potato (~ 0.84G) (Potato Genome Sequencing Consortium 2011), and eggplant (~ 0.83 Gb) (Hideki et al. 2014).

The chromosome number of *L. ruthenicum* is 2n = 24(Chen et al,2008), which is same to the most of Solaneceae species, such as cultivated tomato（2n = 2x = 24, The Tomato Genome Consortium 2012), pepper（2n = 2x = 24, Cheng et al. 2014; Seungill et al. 2014), eggplant (*Solanum melongena* L.) (2n = 2x =

24, Hideki et al. 2014), potato (2n = 4x = 48, Potato Genome Sequencing Consortium 2011), tobacco (2n = 4x = 48, Sierro et al. 2014), but different from *Nicotiana benthamiana*) (x = 19, Bombarely et al. 2012) and Petunia (x = 7, Conia et al. 2010). As above mentioned, the majorities of Solanaceae plants are diploid and have the same basic chromosome number, according to the Wu and Tanksley (2011), the genome sizes of Solaneceae plants are greatly changed, which may indicated that the large-scale genome replication and chromosome diploidization events did not occur in Solanaceae plants during the long evolution process. Therefore, we speculated that the *L. ruthenicum* shared same chromosome evolution event with most of Solaneceae species.

The high heterozygosity was the reason of fitness and ecological success (Vrijenhoek 1994), and was related with morphological and adaptive differentiation of species. The heterozygosity rate of *L. ruthenicum* is 1.13%, suggested that the structure of *L. ruthenicum* genome has great variation. We speculated that the high heterozygosity of *L. ruthenicum* has resulted from the long evolution and adaptation process. Therefore, given the high heterozygosity of the genome, it is not suitable for genome assembly based on the second-generation sequencing results, and it is recommended to use the third-generation sequencing technology with a longer reading length.

The proportion of repeat sequence distribution were gradually increased from bacteria to eukaryotes, the repeat sequence content of some model organisms was as follows; bacteria less than 1%, beer yeast 3.4%, arabidopsis 13–14%, *Caenorhabditis elegans* 16.5%, *Drosophila melanoderma* 33.7%, mouse 38%, human 50%, corn 77%) (Ai 2008).

The repeat rate of *L. ruthenicum* was estimated as 73.13%, which was higher than the proportion in potato (62.2%) (Potato Genome Sequencing Consortium 2011), but was lower than the proportion in pepper (81%) (Varshney et al. 2012). According to the Uozu (1997), the number of repetitive sequence contributed to the nuclear DNA content. Therefore, the different proportions of the repetitive elements caused the genome size variation of same Solanceae family.

Repetitive sequences played the important role in evolution process, they were expanded and enriched the genetic information (Eichler and Sankoff 2003), protect coding sequences (Cangiano and Volpe 1993), at the same time, they were physically determined the chromosome structure, and influenced transcriptional regulation, played an important role in genome differentiation during speciation (Tang 2011). Therefore, we believed that the high proportion of repetitive sequence was the result of long evolution process of *L. ruthenicum*, and had the contributor of relatively great genome size.

The trinucleotide repeat unit accounted for larger proportion, while the dinucleotide repeat unit occupied larger proportion in *L. ruthenicum*. At the same time, the dominant repeat motif was also different, the GTT/CAA, ACA, and ATC were dominant repeat motifs in *L. babarum* genome, while the motifs of AT/AT, AC/GT and AG/CT were the dominant repeat motifs in *L. ruthenicum* (Dang et al. 2016). The mutation of dinucleotide repeat could cause to genetic instability and thus generate genetic diversity (Hammock 2005; Oki et al 1999). Therefore, we speculated that the genome of *L. ruthenicum* instability than *L. babarum*. The SSR characters of genome are same to the transcriptome of *L. ruthenicum* in which

dinucleotide account for larger proportion and AG/CT, AG/CT, AT/AT and AC/GT were the dominant repeat motifs (Hao et al. 2019).

Conclusions

In this study, we surveyed the genomic information of *L. ruthenicum*. The results showed the estimated genome size of *L. ruthenicum* was 3,297.65 Mb, nearly similar with other species in Solanacece. The heterozygosity rate of *L. ruthenicum* was higher (1.13%), suggested it has great variation, and not suitable for genome assembly based on the second-generation sequencing. Also, we identified 958,619 SSR markers, which could be used in next cultivar identification,population genetics. The resources provided in this research could fill the gaps of genome information in *Lycium* and accelerate the genomic and genetic studies, such as drought and salt tolerance mechanism, active substances biosynthesis mechanism, key gene resources mining, evolutionary analysis and breeding.

Material And Methods

A tissue cultured-seedling of *L. ruthenicum* was used for genome survey. The selected plant was cultivated for one week in clean water before sequencing.

Genomic DNA was extracted from the whole plant using DNA extraction kit (Illumina, USA), after elementary detection by agarose gel electrophoresis,the DNA concentration was detected based on the Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA), DNA integrity was detected using Agilent 2100 (Agilent Technologies, Palo Alto, Calif.), Total DNA concentrations and purity was determined using a Nanodrop 2000c spectrophotometer (Thermo-Scientific, Waltham, MA, USA).

Qualified genomic DNA was used for sequencing library construction according to the protocol (Illumina, Beijing, China). Pair-end sequencing was preceded according to the standard procedure (Illumina, Beijing, China). In order to ensure the quality of information analysis, the raw data was further filtered by removing the adapter, 10% N, and low quality (less than 5) bases of all raw reads. After filtering and correction of the raw data, clean reads were obtained. All of the clean reads were used for following bio-informational analysis.

The genome size was evaluated based on the Kmer = 17 analyses, it means the sequencing data were successively segmented by 17nt. The depth frequency of K-mer was assumed to conform to the Poisson distribution, and all K-mer which was extracted from reads one by one could cover the whole genome. The K-mer frequency distribution was calculated from all sequencing data, and the estimated K-mer depth was calculated to make the K-mer distribution curve. The genome size was evaluated based on the formula G = N/C base, where N base is the total number of bases of all sequence, C base is desired depth of the covering base.

The heterozygous and homozygous K-mer in the specific region of reference genome, combined with K-mer coverage of repeated sequences, and other negative binomial factors were formed a comprehensive

model to describe the K-mer distribution curve. The heterozygosity of the genome was determined by the ratio of heterozygosity peak to homozygosity peak.

The Soap-denovo software was used for genome assemble based on the K-mer = 41 with default parameters (Liu et al. 2012). The specific process is as follows; 1, the overlaps of reading sequences were represented using de Bruijn graph; 2, erroneous connection on the graphs were removed through clipping tips, removing low coverage links, resolving tiny repeats and merging bubbles; 3, break at repeat boundaries and output contigs; 4, scaffold construction based on Pair -end relationships of contigs; 5, gap closure of scaffolds were filled with reads.

SSRs were detected based on the Micro-Satellite (MISA) script (www.Pgrc.ipkgatersleben.de/misa), after detecting of all the SSRs in the DNA sequences, the number of SSRs for mono-, di-, tri-, tetra-, penta-, and hexa-nucleotides repeat motifs were identified respectively, according to the definition of microsatellites that the unit size were 10, 6, 5, 5, 5, and 5, respectively.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared that they have no competing interests.

Funding

This research was supported by the National Key Research and Development Program (2018YF00406604), the National Natural Science Foundation of China (31660221).

Authors' contributions

Defang Zhang has designed and performed these experiment, data analysis, results collation, writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Authors' information (optional)

Defang Zhang; the corresponding author

The e-mail address: defangstart2011@163.com

Telephone number: 086-18709711495

References

1. Ai DY. The significance of repeated sequences in genomes. Chem Life. 2008. (3):343–345.
2. Anthony B, Federico S, Bolger ME, et al. The genome of the stress-tolerant wild tomato species *Solanum pennellii*. Nat Genet. 2014;46(9):1034–8.
3. Aureliano B, Michel M, Avichai A, et al. Insight into the evolution of the Solanaceae from the parental genomes of petunia hybrida. Nat Plants. 2016;2(6):16074.
4. Bennett MD, Smith JB. Nuclear DNA amounts in angiosperms. Philosoph Transact Royal Soci of London B. 1976;274:227–74.
5. Bennett MD, Smith JB, Heslop-Harrison JS. Nuclear DNA amounts in angiosperms. Proceeding Roy Soci London B. 1982. 216: 179–199.
6. Biology and Soil. Effects of plantation of *Lycium ruthenicumon* the soil salt distribution in the minqin basin. J Desert Res. 2013;33(5):1406–12.
7. Bombarely A, Rosli HG, Vrebalov J, et al. A draft genome sequence of *Nicotiana benthamiana* to enhance molecular plant-microbe biology research. Mol Plant Microbe. 2012;25(12):1523–30.
8. Cangiano G, Volpe AL. Repetitive DNA sequences located in the terminal portion of the Caenorhabditis elegans chromosomes. Nucleic Acids Res. 1993;21(5):1133.
9. Chen HK, Cao JM, Ren X, et al. Karyotype analysis of the Chromosome of *Lycium ruthenicum* Murr. Northern Horticul. 2008;7:207–9.
10. Chen HQ, Zhao WH. Effect of NaCl stress on seed germination of *Lycium ruthenicum* Murr. Agr Sci Tech. 2010;11(4):37–8.
11. Cheng Q, Changshui Y, Yaou S, et al. Whole-genome sequencing of cultivated and wild peppers provides insights into Capsicum domestication and specialization. P Natl Acad Sci USA. 2014;111(14):5135–40.
12. Conia J, Bergounioux C, Perennes C, et al. Flow cytometric analysis and sorting of plant chromosomes from petunia hybrida protoplasts. Cytom Part A. 2010;8(5):500–8.
13. Dang SF, Wang ZL, Zhang DF, et al. Characteristic analysis of microsatellites of *Lycium barbarum*. J Northwest Fore Uni. 2016.

14. Dong JZ, Yang JJ, Wang Y Resources of *Lycium* species and related research progress. J Chinese Materia Medi. 2008.33(18): 2020.
15. Editorial committee of flora of China. Chinese academy of sciences. Flora of China. 1996. 67(1):8. Beijing: science press.
16. Eichler EE, Sankoff D. Structural dynamics of eukaryotic chromosome evolution. Science. 2003;301(5634):793–7.
17. Hammock. and E A. D. Microsatellite instability generates diversity in brain and socio behavioral traits. Science. 2005;308(5728):1630–4.
18. Hao GQ, Qi YY, Zhang DF, et al. Characteristics Analysis of SSR in *Lycium ruthenicum* Murr. Transcriptome and Primer Design. Plant Mole Breeding. 2019;13:4342–50.
19. He WG, Nasongcaoketu W, Chun-Huan WU, et al. The composition and distribution characteristics of *Lycium ruthenicum* shrub and root system in Yanqi basin. Xinjiang. Pratacultural Science. 2015.
20. Hideki H, Kenta S, Koji M, et al. Draft Genome Sequence of Eggplant (*Solanum melongena* L.): the Representative Solanum Species Indigenous to the Old World. DNA Research An International J Rapid Publi Rep Gene Genom. 2014;21(6):649–60.
21. Jalali GA, Akbarian H, Rhoades C, et al. The effect of the halophytic shrub *Lycium ruthenicum*(Mutt)on selected soil properties of a desert ecosystem in central Iran. Pol J Ecol. 2012;60(4):845–50.
22. Jia MR, Li XW. Chinese ethnopharmacology. Beijing, China medical science and technology press. 2005.p, 112–115.
23. Liu CM, Wong T, Wu E, et al. SOAP3: ultra-fast GPU-based parallel alignment tool for short reads. Bioinform. 2012;28(6):878–9.
24. Liu Z, Dang J, Wang Q, et al. Optimization of polysaccharides from Lyciumruthenicum fruit using RSM and its anti-oxidant activity. Int J Biol Macromol. 2013;61(10):127–34.
25. Liu Z, Shu Q, Wang L, et al. Genetic diversity of the endangered and medically important *Lycium ruthenicum* murr. revealed by sequence-related amplified polymorphism (SRAP) markers. Biochem System Ecol. 2012. 45.
26. Lv X, Wang C, Cheng Y, et al. Isolation and structural characterization of a polysaccharide LRP4-A from *Lycium ruthenicum* Murr. Carbohyd Res. 2013;365(2):20–5.
27. Nicolas S, Battey JND, Sonia O, et al. Reference genomes and transcriptomes of *Nicotiana sylvestris* and *Nicotiana tomentosiformis*. Genome Biol. 2013;14(6):1–17.
28. Oki E, Oda S, Maehara Y, et al. Mutated gene-specific phenotypes of dinucleotide repeat instability in human colorectal carcinoma cell lines deficient in DNA mismatch repair. Oncogene. 1999;18(12):2143–7.
29. Potato Genome Sequencing Consortium. Genome sequence and analysis of the tuber crop potato. Nature. 2011;475(7355):189–95.

30. Seungill K, Minkyu P, Seon-In Y, et al. Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet. 2014;46(3):270–8.
31. Sheng-Lian G. Physio characteristics of lycium ruthenicum under different soil water conditions. J Northwest Fore Uni. 2012;27(1):6–10.
32. Sierro N, Battey JND, Ouadi S, et al. The tobacco genome sequence and its comparison with those of tomato and potato. Nat Commun. 2014;5(5):3833–3.
33. Soltis DE, Soltis PS, Bennett MD, et al. Evolution of genome size in the angiosperms. Am J Bot. 2003;90:1596–603.
34. Tang SJ. Chromatin Organization by Repetitive Elements (CORE): A Genomic Principle for the Higher-Order Structure of Chromosomes. Genes. 2011;2(3):502–15.
35. The Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. Nature. 2012;485(7400):635–41.
36. Uozu S, Ikehashi H, Ohmido N, et al. Repetitive sequences: cause for variation in genome size and chromosome morphology in the genus Oryza. Plant Mol Biol. 1997;35(6):791–9.
37. Varshney RK, Wenbin C, Yupeng L, et al. Draft genome sequence of pigeonpea (Cajanus cajan), an orphan legume crop of resource-poor farmers. Nat Biotech. 2012. 30(1). 83-89
38. Vrijenhoek RC. UNISEXUAL FISH: Model Systems for Studying Ecology and Evolution. Ann Rev Ecol S. 1994;25:71–96.
39. Wang EJ, Li SJ, Han DH, et al. Effect of neutral and alkaline salt stresses on germination and seedling growth of *Lycium ruthenium*. Agri Res Arid Areas. 2014.
40. Wu F, Tanksley SD. Chromosomal evolution in the plant family Solanaceae. BMC Genom. 2010;11(3):1–11.
41. Yabin D, Fan C, Xingchen Y, et al. Protective effect of lycium ruthenicum murr. against radiation injury in mice. Int J Env Res Pub He. 2015;12(7):8332–47.
42. Zheng J, Ding C, Wang L, et al. Anthocyanins composition and antioxidant activity of wild *Lycium ruthenicum* Murr. from Qinghai-Tibet Plateau. Food Chem. 2011;126(3):859–65.

Figures

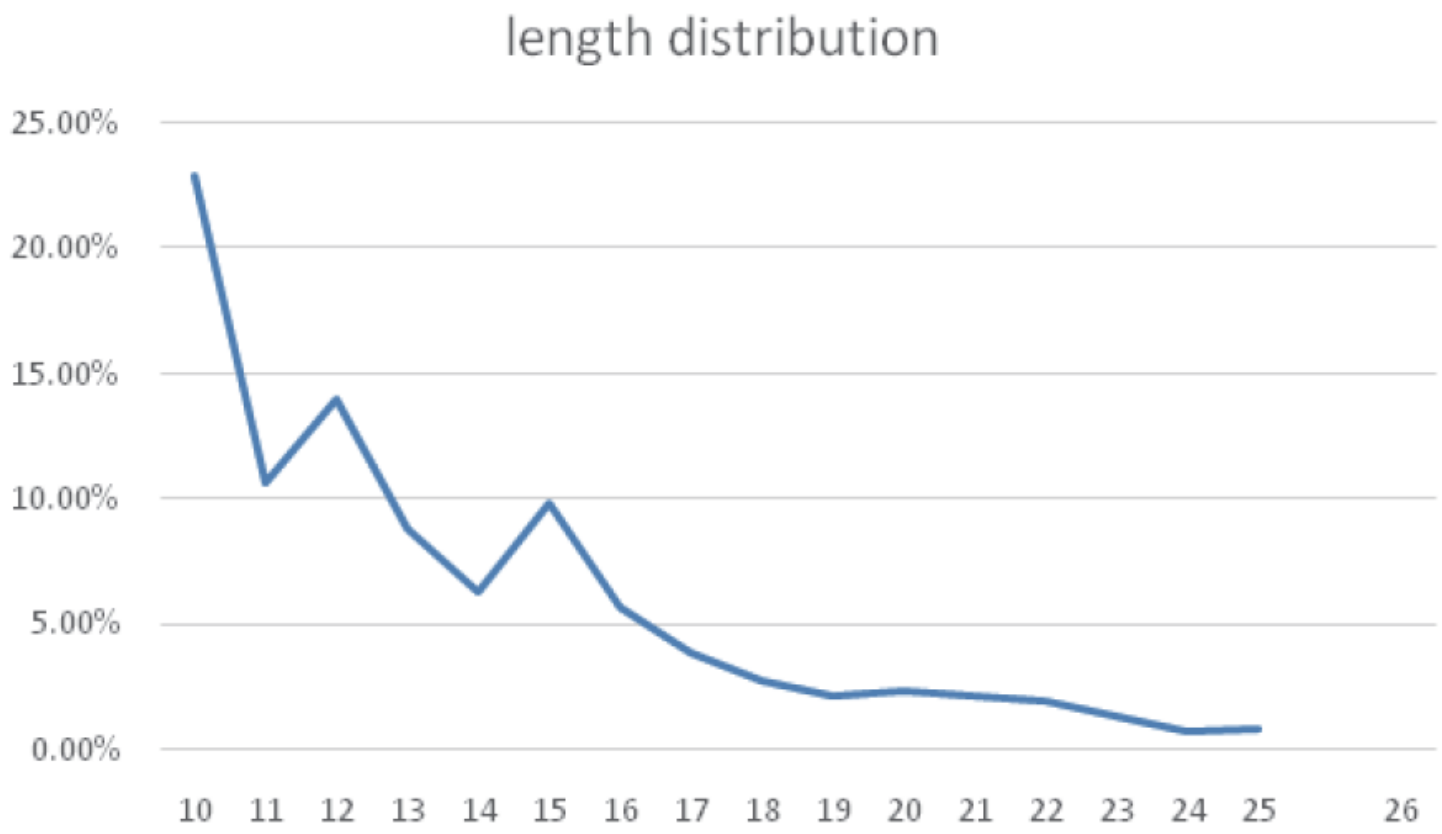

Figure 1

Length distribution and percentage of microsatellites in L. ruthenicum.