Marmara Fen Bilimleri Dergisi 2018, 3: 299-304
DOI: 10.7240/marufbd.395068

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Transferability of Barley Retrotransposons (*Sukkula* and *Nikita*) to Investigate Genetic Structure of *Pimpinella anisum* L.

Arpa Retrotranspozonlarının (Sukkula ve Nikita) Pimpinella Anisum L.'nin Genetik Yapısını İncelemeye Aktarılabilirliği

Sevgi MARAKLI[1]

[1]*Amasya University, Faculty of Arts and Sciences, Department of Biology, 05100, Amasya, Turkey*

Abstract

Transferability of barley retrotransposons (*Nikita* and *Sukkula*) were examined in *Pimpinella anisum* L. seeds by using a retrotransposon-based molecular marker: IRAP (inter-retrotransposon amplified polymorphism). Furthermore, transposons' sequences identified in medically important plants were obtained form NCBI, and multiple alignment analyses were performed to investigate the evolutionary relationships. These two retrotransposons were identified in *Pimpinella anisum* L., showing homomorphic band profiles. In addition, limited similar sequences were detected as a result of clustal analyses. Till date, no study about retrotransposons evaluation using IRAP as molecular marker has been published in aniseed. Our results are expected to contribute a new perspective for genome architect of medically important plants in addition to aniseed.

Keywords: Aniseed, IRAP, Medical Plants, Mobile Genetic Elements

Öz

Arpa retrotranspozonlarının (Nikita ve Sukkula) aktarılabilirliği, retrotranspozon temelli bir moleküler markır olan IRAP (retrotranspozon-arası çoğaltılmış polimorfizm) yöntemi kullanılarak Pimpinella anisum L. tohumlarında incelendi. Ayrıca, tıbbi olarak önemli bitkilerde tanımlanan transpozonların dizileri, NCBI'dan elde edildi ve evrimsel ilişkileri araştırmak için çoklu hizalama analizleri yapıldı. Pimpinella anisum L. bitkisinde tanımlanan bu iki retrotranspozon homomorfik bant profili gösterdi. Bununla birlikte, çoklu hizalama analizleri sonucunda sınırlı sayıda benzer diziler tespit edildi. Bugüne kadar, anasonda IRAP gibi bir moleküler markır kullanılarak retrotranspozon değerlendirmesi yapan bir çalışma yayınlanmamıştır. Sonuçlarımızın, anason ile birlikte tıbbi açıdan önemli bitkilerin genom yapısının anlaşılması için yeni bir bakış açısına katkıda bulunması bekleniyor.

Anahtar Kelimeler: Anason, IRAP, Tıbbi Bitkiler, Hareketli Genetik Elementler

I. INTRODUCTION

Pimpinella anisum L. (aniseed) belonging to Apiaceae family is an annual herb with white flowers and small green/yellow seeds, cultivating Europe, Asian countries and Latin America in addition to many other warm regions of the world [1, 2]. The seeds have been commonly used for folk medicine, pharmacy, food industry and even as a spice [3]. There are many reports about antibacterial [4], anti-oxidative [5], antitoxicity [6] and also anticancer [7] effects of essential oils in aniseed. On the other hand, there is still some concern related to the safety and efficiency. DNA technology/molecular markers are used to prevent these problems. For this purpose, there are many studies related to DNA markers for identification of herbal medicinal species and their adulterants, investigation of genetic changes as a result of biotic/abiotic stress DNA fingerprinting among species and different tissues of the same plant, and evolutionary relationships [8]. One class of these markers is developed on the basis of retrotransposon sequences. Retrotransposons are mobile genetic elements, moving via an

Sorumlu Yazar/Corresponding Author: Sevgi MARAKLI, **Tel:** 90 358 211 50 05**, e-posta:** sevgi.marakli@amasya.edu.tr
Gönderilme/Submitted: 14.02.2018, **Düzenleme/Revised:** 25.06.2018, **Kabul/Accepted:** 11.08.2018

RNA intermediate in genome. Retrotransposon-based molecular markers are codominant, ubiquitous, highly abundant and randomly distributed with high copy numbers in plant genomes [9-12]. Inter-retrotransposon amplified polymorphism (IRAP) is one of the retrotransposon-based molecular markers, amplifying genomic distance between two long terminal repeats (LTRs) found in both two ends of the retrotransposon. In this method, polymorphisms can be calculated by the presence or absence of the PCR product [13]. Sequences of identical LTR retrotransposons indicate the relatedness among species [14]. Therefore, LTR primers designed according to a species can be used to amplify DNA of others. As a result, IRAP gives very valuable information related to the genomes of different species [15, 16].

The major objective of this study was to detect *Nikita* and *Sukkula* retrotransposons, identified in barley [17], in aniseed genome by using IRAP technique. Moreover, different transposons described in medically important plant families were also investigated to evaluate evolutionary relationships.

II. MATERIALS AND METHODS

2.1 Genomic DNA Isolation and IRAP-PCR

Genomic DNAs were isolated from seven *Pimpinella anisum* L. seeds according to Kidwell and Osborn [18] with minor modifications. The quality and the quantity of gDNA was controlled on 1% agarose gel and a spectrophotometer, respectively. IRAP-PCR was performed with *Nikita* and *Sukkula* specific primers (5'ACCCCTCTAGGCGACATCC3' for *Nikita* and 3'GGAACGTCGGCATCGGGCTG5' for *Sukkula*) [19]. Amplification reactions were carried out in 20 µl reaction mixtures, including 9.8 µl of nuclease-free dH_2O, 2 µl of reaction buffer (K1071, Fermentas), 2 µl of $MgCl_2$ (2.5 mM) , 0.6 µl dNTP mixture (0.3 mM), 2 µl of primer (1 µM/µL), 3 µl of 20 ng/µl template genomic DNA (3 ng/µl) and 0.6 µl of enzyme (0.15 U/µl). The values given in parenthesis indicated the final concentrations of PCR components. The amplification conditions were set up as an initial denaturation step at 94ºC for 3 min followed by 30 cycles of 94ºC for 30 s, annealing for 30 s (50ºC for *Nikita* and 56ºC for *Sukkula*) and 72ºC for 3 min. The reaction was completed by a final extension step at 72ºC for 10 min. All the experiments were performed with independent biological replicates (seven seeds). PCR products were resolved on 1% agarose gel in 1X Tris–Borate–EDTA at 120 V for 120 min and photographed on a UV transilluminator. Molecular weight marker (GeneRuler™ 1 kb DNA Ladder, SM0312, Fermentas) was also used to determine the size of amplicons. After agarose gel electrophoresis, gels were photographed on a UV transilluminator. Band profiles among samples for *Nikita* and *Sukkula* were examined.

2.2 Multiple Alignment Analyses

Different transposons sequences belonging to the seven medically important plant families were obtained from NCBI (The National Center for Biotechnology Information – www.ncbi.nlm.nih.gov) (Table 1). Then, multiple alignment analyses were performed with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo) and limited similar sequences were obtained. Therefore, it was not enough to construct a phylogenetic tree.

Table 1. Analysed plants and transposons' sequences

Accession number	Family Name	Plant name	Transposon
AY585677.3	*Alliaceae*	*Allium cepa*	*PINK*
KM434203.1		*Allium cepa*	*AcCACTA1*
KM434202.1		*Allium cepa*	*AcCOPIA1*
EF192476.2		*Allium cepa*	*hAT1*
DQ250807.1	*Apiceae*	*Daucus carota*	*PIF-like DcMaster-1*
DQ250806.1		*Daucus carota*	*PIF-like DcMaster-a*
AB071213.1		*Daucus carota*	*Tdc B2-2*
AB001569.1		*Daucus carota*	*Tdc1*
DQ229838.1	*Asteraceae*	*Helianthus annuus* cultivar R112	*Gypsy*-like retrotransposon
D37795.1	*Convolvulaceae*	*Ipomoea nil*	*Tpn1*
AB073921.1		*Ipomoea nil*	*Tpn8*
EU009625.1	*Cucurbitaceae*	*Citrullus lanatus* var. lanatus	*Cila-1*
AM040263.2	*Rutaceae*	*Citrus sinensis*	*CIRE1.1*
AF279585.2	*Solanaceae*	*Lycopersicon chilense*	*TLC1.1*
AF228701.1		*Lycopersicon peruvianum*	*Retrolyc1-1*
X13777.1		*Nicotiana tabacum*	*Tnt 1-94*
U91987.1		*Solanum tuberosum*	*Potten1*

III. RESULTS

3.1 Nikita and Sukkula were Identified in Aniseed

Nikita IRAP-PCR analyses of seven *Pimpinella anisum* L. seeds were demonstrated homomorphic band profiles,

ranging from 250 bp to 6000 bp. There was no polymorphic band among samples (Figure 1).

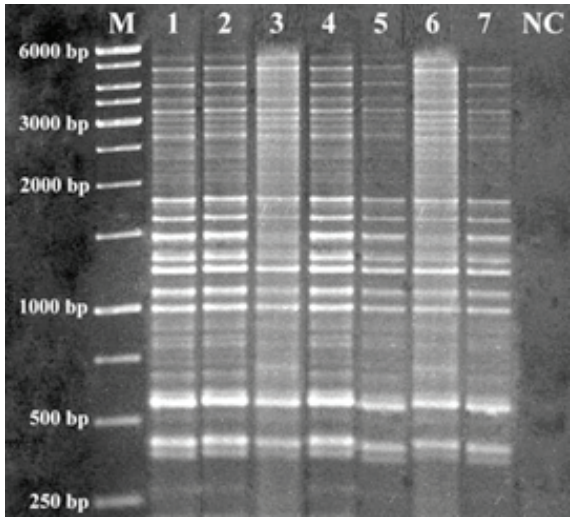

Figure 1. IRAP-PCR results of *Nikita*. M, marker; NC, negative control. Numbers shows seven different seeds of *Pimpinella anisum* L.

In addition to *Nikita*, another retrotransposon, *Sukkula* also showed only homomorphic bands among samples (Figure 2).

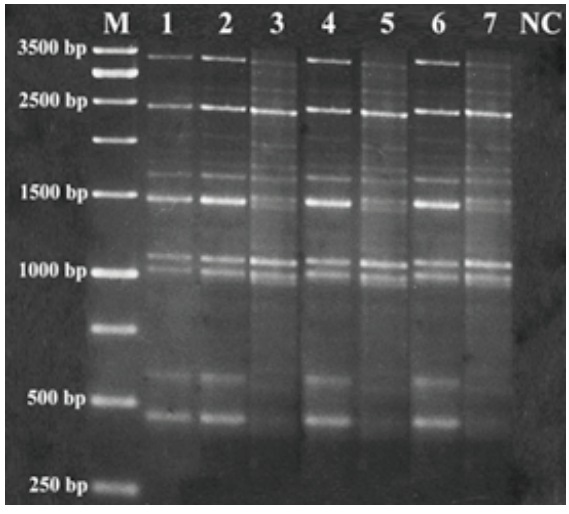

Figure 2. IRAP-PCR results of *Sukkula*. M, marker; NC, negative control. Numbers shows seven different seeds of *Pimpinella anisum* L.

Sukkula band profiles were different when compared to *Nikita*, indicating fewer bands with the length between 500 and 3500 bp. Furthermore, some bands with the same base pairs were observed in both *Nikita* and *Sukkula* (Figure 3).

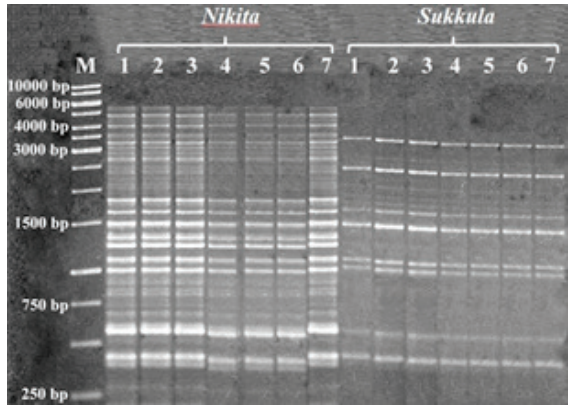

Figure 3. IRAP-PCR results of *Nikita* and *Sukkula*. M, marker; NC, negative control. Numbers shows seven different seeds of *Pimpinella anisum* L.

3.2 Clustal Analyses Indicated Very Few Similar Sequences

We observed limited similarity (a) similar transposon sequences belonging to the same plant species (such as *PIF-like DcMaster-1* and *PIF-like DcMaster-a* in *Daucus carota*), (b) different transposon sequences found in same plant species (such as *Alliaceae* family), in addition to different transposons's sequences in different families (Figure. 4).

Figure 4. Alignment of the deduced sequences obtained from seven families. Every four nucleotides indicated in different colours

IV. DISCUSSION

There are many reports related to the essential oils of aniseed characterised by antispasmodic, antioxidant, antimicrobial, insecticidal, and antifungal effects [20]. On the other hand, there is no study to investigate retrotransposon movements in aniseed. In this study, barley-specific retrotransposons (*Nikita* and *Sukkula*) were detected in aniseed genome for the first time, founding homomorphic band profiles.

IRAP markers were used for analysing polymorphism in different populations. One of them was performed by Boronnikova and Kalendar [21], observing 125 polymorphic IRAP markers in *Adonis vernalis* populations. Moreover, Soorni et al. [22] investigated the same retrotransposons' movements in another medical plant, *Leonurus cardiaca* L., reporting ≥80% polymorphism among samples. *Nikita* retrotransposon movements in *Avena species* also analysed by Tomás et al. [23]. Different obtaining results could be depended on plant species, different transposons and environmental conditions. Furthermore, a specific retrotransposon could be found in different plant species even humans with different polymorphism ratios [15, 16].

In addition to retrotransposons, many DNA barcodes such as *rbcL, matK, trnH–psbA* and ITS has been widely studied to evaluate biodiversity and conservation. Furthermore, they are also a reliable tool for species identification in addition to safety and quality control [24]. Especially, ITS2 DNA barcode is commonly used for identification of medicinal plants [25, 26]. In addition, nuclear ribosomal DNA (nrDNA) ITS region is also a popular marker, comprising the database for Apiaceae subfamily Apioideae [27]. Wang et al. [28] studied with nrDNA ITS and cpDNA intron sequence data to understand evolutionary relations between *Pimpinella* and related genera (Apiaceae). Furthermore, ITS sequences of *Pimpinella pruatjan* were investigated to identify the relationships among other *Pimpinella* species [29].

Other molecular marker techniques have been also performed to assess genetic diversity. Wang et al. [30] investigate the genetic diversity of *Rheum officinale* by using ISSR. They concluded that diversity was high at the species level, whereas low at the population level. The genetic diversity of *Trigonella foenum-graecum* was also investigated using RAPD and ISSR markers in addition to nrDNA and ITS barcodes [31, 32]. Moreover, similar to our study, Kumar et al. [33] investigated *Daucus carota* microsatellite markers in another species, *Cuminum cyminum*.

Analysing of suitable DNA barcodes for every species play an important role in diversity analyses [8]. Transposable elements are source of genetic and epigenetic variabilities and so important drivers of evolution [34]. LTR retrotransposon transferability among species could be as a result of analogous to virus capsid [35]. Sun et al. [36] studied with the transferability of pear IRAP to apples and other Rosaceae species. Different from our results, they reported that polymorphism was very high, ranging from 87.5 to 100%. The transferability of DNA markers depends on genome similarity, showing genome collinearity and evolutionary relationships species [37]. Therefore, molecular markers have been commonly used for genetic reports because of interspecific and intergeneric transferability [38]. To our knowledge, this is the first report about retrotransposon-based marker, IRAP, for identifying *Nikita* and *Sukkula* in aniseed genome. The study revealed that both two retrotransposons were found in aniseed, indicating homomorphic band profiles. Retrotransposons' markers investigations have commonly used for knowledge of diversity. Therefore, our findings might contribute valuable information related to genomes of medical plants.

REFERENCES

[1] Gülçın, İ., Oktay, M., Kıreççı, E., & Küfrevıoğlu, Ö.İ., (2003). Screening of Antioxidant and Antimicrobial Activities of Anise (*Pimpinella anisum* L.) Seed Extracts. *Food Chemistry*, 83(3), 371-382.

[2] Rodrigues, V.M., Rosa, P.T., Marques, M.O., Petenate, A.J., & Meireles, M.A.A., (2003). Supercritical Extraction of Essential Oil From Aniseed (*Pimpinella anisum* L) Using CO_2: Solubility, Kinetics, and Composition Data. *Journal of Agricultural and Food Chemistry*, 51(6), 1518-1523.

[3] Samojlik, I., Mijatović, V., Petković, S., Škrbić, B., & Božin, B., (2012). The Influence of Essential Oil of Aniseed (*Pimpinella anisum*, L.) on Drug Effects on the Central Nervous System. *Fitoterapia*, 83(8), 1466-1473.

[4] Ibrahim, M.K., Mattar, Z.A., Abdel-Khalek, H.H., & Azzam, Y.M., (2017). Evaluation of Antibacterial Efficacy of Anise Wastes Against Some Multidrug Resistant Bacterial Isolates. *Journal of Radiation Research and Applied Sciences*, 10(1), 34-43.

[5] Koriem, K.M., Arbid, M.S., & El-Gendy, N.F., (2016). The Protective Role of Anise Oil in Oxidative Stress and Genotoxicity Produced In Favism. *Journal of Dietary Supplements*, 13(5), 505-521.

[6] Jamshidzadeh, A., Heidari, R., Razmjou, M., Karimi, F., Moein, M.R., Farshad, O., Akbarizadeh, A.R. & Shayesteh, M. R. H., (2015). An *in vivo* and *in vitro* Investigation on Hepatoprotective Effects of *Pimpinella anisum* Seed Essential Oil and Extracts Against Carbon Tetrachloride-Induced Toxicity. *Iranian Journal of Basic Medical Sciences*, 18(2), 205-211.

[7] Asadi, M.H., & Rahamooz-Haghighi, S., (2016). Anti-Proliferative Effect of the Extracts and Essential Oil of *Pimpinella*

anisum on Gastric Cancer Cells. *Journal of HerbMed Pharmacology*, 5(4), 157-161.

[8] Li, L., Josef, B.A., Liu, B., Zheng, S., Huang, L., & Chen, S., (2017). Three-Dimensional Evaluation on Ecotypic Diversity of Traditional Chinese Medicine: A Case Study of *Artemisia annua* L. *Frontiers in Plant Science*, 8, 1225.

[9] Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J.L., Capy, P., Chalhoub, B., Flavell, A., Leroy, P., Morgante, M., Panaud, O., Paux, E., SanMiguel, P., & Paux, E., (2007). A Unified Classification System for Eukaryotic Transposable Elements. *Nature Reviews Genetics*, 8(12), 973-982.

[10] Schulman, A.H., Flavell, A.J., Paux, E., Ellis, T.H.N., (2012). The application of LTR retrotransposons as molecular markers in plants. In: Bigot, Y. (ed.), *Mobile Genetic Elements: Protocols and Genomic Applications*. Humana Press, 115-153.

[11] Gozukirmizi, N., Temel, A., Marakli, S., Yilmaz, S., (2016). Transposon Activity in Plant Genomes. In: Hakeem, H.R., Tombuloglu, H., Tombuloglu, G. (eds.), *Plant Omics: Trends and Applications.* Springer-Verlag, Springer International Publishing Switzerland, 83-108.

[12] Gozukirmizi, N., Yilmaz, S., Marakli, S., Temel, A., (2015). Retrotransposon-Based Molecular Markers; Tools for Variation Analyses in plants. In: Taski-Adjukovic, K. (ed.), *Applications of Molecular Markers in Plant Genome Analysis and Breeding*. Research Signpost, Kerala, 19-45.

[13] Kalendar, R., (2011). The Use of Retrotransposon-Based Molecular Markers to Analyze Genetic Diversity. *Ratarstvo i Povrtarstvo*, 48, 261-274.

[14] Kalendar, R., Tanskanen, J., Chang, W., Antonius, K., Sela, H., Peleg, O., & Schulman, A.H., (2008). Cassandra Retrotransposons Carry Independently Transcribed 5S RNA. *Proceedings of the National Academy of Sciences*, 105(15), 5833-5838.

[15] Cakmak, B., Marakli, S., & Gozukirmizi, N., (2015). *SIRE1* Retrotransposons in Barley (*Hordeum vulgare* L.). *Russian Journal of Genetics*, 51(7), 661-672.

[16] Cakmak, B., Marakli, S., & Gozukirmizi, N., (2017). *Sukkula* Retrotransposon Movements in the Human Genome. *Biotechnology & Biotechnological Equipment*, 31(4), 756-760.

[17] Shirasu, K., Schulman, A.H., Lahaye, T., & Schulze-Lefert, P., (2000). A Contiguous 66-kb Barley DNA Sequence Provides Evidence for Reversible Genome Expansion. *Genome Research*, 10(7), 908-915.

[18] Kidwell, K.K., Osborn, T.C., (1992). Simple Plant DNA Isolation Procedures, In: Beckmann, J.S., Osborn, T.C. (eds.) *Plant Genomes: Methods for Genetic and Physical Mapping*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 1-13.

[19] Leigh, F., Kalendar, R., Lea, V., Lee, D., Donini, P., & Schulman, A. H., (2003). Comparison of the Utility of Barley Retrotransposon Families for Genetic Analysis by Molecular Marker Techniques. *Molecular Genetics and Genomics*, 269(4), 464-474.

[20] Shojaii, A., & Abdollahi Fard, M., (2012). Review of Pharmacological Properties and Chemical Constituents of *Pimpinella anisum*. *ISRN pharmaceutics*,

[21] Boronnikova, S.V., & Kalendar, R. N., (2010). Using IRAP Markers for Analysis of Genetic Variability in Populations of Resource and Rare Species of Plants. *Russian Journal of Genetics*, 46(1), 36-42.

[22] Soorni, A., Nazeri, V., Fattahi, R., & Khadivi-Khub, A., (2013). DNA Fingerprinting of *Leonurus cardiaca* L. Germplasm in Iran Using Amplified Fragment Length Polymorphism and Inter-Retrotransposon Amplified Polymorphism. *Biochemical Systematics and Ecology*, 50, 438-447.

[23] Tomás, D., Rodrigues, J., Varela, A., Veloso, M.M., Viegas, W., & Silva, M., (2016). Use of Repetitive Sequences for Molecular and Cytogenetic Characterization of *Avena* species From Portugal. *International Journal of Molecular Sciences*, 17(2), 203.

[24] Li, M., Cao, H., But, P.P.H., & Shaw, P.C., (2011). Identification of Herbal Medicinal Materials Using DNA Barcodes. *Journal of Systematics and Evolution*, 49(3), 271-283.

[25] Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y. Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Leon, C., (2010). Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species. *PloS One*, 5(1), e8613.

[26] Gao, T., Yao, H., Song, J., Liu, C., Zhu, Y., Ma, X., Pang X., Xu, H., & Chen, S., (2010). Identification of Medicinal Plants in the Family Fabaceae Using a Potential DNA Barcode ITS2. *Journal of Ethnopharmacology*, 130(1), 116-121.

[27] Downie, S.R., Spalik, K., Katz-Downie, D.S., & Reduron, J.P., (2010). Major Clades within Apiaceae Subfamily Apioideae as Inferred by Phylogenetic Analysis of nrDNA ITS Sequences. *Plant Diversity and Evolution*, 128(1-2), 111-136.

[28] Wang, Z.X., Downie, S.R., Tan, J.B., Liao, C.Y., Yu,Y., & He, X.J., (2014). Molecular Phylogenetics of *Pimpinella* and Allied Genera (Apiaceae), with Emphasis on Chinese Native Species, Inferred From nrDNA ITS and cpDNA Intron Sequence Data. *Nordic Journal of Botany*, 32(5), 642-657.

[29] Nurcahyanti, A.D., Nasser, I.J., Sporer, F., Graf, J., Bermawie, N., Reichling, J., & Wink, M., (2016). Chemical Composition of the Essential Oil From Aerial Parts of Javanian *Pimpinella pruatjan* Molk. and Its Molecular Phylogeny. *Diversity*, 8(3), 15.

[30] Wang, X.M., Hou, X.Q., Zhang, Y.Q., Yang, R., Feng, S. F., Li, Y., & Ren, Y., (2012). Genetic Diversity of the Endemic and Medicinally Important Plant *Rheum officinale* as Revealed by Inter-Simpe Sequence Repeat (ISSR) Markers. *International Journal of Molecular Sciences*, 13(3), 3900-3915.

[31] Kakani, R.K., Singh, S.K., Pancholy, A., Meena, R.S., Pathak, R., & Raturi, A., (2011). Assessment of Genetic Diversity in *Trigonella foenum-graecum* Based On Nuclear Ribosomal

DNA, Internal Transcribed Spacer and RAPD Analysis. *Plant Molecular Biology Reporter*, 29(2), 315-323.

[32] Sindhu, A., Tehlan, S.K., & Chaudhury, A., (2017). Analysis of Genetic Diversity Among Medicinal Therapist *Trigonella foenum-graecum* L. Genotypes Through RAPD and SSR Markers. *Acta Physiologiae Plantarum*, 39(4), 100.

[33] Kumar, S., Mahendi, H.A., Fougat, R.S., Sakure, A.A., & Mistry, J.G., (2014). Transferability of Carrot (*Daucus carota*) Microsatellite Markers to Cumin (*Cuminum cyminum*). *International Journal Seed Spices*, 4(1), 88-90.

[34] Zhang, Y., Fan, C., Li, S., Chen, Y., Wang, R.R.C., Zhang, X., Han, F., & Hu, Z., (2017). The diversity of Sequence and Chromosomal Distribution of New Transposable Element-Related Segments in the Rye Genome Revealed by FISH and Lineage Annotation. *Frontiers in Plant Science*, 8, 1706.

[35] Wallau, G.L., Ortiz, M.F., & Loreto, E.L.S., (2012). Horizontal Transposon Transfer in Eukarya: Detection, Bias, and Perspectives. *Genome Biology and Evolution*, 4(8), 801-811.

[36] Sun, J., Yin, H., Li, L., Song, Y., Fan, L., Zhang, S., & Wu, J., (2015). Evaluation of New IRAP Markers of Pear and Their Potential Application in Differentiating Bud Sports and Other Rosaceae Species. Tree Genetics & Genomes, 11, 25.

[37] Zhang, M., Fan, L., Liu, Q., Song, Y., Wei, S., Zhang, S., & Wu, J., (2013). A Novel Set of EST-Derived SSR Markers for Pear and Cross-Species Transferability in Rosaceae. *Plant Molecular Biology Reporter*, 32, 290-302.

[38] Liang, Y., Lenz, R.R., & Dai, W., (2016). Development of Retrotransposon-Based Molecular Markers and Their Application in Genetic Mapping in Chokecherry (*Prunus virginiana* L.). *Molecular Breeding*, 36, 109.