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CLONING AND SEQUENCING OF POTATO *trnI* AND *trnA* PLASTID DNA FRAGMENTS FOR DEVELOPING HOMOLOGOUS PLASTID TRANSFORMATION VECTOR

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ABSTRACT

For developing plastid transformation vector, based on homologous targeting regions of potato (*Solanum tuberosum* L.), the *trnI* and *trnA* intergenic regions of the plastome from the commercial potato cultivar Kufri Bahar was PCR amplified, cloned and sequenced. Isolation and cloning of potato *trnI* and *trnA* cpDNA fragments into pDrive cloning vector yielded plasmid pD*trnI* and pD*trnA* with cpDNA fragments of approximately 800 bp and 1000bp, respectively. Sequence alignment using Clustal W revealed 98% sequence homology with the corresponding fragment of *Nicotiana tabacum* L. (GenBank Accession no. Z00044). The most remarkable difference was 9 bp insertion from 647-655 in potato plastome that was missing in tobacco. Similarly, sequence alignment of potato *trnA* region showed 100% identity to the respective fragment of *Nicotiana tabacum* L., except an insertion of 102 nucleotides from 700-801. The observed differences in the sequence homology between potato and tobacco plastome might be responsible for lower transformation efficiency in potato using tobacco-based vector. The presence of unique restriction enzyme sites within the intergenic region of both fragments will make possible the construction of potato specific plastid transformation vector. The cloned *trnI*/*trnA* region of the potato plastome will contribute towards designing plastid transformation vector with better transformation efficiency.

Keywords: Chloroplast DNA, Plastid transformation, Plastome, Potato, Tobacco.

1. INTRODUCTION

The plastid genome is an attractive target for genetic engineering in higher plants which offers several unique advantages over nuclear transformation including maternal inheritance of transgene, no position effect as the genes of interest are introduced into the plastome via homologous recombination and high level of foreign protein expression [1-3]. Plastid transformation in higher plant was first successfully reported in tobacco using mutant plastid 16S ribosomal RNA gene for selection of transformants [4]. Plastid transformation has been reported in many other higher plants such as *Arabidopsis thaliana* L. [5], potato [6-9], rapeseed [10], rice [11,12], tomato [13-14], soybean [15], carrot [16], cotton [17], wheat [18], eggplant [19,20] and many more. Recently plastid transformation has been reported in bitter melon [21], medicinal plant (*Artemisia annua* L.) [22] and licorice weed (*Scoparia dulcisi* L.) [23]. However, plastid transformation is routinely reported in tobacco and the efficiency of transformation is much lower in other plants than in tobacco [24]. Due to this success and specially because *Arabidopsis*, potato and tomato were transformed with homeologous vectors (not species-specific vectors), the plastid genomes of important crop species have received increasing attention with the possibility to develop chloroplast transformation systems to introduce agronomically important traits for plant improvement into the chloroplast genome [25] .

The chloroplast genome generally has a highly conserved organization among different plant species, having two identical copies of a 20 to 30 kb inverted repeat region (IRA and IRB) separating a large single copy (LSC) region and a small single copy (SSC) region [26]. Stability of plastid transformation depends on the integration of foreign gene into the plastid genome by homologous recombination of flanking sequences used in plastid transformation vectors. Hence the introduced gene must be flanked by sequences homologous to the plastid genome.

Identification of spacer regions to integrate transgenes and endogenous regulatory sequences that support optimal expression is the first step in construction of plastid transformation vectors [4]. Plastid vectors are based on homologous targeting of left and right plastid targeting regions. The site of insertion in the plastid genome is determined by the choice of insertion in the plastid DNA segment flanking the selectable marker gene and gene of interest. Insertion of foreign DNA is successful at 16 sites distributed among plastid genome [24]. Most commonly used site for integration of transgene is *trnI-trnA* intergenic region. These two tRNAs are located between small (*rrn16*) and large (*rrn23*) rRNA subunits and operon is transcribed from promoters upstream of *rrn16*. The foreign gene expression at this site is highest ever reported [27, 28].

Plastid transformation vector also carry origin of replication (*OriA*) which is present within *trnI* flanking region [29, 30], which enhances the probability of transgene integration and achieve homoplasmy even in the first round of selection [31]. Plastid transformation vectors utilize spectinomycin and streptomycin resistance as a selectable marker, based either on integration of 16S-rRNA (*rrn16*) nucleotide sequences containing point mutations [4] or the expression of aminoglycoside-3′-adenyltransferase (*aadA* gene), that inactivates spectinomycin and streptomycin by adenylation [32]. The use of *aadA* gene to confer spectinomycin and streptomycin resistance has been by far the most reliable and commonly used selectable marker for the production of plastid transformants, even though alternative selectable markers have also been reported [33-35].

As chloroplast transformation occurs by homologous recombination, it is very important to know the sequence of the chloroplast DNA (cpDNA) to which the gene of interest would be specifically targeted. Since the first chloroplast genome from tobacco was sequenced in 1986 [36], over 800 complete chloroplast genome including 300 crop and trees have been sequenced and submitted in the National Centre for Biotechnology Information (NCBI) organelle genome database [37]. Comparative analyses of these genomes show a remarkably conserved structure and coding capacity across large phylogenetic distances among different plant species [38].

A universal vector that utilizes the plastid DNA flanking sequences of one plant species to transform another species of unknown genome sequences [3] has been used to transform potato and tomato plastid genome using flanking sequences from tobacco [6,7,13]. However, the transformation efficiency is lowered because the flanking sequences are not completely homologous. The significant differences in transformation frequencies between potato and tobacco have been reported. In general, transformation frequency of about one event per bombarded plate has been reported in tobacco [32], in comparison to one event from 15-30 bombarded plates in potato [6]. The lower transformation efficiency may be due to poor homologous recombination obtained with tobacco flanking sequences. Valkov et al. [9] reported one transplastomic shoot per bombardment to that usually achieved in tobacco with transformation vector carrying homologous potato flanking sequences. Since plastid transformation vector containing tobacco *trnI* and *trnA* intergenic region was used to transform closely related members of solanaceous family, hence efforts were made in the present study to clone and sequence potato *trnI* and *trnA* plastid DNA fragments for developing potato-specific plastid transformation vector with homologous target sequence bordering the gene of interest to overcome the lower transformation efficiency obtained with tobacco specific transformation vector.

2. MATERIAL AND METHODS

2.1. Cloning of *trnI* and *trnA* fragments of potato plastome

For cloning of *trnI* and *trnA* fragments of potato plastome, genomic DNA of Indian potato cultivar Kufri Bahar was isolated from fresh leaf tissue (100 mg) by CTAB method [39]. Then *trnI* and *trnA* repeat regions of potato plastome were PCR amplified using tobacco sequence as primers (GenBank Accession no. Z00044). *trnI* region of potato was amplified using *trnI* F 5‘ GCT AAT GCT TGT TGG GTA TTT TG 3’ and *trnI* R 5‘ TTC AAA CCT GCT CCC ATT TCG AG 3’ primers. Similarly, *trnA* region of potato was amplified using *trnA* F 5‘ GAC CGT AGG TGC GAT GAT TTA CT 3’and *trnA* R 5‘ AGT TGG AGA TAA GCG GAC TCG AA 3’ primers. The reaction conditions for amplification of *trnI* and *trnA* region of potato plastome were as follows, denaturation at 94°C for 5 min, then 35 cycles consisting of 94°C for 1 min, 55°C for 30 sec, 72°C for 1 min, followed by 10 min final extension at 72°C. The amplified product was run in 0.8% agarose gel at 90 V and the amplified fragments were excised from the agarose gel with the sterile blade and eluted by the gel extraction kit (Quiagen). Ligation of *trnI* and *trnA*

fragments in PCR cloning vector pDrive (Quiagen) was done using rapid ligation kit (MBI Fermentas). Ligated plasmids were used to transform *E. coli* JM109 competent cells and plated on ampicillin containing medium. The colonies obtained after transformation were screened for the presence of the insert. Confirmation of ligation was done by restricting the plasmid of selected colonies with restriction enzyme *Eco*RI. Cloning of potato *trnI* and *trnA* cpDNA fragments into pDrive cloning vector yielded plasmid pD*trnI* and pD*trnA*.

2.2. Sequencing of cloned *trnI* and *trnA* potato plastome region

Sequencing of cloned *trnI* and *trnA* potato plastome region was done using M13 F 5‘ GTA AAA CGA CGG CCA GT 3’and M13 R 5‘ AAC AGC TAT GAC CAT G 3’ and gene specific internal primers *trnI* IF 5‘ GAG CAG GTT TGA AAA AGG ATC TTA 3’ and *trnA* IF 5‘ AGA TAC TAT CAT TAC CGC CTG GAC 3’ using ABI Prism 310 Genetic Analyzer (Applied Biosystems). The electronic format of sequenced data was assembled using program package Navigator and sequence analysis was done.

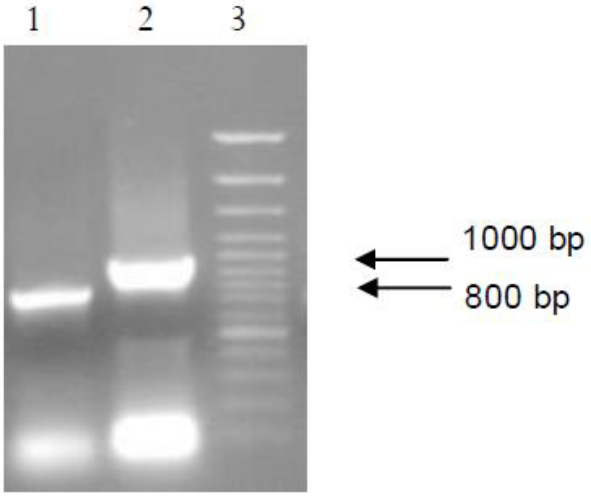
2.3. Sequence Analysis

Sequence analysis was carried out using tools of European Bioinformatics Institute [40]. The assembled potato cpDNA sequences were compared with the chloroplast genome sequence of tobacco (*Nicotiana tobacumi* L.) using BLAST 2.0 algorithm [41]. Potato *trnI* and *trnA* nucleotide sequences were aligned with the corresponding sequences from tobacco with Clustal W.

3. RESULTS AND DISCUSSION

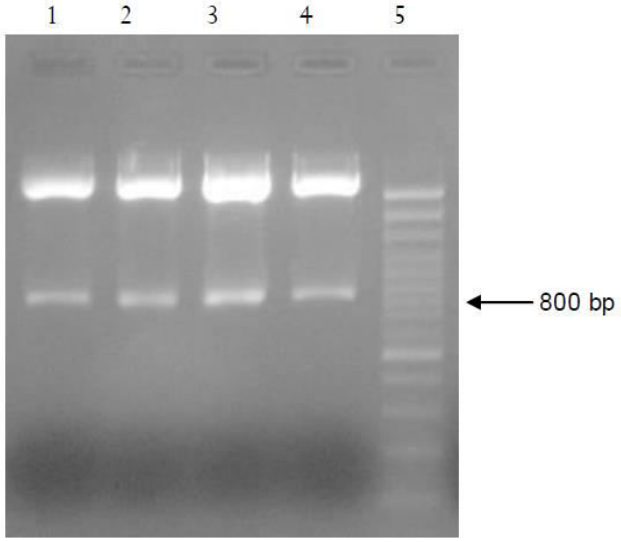
For developing plastid transformation vector based on homologous targeting regions of potato (*Solanum tuberosum* L.), the genomic DNA of popular Indian potato cultivar Kufri Bahar was isolated and the *trnI* and *trnA* intergenic regions of the potato plastome was PCR amplified using primers based on tobacco plastome sequence (GenBank Accession no. Z00044). These two homologous fragments were cloned in PCR cloning vector pDrive (Quiagen) and used as left and right targeting borders of the potato transformation vector. Expected bands of approximately 800 bp with *trnI* primers and 1000bp band with *trnA* primers were observed on separation of amplified product in 0.8% agarose gel (Fig. 1).

The amplified fragments were excised from the agarose gel and cloned into pDrive PCR cloning vector as *Eco*RI/*Eco*RI fragment resulting in plasmid pD*trnI* and pD*trnA* with cpDNA fragments of approximately 800bp and 1000bp respectively. Ligation of *trnI* and *trnA* fragments was confirmed by restricting the recombinant pDrive vector with *Eco*RI restriction enzyme (Fig.2 & 3).



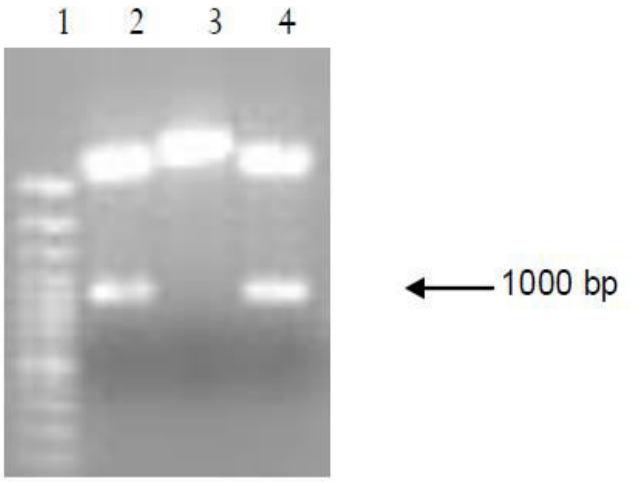
Lane 1: PCR product of trnI region of potato plastome, Lane 2: PCR product of trnA region of potato plastome, Lane 3: GeneRular 100 bp DNA Ladder Plus (Fermantas).

Fig. 1: Agarose gel electrophoresis of amplified *trnI* and *trnA* region of potato plastome.



Lane 1-4, EcoRI restricted product of trnI cloned in pDrive; Lane 5, Marker (GeneRuler 100 bp DNA Ladder Plus).

Fig. 2: Restriction analysis of *trnI* region of potato plastome cloned in pDrive



Lane 1, Marker (GeneRuler 100 bp DNA Ladder Plus); Lane2-4, EcoRI restricted product of trnA cloned in pDrive

Fig. 3: Restriction analysis of *trnA* region of potato plastome cloned in pDrive

The cloned *trnI* and *trnA* repeat regions of potato plastome were sequenced using M13 and gene specific internal primers. Sequences obtained were submitted in Genbank (GenBank accession number FJ237370 for potato *trnI* and FJ237371 for potato *trnA*). When multiple sequence alignment and BLAST search for cloned potato *trnI* was done with members of solanaceae family , it was found that it showed 98% identity with *Nicotiana tabacum* L. chloroplast genome DNA (GenBank accession number Z00044), 98% identity with *Nicotiana tabacum* L., 16S ribosomal RNA gene (accession no. DQ001747), 100% identity with *Solanum tuberosum* L. cultivar Desiree Chloroplast complete genome (accession no. DQ386163), 99% identity with *Solanum tuberosum* L. 16S ribosomal RNA genes (accession no. DQ001745) and 99% identity with *Solanum lycopersicum* complete chloroplast genome, cultivar IPA-6 (accession no. AM087200). The sequence alignment for cloned potato *trnI* and tobacco are shown in table 1.

The most remarkable difference was 9 bp nucleotides insertion from 647-655 in potato plastome that was found missing in tobacco. Insertion of nucleotide in potato plastome may be due to DNA polymerase slippage during DNA replication. Similarly, multiple sequence alignment and BLAST search for cloned potato *trnA* region showed 100% identity with *Nicotiana tabacum* L. chloroplast genome DNA (accession no. Z00044) and 100% identity with *Nicotiana tabacum* L., 16S ribosomal RNA gene (accession no. DQ001747), except an insertion of 102 nucleotides from 700-801 in potato, 99% identity with *Solanum tuberosum* L. cultivar Desiree Chloroplast complete genome (accession no. DQ386163), 99% identity with *Solanum tuberosum* L. 16S ribosomal RNA genes (accession no. DQ001745) and 99% identity with *Solanum lycopersicum* L. complete chloroplast genome, cultivar IPA-6 (accession no. AM087200). The sequence alignment for cloned potato *trnA* and tobacco are shown in table 2.

Table 1: Alignment of *trn*I repeat region of potato and tobacco, showing 9 bp insertion within potato cv. Kufri Bahar (KB)

KB	GCTAATGCTTGTTGGGTATTTTGGTTTGACACTGCTTCACACCCCCAAAA 50
NT	GCTAATGCTTGTTGGGTATTTTGGTTTGACACTGCTTCACACCCCCAAAA 50
KB	AAAAGAAGGGAGCTACGTCTGAGTTAAACTTGGAGATGGAAGTCTTCTTT 100
NT	AAAAGAAGGGAGCTACGTCTGAGTTAAACTTGGAGATGGAAGTCTTCTTT 100
KB	CCTTTCTCGACGGTGAAGTAAGACCAAGCTCATGAGCTTATTATCCTAGG 150
NT	CCTTTCTCGACGGTGAAGTAAGACCAAGCTCATGAGCTTATTATCCTAGG 150
KB	TCGGAACAAGTTGATAGGAtCCCCTTTTTTTACGTCCCtATGTTCCCCC-G 199
NT	TCGGAACAAGTTGATAGGAcCCCCTTTTTTACGTCCCcATGTTCCCCCCG 200
KB	TGTGGCGACATGGGGGCGAAAAAAGGAAAGAGAGGGATGGGGTTTCTCTC 249
NT	TGTGGCGACATGGGGGCGAAAAAAGGAAAGAGAGGGATGGGGTTTCTCTC 250
KB	GCTTTTGGCATAGCGGGCCCCCAGTGGGAGGCTCGCACGACGGGCTATTA 299
NT	GCTTTTGGCATAGCGGGCCCCCAGTGGGAGGCTCGCACGACGGGCTATTA 300
KB	GCTCAGTGGTAGAGCGCGCCCCTGATAATTGCGTCGTTGTGCCTGGGCTG 349
NT	GCTCAGTGGTAGAGCGCGCCCCTGATAATTGCGTCGTTGTGCCTGGGCTG 350
KB	TGAGGGCTCTCAGCCACATGGATAGTTCAATGTGCTCATCGGCGCCTGAC 399
NT	TGAGGGCTCTCAGCCACATGGATAGTTCAATGTGCTCATCGGCGCCTGAC 400

KB	CCTGAGATGTGGATCATCCAAGGCACATTAGCATGGCGTACTCCTCCTGT 449
NT	CCTGAGATGTGGATCATCCAAGGCACATTAGCATGGCGTACTCCTCCTGT 450
KB	TCGAACCGGGGTTTGAAACCAAACTCCTCCTCAGGAGGATAGATGGGGCG 499
NT	TCGAACCGGGGTTTGAAACCAAACTCCTCCTCAGGAGGATAGATGGGGCG 500
KB	ATTCGGGTGAGATCCAATGTAGATCCAACTTTCGATTCACTCGTGGGATC 549
NT	ATTCGGGTGAGATCCAATGTAGATCCAACTTTCGATTCACTCGTGGGATC 550
KB	CGGGCGGTCCGGGGGGGGACCACCACGGCTCCTCTCTTCTCGAGAATCCAT 599
NT	CGGGCGGTCCGGGGGGGGACCACCACGGCTCCTCTCTTCTCGAGAATCCAT 600
KB	ACATCCCTTATCAGTGTATGGACAGCTATCTCTCGAGCACAGGTTTAGGT 649
NT	ACATCCCTTATCAGTGTATGGACAGCTATCTCTCGAGCACAGGTTTAG--- 648
KB	TCGGCCTCAATGGGAAAATAAAATGGAGCACCTAACAACGCATCTTCACA 699
NT	----------CAATGGGAAAATAAAATGGAGCACCTAACAACGCATCTTCACA 691
KB	GACCAAGAACTACGAGATCaCCCCTTTCATTCTGGGGTGACGGAGGGATC 749
NT	GACCAAGAACTACGAGATCgCCCCTTTCATTCTGGGGTGACGGAGGGATC 741
KB	GTACCATTCGAGCCGTTTTTTTCTTGACTCGAAATGGGAGCAGGTTTGAA 799
NT	GTACCATTCGAGCCGTTTTTTTCTTGACTCGAAATGGGAGCAGGTTTGAA 791

Table 2: Alignment of *trn*A repeat region of potato and tobacco, showing 102 bp insertion within potato cv. Kufri Bahar (KB)

KB	GACCGTAGGTGCGATGATTTACTTCACGGGCGAGGTCTCTGGTTCAAGTC 50
NT	GACCGTAGGTGCGATGATTTACTTCACGGGCGAGGTCTCTGGTTCAAGTC 50
KB	CAGGATGGCCCAGCTGCGCCAGGGAAAAGAATAGAAGAAGCATCTGACTA 100
NT	CAGGATGGCCCAGCTGCGCCAGGGAAAAGAATAGAAGAAGCATCTGACTA 100
KB	CTTCATGCATGCTCCACTTGGCTCGGGGGGATATAGCTCAGTTGGTAGAG 150
NT	CTTCATGCATGCTCCACTTGGCTCGGGGGGATATAGCTCAGTTGGTAGAG 150
KB	CTCCGCTCTTGCAATTGGGTCGTTGCGATTACGGGTTGGATGTCTAATTG 200
NT	CTCCGCTCTTGCAATTGGGTCGTTGCGATTACGGGTTGGATGTCTAATTG 200
KB	TCCAGGCGGTAATGATAGTATCTTGTACCTGAACCGGTGGCTCACTTTTT 250
NT	TCCAGGCGGTAATGATAGTATCTTGTACCTGAACCGGTGGCTCACTTTTT 250
KB	CTAAGTAATGGGGAAGAGGACCGAAACGTGCCACTGAAAGACTCTACTGA 300
NT	CTAAGTAATGGGGAAGAGGACCGAAACGTGCCACTGAAAGACTCTACTGA 300
KB	GACAAAGATGGGCTGTCAAGAACGTAGAGGAGGTAGGATGGGCAGTTGGT 350
NT	GACAAAGATGGGCTGTCAAGAACGTAGAGGAGGTAGGATGGGCAGTTGGT 350
KB	CAGATCTAGTATGGATCGTACATGGACGGTAGTTGGAGTCGGCGGCTCTC 400
NT	CAGATCTAGTATGGATCGTACATGGACGGTAGTTGGAGTCGGCGGCTCTC 400
KB	CCAGGGTTCCCTCATCTGAGAcCTCTGGGGAAGAGGATCAAGTTGGCCCT 450
NT	CCAGGGTTCCCTCATCTGAGAtCTCTGGGGAAGAGGATCAAGTTGGCCCT 450
KB	TGCGAACAGCTTGATGCACTATCTCCCTTCAACCCTTTGAGCGAAATGCG 500
NT	TGCGAACAGCTTGATGCACTATCTCCCTTCAACCCTTTGAGCGAAATGCG 500
KB	GCAAAAGAAAAGGAAGGAAAATCCATGGACCGACCCCATCATCTCCACCC 550
NT	GCAAAAGAAAAGGAAGGAAAATCCATGGACCGACCCCATCATCTCCACCC 550
KB	CGTAGGAACTACGAGATCACCCCAAGGACGCCTTCGGCATCCAGGGGTCA 600
NT	CGTAGGAACTACGAGATCACCCCAAGGACGCCTTCGGCATCCAGGGGTCA 600
KB	CGGACCGACCATAGAACCCTGTTCAATAAGTGGAACGCATTAGCTGTCCG 650
NT	CGGACCGACCATAGAACCCTGTTCAATAAGTGGAACGCATTAGCTGTCCG 650
KB	CTCTCAGGTTGGGCAGTCAGGGTCGGAGAAGGGCAATGACTCATTCTTAA 700
NT	CTCTCAGGTTGGGCAGTCAGGGTCGGAGAAGGGCAATGACTCATTCTTA- 699
KB	AACCAGCGTTCTTAAGACCAAAGAGTCGGGCGGAAGGGGGGGAAAGCCCT 750

NT	---------- ---------- ---------- ---------- ----------
KB	CCGTTCCTGGTTCTCCTGTAGTTGGATCCTCCGGAACCACAAGAATCCTT 800
NT	----------
KB	AGTTAGAATGGGATTCCAACTCAGCACCTTTTGAGTGAGATTTTGAGAAG 850
NT	--GTTAGAATGGGATTCCAACTCAGCACCTTTTGAGTGAGATTTTGAGAAG 748
KB	AGTTGCTCTTTGGAGAGCACAGTACGATGAAAGTTGTAAGCTGTGTTCGG 900
NT	AGTTGCTCTTTGGAGAGCACAGTACGATGAAAGTTGTAAGCTGTGTTCGG 798
KB	GGGGGAGTTATTGTCTATCGTTGGCCTCTATGGTAGAATCAGTCGGGGGA 950
NT	GGGGGAGTTATTGTCTATCGTTGGCCTCTATGGTAGAATCAGTCGGGGGA 848
KB	CCTGAGAGGCGGTGGTTTACCCTGCGGCGGATGTCAGCGGTTCGAGTCCG 1000
NT	CCTGAGAGGCGGTGGTTTACCCTGCGGCGGATGTCAGCGGTTCGAGTCCG 898
KB	CTTATCTCCAACT 1013
NT	CTTATCTCCAACT 911

One possible explanation is that at some point in evolution, this 102 bp fragment was deleted from tobacco plastid DNA by either recombination or strand slippage during DNA replication [42]. The observed differences in the sequence homology between potato and tobacco plastome might be responsible for lower transformation efficiency in potato using tobacco-based vector. Further, *trnI* and *trnA* intergenic regions were analysed for the presence of unique restriction enzymes to facilitate insertion of transgene. *Apa*I is the potential insertion site within 800 bp fragment of *trnI* intergenic region, while *Pvu*II, *Sph*I and *Sac*I are the unique restriction sites within 1000 bp *trnA* intergenic region. The presence of unique restriction sites within the intergenic regions of both the fragments will make possible the construction of potato specific plastid transformation vector. Since plastid transformation with tobacco-specific plastid transformation vector containning *trnI* and *trnA* was used to transform closely related members of solanaceae family like potato and tomato [6, 7, 13], however, the transformation efficiency with tobacco-specific plastid transformation vector is lowered because the flanking sequences are not completely homologous as it was observed in the present study. This region can play significant role in homologous recombination. Hence *trnI* and *trnA* coding region of potato plastome of cultivar Kufri Bahar will contribute towards developing potato specific plastid transformation vector with better transformation efficiency.

4. CONCLUSION

Plastid transformation with tobacco-specific plastid transformation vector containing *trnI* and *trnA* was used to transform closely related members of solanaceae family like potato and tomato. However, the transformation efficiency is lowered because the flanking sequences are not completely homologous. This intergenic region *trnI- trnA* plays significant role in homologous recombination. The differences which we observed in the sequence homology between potato and tobacco *trn*I and *trn*A intergenic region might be responsible for lower transformation efficiency in potato using tobacco-based vector. The presence of unique restriction enzyme sites within the intergenic region of both fragments will make possible the construction of potato specific plastid transformation vector. Hence *trn*I and *trn*A coding region of potato plastome of cultivar Kufri Bahar may be utilized for developing potato specific plastid transformation vector with better transformation efficiency.

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6. REFERENCES

1. Maliga P. *Trends Biotechnol*, 1993; **11:**101-107.
2. Bock R, *J Mol Bio*, 2001; **312:**425-438.
3. Daniell H, Datta R, Varma S, Gray S, Lee SB. *Nat Biotechnol*, 1998; **16:**345-348.
4. Svab Z, Hajdukiewicz P, Maliga P. *Proc Natl Acad Sci USA*, 1990; **87:**8526-8530.
5. Sikdar SR, Serino G, Chaudhari S, Maliga P. *Plant Cell Rep*, 1998; **18:**20-24.

6. Sidorov VA, Kasten D, Pang SZ, Hajdukiewicz PTJ, Staub JM, Nehra NS. *Plant J*, 1999; **19:**209-216.
7. Nguyen TT, Nugent G, Cardi T, Dix PJ. *Plant Sci*, 2005; **168:**1495-1500.
8. Valkov VT, Gargano D, Manna C, Formisano G, Dix PJ, Gray CJ et al. *Transgenic Res*, 2011; **20:** 137-151.
9. Valkov VT, Gargano D, Scotti N, Cardi T. *Methods Mol Bio*, 2014; **1132:**295-303.
10. Cheng L, Li HP, Qu B, Huang T, Tu JX, Fu TD , Liao YC. *Plant Cell Rep*, 2010; **29:**371-381.
11. Khan MS, Maliga P. *Nat Biotechnol*, 1999; **17:**910-915.
12. Lee SM, Kang K, Chung H, Yoo SH, Xu XM, Lee SB et al. *Mol Cells,* 2006; **21:**401-410.
13. Ruf S, Hermann M, Breger IJ, Carrer H, Bock R. *Nat Biotechnol*, 2001; **19:**870-875.
14. Apel W, Bock R. *Plant Physiol*, 2009; **151:**59-66.
15. Dufourmantel N, Pelissier B, Garcon F, Peltier G, Ferullo JM, Tissot G. *Plant Mol Biol*, 2004; **55:**479-489.
16. Kumar S, Dhingra A, Daniell H, *Plant Physiol,* 2004a; **136:**2843-2854.
17. Kumar S, Dhingra A, Daniell H. *Plant Mol Biol*, 2004b; **56:**203-216.
18. Cui C, Song F, Tan Y, Zhou X, Zhao W, Ma F, *et al.*, *Acta Biochim Biophys Sin*, 2011; **43:**284-291.
19. Singh AK, Verma SS, Bansal KC. *Transgenic Res*, 2010; **19:**113-119.
20. Bansal KC, Singh AK. *Methods Mol Bio*, 2014; **1132:**305-316.
21. Narra M, Kota S, Velivela Y, Ellendula R, Allini V, Abbagani S, *3 Biotech*, 2018; **8:**140.
22. Kaushal C, Abdin MZ, Kumar S. *Plant Biotechnol J*, 2020; **18:**2155-2157.
23. Kota S, Hao Q, Narra M, Anumula V, Rao AV, Hu Z, et al. *J Plant Biotechnol*, 2019; **46:**323-330.
24. Maliga P, *Annu Rev Plant Biol*, 2004; **55:**289-313.
25. Daniell H, Kumar S, Dufourmantel N. *Trends Biotechnol*, 2005; **23:**238-245.
26. Jansen RK, Raubeson LA, Boore JL, DePamphilis CW, Chumley TW, Rosemarie C. et al., *Meth Enzymol*, 2005; **395:**348-384.
27. De Cosa B, Moar W, Lee S B, Miller M, Daniell H. *Nat Biotechnol*, 2001; **19:**71-74.
28. Ruhlman T, Verma D, Samson N, Daniell H. *Plant Physiol*, 2010; **152:**2088-2104.
29. Kunnimalaiyaan M, Nelson BL. *Nucleic Acid Res*, 1997; **25:**3681-3686.
30. Lugo SK, Kunnimalaiyaan M, Singh NK, Nelson BL. *Plant Sci*, 2004; **166:**151-161.
31. Guda C, Lee SB, Daniell H. *Plant Cell Rep*, 2000; **19:**257-262.
32. Svab Z, Maliga P. *Proc Natl Acad Sci USA*, 1993; **90:**913-917.
33. Carrer H, Hockenberry TN, Svab Z, Maliga P. *Mol Gen Genet*, 1993; **241:**49-56.
34. Carrer H, Maliga P. *Bio/Technology*, 1995; **13:**791-794.
35. Daniell H, Muthukumar B, Lee SB. *Curr Genet*, 2001; **39:**109-116.
36. Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, et al. *EMBO J*, 1986; **5:**2043-2049.
37. Daniell H, Lin S, Yu Ming, Chang WJ. *Genome Biol*, 2016; **17:**134.
38. Calsa JT, Carraro DM, Benatti MR, Barbosa AC, Kitajima JP, Carrer H. *Curr Genet*, 2004; **46:**366-373.
39. Murray MG, Thompson WF. *Nucleic Acids Res*, 1980; **8:**4321-4325.
40. EMBL.EBI.www.ebi.ac.uk.
41. NCBI-www.ncbinlm.nih.gov/BLAST.
42. Berger IJ, Carravo DM, Bock R, Azevedo RA, Carrer H. *Braz J Plant Physiol*, 2005; **17:**239-246.