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DEVELOPMENT AMPLIFICATION CONCENSUS GENETIC MARKERS IN *BETULA LUMINIFERA* BASED ON BIRCH EST DATABASE

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ABSTRACT

By designing primers in the conserved coding sequences flanking a polymorphic site, it is possible to exploit a PCR-based marker called amplified consensus genetic marker (ACGM), which is transferable among closed species. The purpose of this paper was to investigate the feasibility of developing ACGMs in *Betula luminifera* using birch data. Based on bitch EST data, 109 ACGM candidate primers were developed. Two *B. luminifera* varieties including Lin'an5 and Sichuan4 which represent two different ecotype were used to verify the candidate ACGM markers. By aligning 3 028 EST sequences from birch with the CDS from Abidopsis using blast, 1227 ESTs could be align successfully. Of these 1227 ESTs, 976 (79.5 %) can design primer in flanking split. We successfully obtained 656 e-PCR products from those 976 putative ACGM loci. And 109 candidates ACGM markers were selected and tested, 105 (96.3 %) yielded stable and clear PCR products in birch. Of those 105 markers, 95 (90.5 %) can work well in at least one *B. luminifera* genotype. The result showed that ACGM marker could be a good choice for those species species with scarce available sequence data.

KEYWORDS: Development, ACGM, markers, *Betula luminifera*, birch.

INTRODUCTION

Molecular markers have been broad used in genetic research, gene clone, comparative genomics and molecular assisted selection. Many types of molecular markers have been developed since restriction fragment length polymorphisms (RFLP) were developed in 1980 (Botstein et al. 1980). Up to now more powerful and available markers are those based on polymerase chain

reaction (PCR) techniques. In short, there are two types of PCR-based markers; one is random primer markers which can be used in nearly all kinds of species, examples are randomly amplified polymorphic DNAs (RAPDs) (Williams et al. 1990) and amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995). The other type is special primers markers such as SSR (simple sequence repeat), which must developed from and used in target species (Becker and Heun 1995). Although the utility of SSRs in genetics studies is well established, the isolation and characterization of such markers via traditional methods are costly and time consuming, making the *de novo* development of SSRs unrealistic for some taxa (Pashley et al. 2006). Random primer markers are more flexible because they can be used in nearly all kinds of species. But their reliability, especially in RAPDs, is in doubt in some extent.

Up to now, tree biology research, in contrast with the others, lags behind. The databases of tree sequence data are limited. For many tree species, there is no DNA sequence information. This lack of data makes it difficult to develop locus specific primers for those species. There is narrow choice of markers for biology research in those species. It is useful and necessary to develop and test more markers for those species.

Amplified consensus genetic marker (ACGM) is a PCR-based marker with primers designed in conservative regions of coding sequence (Fourmann et al. 2002). By exploring the consensus region between closed species, it is possible to develop ACGM markers for those species that have sufficient expressed sequence (Lu et al. 2006a). With the recent progress made in large-scale plant functional genome sequencing project, a great amount of express sequence tag (EST) data is becoming available. By searching consensus region in ESTs between closed-related species, it is possible to develop ACGM markers, and these markers can be transferable among closed species.

But still there are lots of species short of EST database. On the other hand, it is those species, which need more efficient molecular markers to support its biology research. According to Wang et al. (2005), EST-derived markers are likely to be conserved across a broader taxonomic than any other sorts of marker. So for those species with scarce available sequence data, it is feasible to develop ACGM markers with their closed species EST database. Yang et al. (2007) exploit candidate ILP (intron length polymorphism) markers by comparing dicot EST with genome of Arabidopsis or by comparing monocot with Rice. The research shows it can work well to search homologous between really far genetic distant species.

Betula luminifera is deciduous tree, widely occurring at temperate of China. As lots of other tree species, its biology and sequence data are limited. Birches (*Betula platyphylla* Suk), having lots of sequencing information and biology database, is closed to *B. luminifera*. By comparing birch EST with Arabidopsis to search conserved region, we can exploit ACGM marker for *B. luminifera*. This paper provides a case study of the utility of freely available birches EST resources for the development of ACGM markers necessary for the genetics analysis of *B. luminifera*. Therefore ACGM system could be another way to afford primer and genomic information to *B. luminifera*.

MATERIAL AND METHODS

Search of putative ACGM

In totally, 3028 (*Betula platyphylla* Suk) EST sequences of birch-released by the plant GDB (http://www.plantgdb.org) were downloaded. In addition, the genome, cDNA and CDS (coding sequence) data of Arabidopsis (*Arabidopsis thaliana* ecotype Columbia) were downloaded from http://www.arabidopsis.org/.

We developed a pipeline using Perl script to search conserved region between birch and

Arabidopsis and exploit candidate ACGM markers. The initial step was to identify the most likely conserved EST of the available EST sequences in birch by aligning the EST sequences of birch with the CDS of Arabidopsis using BLASTN.30. We used a high E-value (10-20) for the BLASTN to remove paralogues. Then we used the program BLAT to make sure the split during align each EST with its corresponding Arabidopsis gene. In order to include more putative polymorphism, putative ACGM should be those EST sequence having one more split. For detect by gel clearly, each split length should be more than 20bp.

Exploitation of candidate ACGM by electronic PCR

To exploit candidate ACGM markers from the putative ACGMs identified by BLASTN and Blat, we designed PCR primers based on the EST sequences corresponding to the flanking splits using ePrime3 (http://www.hgmp.mrc.ac.uk/). For convenience, we used a 200 bp cDNA sequence with 100 bp on each side of the target split for the primer design for each ACGM. We then tested the designed primers by electronic PCR (e-PCR, Schuler 1997) on the birch EST sequence. To increase the quality and usability of the in silicon exploited ACGM markers, we required exact matches between primers and templates and set a 300 bp margin on the product size for the e-PCR. We took a putative ACGM locus as a candidate ACGM marker. Those were successfully and uniquely detected by the e-PCR were selected as candidate primer and named it with the abbreviation BLA (for *B. luminifera* ACGM) followed by a unique number (BLA80).

Verification and evaluation of ACGM markers in birth by experiment

One birch, as well as two *B. luminifera* varieties including Lin'an5 and Sichuan4 which represent two different ecotypes, were used to verify the candidate SSR markers. Total genomic DNA was isolated from 200 mg of fresh leaf tissue using CTAB method (Murray and Thompson 1980).

All primers used were synthesized by Nanjing Jinsite Biological Engineering & Technology Company in China. PCR was performed in 20 μL reactions containing

50 ng of template DNA, 0.5 μmol.L^{-1} of each primer, 200 μmol.L^{-1} of each dNTP, 1.5 mmol.L^{-1} of $MgCl_2$, 1 unit of Taq polymerase, and 2 μL of 10 × PCR reaction buffer. A touchdown PCR program (Don et al. 1991) was used: 5 min at 95 °C; 10 cycles of 30 s at 95 °C, 30 s at 580 °C minus 0.3 °C/cycle, 1 min at 72 °C ; 20 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C; and 7 min at 72 °C for a final extension. For those primer pairs that did not generate good amplification results, the initial annealing temperatures were adjusted from 55 °C to 60 °C. Each of the primer pairs was tested twice to confirm the repeatability of the observed bands in each genotype. PCR products were separated on agarose gel. Gels were stained with Ethidium Bromide for visualizing DNA bands.

Sequencing PCR product

To confirm the PCR product amplified in *B. luminifera* were homologuous to the birch genes where the loci were first identified, a band yielded by primer BL26 in Lin'an was isolated, purified and sequenced.

RESULTS

Candidate ACGM markers

By aligning 3 028 EST sequences from birch with the CDS from Abidopsis using blast, 1227 ESTs could be align successfully. This result showed that about 40 % have some homologous

between birch and Arabidopsis. This ratio may be high than the ture situation because of the repeat sequence among those ESTs. In order to include some polymorphic loci we selected ESTs with at least one split during align with Arabidopsis using BLAT. The split may result from two ways, one is intron and the other is mismatch yield from DNA sequence variation. Either of this should be candidate of polymorphism. Therefore 976 (79.5 %) ESTs were selected and designed primer in flanking split. We successfully obtained 656 e-PCR products from those 976 putative ACGM loci. Although we designed the primers based on the ESTs from birch, we failed to acquire e-PCR products from about 1/3 putative ACGM loci in birch, probably due to the several constraint conditions set for the primer design and e-PCR. Among those products, there were 441 primer pairs detecting either multiple BAC clones located on different chromosomes and appearing to have multiple copies in Arabidopsis, or overlap of ESTs obtained same PCR product. Multiple-copy is not desirable for molecular markers, we discarded these primer pairs. At last, 109 candidate ACGM markers are selected.

ACGM markers exploited by experiment

Because the primers were designed from birch EST data, they must be tested for application in *B. luminifera* by experiment. All primers were tested on birch DNA preparations first (Fig. 1). All primers were detected in birch first (Fig. 1). Of the 109 candidate ACGM markers tested, 105 (96.3 %) yielded stable and clear PCR products as expected in birch. Then we tested the utility of those 105 markers in *B. luminifera* (Fig. 2). The result showed 95 (90.5 %) yielded stable and clear PCR products in at least one *B. luminifera* genotype. The primers of ACGM markers and their homologous gene in Arabidopsis with which they were aligned are shown in Tab. 1. Besides, the Arabidopsis genes' id and function are given

Fig. 1: Primers screened in Birch separated by electrophoresis on 1 % agrose gel (M representing DNA land Marker DL2000)

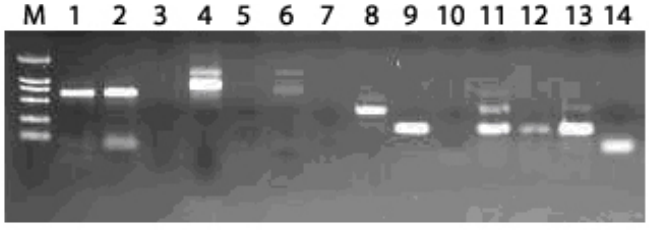

Fig. 2: Primers screened in B. luminifera separated by electrophoresis on 1 % agrose gel (M representing DNA land Marker DL2000)

Tab. 1: 95 ACGM markers developed for B. luminifera

Primer' name	Former (5'-3')	Reverse(5'-3')	Arabidopsis' number	Description
BLA1	ATCTCCGCTTT CTTCGTT	GAGGAACCAA ACCACCAA	AT3G09350	armadillo/beta-catenin repeat family protein; β - catenin
BLA2	CCCCAAACCTA ACCCTAA	CCTAAGGACG ATGATAACT	AT3G62840	similar to small nuclear ribonucleoprotein D2
BLA3	TTTTATGGAGGC TCAGGT	CAGAGTCGGAT GATGAAAT	AT5G22000	encodes a RING-type E3 ubiquitin ligase implicated in gametogenesis.
BLA4	CTAATCATCCTTC AGATCCCTC	TAAGAAGGGTC CCAAACA	AT5G11280	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G80200.1)
BLA5	GGCATTGTCAAC GAGGCT	CCAAGCCATAGT ACATCA	AT4G14305	similar to PMP22
BLA6	TACCTGAAGCGG GAGACA	TGAAAATACTGG AACCCT	AT4G30220	RUXF
BLA7	GGGACCTCTGGC TTATTT	CCATTCCAAGG GCAGACA	AT2G34160	nucleic acid binding
BLA8	CACTCCAGAACTG CGTGCCT	CCATTAGATACC GCTCAA	AT3G60210	similar to CHL-CPN10
BLA9	CGAAGGGAGCAAC AGCCAGAA	TTCTGAAAGTAG TGCCTCC	AT2G47170	Gene encoding ADP-ribosylation factor
BLA10	TCTGTACGCTACT TTCATC	ACGGAGCACGAG GTGGAGA	AT4G05320	One of five polyubiquitin genes in A. thaliana.
BLA11	TCCTCGGAACTT CAGATT	TTCCACCACTCC AGTTTCAT	AT3G52560	MMZ4/UEV1D encodes a protein
BLA12	TCGTGACGGGTC GTGGAT	AGACACCACCAA GCCAGT	AT2G39960	microsomal signal peptidase 25 kDa subunit
BLA13	GAAAGCCAAGATT CAAGAC	GTGGGATACCCT CCTTAT	AT4G05320	One of five polyubiquitin genes in A.
BLA14	CCATCCGCAAGAA CGGCTAC	CCTTGGTGTTCC CATTTT	AT1G13950	Encodes eukaryotic translation initiation factor 5A (EIF-5A).
BLA15	CACCGATGACGC CTCCACT	AGCATTGTTAGCC TCTTC	AT2G27450	Encodes N-carbamoylputrescine amidohydrolase
BLA16	GCATTCGTCGC ACATTCT	CCACCACCGTTTG CGAGT	AT3G61110	Arabidopsis ribosomal protein
BLA17	GACCCGAAGA AGAACTGG	CTCCTGAAGGG TGTTTGC	AT5G25450	ubiquinol-cytochrome C reductase complex 14 kDa protein
BLA18	TTTGCGTTGTTG CTTTCC	TCAACCTCGTT ATGCTGT	AT2G34160	nucleic acid binding
BLA19	ACGATGTCGGAC GAGGAG	TGGGACGAGG GAACGAAT	AT1G13950	Encodes eukaryotic translation initiation factor 5A
BLA20	TAGCGGCTCAGA CTCGGCAATA	CTTTGTTTCAGCC TTTGC	AT5G08300	succinyl-CoA ligase (GDP-forming) alpha-chain
BLA22	AAATGTCGGACGA GGAAC	TGGGAGGAAG GAACAATA	AT1G69410	eukaryotic translation initiation factor 5A
BLA23	GGAGACAGCCGCA GAGGAGA	TGCCCATCAATGTA TTCTTC	AT4G30220 form 16-267	RUXF
BLA26	CCCTCGCCGACTA CAACA	CCGTCTTCCGAG TTGCTT	AT4G05320 form 1-374	One of five polyubiquitin genes
BLA27	TTTTCCCAAGCAC GAAGTAA	TCAAGCCCATCA CCTCCC	AT5G23540	26S proteasome regulatory subunit
BLA28	TGCTCCACCGCTT ACTACTGCC	GCCATTACGCAT TTGTCC	AT3G60820	Encodes 20S proteasome beta subunit PBF1 (PBF1)
BLA29	TTCCCAAGCACGA AGTAAA	CAAGCCCATCA CCTCCAT	AT5G23540	26S proteasome regulatory subunit
BLA30	CGATTCCACCAAG CAACT	CATCGGGCTTT CCTCCTT	AT5G42890	sterol carrier protein 2 (SCP-2) family protein
BLA31	AAACAATGGTGC GAGTGA	TAGCCTTCCAT TCAGTTC	AT1G07770	Encodes cytoplasmic ribosomal protein S15a
BLA32	TGTGCCAGGGTT CGTTGA	AGTGCCCAGTCC TTTCTC	AT1G65980	thioredoxin-dependent peroxidase
BLA34	CTCTGCTTGGGCT TCTGT	CCTTGTTGCTC ACATCCC	AT4G28830	methyltransferase
BLA35	AGCCATCAACCAC CGCAGCAC	ACCTACAACACG CTCTGC	AT1G06390	encodes a GSK3/shaggy-like protein kinase
BLA36	GAGGAAACTAAGC AACCCAA	TGGCACGCTATC TTCTCG	AT4G18730	encodes a cytosolic ribosomal protein L16
BLA37	GGGACCCATACTT GAAAC	CTCGCTTGTTG GGTTCAG	AT1G53310	Encodes one of four Arabidopsis phosphoenolpyruvate carboxylase proteins
BLA38	ACTGGCAAGACC ATCACC	CGGAGCACCAAG TGAAGG	AT4G053 20 form 1-427	ubiquitin
BLA40	TAGCGGCTCAGA CTCGGCAATA	TTCTGTTCCACC TTTCTTT	AT5G08300	succinyl-CoA ligase (GDP-forming) alpha-chain
BLA42	CTTGGCAACAGT CAGGGTG	GCAGGATTGAAA TGTGGC	AT1G08830 form 101-356	Encodes a cytosolic copper/zinc superoxide dismutase CSD1
BLA43	GAGAAAACTAT GGAGCAG	GCAGACAGGTC GGCATAG	AT4G09320	nucleoside diphosphate kinase type 1 (NDPK1) gene, complete

BLA44	ATTCCCTACAA CAAATCAAACC	CTTTGCTCGTTC CACCTC	AT5G57815	cytochrome c oxidase subunit 6b, putative
BLA45	CCTTCTCGTC CGATTCCA	ATCTATGTGAAA CGCAAC	AT5G03290	isocitrate dehydrogenase
BLA48	CCCCAAGGAG CAATCACA	AGAAGTGTCGGT CAAAGC	AT1G26470	similar to Os05g0512500
BLA50	CAAGGCAAAG ATTCAAGAT	CACGAAGCCTCA GACAAG	AT2G36170 form 1-360	60S ribosomal protein L40 (RPL40A)
BLA51	GGTCTATCCC TCTGGTTG	GGAACACTGCC CTTGGAG	AT5G09810	Member of Actin gene family
BLA52	GCCAAGGAGA TCGTTTCG	ATCGCTTTCAGT GTCCAA	AT5G40370	glutaredoxin, putative; Identical to Glutaredoxin-C2 (GRXC2)
BLA53	CGCACCGTTCTT CGGCTTCC	ACTTCATCACC AGCTCCG	AT2G16510	vacuolar ATP synthase 16 kDa proteolipid subunit 5
BLA54	GGAAAGAGCAGA GCGGCAACA	GCCTTAGGAGC CTTCTTC	AT4G26230	60S ribosomal protein L31 (RPL31B)
BLA55	GCAAGAACAAGGA ATGCGAGAA	GGGTTTGGTCT GTCCTCC	AT4G14320	60S ribosomal protein L36a/L44 (RPL36aB)
BLA58	AAGCAGCACTCA GACTAAA	CAAAGCGTGTT CAATCTG	AT1G79210	20S proteasome alpha subunit B
BLA59	AAAGGCACTCAA ACATAC	GCCGGAATGACT TTCAGCACAT	AT4G30800	40S ribosomal protein S11 (RPS11B)
BLA60	ACCCAGGCAGTT GAAGAA	TCGGGTCATTG TTTCTCA	AT5G28050	cytidine/deoxycytidylate deaminase family protein
BLA61	ACGCCTCCACTA ATGTCG	AAATCCTCCCTT TCTGCCTGACAGA	AT2G27450	Encodes N-carbamoylputrescine amidohydrolase
BLA62	CCCTCAAGTGCT TACAAT	TCTCCACCAGAT GATAGTCC	AT4G35090	Encodes a peroxisomal catalase
BLA63	ATGCTGTAGGCG ATGTGG	CCCTCCTCACTT GCCTTA	AT5G03630	ATMDAR2
BLA65	GACCCGTACATC GTCGTCA	ATTGGAACATTA GGGTCT	AT3G17980	C2 domain-containing protein
BLA66	AGATCAATGAGC CACTGAAGC	GTGGTGGAAAT GATGTGA	AT2G30980	Encodes a GSK3-linke protein kinase
BLA67	TGTGCTTGGGA AGATGAG	AGCAGCCATCA TTCCAAA	AT4G27270	quinone reductase family protein
BLA68	TCTACAAAGAAT GGACGGGACT	CCAGGATGGAA ACGGGTG	AT2G16930	ribosomal protein L27 family protein
BLA69	AGATAGCCATGT CGAGCAC	CATTTGCCTGA GGTGGTG	AT1G77120	Catalyzes the reduction of acetaldehyde using NADH as reductant
BLA70	TTTGTTAGGGC TTCTTTG	CAGATTTATGG GTTGAGT	AT4G30330	small nuclear ribonucleoprotein E, putative
BLA71	CTCCCGATTAT CCATTCA	GGGCTCCACTG TTCTTTA	AT3G08690	UBC11
BLA72	CTCTATCGGTT CTACCTTCTGA	CTTGTAGCCGAG GATTGT	AT3G55750	60S ribosomal protein L35a (RPL35aD)
BLA73	AACGAGGCTTG GAGGAAA	CCATCAACTTGT GGAGGA	AT4G14305	similar to PMP22
BLA74	AAATGGTTGCG GTGAAGA	AACGGAGGACGG TCTTGT	AT1G77940	60S ribosomal protein L30 (RPL30B)
BLA75	TACAATCGCCG CTTCGTC	TGCCTCCCCTAA CAAAAT	AT4G29390	40S ribosomal protein S30 (RPS30B)
BLA76	CTGCTCCAATG GGAAAGG	CAGCGACTCTTC TGGAGATGGA	AT1G52300	60S ribosomal protein L37 (RPL37B)
BLA77	AGGGTATTTCG GCTTCTG	GACCCTTCTTAG CAAACT	AT4G00100	Encodes a cytoplasmic ribosomal protein S13 homologue involved in early leaf development
BLA78	TAACCGAATAAA GGTCATC	AATGCTAATCC TCCTTTC	AT2G29140	APUM3
BLA79	ATCATCAAATTG CTACTGG	TAGCCTTCCAT TCAGTTC	AT3G46040	TCP20
BLA80	CGCACAGAGCTA GGGTTT	TACCGCATACACG GCAAG	AT4G33865	40S ribosomal protein S29 (RPS29C)
BLA81	GCTATGCCACCA AGTCCA	GTTACAGGGCACT TGGGT	AT3G28900	60S ribosomal protein L34 (RPL34C)
BLA83	CCCAGGCAAAG CAACTGT	GTGCCATAAGGTA TTTAGTTTC	AT3G59990	Encodes a MAP2 like methionine aminopeptidase
BLA84	CGAATAAAGGT TCATCTG	AATGCTAATCCTC CTTTC	AT2G29140	APUM3
BLA85	AGAACAGTAGC GGGGAGT	ATCCCACTTGTTT CCAATCACC	AT1G61700	DNA-directed RNA polymerase II, putative (RPB10)
BLA86	TGGTGGCGAGG GTTGCTCT	AGTGTCGTACTTA AACAT	AT1G13440	GAPC-2; glyceraldehyde-3-phosphate dehydrogenase
BLA87	GGAAGGTCAAG ATCGGAATC	GTAGTCGGTGGTG ATGAA	AT3G04120	encodes cytosolic GADPH
BLA88	GACATCGCCGT CTTCTTC	TGTTGGTTTGGTA GAGCC	AT5G20020	A member of RAN GTPase gene family
BLA89	GCTGTGCCAAG TGGTGCT	CACAGCCTTGAGG ACACC	AT2G36530	enolase

BLA90	GGTCTTGATGC TGCTGGTA	AGTCTCCACATTG AACCC	AT1G23490	Gene encoding ADP-ribosylation factor
BLA91	CCTAAGACGAA GAAAACA	TATCCTTGCCTTT CTTGT	AT4G14320	60S ribosomal protein L36a/L44 (RPL36aB)
BLA93	GCAGCTTCTT CGAGACAA	CACGCTTAGATGC TTCCTTA	AT1G78300	G-box binding factor GF14 omega encoding a 14-3-3 protein
BLA94	CCAGTTGCCTG CGGAGTA	AAATCTTGGTTT GGGTTTC	AT3G17000	UBC32 (UBIQUITIN-CONJUGATING ENZYME 32); ubiquitin-protein ligase; similar to UBC34 (UBIQUITIN-CONJUGATING ENZYME 34)
BLA95	ATGAGGACCAT TATCGTT	GGTATGTTTGAGT GCCTTT	AT4G30800	40S ribosomal protein S11 (RPS11B)
BLA96	TTTCCAAGCAG GAGTTTA	ATGGTAACCTT TCGTGGA	AT3G12760	similar to calcium ion binding [Arabidopsis thaliana] (TAIR:AT1G15860.1); similar to SM10 [Nicotiana tabacum] (GB:ABI49160.1)
BLA97	TCCCTACAGG AAAGACAG	TGGCACATCC AGGACACC	AT5G67520	adenylylsulfate kinase, putative; similar to AKN2 (APS-KINASE 2), ATP binding / kinase/ transferase, transferring phosphorus-
BLA98	GGACAAGAA GCTCCAGAT	CATAAACTGGT CAAACCC	AT3G11500	small nuclear ribonucleoprotein G, putative / snRNP-G, putative / Sm protein G, putative; Identical to Probable small nuclear
BLA99	CTCATTTGGC TGTTTTCT	TGTTGGTTTG GTAGAGCC	AT5G20020	A member of RAN GTPase gene family. Encodes a small soluble GTP-binding protein. Likely to be involved in nuclear translocat
BLA100	AGGCATACTTC AAGCGGTTTC	GCCCGATAGT CTGTCTTCC	AT5G39740	60S ribosomal protein L5 (RPL5B)
BLA101	TTTTCAGTTG CGACGATG	CGTCAGACTG GCGAGCAT	AT3G61860	encodes an arginine/serine-rich splicing factor. transcript is alternatively spliced and is differentially expressed in diffe
BLA102	AATGGGTTCA GAGTATCTT	ATCCGAGCCC TAATCACA	AT3G60190	At3g60190 encodes Arabidopsis dynamin-related protein 1E, DRP1E, also known as EDR3, ADL4 and ADL1E, which is 624 amino acid
BLA103	GAGAACTGCCA AAAGAGC	TCCTCACCCT TGCTGTAT	AT5G59890	actin depolymerizing factor 4 (ADF4) mRNA, complete cds
BLA104	CGTCAAGTGC CAGGGTTG	CCACCACCGT TTGCGAGT	AT3G61110	Arabidopsis ribosomal protein
BLA105	CCTGGTAGGT CTGTTTGA	TGATGGTAAC CCGCTTGG	AT4G40040	histone H3.2; Identical to Histone H3.3 (HTR8) [Arabidopsis Thaliana] (GB:P59169;GB:Q6NR95); similar to histone H3
BLA106	CTCATTCGTC GTCACCAT	CAGAAGCCGA AATACCCT	AT4G00100	Encodes a cytoplasmic ribosomal protein S13 homologue involved in early leaf development
BLA107	GCGGATTACAA CATTCAGA	CACGAAGCCT CAGAACAA	AT2G36170	ubiquitin extension protein 2 (UBQ2) / 60S ribosomal protein L40 (RPL40A); Identical to Ubiquitin (UBQ16)
BLA108	GAAAGCAGGA TTTTGTAG	TGGGCGGAAG GATGAAAA	AT1G16720	Encodes HCF173, a protein with weak similarities to the superfamily of the short-chain dehydrogenases/ reductases.
BLA109	CCCTTCCTCTG CTTCTTC	TCCACCTGTG ATTGCTCC	AT1G26470	similar to Os05g0512500 [Oryza sativa (japonica cultivar-group)] (GB:NP_001056022.1); contains InterPro domain CT20

Homologous test

To examine the homologous of ACGM marks amplified in *B. luminifera* using primers derived from the EST sequence in birch, we randomly selected one PCR product yielded by primer BL26 in Lin'an variety for DNA sequencing. BLAST (http://www.Arabidopsis.org/Blast) result of the 172 bp fragment showed that the sequence shared 88 % identity with the EST that generated the ACGM primer (BL26), which met our expectations (Fig. 3).

CTCCGCGGCGGGAtGCAGATCTTCGTCAAAACCCTAACCGGGAAGACCATCAcTCTCGA

GGTCGAGAGTTCCGACACCATCGACAACGTCAAGGCTAAGATCCAAGACAAGGAAGG

CATCCCTCCGGATCAGCAGCGATTGATCTTCGCCGGAAAGCAACTCGGAAGACGGA

Fig. 3: Nucleotide sequence of PCR produce yielded by primer BL26

DISCUSSION

Good molecular markers are useful tools for genetic and biology studies. ACGM is gene marker, that is the primer on expressed sequence. So, fragments generated form these primers are parts of gene sequences. Polymorphism yield from ACGMs can directly reflect variation within genes (Gale and Devos 1998). Therefore, the maps constructed with ACGM markers would be more valuable for genetic studies. However the polymorphic level could be low for its conservation. A higher estimate of the polymorphic level of ACGM markers could probably be obtained if methods of DNA fragment analysis with higher resolution capacity (denaturing PAGE, usually used for SSR analysis) were adopted.

ACGM marker would be more useful for comparative genomic study because it was designed in conserved expressed region. Therefore it could be used in wide range and shown linear relationship among different genomes within same genus (Rong et al. 2005). According to Lu et al. (2006b), ACGMs exploited by rice data have a good transferability among several main species of Gramineae.

The research of plant biology, as with all areas of biology, has undergone dramatic changes in the past decade since the development high-throughput methods for sequence determination. In recent years, high-density oligonucleotide re-sequencing microarrarys and next-generation sequencing technologies have resulted in a considerable increase in the amount of available genome sequence data (http://www.ncbi.nlm.nih.gov). However, there are still lots of species, especially woody plants, which have little or no publicly available sequence information. And at present, it is not feasible to sequence the genome of all the species. However, with the help of complete genomic sequence information of model plants (rice and Arabidopsis), it is possible to predict some information for other plants. Therefore, ACGM marker could be a good choice for those species.

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REFERENCES

1. Becker, J., Heun M., 1995: Barley microsatellites: Allele variation and mapping. Plant Molecular Biology 27(4): 835-845.
2. Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980: Construction of agenetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet . 32(3): 314-331.
3. Don, R.H., Cox, P.T., Wainwright, B.J., Baker, K., Mattick, J.S., 1991: 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 19(14): 4008.
4. Fourmann, M., Barret, P., Froger, N., Baron, C., Charlot, F., Delourme, R., Brunel, D., 2002: From *Arabidopsis thaliana* to *Brassica napus*: Development of amplified consensus genetic markers (ACGM) for construction of a gene map. Theor. Appl. Genet. 105(8): 1196-1206.

5. Gale, M.D., Devos, K.M., 1998: Comparative genetics in the grass. Proc. Natl. Acad. Sci. USA 95(5): 1971-1974.
6. Lu, Y., Wang, X., Huang, W., Xiao, T., Zheng, Y., Wu, W., 2006a: Development of amplified consensus genetic markers in Gramineae based on rice intron length polymorphisms. Scientia Agricultura Sinica 39(3): 433-439 (in Chinese with an English abstract).
7. Lu, Y., Ye, Z., Wu, W., 2006b: Analysis of the phylogenetic relationships among several species of gramineae using ACGM markers. Acta Genetica Sinica 33(12): 1127–1131.
8. Murray, M.G.., Thompson, W.F., 1980: Rapid isolation of highmolecular-weight plant DNA. Nucleic Acids Res. 8(19): 4321-4325.
9. Pashley, C.H., Ellis, J.R., Mccauley, D.E, Burke, J.M., 2006: EST Database as a source for molecular markers: lessons from Helianthus. Journal of heredity 97(4): 381-388.
10. Rong, J., Bowers, J.E., Schulze, S.R., Waghmare, V.N., Rogers, C.J., Pierce, G.J., Zhang, H., Estill, J.C., Paterson, A.H., 2005: Comparative genomics of Gossypium and Arabidopsis: Uraveling the consequences of both ancient an rece polypolidy. Genome Research 15(9): 1198-1210.
11. Schuler, G.D., 1997: Sequence mapping by electronic PCR. Genome Res. 7: 541–550.
12. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., 1995: AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23(21): 4407-4414.
13. Wang, X., Zhao, X., Zhu, J., Wu, W., 2005: Genome-wide investigation of intron length polymorphisms and their potential as molecular markers in rice (*Oryza sativa* L.). DNA Research 12(6): 417-427.
14. Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990: DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18(22): 6531-6535.
15. Yang, L., Jin, G., Zhao, X., Zheng, Y., Xu, Z., Wu, W., 2007: PIP: A database of potential intron polymorphism markers. Bioinformatics 23(16): 2174-2177.

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