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INTEGRATION OF TRAP MARKERS ONTO A SUNFLOWER SSR MARKER LINKAGE MAP CONSTRUCTED FROM 92 RECOMBINANT INBRED LINES

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SUMMARY

The target region amplification polymorphism (TRAP) marker technique was employed to expand the published sunflower simple sequence repeat (SSR) linkage map constructed from a recombinant inbred population derived from the cross of RHA 280 × RHA 801. A previous report described the mapping of 183 TRAP markers generated by using fixed primers designed against the conserved *Arabidopsis*-type telomere sequence repeat into the above mentioned map of 577 SSR markers. Thirty-two markers were mapped to the outermost positions of the linkage groups, defining 21 of the 34 linkage group ends. This paper reports the integration of an additional 220 TRAP markers onto the same map. These newly added TRAP markers were generated by 23 combinations with fixed primers designed against selected sunflower ESTs showing homology with components of plant disease resistance genes and homeobox genes. The resulting map consists of 980 markers in 17 linkage groups (LG) and the total length is 1920 centiMorgan (cM). Although the average distance between two markers is less than two cM, there are four gaps larger than 20 cM (one in LG2, one in LG4, and two in LG6). The gaps could be due to the lack of polymorphism between the two parental lines in these chromosome regions with respect to the type of markers used in this study.

Key words: **target region amplification polymorphism, genome map, linkage group ends**

INTRODUCTION

More than ten linkage maps of various molecular markers have been constructed for the sunflower genome by different laboratories in North America and

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Europe (Berry *et al.*, 1995, 1996, 1997; Gentzbittel *et al.*, 1995, 1999; Jan *et al.*, 1998; Rieseberg *et al.*, 1993; Rieseberg, 1998, Peerbolte and Peleman 1996; Gedil *et al.*, 2001; Langar *et al.*, 2003; Tang *et al.*, 2002; Yu *et al.*, 2003; Hu *et al.*, 2004). These maps provided useful information on the genomic organization of this important oil crop. The objectives of this study are to further saturate the public sunflower map with a fairly new type of DNA-based marker, the TRAP (target region amplification polymorphism) marker (Hu and Vick, 2003) in order to better characterize the genome and to generate useful markers for genetic improvement of sunflower through marker-assisted breeding.

MATERIALS AND METHODS

Mapping population

A recombinant inbred population was kindly provided by Steve Knapp. This population was derived from the cross of RHA 280 × RHA 801 and a linkage map of 577 simple sequence repeat (SSR) markers was constructed from this population (Tang *et al.*, 2002; Yu *et al.*, 2003). In addition, 21 of the 34 linkage group ends were defined with the *Arabidopsis*-type telomere sequence repeat-derived markers (Hu, 2006).

Table 1: Information of the fixed primers used in the current study

Name	Sequence	Homology by BLAST search
QHB18F12b	TCTTCAGTTTGGATAGGC	NBS/LRR
ORS323R	CGGGAAACTAGGATCAGAGG	SSR
QHG17L13R	TGTTCATGTTCCTTGCAT	sunflower homeobox gene
QHG17L13L	TGGCTGTTTGAACACTTT	sunflower homeobox gene
QHF6H21R	CTGCTGCTGTTGAAGTTG	sunflower homeobox gene
QGAH07L	AAGGATTCGGACAAACAT	Lettuce homeobox gene
QHF6H21L	CTGCTGCTGTTGAAGTTG	sunflower homeobox gene
QGB9J18L	TGGACTTCAACCAAGACA	Lettuce homeobox gene
ALSR1	CATATTGAGGCTTTGGCATTC	ALS
QHB37D06a	ATCAGTTCATTAGGGCAC	LRR
QHA20I01a	CCGAGTTGGTATGCTTGT	LRR
QHA14H20a	CGAATCTCCACTAAACCC	Kinase
QHA13J07a	CGATCTAGAATCCAAGCC	Kinase
QHA12P24b	CTCCAGTCTGACCCGTTG	Kinase
QHA21B09a	TGTCATTCAATTCGGTGC	LRR
QHB18I19b	CTGCCAAGTGAAAACGCT	NBS
QHA10B18a	GCAAACAAGTTCCTGGCT	Kinase
QHA10B18b	GTTTGCCTTTAAGAACCG	Kinase
QHG14I14F1	CGTGGAAGCATCTAGACA	delta-8 sphingolipid desaturase
	TCTTCAGTTTGGATAGGC	NBS/LRR
ORS333R	CGGTTAAGATGGTTCAGTTGG	SSR
ORS523R	TCGAGAAAGAAAGAGAGAGTGTGA	SSR
QGA7H07R	CACCATTGGCTTCCATAG	Lettuce homeobox gene

TRAP marker generation

The TRAP protocol of Hu and Vick (2003) was used with the following modifications: 1) molar ratio of fixed primer to arbitrary primer was changed from 30:1 to 10:1, and 2) the annealing temperature of the first five cycles was changed from 35ºC to 40ºC and for the last 30 cycles it was changed from 50ºC to 53ºC. The fixed primers were designed against sunflower expressed sequence tag (EST) sequences showing homology with the components of plant disease resistance genes, homeobox genes, and an acetolactate synthase gene. Three SSR primers were also used in this study (Table 1). The name of the fixed primer contains the sequence ID of the EST from which the primer was designed in the *Compositae* Genome initiative database http://cgpdb.ucdavis.edu/database, accessed on January 20, 2007). A total of 23 different primer combinations coded as TRAP01 to TRAP25 (Table 2) were used to generate TRAP markers.

Table 2: Primer combinations used for TRAP marker generation

Primer pair code	Fixed primer	Arbitrary primers	
TRAP01	QHB18F12b	Trap03-700	Trap13-800
TRAP02	ORS323R	Trap03-700	Sa17-800
TRAP03	QHG17L13R	Sa12-700	Ga5-800
TRAP04	QHG17L13L	Sa12-700	Ga5-800
TRAP05	QHF6H21R	Sa12-700	Ga5-800
TRAP06	QGAH07L	Sa4-700	Ga3-800
TRAP07	QHF5H21L	Sa12-700	Ga5-800
TRAP08	QGB9J18L	Sa4-700	Ga3-800
TRAP09	ALSR1	Sa12-700	Ga5-800
TRAP11	QHB37D06a	Trap03-700	Sa17-800
TRAP12	QHA20I01a	Trap03-700	Sa17-800
TRAP13	QHA14H20a	Trap03-700	Sa17-800
TRAP14	QHA13J07a	Trap03-700	Sa17-800
TRAP15	QHA12P24b	Trap03-700	Sa17-800
TRAP16	QHA21B09a	Trap03-700	Sa17-800
TRAP17	QHB18I19b	Trap03-700	Sa17-800
TRAP18	QHA10B18a	Trap03-700	Sa17-800
TRAP19	QHA10B18b	Sa12-700	Ga5-800
TRAP20	QHG14I14F1	Sa12-700	Ga5-800
TRAP21	QHB18F12b	Trap03-700	Sa17-800
TRAP23	ORS333R	Trap03-700	Sa17-800
TRAP24	ORS523R	Trap03-700	Sa17-800
TRAP25	QGA7H07R	Sa4-700	Ga3-800

Polymorphic markers were manually scored. Each marker was named by following the nomenclature proposed earlier (Hu *et al.*, 2004). The name of each scored marker consists of two parts: 1) the primer combination code, and 2) the fragment size in base pairs. Since almost all the scored TRAP fragments in this

study are under 1000 bp, we use the last three digits of the marker name to indicate the size. The numeral, 7 or 8, between primer combination code and the fragment size indicates the IR dyes of the labeled arbitrary primers which generate the image in the 700 nm or 800 nm channels of the Li-Cor Genotyper, respectively.

Map construction

MapMaker program (Lander *et al.*, 1987) was used to add these TRAP markers to the published map. The data set containing both SSR and TRAP markers was run with the genotype data of individual, established linkage groups of the population. First, the GROUP command was used to determine the linkage relationship of the markers to the existing linkage groups with a minimum LOD score of 3.5; then the TRY command was used to place each of the added TRAP markers to the most likely position in the linkage group.

RESULTS AND DISCUSSION

A total of 23 sets TRAP reactions were run using 22 fixed primers and seven arbitrary primers. All 46 primer combinations worked. Figure 1 shows the profile amplified by QHG14I14f1/Sa12-700 and QHG14I14f1/Ga5-800. The average number of scorable polymorphic markers per primer combination was 6.4 with a wide range of one to 14.

The added TRAP markers integrated well with the previously mapped SSR and TRAP markers and were spread over the genome. Of the 296 polymorphic markers amplified by 46 primer combinations from the RIL population, 220 were added successfully to the map. The resulting map (Figure 2) consists of 980 markers in 17 linkage groups (LG) and the total length is 1920 centiMorgan (cM). None of the 220 markers was placed outside of the telomeric positions defined by the conserved *Arabidopsis*-type telomere sequence repeat-derived markers (Hu, 2006).

Although the average distance between two markers is less than two cM, there are four gaps larger than 20 cM (one in LG2, one in LG4, and two in LG6). The gaps could be due to the lack of polymorphism between the two parental lines in these chromosome regions with respect to the type of markers used in this study.

More than ten *Pl* genes conferring resistance to different races of downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) have been reported in the sunflower genome. Among them, Pl_1, Pl_2, Pl_6 *and* Pl_7 were mapped on LG8 (Slabaugh *et al.*, 2003); Pl_5 and Pl_8 were mapped on LG13 (Slabaugh *et al.*, 2003; Radwan *et al.*, 2003). A TRAP marker associated with the downy mildew resistance in PI 468435 was developed (Hu *et al.*, 2004) with a fixed primer designed against a sunflower EST that has homology with *RPS2*, the first disease resistance gene cloned from *Arabidopsis*. Seven polymorphic markers amplified by the same primer combinations were added to the current map. Two markers, TRAP018749 and TRAP017445, were mapped to the approximate positions of *HaRGC1* on LG8 and

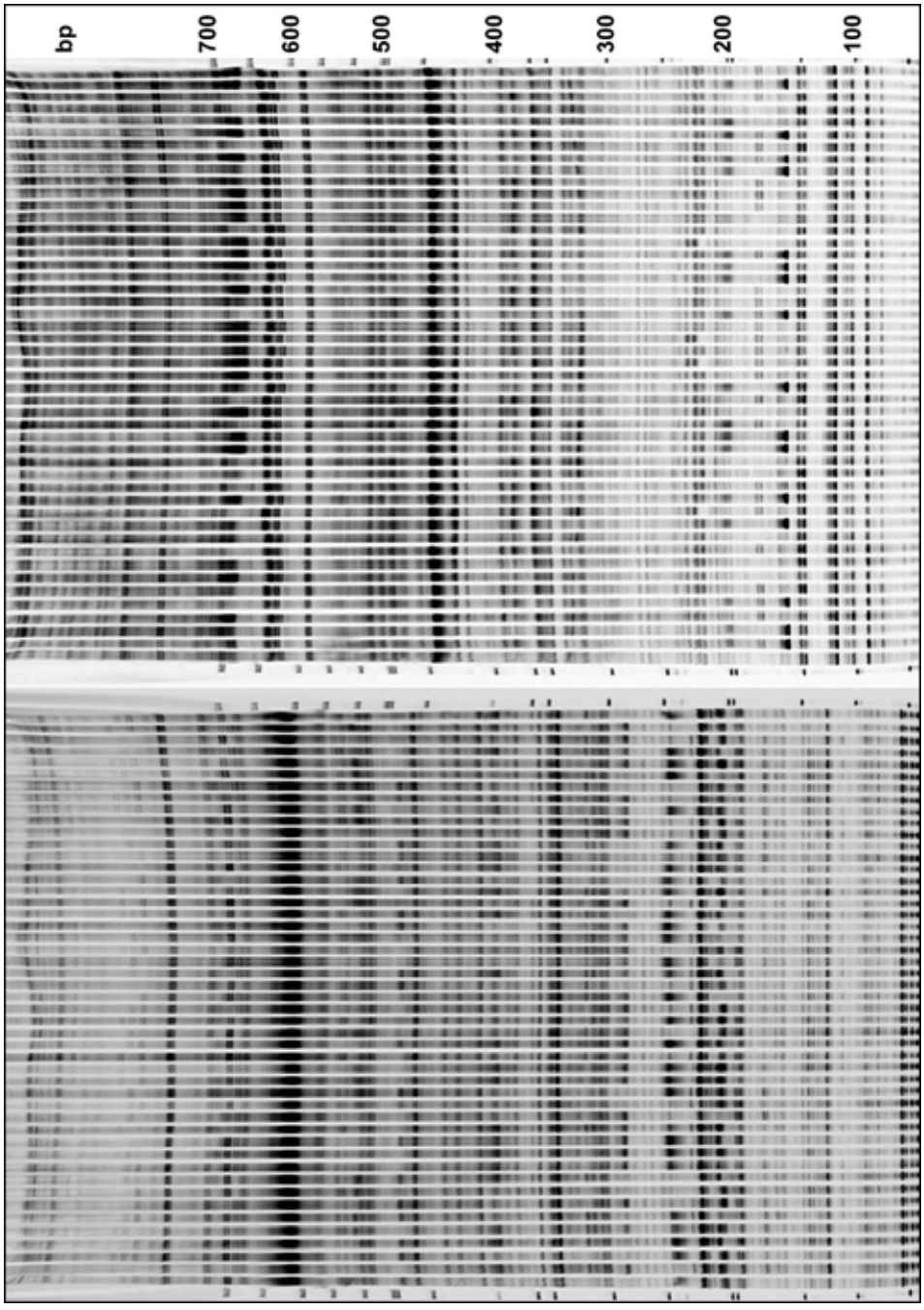

Figure 1: The TRAP profile amplified by QHG14I14f1/Sa12-700 (left) and QHG14I14f1/Ga5-800 (right)

Ha-1W41 on LG13, respectively. These results suggested that TRAP has potential to target genomic regions harboring the functional genes controlling the phenotype of interest.

The TRAP pattern of the same primer combination is basically the same between two sunflower mapping populations with different polymorphic markers. However, the same polymorphic marker could be identified across populations. This group of markers will enable us to align the linkage maps constructed from different RIL populations.

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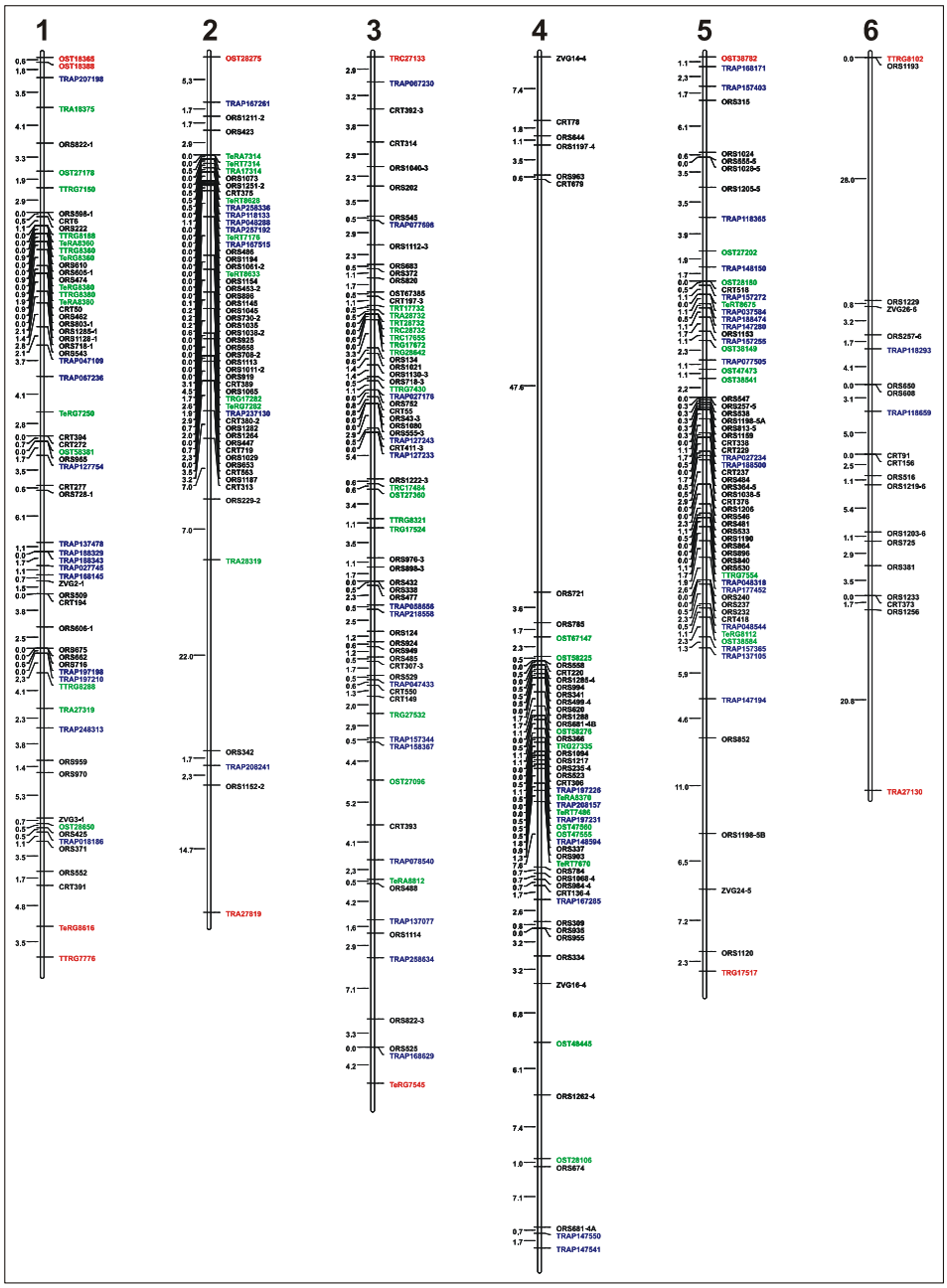

Figure 2: ***Part I*** *A linkage map consisting of previously mapped SSR markers (black), telomeric repeat derived markers (markers added to interstitial positions are in green and to the termini positions in red) and the 220 newly added markers (blue).*

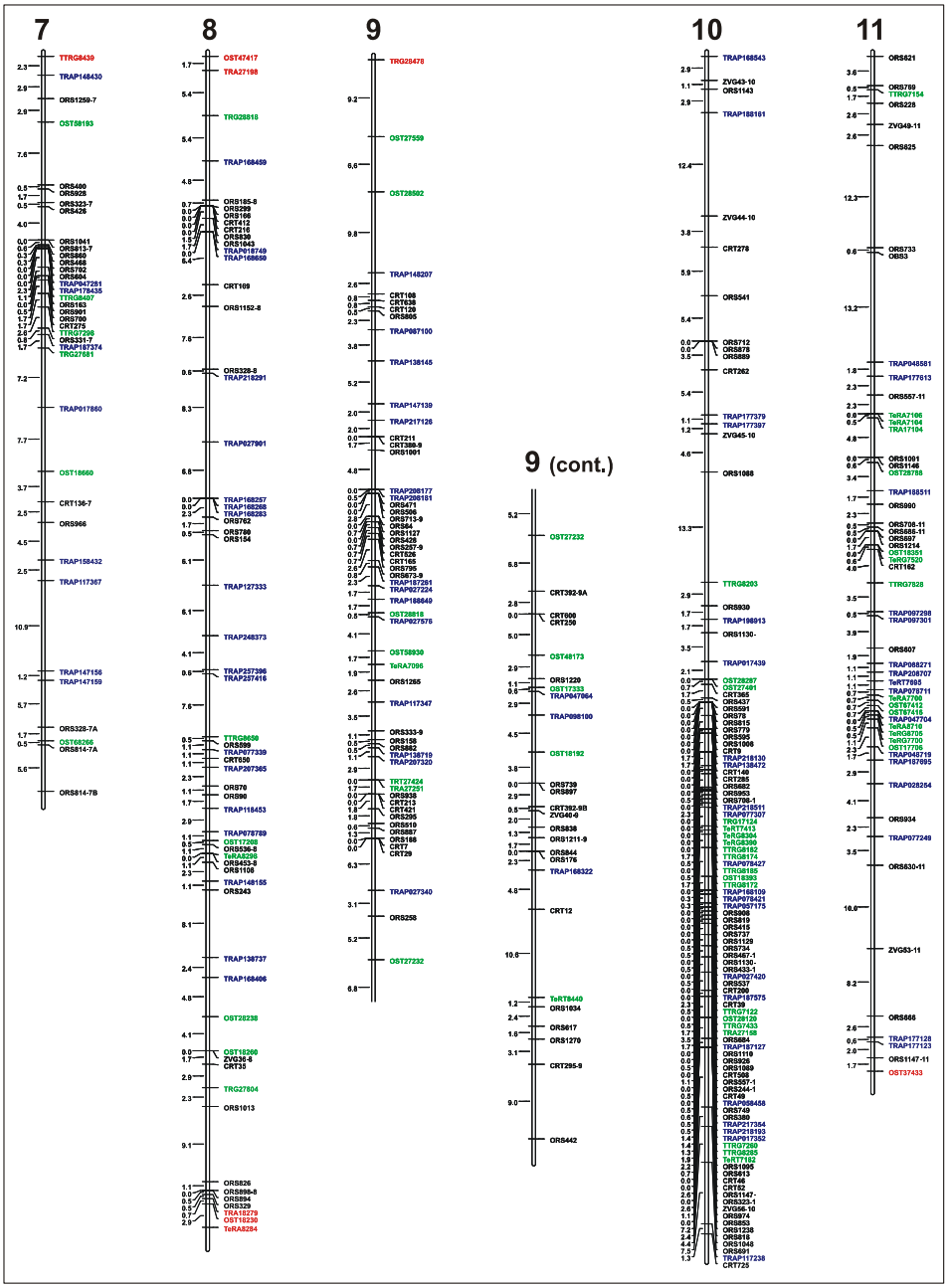

Figure 2: ***Part II*** *A linkage map consisting of previously mapped SSR markers (black), telomeric repeat derived markers (markers added to interstitial positions are in green and to the termini positions in red) and the 220 newly added markers (blue).*

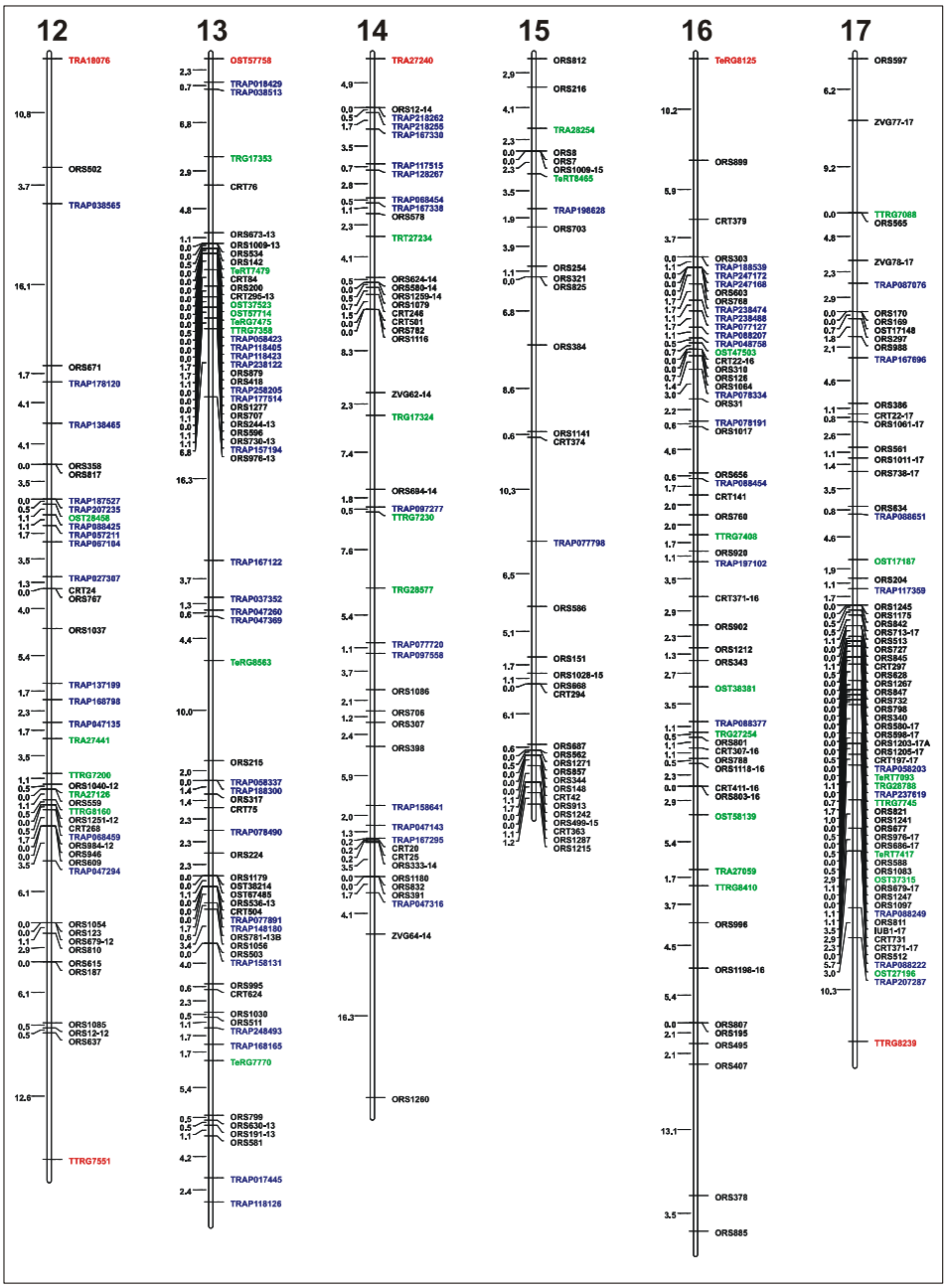

Figure 2: ***Part III*** *A linkage map consisting of previously mapped SSR markers (black), telomeric repeat derived markers (markers added to interstitial positions are in green and to the termini positions in red) and the 220 newly added markers (blue).*

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INTEGRACIÓN DE LOS MARCADORES TRAP EN EL MAPA DE LIGAMIENTO DE MARCADORES SSR DE GIRASOL, CONSTRUIDO CON AYUDA DE 92 LÍNEAS CONSANGUÍNEAS RECOMBINANTES

RESUMEN

La técnica de marcadores TRAP (Target region amplification polymorphism) fue utilizada para amplificar adicionalmente el mapa de ligamiento de girasol SSR (simple sequence repeat) antes publicada, construida sobre la base de la población consanguínea recombinante, obtenida del cruzamiento de RHA 280 × RHA 801. En la comunicación anterior, fue descrito el mapado 183 de los marcadores TRAP, generados por la utilización de los primers fijos, desarrollados según las secuencias repetibles de telómero conservativos, tipo *Arabidopsis*, en el mapa arriba citado, que contiene 577 marcadores SSR. Treintaidós marcadores han sido mapados en las posiciones exteriores de los grupos de ligamiento, así que con ellos están definidos 21 de 34 extremos de los grupos de ligamiento. En este trabajo fue presentada la integración de 220 marcadores TRAP adicionales en el mapa arriba citado. Estos nuevos introducidos marcadores TRAP fueron generados sobre la base de 23 combinaciones con los primers fijos, desarrollados según ciertos EST de girasol, mostrando homología con las componentes del gen de resistencia hacia las enfermedades y los genes homeobox. El mapa construido resultante está compuesto por 980 marcadores colocados en 17 grupos de ligamiento (LG) y su longitud total es 1920 centiMorgans (cM). Aunque la longitud promedia entre dos marcadores es menor de 2 cM, se han observado 4 distancias mayores de 20 cM (una en el grupo LG2, una en el grupo LG4 y dos en el grupo LG6). Estas distancias podrían provenir como resultado de polimorfismo insuficiente entre las líneas parentales en las regiones cromosómicas citadas, teniendo en cuenta el tipo de marcadores utilizados en este estudio.

INTÉGRATION DE MARQUEURS TRAP SUR UNE CARTE DE LIAISON DE MARQUEURS SSR CONSTRUITE À L'AIDE DE 92 SOUCHES PURES RECOMBINANTES

RÉSUMÉ

La technique de marqueurs TRAP (Target Region Amplification Polymorphism) a été utilisée pour développer la carte de liaison SSR (Simple Sequence Repeat) du tournesol construite à partir d'une population autogame recombinante obtenue du croisement RHA 280 × RHA 801. Dans un article précédent, on avait décrit la cartographie de 183 marqueurs TRAP générés par l'utilisation d'amorces fixes développées selon des séquences conserves répétables de télomères de type *Arabidopsis* dans la carte mentionnée ci-dessus qui contient de 557 marqueurs SSR. Trente-deux marqueurs ont été cartographiés aux positions extrêmes des groupes de liaison

de sorte que 21 des 34 extrémités des groupes de liaison leur sont déterminés. Cet article présente l'intégration de 220 marqueurs TRAP additionnels sur la même carte. Ces marqueurs TRAP nouvellement ajoutés ont été générés par 23 combinaisons avec amorces fixes développées à partir de tournesols EST montrant une homologie avec des composantes de gènes résistants aux maladies des plantes et aux gènes de boîte homéotique. La carte obtenue est faite de 980 marqueurs placés dans 17 groupes de liaison (LG) et leur longueur totale est de 1920 centiMorgan (cM) Bien que la longueur moyenne entre deux marqueurs soit de moins de 2 cM, on a observé 4 distances plus longues que 20 cM (l'une dans le groupe LG2, l'autre dans le groupe LG4 et deux dans le groupe LG6). Ces distances peuvent être dues au manque de polymorphisme entre deux lignées parentales dans ces régions chromosomiques étant donné le type de marqueurs utilisés dans cette étude.