## ONLINE RESOURCES

**Genomic restructuring in $F_1$ *Hordeum chilense* x durum wheat hybrids and corresponding hexaploid tritordeum lines revealed by DNA fingerprinting analyses**

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**Short running title:** Molecular rearrangements in $F_1$ hybrids and tritordeum

**Keywords.** Allopolyploidy; inter-primer binding site (iPBS) markers; inter-retrotransposon amplified polymorphism (IRAP); inter-simple sequence repeats (ISSRs); retrotransposon-microsatellite amplified polymorphism (REMAP); Triticeae.

**Abstract**

Allopolyploidization induces irreversible changes and some of which can be detected by molecular markers. Such alterations may occur at the hybrid stage and/or after polyploidization. In this study we aimed to determine the exact stage at which they occur during the synthesis of hexaploid tritordeum. We characterized two $F_1$ hybrids, genomic constitution $H^{ch}AB$ (2n=3x=21), derived from crosses between *Hordeum chilense* (lines H1 and H75; $H^{ch}H^{ch}$; 2n=2x=14) and durum wheat (line T846; AABB; 2n=4x=28), their corresponding tritordeum lines ($H^{ch}H^{ch}AABB$; 2n=6x=42) produced through chromosome doubling of the sterile $F_1$ hybrids, and the parental species using four molecular marker systems. The inter-retrotransposon amplified polymorphism (IRAP), retrotransposon-microsatellite amplified polymorphism (REMAP), inter-simple sequence repeat (ISSR), and inter-primer binding site (iPBS) markers were suitable for DNA fingerprinting, and also detected molecular rearrangements in both $F_1$ hybrids and/or respective tritordeums relative to their parents. The number of bands of wheat-origin inherited by the descendants of both crosses was almost the double of those of *H. chilense*-origin. The rearrangements consisted of two novel bands detected in the $F_1$ hybrid H75xT846 and/or its tritordeum, and lost parental bands (only amplified in one or both parents, not transmitted to the descendants). The number of lost parental bands of *H. chilense*-origin and of wheat-origin was almost equal. The novel and lost parental bands were mostly detected in $F_1$ hybrids revealing that the genomic rearrangements took place at the hybrid stage.



**Introduction**

The key role of polyploidy in the evolution of plants has been long recognized by biologists (see Matsuoka *et al*. 2014 for a review). Allopolyploidy consists in two major events starting with the hybridization of two or more divergent genomes into a single nucleus, followed by polyploidization (Stebbins 1971). The nucleus of a newly formed allopolyploid responds to

allopolyploidization with several changes at the chromosomal (Leitch and Bennett 1997) and DNA sequence levels (Song *et al*. 1995; Wendel *et al*. 1995). Additionally, alterations of gene expression and regulation (Scheid *et al*. 1996; Comai *et al*. 2000); transposon activity (Matzke and Matzke 1998); amplification, segregation and elimination of highly repetitive (Salina *et al*. 2000); and low-copy sequences (Feldman *et al*. 1997; Liu *et al*. 1998) were also reported.

Over the years, the occurrence of rearrangements was ascribed to various allopolyploid species such as, the wheat (*Aegilops-Triticum*) group (Ozkan *et al*. 2001, 2003; Shaked *et al*. 2001), triticale and wheat-rye addition lines (Bento *et al*. 2008, 2010), and newly formed allotetraploids *Cucumis* x *hytivus* Chen & Kirkbride; (Chen *et al*. 2007; Jiang *et al*. 2011) amongst others.

Tritordeum (x *Tritordeum* Ascherson et Graebner) is the synthetic allopolyploid produced by crossing wild barley (*Hordeum chilense* Roem. et Schult.) and cultivated durum wheat, followed by the chromosome doubling of the sterile $F_1$ interspecific hybrids with colchicine (Martín and Sánchez-Monge Laguna 1982). This synthetic allopolyploid has been considered a potential new crop since it shows good agronomic performance, chromosomal stability, fertility, among other interesting traits (Alvarez *et al*. 1992; Martín *et al*. 1999; Atienza *et al*. 2007; Mellado-Ortega and Hornero-Méndez 2012; Navas-Lopez *et al*. 2014). The tritordeum accessions have been characterized by cytogenetic tools and with a small set of DNA markers (Castilho *et al*. 2013). However, the recent development of diversity array technology (DArT) in *H. chilense* will offer new possibilities for screening a large number of tritordeum accessions (Castilho *et al*. 2013).

The screening of tritordeum accessions is needed due to the possible occurrence of rearrangements induced by allopolyploidization. The level and type of DNA sequences involved in genomic restructuring induced by allopolyploidization, remains to be determined.

However, the use of inter-retrotransposon amplified polymorphism (IRAP), retrotransposon-microsatellite amplified polymorphism (REMAP) and inter-simple sequence repeat (ISSR) markers have provided valuable information about genetic rearrangements in triticale (Bento *et al*. 2008), wheat-rye addition lines (Bento *et al*. 2010) and hexaploid tritordeum (Cabo *et al*. 2014a). In our previous work developed in newly formed hexaploid tritordeum (Cabo *et al*. 2014a, 2014b), it was not possible to determine if the rearrangements occurred early at the hybrid stage or after the genomes duplication since no interspecific $F_1$ hybrids were included.

The main limitation of most of the retrotransposon (RTN)-based markers is the previous need of sequence knowledge for specific primer design (Wegsheider *et al*. 2009). Since the reverse transcription of the RNA intermediate of LTR-RTNs starts at the 5′ end of the internal domain, referred to as the primer binding site (PBS) (Havecker *et al*. 2004), the use of a single primer based on conserved PBS sequences may allow the production of inter-primer binding site (iPBS) markers (Kalendar *et al.* 2010). The primer anneals to the PBS of two proximal long terminal repeat (LTR)-RTNs with head-to-head orientation, amplifying the two complete LTRs and the genomic DNA region between them (Kalendar *et al.* 2010). The iPBS markers are useful for the detection of polymorphism among individuals and isolation of RTNs (Kalendar *et al*. 2010; Poczai *et al*. 2013).

With the present work, we aim to perform the DNA fingerprinting of two $F_1$ *H. chilense* x durum wheat hybrids ($H^{ch}AB$; 2n=3x=21), their corresponding tritordeum lines ($H^{ch}H^{ch}AABB$; 2n=6x=42) and parental species using IRAP, REMAP, ISSR and iPBS markers, and to screen for potential molecular rearrangements during the synthesis of hexaploid tritordeum.

## Materials and methods

### *Plant material*

The plant material used in this work is presented in table 1.

The exact parental lines of the $F_1$ interspecific hybrids and corresponding tritordeum lines (synthetic allopolyploids) were highly inbred and resulted from several years of self-fertilization. Thus, only one plant per parental line was used.

Due to the reduced rates of successful hybridization and survival of the resultant $F_1$ hybrid plants (even under greenhouse conditions), only one $F_1$ hybrid plant was produced per interspecific cross and used in this study. The two tritordeum (HT) lines studied here constitute the first generation after polyploidization (S1 individuals), and produced viable seeds. A single HT plant per cross was also analyzed. The plant material was obtained by Antonio Martín at the IAS-CSIC (Córdoba, Spain).

Young leaves of six to eight week old plants of the parental species, $F_1$ hybrids and HT lines were collected, immediately frozen in liquid nitrogen, and maintained at -80 ºC till the extraction of genomic DNA.

***Fluorescence* in situ *hybridization (FISH) experiments***

Before starting the molecular analyses, the success of the interspecific crosses was confirmed by the identification of the parental genomes using the Fluorescence *in situ* hybridization (FISH) technique. Thus, the fixed root-tips from germinated seeds of hexaploid tritordeum (HT lines A and B) were used for the preparation of mitotic chromosome spreads according to Lima-Brito *et al.* (1997). Then the chromosome spreads were hybridized with genomic DNA of *H. chilense* labelled with biotin-16-dUTP (Roche Applied Science, Mannheim, Germany) and 45S rDNA sequence, pTa71 (Gerlach and Bedbrook 1979) labelled with digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany). Both probes were labelled by nick translation. The FISH experiments were developed according to Schwarzacher and Heslop-Harrison (2000).

***Isolation of genomic DNA and amplification of molecular markers***

The genomic DNA was extracted from the frozen leaves using the CTAB method of Doyle and Doyle (1987). The DNA samples were quantified in the spectrophotometer Nanodrop™ ND-1000 (Thermo Scientific) and their integrity was evaluated after electrophoresis on 0.8% agarose gels stained with ethidium bromide. The DNA samples were diluted to working solutions with a concentration of 40ng/μL for the further amplification of molecular markers.

Different primers (table 2) were tested in this study for the amplification of IRAP, REMAP, ISSR, and iPBS markers using a total of 25 combinations (table 3) previously successful in some Triticeae species, including those studied here.

The amplification and visualization of IRAP, REMAP and ISSR markers were performed according to the conditions described by Cabo *et al.* (2014a). The same conditions and procedures were followed for the production of iPBS markers with the single primer *F0100*.

To assess if the rearranged bands detected in the F1 hybrids and corresponding tritordeums are an effective result of polyploidization and to discard the hypothesis of being generated by competition between primer hybridization sites of one parent or the other, control PCR experiments containing the mixture of the DNAs of the parental lines were performed.

Each band was considered an IRAP, REMAP, ISSR or iPBS marker. Although there is the potential for the occurrence of homoplasy among different genera, for the four dominant marker systems we assumed that similar sized bands among the studied individuals produced with the same combination of primers, would correspond to the same *locus*.

Each PCR reaction was repeated at least twice and only reproducible bands were considered for the presence (1)/absence (0) analysis.

Since the amplification conditions were the same for the four marker systems, in each REMAP matrix, bands with similar molecular weight to ISSRs and/ or IRAPs produced with

the same SSR or LTR primers, respectively, were discarded, to ensure the analysis of effective REMAPs, as suggested by Kalendar *et al*. (1999).

**Results**

Figure 1 shows root-tip metaphase cells of tritordeum (HT line A and HT line B) with chromosome constitution $H^{ch}H^{ch}AABB$ (2n=6x=42). The 14 chromosomes originating from *H. chilense* were strongly hybridized with the *H. chilense* genomic DNA probe (green). The 28 wheat-origin chromosomes are also observed (blue). The pTa71 probe identified eight *major* 18S-25S rDNA sites (red) per metaphase cell of tritordeum, four being of wheat-origin (chromosome pairs 1B and 6B) and four of *H. chilense*-origin (chromosome pairs $5H^{ch}$ and $6H^{ch}$) (figure 1).

Among 25 combinations of primers tested, 14 were selected based on the successful amplification and/or production of clear bands (table 4).

The LTR primers *Sukkula* and *3'LTR-BARE1* produced IRAPs when used alone (table 4). Also, the single PBS primer *F0100* generated iPBS markers (table 4).

The highest total percentages of REMAP and IRAP polymorphism were observed among the parental lines and descendants of cross B (table 4). The highest percentage values of ISSR and iPBS polymorphism were detected in cross A (table 4).

The different cases of polymorphic bands, such as those shared among each parent, the $F_1$ hybrid and/or respective tritordeum (HT), as well as the rearranged bands (novel and lost parental bands) are indicated in table 5. Considering the two crosses and all marker systems, the fingerprinting analyses revealed that the bands of wheat-origin inherited by the descendants ($F_1$ hybrids and HTs) of each cross (61) were almost the double of those of *H. chilense*-origin (32). Regarding the IRAP markers of cross A, the number of bands inherited by both the $F_1$ hybrid H1 x T846 and its respective HT with origin in the female parent (19) was slightly higher than that of wheat-origin (17) (table 5). Most of the markers were

inherited by both the $F_1$ hybrid and its corresponding HT, but six *H. chilense*-origin markers and five wheat-origin bands were only transmitted to the $F_1$ hybrids being absent in tritordeum (table 5).

Considering the lost parental bands (not transmitted to the $F_1$ hybrid neither to the amphiploid tritordeum) the number of bands with *H. chilense*-origin (16) was similar to those of wheat-origin (13) (table 5). Such feature was verified with the four marker systems (table 5). Two novel bands (absent in the parental species) were detected (table 5; figure 2). One novel REMAP band (~900bp) was amplified in the $F_1$ hybrid B and respective tritordeum, and one novel IRAP (~400bp) was exclusively amplified in the $F_1$ hybrid B (table 5; figure 2; figure S2).

## Discussion

FISH technique confirmed the success of interspecific crosses and genome duplication since it was possible to differentiate the parental genomes and to localize physically the rDNA *loci* on mitotic chromosome spreads of both tritordeum lines (HT line A and HT line B). The same results were previously obtained in other lines of hexaploid tritordeum by Lima-Brito *et al*. (1998) and Cabo *et al*. (2014a, 2014b).

An intraspecific analysis of the IRAP molecular patterns achieved among three individual plants of each parental line, *H. chilense* (lines H1 and H75) and durum wheat line T846 was performed using the LTR primer *Sukkula* (figure S1). All plants of the same parental line presented identical IRAP patterns, confirming that they were highly inbred, and justifying the use of a single plant per parental line in the following molecular analyses. Furthermore, the IRAP, REMAP and ISSR profiles obtained for the mixture of the parental genomes, and their comparison with those produced individually in wheat, *H. chilense*, $F_1$ hybrid and corresponding tritordeum, evidenced that the rearranged bands (novel and/or lost

parental bands) resulted from hybridization and/or polyploidization (figure S2). Thus, any detectable polymorphism in the $F_1$ hybrids or tritordeums was related to genomic rearrangements induced by hybridization and/or polyploidization.

Several investigations have been focused on genomic changes induced by allopolyploidization, but the involved mechanisms can remain elusive when the exact parental species of the allopolyploids are unknown (Wendel 2000). Here and in our previous studies (Cabo *et al*. 2014a, 2014b), the exact parental species of the newly formed hexaploid tritordeums or $F_1$ hybrids are used for comparison.

The present study revealed: (i) high percentage values of REMAP and IRAP polymorphism among the $F_1$ hybrids, corresponding tritordeums and their parental species; (ii) a higher number of markers with wheat-origin inherited by the $F_1$ hybrids and tritordeums; (iii) the number of lost parental bands (not transmitted to the descendants of each cross) with *H. chilense*-origin was similar to that of wheat-origin; (iv) and the amplification of two novel bands (tables 4 and 5). The IRAP data obtained in cross A (table 5) constitute an exception to the case (ii) because the higher number of IRAPs inherited by both the $F_1$ hybrid A and HT line A, had origin in the female parent, *H. chilense*. Such molecular results could be explained by the use of LTR primers that were designed for the RTN family *BARE-1* of *Hordeum vulgare* L. (Kalendar *et al*. 1999) and by the preferential elimination of SSR sequences of *H. chilense*-origin for the homogenization of the divergent parental genomes. In other tritordeum lines the number of IRAPs of *H. chilense*-origin was almost the double of those of wheat-origin (Cabo *et al*. 2014a) and these molecular results were supported by cytogenetic analyses that showed the inheritance of SSR-rich regions with wheat-origin by tritordeum (Cabo *et al*. 2014c).

The loss of parental bands as well as the appearance of novel bands in the $F_1$ hybrids and/or corresponding tritordeum lines can be assumed as evidence of genomic restructuring (table 5).

As far as we know, this study constitutes the first use of iPBS markers for detection of genomic restructuring in $F_1$ interspecific hybrids and/or allopolyploids. Such markers enabled the detection of one lost parental band (table 5), and could be useful not only for the study of interspecific hybrids and allopolyploids of Triticeae tribe but also in other plant species regarding the use of a conserved PBS sequence as primer. According to Kalendar *et al*. (2010), the iPBS markers are applicable to any organism with RTNs containing PBS sites complementary to tRNA.

In this study, the loss of parental bands in both the $F_1$ hybrids and tritordeum lines was more frequent than the appearance of novel bands (table 5). Only one novel IRAP band in the $F_1$ hybrid B ($F_1$ H75xT846 hybrid), and one novel REMAP band in both $F_1$ hybrid B and tritordeum line B were detected (table 5). The novel REMAP band was detected with primers *Sukkula* + *8081* which demonstrated that some rearrangements occurred during or after hybridization in the $F_1$ hybrid B, being maintained in the HT line B. On the other hand, the novel IRAP band was not amplified in tritordeum, being exclusively amplified in the $F_1$ hybrid B (table 5; figure 2; figure S2).

The number of rDNA *loci* observed in hexaploid tritordeum (eight), detected by the probe pTa71, constitutes an additive pattern since it results from the inheritance of four rDNA *loci* of wheat-origin and four rDNA *loci* of *H. chilense*-origin (see figure 1). The detection of lost parental bands and novel bands in the $F_1$ hybrids and/or corresponding amphiploids (table 5) constitute exceptions to the expected additive pattern of the parental REMAP, IRAP, ISSR (figure S2), and iPBS markers.

The occurrence of genomic restructuring was previously detected in other newly formed tritordeum lines not only using REMAP, IRAP and ISSR (Cabo *et al*. 2014a), but also the Start Codon Targeted (SCoT) markers (Cabo *et al*. 2014b). Novel and lost parental bands were also revealed by these markers in other allopolyploids of the Triticeae tribe (Bento *et al*. 2008, 2010). The rearranged bands detected so far in both triticale and tritordeum have been considered as being induced by polyploidization (Bento *et al*. 2008, 2010; Cabo *et al*. 2014a, 2014b). Here, due to the inclusion of the $F_1$ hybrids, we were able to verify the early occurrence of genomic restructuring at the hybrid stage, suggesting that the observed molecular rearrangements were induced at the hybridization step. Additionally, such rearrangements seem to involve RTN and/or SSR regions, once they were detected with IRAP, REMAP, ISSR and iPBS markers. The early occurrence of genomic rearrangements (in the $F_1$ generation) was previously reported in plant species of the genera *Brassica* (Song *et al*. 1995), *Cucumis* (Chen *et al*. 2007; Jiang *et al*. 2011), *Nicotiana* (Petit *et al*. 2010) and *Arabidopsis* (Bento *et al*. 2013).

The different results in comparison to our previous studies can be explained by the use of distinct lines of tritordeum, durum wheat and *H. chilense*, and by the high genetic variability among lines of *H. chilense* (Prieto *et al*. 2004; Marín *et al*. 2008). These assumptions could also explain why some combinations of primers that were previously successful, did not amplify IRAP and REMAP markers in the present work. Nonetheless, two interesting features have been reiterated through our studies of newly formed tritordeum namely, the non-random elimination of sequences of *H. chilense*-origin, and the preferential inheritance of markers with wheat origin, independently of the tritordeum line studied. A parallelism could be made with studies developed in triticale and wheat-rye addition lines (Bento *et al*. 2008, 2010), where the non-random elimination of bands of rye origin genome was reported. These authors studied genomic rearrangements in the seven wheat-rye addition

lines and triticale, and explained that the degree of genomic reshuffling increases with the reduction of alien (rye) chromatin introgressed into the wheat background (Bento *et al*. 2008, 2010). The rearranged RTN and SSR sequences seem to be preferentially allocated in major heterochromatic domains, and affect mainly the genome with more reduced nuclear DNA content, as does rye genome in triticale (Bento *et al*. 2008). Similarly, the genome with reduced nuclear DNA content in hexaploid tritordeum is *H. chilense*, justifying the preferential elimination of its markers in both the $F_1$ hybrids and corresponding tritordeum lines.

Overall, our molecular data demonstrated that the rapid and non-random elimination of repetitive DNA sequences probably occurs early at the $F_1$ hybrid stage. The non-random elimination of sequences might constitute a way to homogenize the divergent genomes of new allopolyploids, improving their diploid-like behavior, ensuring their fertility and success as new species in nature (Feldman *et al*. 1997; Comai 2000). Other authors proposed that in the first generation of a new allopolyploid, the elimination events are more frequent than the genetic gain (Song *et al*. 1995; Ozkan *et al*. 2001; Shaked *et al*. 2001; Kashkush *et al*. 2002; Chen *et al*. 2007; Bento *et al*. 2008, 2010). Although the present results agreed with this assumption, two novel bands were detected once; but, this should not be considered as a rule. Different studies have demonstrated that genomic restructuring mostly involves sequences associated with RTNs and SSR-rich regions. These genomic regions do not obey a predictive mechanism of elimination or genetic gain throughout allopolyploidization. In fact, novel SSR regions may arise in response to allopolyploidization (Cabo et al. 2014c). We could not discard the hypothesis that the amount of genetic gain could be undetectable with the approaches used, and it is probably easier to detect sequences elimination. In addition, the procedures of homogenization are highly dependent on the level of divergence between or among the genomes merging in the nascent allopolyploid, and on the genotype of the parental

species. Further studies, in this and other synthetic allopolyploids, should be performed through successive generations after polyploidization, based on these or other approaches before establishing a trend.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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**Table 1.** Plant material.

| **Plant material** | **Designation** | **Genomic constitution** |
|---|---|---|
| *H. chilense* line H1 | Female parent of cross A | 2n=2x=14 ($H^{ch}H^{ch}$) |
| *H. chilense* line H75 | Female parent of cross B | 2n=2x=14 ($H^{ch}H^{ch}$) |
| *T. turgidum* line T846 | Male parent of crosses A and B | 2n=4x=28 (AABB) |
| $F_1$ hybrid H1xT846 | $F_1$ hybrid A | 2n=3x=21 ($H^{ch}$AB) |
| $F_1$ hybrid H75xT846 | $F_1$ hybrid B | 2n=3x=21 ($H^{ch}$AB) |
| Tritordeum H1xT846 | HT line A (synthetic allopolyploid) | 2n=6x=42 ($H^{ch}H^{ch}$AABB) |
| Tritordeum H75xT846 | HT line B (synthetic allopolyploid) | 2n=6x=42 ($H^{ch}H^{ch}$AABB |

**Table 2.** Primers for IRAP, REMAP, ISSR and iPBS markers amplification.

| Primer | RTN source and orientation | Origin | GenBank accession | Sequence 5'→3' | Reference |
|---|---|---|---|---|---|
| **LTR** | | | | | |
| 3' LTR | *BARE-1*→ | *H. vulgare* | Z17327 | TGTTTCCCATGCGACGTTCCCCAACA | Teo *et al*. (2005) |
| 5' LTR2 | *BARE-1*← | *H. vulgare* | Z17327 | ATCATTGCCTCTAGGGCATAATTC | Teo *et al*. (2005) |
| LTR 6149 | *BARE-1*→ | *H. vulgare* | Z17327 | CTCGCTCGCCCACTACATCAACCGCGTTTATT | Kalendar *et al*. (1999) |
| LTR 6150 | *BARE-1*← | *H. vulgare* | Z17327 | CTGGTTCGGCCCATGTCTATGTATCCACACATGGTA | Kalendar *et al*. (1999) |
| LTR 7286 | *BARE-1*← | *H. vulgare* | Z17327 | GGAATTCATAGCATGGATAATAAACGATTATC | Kalendar *et al*. (1999) |
| Nikita | *Nikita*→ | *T. turgidum* | AY078073<br>AY078074<br>AY078075 | CGCATTTGTTCAAGCCTAAACC | Teo *et al*. (2005) |
| Sukkula | *Sukkula*→ | *H. vulgare* | AY034376 | GATAGGGTCGCATCTTGGGCGTGAC | Teo *et al*. (2005) |
| Stowaway | *Stowaway*→ | *H. vulgare* | | CTTATATTTAGGAACGGAGGGAGT | Bento *et al*. (2008) |
| **SSR** | | | | | |
| 8081 | | | | (GA)9C | Kalendar *et al*. (1999) |
| 8082 | | | | (CT)9G | Kalendar *et al*. (1999) |
| 8564 | | | | (CAC)7T | Kalendar *et al*. (1999) |
| **PBS** | | | | | |
| *F0100* | *F0100*→ | universal RTN-based sequence | | TAGGTCGGAACAGGCTCTGATACCA | Kalendar *et al*. (2008)<br>Wegscheider *et al*. (2009) |

**Table 3.** Combinations of primers tested for the amplification of IRAP, REMAP, ISSR and iPBS markers.

| Marker | Combination of primers |
|---|---|
| IRAP | *Sukkula* |
| | *Nikita* + *Sukkula* |
| | *5'LTR2-BARE-1* + *Sukkula* |
| | *LTR 6149* + *Sukkula* |
| | *LTR 6150* + *Sukkula* |
| | *3'LTR-BARE-1* |
| | *Stowaway* |
| REMAP | *Sukkula* + *3'LTR-BARE-1* |
| | *Sukkula* + *Stowaway* |
| | *Sukkula* + *8081* |
| | *Sukkula* + *8082* |
| | *Sukkula* + *8564* |
| | *Nikita* + *8081* |
| | *Nikita* + *8082* |
| | *LTR 7286* + *8081* |
| | *LTR 7286* + *8082* |
| | *LTR 7286* + *8564* |
| | *3'LTR-BARE-1* + *8081* |
| | *3'LTR-BARE-1* + *8654* |
| | *F0100* + *8081* |
| | *Stowaway* + *8081* |
| ISSR | *SSR 8081* [$(GA)_9C$] |
| | *SSR 8082* [$(CT)_9G$] |
| | *SSR 8564* [$(CAC)_7T$] |
| iPBS | *F0100* |

**Table 4.** REMAP, IRAP, ISSR and iPBS data achieved per combination of primers in each cross. T - Total number of amplified bands; M - number of monomorphic bands; P - number of polymorphic bands, and %P - percentage of polymorphism (calculated by P/T x 100).

| Marker | Cross | Primers | T | M | P | %P |
|---|---|---|---|---|---|---|
| REMAP | A | | | | | |
| | (H1xT846) | *Sukkula+8081* | 6 | 1 | 5 | 83.33 |
| | | *Sukkula+8082* | 4 | 0 | 4 | 100 |
| | | *Sukkula+8564* | 4 | 2 | 2 | 50.0 |
| | | *3'LTR+8081* | 5 | 1 | 4 | 80.00 |
| | | *3'LTR+8564* | 3 | 2 | 1 | 33.33 |
| | | *Nikita+8081* | 6 | 3 | 3 | 50.00 |
| | | TOTAL | 28 | 9 | 19 | 67.86 |
| | B | | | | | |
| | (H75xT846) | *Sukkula+8081* | 3 | 0 | 3 | 100 |
| | | *Sukkula+8082* | 7 | 0 | 7 | 100 |
| | | *Sukkula+8564* | 10 | 0 | 10 | 100 |
| | | *3'LTR+8081* | 6 | 2 | 4 | 66.67 |
| | | *3'LTR+8564* | 6 | 0 | 6 | 100 |
| | | *Nikita+8081* | 10 | 6 | 4 | 40.00 |
| | | TOTAL | 42 | 8 | 34 | 80.95 |
| IRAP | A | | | | | |
| | (H1xT846) | *Sukkula* | 21 | 9 | 12 | 57.14 |
| | | *3'LTR-BARE1* | 10 | 9 | 1 | 10.00 |
| | | *Sukkula+Nikita* | 6 | 1 | 5 | 83.33 |
| | | *Sukkula+3'LTR* | 8 | 5 | 3 | 37.50 |
| | | TOTAL | 45 | 24 | 21 | 46.67 |
| | B | | | | | |
| | (H75xT846) | *Sukkula* | 13 | 1 | 12 | 92.30 |
| | | *3'LTR-BARE1* | 7 | 5 | 2 | 28.57 |
| | | *Sukkula+Nikita* | 10 | 1 | 9 | 90.00 |
| | | *Sukkula+3'LTR* | 6 | 2 | 4 | 66.67 |
| | | TOTAL | 36 | 9 | 27 | 75.00 |
| ISSR | A | | | | | |
| | (H1xT846) | *8081* | 8 | 2 | 6 | 75.00 |
| | | *8082* | 10 | 3 | 7 | 70.00 |
| | | *8564* | 6 | 4 | 2 | 33.33 |
| | | TOTAL | 26 | 9 | 17 | 65.38 |
| | B | | | | | |
| | (H75xT846) | *8081* | 7 | 1 | 6 | 85.71 |
| | | *8082* | 6 | 4 | 2 | 33.33 |
| | | *8564* | 9 | 6 | 3 | 33.33 |
| | | TOTAL | 22 | 11 | 11 | 50.00 |
| iPBS | A | | | | | |
| | (H1xT846) | *F0100* | 6 | 3 | 3 | 50.00 |
| | B | | | | | |
| | (H75xT846) | *F0100* | 12 | 9 | 3 | 25.00 |

**Table 5.** Polymorphic REMAP, IRAP, ISSR and iPBS markers among each parent ($H^{ch}$ – line H1 or H75; wheat - line T846), the $F_1$ hybrid ($F_1$) and/or respective tritordeum (HT), and rearranged bands (novel and lost parental bands) detected per cross and combination of primers.

| Marker | Cross | Primers | Number of polymorphic bands | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Common to $H^{ch}$ and: | | | Common to wheat and: | | | Rearranged bands: | | | | |
| | | | | | | | | | Novel bands | | Lost parental bands | | |
| | | | $F_1$ | HT | $F_1$ and HT | $F_1$ | HT | $F_1$ and HT | $F_1$ | $F_1$ and HT | $H^{ch}$ | wheat | $H^{ch}$ and wheat |
| REMAP | A | | | | | | | | | | | | |
| | (H1xT846) | *Sukkula+8081* | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | | *Sukkula+8082* | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 |
| | | *Sukkula+8564* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| | | *3'LTR+8081* | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| | | *3'LTR+8564* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | | *Nikita+8081* | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| | | TOTAL | 1 | 0 | 5 | 0 | 0 | 8 | 0 | 0 | 1 | 4 | 0 |
| | B | | | | | | | | | | | | |
| | (H75xT846) | *Sukkula+8081* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (900bp) | 1 | 0 | 0 |
| | | *Sukkula+8082* | 1 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 1 | 0 |
| | | *Sukkula+8564* | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 3 | 2 | 1 |
| | | *3'LTR+8081* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| | | *3'LTR+8564* | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 2 | 0 |
| | | *Nikita+8081* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 |
| | | TOTAL | 2 | 0 | 3 | 1 | 0 | 10 | 0 | 1 | 8 | 7 | 2 |

**Table 5.** *continued.*

| Marker | Cross | Primers | Number of polymorphic bands | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Common to $H^{ch}$ and: | | | Common to wheat and: | | | Rearranged bands: | | | | |
| | | | | | | | | | Novel bands | | Lost parental bands | | |
| | | | $F_1$ | HT | $F_1$ and HT | $F_1$ | HT | $F_1$ and HT | $F_1$ | $F_1$ and HT | $H^{ch}$ wheat | $H^{ch}$ and wheat | |
| IRAP | A | | | | | | | | | | | | |
| | (H1xT846) | *Sukkula* | 0 | 0 | 9 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | | *3'LTR-BARE1* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | *Sukkula+Nikita* | 0 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 |
| | | *Sukkula+3'LTR* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 |
| | | TOTAL | 0 | 0 | 11 | 0 | 0 | 7 | 0 | 0 | 3 | 0 | 0 |
| | B | | | | | | | | | | | | |
| | (H75xT846) | *Sukkula* | 2 | 0 | 1 | 4 | 0 | 3 | 1 (400bp) | 0 | 0 | 1 | 0 |
| | | *3'LTR-BARE1* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| | | *Sukkula+Nikita* | 1 | 0 | 5 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | | *Sukkula+3'LTR* | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| | | TOTAL | 3 | 0 | 8 | 4 | 0 | 10 | 1 | 0 | 0 | 1 | 0 |
| ISSR | A | | | | | | | | | | | | |
| | (H1xT846) | *8081* | 0 | 0 | 2 | 0 | 0 | 4 | 0 | 0 | 2 | 0 | 0 |
| | | *8082* | 0 | 0 | 1 | 0 | 1 | 5 | 0 | 0 | 1 | 0 | 0 |
| | | *8564* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| | | TOTAL | 0 | 0 | 3 | 0 | 1 | 11 | 0 | 0 | 3 | 0 | 0 |
| | B | | | | | | | | | | | | |
| | (H75xT846) | *8081* | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 |
| | | *8082* | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| | | *8564* | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | | TOTAL | 0 | 0 | 2 | 0 | 0 | 10 | 0 | 0 | 1 | 0 | 0 |
| iPBS | A | | | | | | | | | | | | |
| | (H1xT846) | *F0100* | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | B | | | | | | | | | | | | |
| | (H75xT846) | *F0100* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |

**Figure 1.** Root-tip metaphases of tritordeum ($H^{ch}H^{ch}AABB$; 2n=42), HT line A (a) and HT line B (b), after FISH experiments probed with genomic DNA of *H. chilense* and pTa71. The 28 wheat-origin chromosomes were counterstained with DAPI (blue). Genomic DNA of *H. chilense* probe labeled uniformly the 14 *H. chilense*-origin chromosomes (green). All nucleolar chromosomes of wheat-origin (pairs 1B and 6B) and *H. chilense*-origin (pairs $5H^{ch}$ and $6H^{ch}$) were identified by the rDNA probe pTa71 (red).

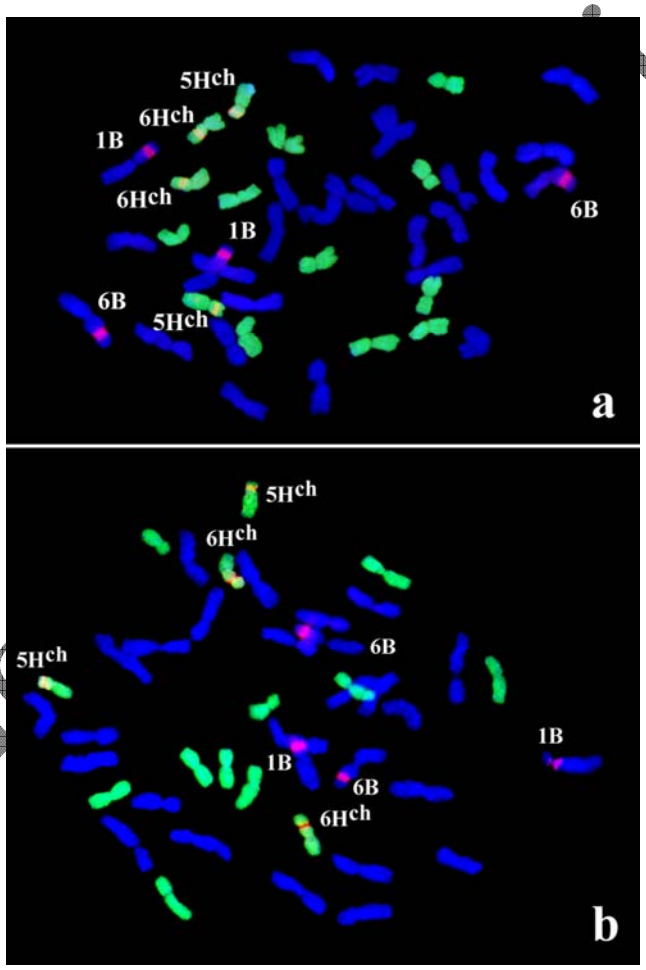

**Figure 2.** Detailed sections of (a) IRAP and (b) REMAP profiles produced by the primer *Sukkula* and primers *Sukkula* + 8081 respectively, in the $F_1$ hybrid B, respective tritordeum HT line B (HTB) and the parents, *H. chilense* (H75) and durum wheat line T846 (W). One novel IRAP with ~400bp (*) was uniquely detected in the $F_1$ hybrid B. One novel REMAP with ~900 bp (*) was amplified in both the $F_1$ hybrid B and corresponding tritordeum (HTB). The arrow indicates a specific band of line H75 that was not transmitted to the descendants of the cross B (lost parental band). M – GeneRuler DNA Ladder Mix (Thermo Scientific).

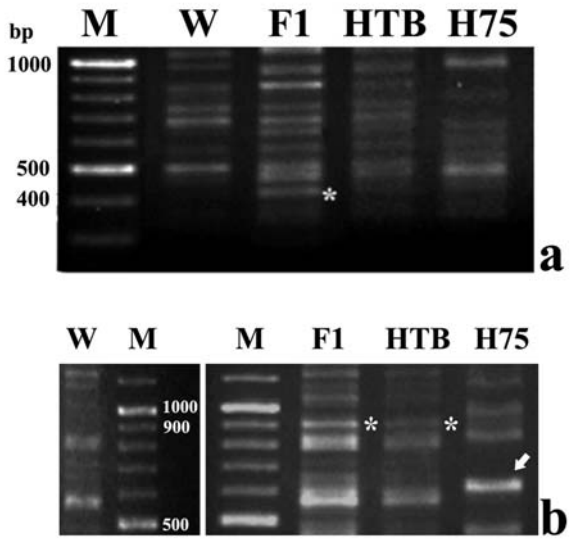

**Figure S1** IRAP molecular patterns produced with the LTR primer *Sukkula* in three individual plants of each parental line: *H. chilense* line H1, durum wheat line T846 and *H. chilense* line H75.

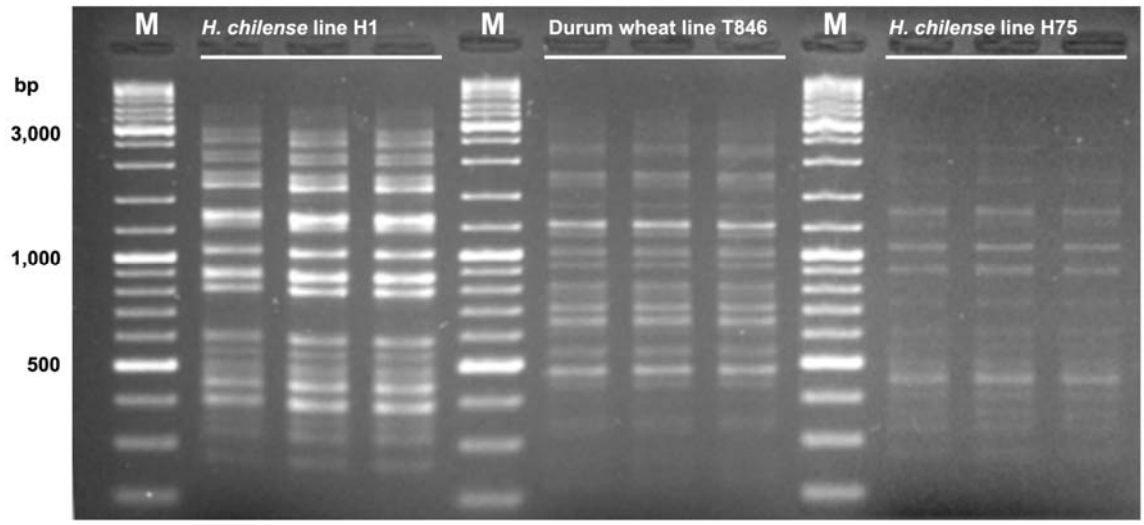

**Figure S2** (a) IRAP, REMAP and ISSR patterns produced with the primers *Sukkula*, *Sukkula* + 8081, and SSR 8081, respectively, in the parental lines, $F_1$ hybrid and tritordeum of cross A. The control (Mix) is composed by the mixture of DNAs of both parents *H. chilense* (line H1) and wheat line T846. (b) IRAP patterns produced with primer *Sukkula* in the parental lines, $F_1$ hybrid and tritordeum of cross B. The control (Mix) is composed by the mixture of DNAs of both parents *H. chilense* (line H75) and wheat line T846. Rearranged bands, such as one lost parental band (*) and one novel IRAP band with 400 bp (arrow) are indicated.