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ORIGINAL ARTICLE

Fingerprinting and genetic purity assessment of F_1 barley hybrids and their salt-tolerant parental lines using nSSR molecular markers

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Abstract

Hybridity and the genuineness of hybrids are prominent characteristics for quality control of seeds and thereby for varietal improvement. In the current study, the cross between two local barley genotypes (Ardhaoui: female; Testour: male) previously identified as susceptible/tolerant to salt stress in Tunisia was achieved. The hybrid genetic purity of the generated F_1 putative hybrids and the fingerprinting of the parents along with their offspring were assessed using a set of 17 nuclear SSR markers. Among the analyzed loci, 11 nSSR were shown polymorphic among the parents and their offspring. Based on the applied 11 polymorphic SSR loci, a total of 28 alleles were detected with an average of 2.54 alleles per locus. The locus *HVM33* presented the highest number of alleles. The highest polymorphism information content value was detected for the locus *HVM33* (0.6713) whereas the lowest PIC value (0.368) was revealed by the loci *BMAC0156*, *EBMAC0970* and *BMAG0013* with a mean value of 0.4619. The probabilities of identical genotypes PI for the 11 microsatellite markers were 8.63×10^{-7}. Banding patterns among parents and hybrids showed polymorphic fragments. The 11 SSR loci had produced unique fingerprints for each analyzed genotype and segregate between the two parental lines and their four hybrids. Parentage analysis confirms the hybrid purity of the four analyzed genotypes. Six Tunisian barley accessions were used as an outgroup in the multivariate analysis to confirm the efficiency of the employed 11 nSSR markers in genetic differentiation among various barley germplasms. Thus, neighbor joining and factorial analysis revealed clearly the discrimination among the parental lines, the four hybrids and the outgroup accessions. Out of the detected polymorphic 11 nuclear SSR markers, a set of five markers (*HVM33*, *WMC1E8*, *BMAC0154*, *BMAC0040* and *BMAG0007*) were shown to be sufficient and informative enough to discriminate among the six genotypes representing the two parental lines and the four hybrids from each others. These five nSSR markers presented the highest number of alleles per locus (A_n), expected heterozygosity (H_e), PIC values and the lowest probabilities of identity (PI). These nSSR loci may be used as referral SSR markers for unambiguous discrimination and genetic purity assessment in barley breeding programs.

Keywords Barley · F_1 hybrids · Parental lines · Genetic purity · NSSR

Introduction

Barley (*Hordeum vulgare*) is a staple crop domesticated since the Neolithic area over 10,000 years ago (Harlan and Zohary 1966; Ullrich 2011). This species is considered as a model plant due to its diploid nature, low chromosome number ($2n = 14$) and facility in cross-breeding (Saisho and Takeda 2011). In Tunisia, barley traditional cultivars have been grown in harsh conditions by marginal farming householders where it faced human selection, evolutionary process and environmental changes (Zoghlami et al. 2011; Ben Romdhane et al. 2017). According to results of Ben Romdhane et al. (2017), Tunisian barley accessions exhibited a high phenotypic and gene diversity and present an untapped reservoir of interesting genes which makes them valuable genetic resources for barley future breeding programs.

Recently, the creation of salt-tolerant barley varieties presented a priority in Tunisian research programs. For this purpose, 21 barley landraces representatives of the genetic diversity of the Tunisian barley germplasm (Zoghlami et al.

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2011) were tested at different salt concentrations (0, 200 and 250 mM) under greenhouse conditions to assess their salinity tolerance (Ben Chikha et al. 2016). The last investigation allowed the identifications of accessions barley with contrasting behavior towards salt stress at germination, tillering and maturation stages (Ben Chikha 2017).

In continuity with the previous report, the current study was launched with the aim of researching quantitative trait loci implied in salt tolerance for Tunisian barley germplasm. Thus, a controlled cross between two local barley parents previously identified as susceptible/tolerant to salt stress (Ben Chikha et al. 2016) was achieved to obtain the F_1 hybrids. Indeed, for efficient gene identification, the crosses are conducted between genetically distinct parents showing a contrasting behavior towards the trait of interest (Collard et al. 2005; Sannemann et al. 2015).

Nonetheless, for further continuation of the breeding research program, genetic purity test is an essential prerequisite of any hybrid seeds (Chetan Kumar et al. 2012). This allowed the confirmation of the cross success and the discrimination between parental lines and hybrids. Indeed, the generated hybrids are prone to contamination by outcrossing with foreign pollens or physical admixtures. Furthermore, the self pollination characterizing barley species may unwittingly occur before the controlled out-crossing which hinder the generation of pure hybrids. Thus, the F_1 hybrid purity has to be verified by accurate tools for future mapping populations (Tamilkumar et al. 2009).

Generally, the hybrid genetic purity of the F_1 seeds is conducted based on the conventional Grow Out Test (GOT) method. GOT is based on morphological characters and was described as time consuming, very tributary of environmental effects, highly vulnerable to errors and requires skilled personnel. As a result, selected genotypes based on morphological traits may not represent true hybrids (Kumar et al. 2014; Bora et al. 2016). Based on these facts, others alternative methods were used such as isozyme, protein and phenol biochemical markers (Kumar et al. 2007). While these last markers are least influenced by the environment as morphological traits, they confront others disadvantages especially low polymorphism rate. Thus, they fail to discriminate among large number of varieties and between closely related genotypes such as between hybrids and parental lines (Kumar et al. 2007).

The emergence of the molecular tools during the last decade has brought an accurate and rapid solution to overcome the drawback of morphological and biochemical markers. DNA fingerprinting of parental lines and their putative hybrids especially when co-dominant markers are used has proved to be an efficient and powerful technique in breeding and management programs. In view of this, DNA molecular markers are still the efficient strategy to accomplish the previous goals. Indeed, DNA molecular markers provide more efficient fingerprinting and genetic purity assessment hybrids and parental line seed purity. Among a large range of molecular markers, microsatellites are the preferred system thanks to their high abundance in genome, uniform distribution throughout the genome, co-dominance inheritance, polymorphism and simple use (Chetan Kumar et al. 2012; Bohra et al. 2017).

These SSR molecular markers have proved their utility in F_1 hybrid purity assessment for others cereal crops such as pearl millet (Kumar et al. 2015) and rice (Bora et al. 2016). Furthermore, SSR fingerprinting method was applied with success for other economic plant species like eucalyptus (Subashini et al. 2014), pigeonpea (Bohra et al. 2017) and melon (Ju-Fen et al. 2008). However, for barley species, rare is documented about the use of molecular markers for hybrid purity testing. The investigations reported in literature were limited to the use of dominant RAPD markers for hybrid purity confirmation (Mylonas et al. 2014).

This present study reports for the first time the use of informative nuclear microsatellites to test the F_1 hybrid purity and to assess the molecular fingerprinting of their parental lines identified earlier as susceptible/tolerant to salt stress. Moreover, among our objectives is to select a minimal set of nuclear microsatellite (nSSR) molecular markers which are able to discriminate among the parents and their hybrids.

Materials and methods

Plant materials and crossing steps

In this study, two Tunisian barley accessions (Ardhaoui: female, Testour: male) previously identified as susceptible/tolerant to soil salinity (Ben Chikha 2017) were crossed at the maturation stage under semi-controlled greenhouse in the Center of Biotechnology of Borj Cedria (CBBC). For the preparation of the female parent, the emasculation was performed by removing the three immature anthers from each spikelet to prevent self-pollination according to the egg-topping method (Pope 1944). The female spike is ready to be pollinated as soon as the spikelets are open (2–7 days after emasculation).

The spike presenting pale yellow stamens (mature) was selected as the male. Once the spikelets were clipped above stamens and exposed for few minutes, the anthers start to shed pollen. Then, these anthers are introduced into female spikelet. When the pollination takes end, female spikelet was covered by a glassine bag. The plants are controlled and enlarged seeds were observed for 7–10 days once the fecundation occurs. The obtained F_1 seeds were collected. In addition to the list of two parents and four F_1 hybrids, six barley accessions were used as an outgroup in the

multivariate analysis based on the applied nSSR analysis to highlight the efficiency of the employed SSR markers in genetic differentiation and discrimination among different barley germplasms (Table 1).

DNA extraction and microsatellites analysis

Genomic DNA of the analyzed genotypes was extracted from leaf tissue of 2-week-old plants as described by Bowers et al. (1993) and modified by Zoghlami et al. (2007). It is noted that the analyzed genomic DNA was extracted from leaves originating from single germinated seed for each investigated accession. Seventeen nuclear microsatellite markers (Liu et al. 1996; Ramsay et al. 2000; Mesfin et al. 2003; Rostoks et al. 2005) were used in the current study to genotype the two parents, the four generated hybrids and the six outgroup accessions (Table 1). DNA amplification was performed as documented in Macaulay et al. (2001). Amplified products were analyzed in 3% agarose gel based on 100 bp DNA ladder. The list of the 17 used nSSR primers and their characteristics are presented in Table 2.

Table 1 List of parental genotypes along with their putative hybrids and six Tunisian barley accessions used as an outgroup in this study

No	Accession	Origin
1	Female parent: Ardhaoui	Medenine
2	Male parent: Testour	Beja
3	H16	Putative hybrid P1xP2
4	H17A	Putative hybrid P1xP2
5	H17B	Putative hybrid P1xP2
6	H18	Putative hybrid P1xP2
7	Mehessen*	Elkef
8	Touiref*	Elkef
9	Mograne*	Zaghouan
10	Echkeul*	Bizerte
11	Utique*	Bizerte
12	Mereth*	Gabes

*Accessions forming the outgroup

Data analysis

The software Identity 4.0 (Wagner and Sefc 1999) was used to assess the number of alleles per locus, the probability of identity per locus PI (Paetkau et al. 1995) and the parentage analysis. The non biased gene diversity H_e (Nei 1978) and the observed heterozygosity H_o along with their standard deviations were calculated applying GENETIX 4.02 computer package (Belkhir 1999). The polymorphic information content PIC was obtained using Cervus 3.0 software (Marshall et al. 1998). The Hybrid purity index for each locus was calculated according to Bohra et al. (2011) by dividing the number of true hybrids (encompassing alleles from both the parents) by the total number of hybrids tested.

Table 2 List of the 17 studied nSSR primers, their characteristics along with their sources and chromosome bin positions

SSR name	Forward primer	Reverse primer	Sources	Chromosome bin position
*HVM33**	ATATTAAAAAAGGTGGAAAGCC	CACGCCCTCTCCCTAGAT	Ramsay et al. (2000)	3H, bin 7.2
HVM36	TCCAGCCGACAATTTCTTG	AGTACTCCGACACCACGTCC	Liu et al. (1996)	2H, bin 3.2
HVM67	GTCGGGCTCCATTGCTCT	CCGGTACCCAGTGACGAC	Liu et al. (1996)	4H, bin 12.2
*WMC1E8**	TCATTCGTTGCAGATACACCAC	TCAATGCCCTTGTTTCTGACCT	Ramsay et al. (2000)	1H, bin 14.2
BMAC0067	AACGTACGAGCTCTTTTTCTA	ATGCCAACTGCTTGTTTAG	Ramsay et al. (2000)	3H, bin 6.1
BMAC0093	CGTTTGGGACGTATCAAT	GGGAGTCTTGAGCCTACTG	Ramsay et al. (2000)	2H, bin 7.1
BMAC0156	AACCGAATGTATTCCTCTGTA	GCCAAACAACTATCGTGTAC	Ramsay et al. (2000)	7H, bin 12.2
*BMAC0154**	CTGGGTGATGAATAGAGTTTC	TATTCTTCAAAAGATGTTCTGC	Ramsay et al. (2000)	1H
EBMAC0970	ACATGTGATACCAAGGCAC	TGCATAGATGATGTGCTTG	Ramsay et al. (2000)	5H
BMAC0134	CCAACTGAGTCGATCTCG	CTTCGTTGCTTCTCTACCTT	Ramsay et al. (2000)	2H, bin 1.2
BMAG0384	TGTGAGTAGTTCACCATAGACC	TGCCATTATCATTGTATTGAA	Ramsay et al. (2000)	4H, bin 6.1
EBMAC0684	TTCCGTTGAGCTTTCATACAC	ATTGAATCCCAACAGACACAA	Rostoks et al. (2005)	2H
BMAC0113	TCAAAAGCCGGTCTAATGCT	GTGCAAAGAAAATGCACAGATAG	Ramsay et al. (2000)	5H, bin 5.1
BMAG0013	AAGGGGAATCAAAATGGGAG	TCGAATAGGTCTCCGAAGAAA	Ramsay et al. (2000)	3H, bin 13.1
BMAG0223	TTAGTCACCCTCAACGGT	CCCCTAACTGCTGTGATG	Ramsay et al. (2000)	5H, bin 9.2
*BMAC0040**	AGCCCGATCAGATTTACG	TTCTCCCTTTGGTCCTTG	Mesfin et al. (2003)	6H
*BMAG0007**	TGAAGGAAGAATAAACAACCAACA	TCCCCTATTATAGTGACGGTGTG	Ramsay et al. (2000)	7H, bin 2.1

*Loci belonging to the five selected nSSR

The software DARwin v5 (Perrier et al. 2003) was used to calculate dissimilarity from allelic data and to build a phylogenetic tree applying the neighbor joining method. The significance of each node was evaluated by bootstrapping data for 1000 replications of the original matrix. The same software was used to perform a factorial analysis representing genetic relationships among the analyzed genotypes.

Results and discussion

Molecular polymorphism and informativeness of the studied SSR loci

In this study, a controlled cross between two local barley parents (Ardhaoui: female, Testour: male) previously identified as susceptible/tolerant to salt stress (Ben Chikha et al. 2016) was achieved to obtain the F_1 hybrids. These two Tunisian barley parents exhibited a contrasting behavior towards the salt stress based on physiological and biochemical traits (Ben Chikha 2017).

Seventeen nSSR markers are presently applied on barley parental genotypes and their putative hybrids to validate the most informative loci for the hybrid purity assessment and the discrimination between the parents and their offspring. The used nSSRs have previously proved their efficiency and ability to characterize and discriminate among 66 barley accessions representing 11 populations of Tunisian barley accessions (Ben Romdhane et al. 2017).

Based on genotyping results, 11 nSSR loci have been shown to be polymorphic among the 17 tested loci (Table 3) while the six loci *HVM36*, *HVM67*, *BMAC0067*, *BMAC0093*, *BMAG0384* and *EBMAC0684* exhibited monomorphic allelic pattern among the genotyped set of barley genotypes and were discarded from the genetic analysis. The polymorphic 11 SSR loci exhibited a total of 28 alleles among the parental and putative hybrids with a mean value of 2.545 alleles per locus. The number of alleles per locus ranges from 2 to 4 (Table 3). The highest number of alleles was detected for the locus *HVM33,* whereas the loci *BMAC0156*, *EBMAC0970*, *BMAC0134*, *BMAG0223*, *BMAG0013* and *BMAC0113* presented 2 alleles per locus. The range of gene diversity vary between 0.530 (*BMAG0013*, *EBMAC0970*, *BMAC0156*) and 0.787 (*HVM33*) with a mean value of 0.610 ± 0.089. The observed heterozygosity vary among the locus from 0.666 (*BMAG0223*) to 1.000 (*HVM33*, *WMC1E8*, *BMAC0040*, *BMAC0113* and *BMAC0134*).

Additionally, the polymorphism information content (PIC) for each locus was measured to underline the informativeness of the used markers. The highest polymorphism information content PIC value was detected for the locus *HVM33* (0.6713), whereas the lowest PIC value 0.368 was recorded for the loci *BMAC0156*, *EBMAC0970* and *BMAG0013* with a mean value of 0.4619. Similarly, the probabilities of identity values (PI) between two genotypes randomly selected were calculated to evaluate the ability of the employed markers to discriminate among the studied genotypes. The lowest PI value was observed for *HVM33* (0.128) while *BMAC0156*, *EBMAC0970* and *BMAG0013* displayed the highest PI values (0.382). Cumulative probability to obtain identical genotypes among the two parents and the four putative hybrids pooling all the 11 nuclear microsatellite loci is 8.63×10^{-7}. This result confirms the high discriminative power of the 11 studied markers set in the investigated genotypes. Furthermore, low cumulative probability level applying SSR loci reflect a considerable genetic polymorphism among the investigated accessions (Ben Romdhane et al. 2016).

The efficiency of the employed microsatellites to discriminate among several genotypes was also reported in Ben Romdhane et al. (2017) with a low PI value 9.649×10^{-24}. In fact, when the same set of nSSR was used to genotype higher number of samples (66 Tunisian barley landraces), higher mean number of alleles was detected (8.095) and

Table 3 Characterization of the 11 polymorphic microsatellite markers used in this study: observed heterozygosity (H_o), expected heterozygosity (H_e), number of alleles per locus (A_n), probability of identity (PI) and the polymorphic information content (PIC)

Locus	H_e	H_o	A_n	PI	PIC
HVM33	0.787	1.000	4	0.128	0.6713
WMC1E8	0.666	1.000	3	0.226	0.5355
BMAC0156	0.530	0.833	2	0.382	0.368
BMAC0154	0.681	0.833	3	0.211	0.5547
EBMAC0970	0.530	0.833	2	0.382	0.368
BMAC0134	0.545	1.000	2	0.375	0.375
BMAC0113	0.545	1.000	2	0.375	0.375
BMAC0013	0.530	0.833	2	0.382	0.368
BMAC0223	0.545	0.666	2	0.375	0.375
BMAC0040	0.666	1.000	3	0.226	0.5355
BMAG0007	0.681	0.833	3	0.211	0.5547
	0.610 ± 0.089	0.893 ± 0.112	2.54	8.63×10^{-7}	0.4619

slightly greater gene diversity was recorded (0.741) (Ben Romdhane et al. 2017). This difference in polymorphism levels is obviously due to the higher number of the tested samples. Moreover, the heterozygous status of the landraces could also explain the larger genetic diversity encompassed in local resources.

Fingerprinting of hybrids and their parental lines and hybrid purity testing

The 11 SSR loci had produced unique fingerprints for each analyzed genotype and segregate between the two parental lines and their four hybrids. Banding patterns among parents and hybrids show polymorphic fragments among parental lines and hybrids. The presence of one allele from each parent in all the studied loci for the putative hybrids confirmed their origin as an offspring of the two parents P1 and P2. Parentage analysis using Identity software (Wagner and Sefc 1999) confirms this result and showed the four genotypes H16, H17A, H17B and H18 as real hybrids of the parents Ardhaoui and Testour. Further, the hybrid purity index was performed pooling all the 11 loci with the aim of identifying the true hybrids and thereby validating the performed crosses. The hybrid purity index calculated by dividing the number of hybrids having one allele from each parent was 100% for the entire tested locus. This confirms the hybrid purity of the four analyzed genotypes. The banding pattern of the most informative nSSR loci *HVM33* for the studied parents and their four hybrids is presented in Fig. 1.

Furthermore, to highlight the ability of the 11 polymorphic nSSR to differentiate among the four F_1 hybrids, their parental genotypes and six barley accessions used as outgroup, a neighbor joining tree was performed on the basis of a genetic dissimilarities matrix (Fig. 2a). The neighbor joining analysis clearly showed the discrimination among the 12 genotypes, especially among a close genetic plant material like the parental lines and the four hybrids. Two groups were differentiated based on the 11 polymorphic nSSR. Group A included the parental lines and the analyzed hybrids, whereas the six accessions presenting the outgroup were clustered in the group B (Fig. 2a).

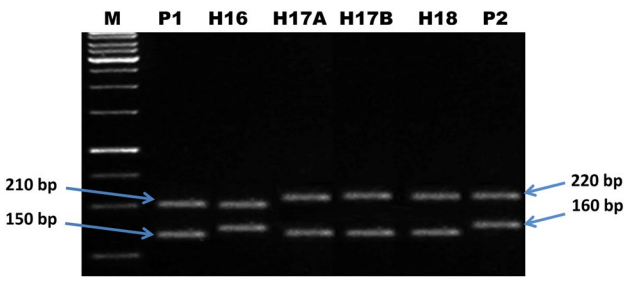

Fig. 1 Banding pattern of the nSSR loci *HVM33* for the four hybrids (H16, H17A, H17B and H18) and their parental lines (P1 and P2). *M* 100 bp DNA ladder

The factorial analysis was implemented to bring more accuracy regarding patterns of genetic relationships among the investigated genotypes using the 11 SSR (Fig. 3a). This analysis has clearly shown the demarcation of parents from the yielded hybrids. Moreover, a discrimination between the cross group (Group A) and the outgroup (Group B) is shown (Fig. 3a).

Hybrid purity tests is crucial in crop hybridization as the genetic purity of the F_1 seeds during the cross between the parental lines could be affected by foreign or self pollen (Subashini et al. 2014). Non-essential genotypes called false hybrids should be removed at an early step of the amelioration strategy (Subashini et al. 2014). Hybrid purity tests are also required where true hybrids are needed to develop genetic linkage maps, QTL research and marker assisted selection (Subashini et al. 2014).

The assessment of genetic purity of hybrids and molecular fingerprinting of their parental lines based on informative SSR markers has been used in other cereal crops such as maize (Hipi et al. 2013), sorghum (Arya et al. 2014), pearl millet (Kumar et al. 2015) and rice (Bora et al. 2016). These reports highlight the utility and efficiency of SSR markers as precise and rapid molecular tool for genetic purity testing, fingerprinting and identification. According to Pali et al. (2014), SSR molecular markers allowed the detection of genetic impurities, which were not detected when the grow-out test (GOT) method was used.

Selection of informative microsatellites for hybridity confirmation

It is known that molecular identification of plant genetic resources is a crucial step in varietal improvement strategies, especially in breeding programs where discrimination of close genetic material becomes sometimes difficult. Molecular characterization is necessary for hybrid purity certification and hybrid seed production and commercialization. In this context, the selection of minimal set of informative molecular markers is of great interest. The use of DNA markers to obtain genotype specific profiles with minimal markers is advantageous for both time and cost (Riahi et al. 2013; Mezhnina and Urbanovich 2017).

Out of the detected polymorphic 11 nuclear SSR markers, a set of five markers (*HVM33*, *WMC1E8*, *BMAC0154*, *BMAC0040* and *BMAG0007*) have been shown sufficient and informative enough to discriminate among the six genotypes representing the two parental lines and the four hybrids from each other. These five SSR markers presented the highest number of alleles per locus (A_n), expected heterozygosity (H_e), PIC values and the lowest probabilities of identity (PI) among the 11 used polymorphic SSR loci (Table 3).

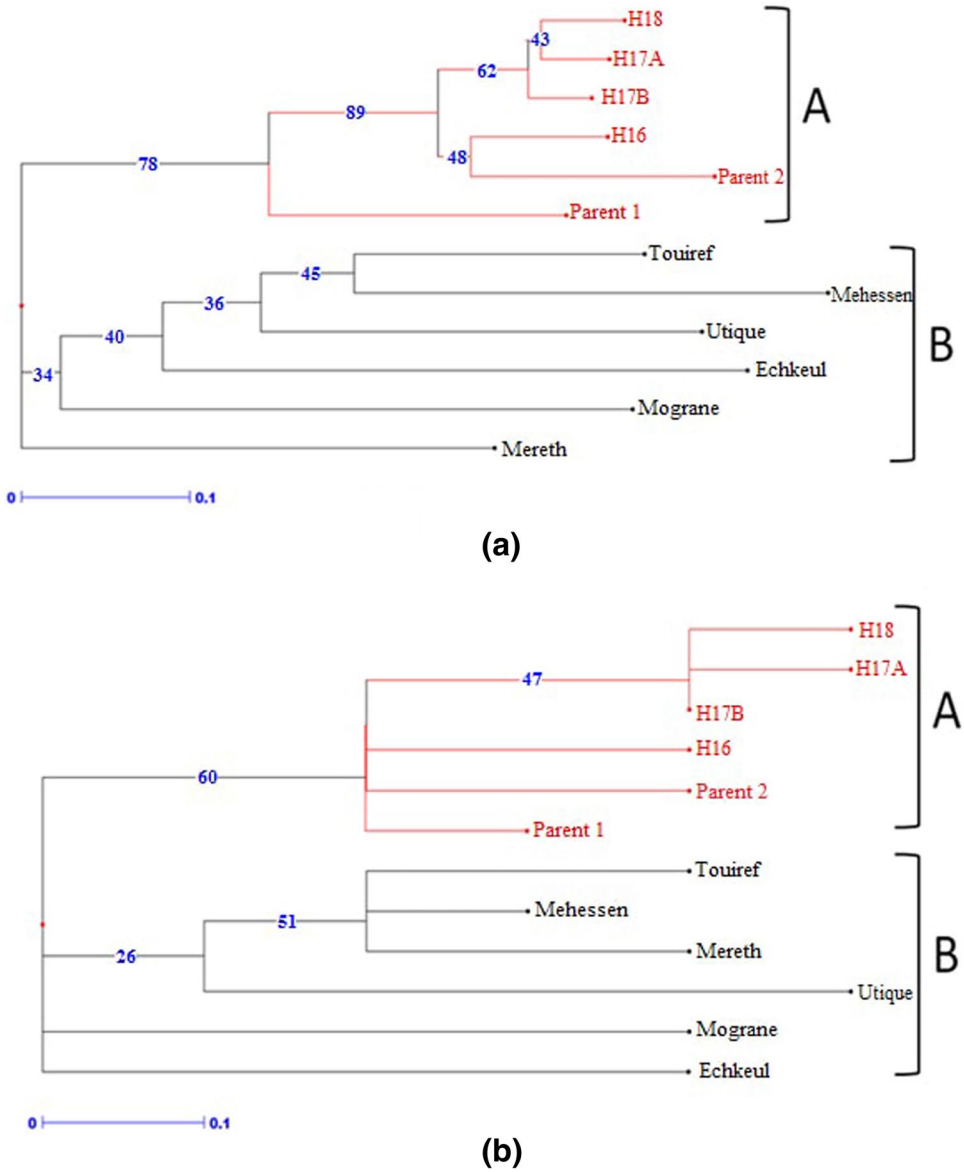

Fig. 2 Neighbor joining tree showing the ability of the 11 SSR loci (**a**) and the set of five SSR loci (**b**) to discriminate among the four F_1 hybrids, their parental genotypes and the six barley accessions used as outgroup

Furthermore, as indicated in Table 2, these five SSR loci are located in four different Barley chromosomes which explain their significant discrimination power to distinguish closely related barley accessions.

Thus, based on our analysis the use of the cited five SSR markers would be enough to differentiate among the 12 barley accessions under investigation.

The informativeness and discriminate power of the set of five SSR molecular markers was confirmed using multivariate analysis. The phylogenetic tree applying the neighbor joining method constructed on the basis of the five set of SSR (Fig. 2b) revealed a clear separation between parental and hybrid genotypes (Group A) and the classification of the six outgroup barley accessions in a different group (Group B). This confirms the high efficiency and informativeness of the selected set of markers.

Similarly, to analyze genetic relationships between the two parental genotypes the four hybrids and the six accessions used as outgroup, a factorial analysis was constructed based on the five selected SSR. The present analysis highlighted the clear classification of the gene pool in two groups A and B according to the axis 1 (Fig. 3b). In group A was found the two parents and the four hybrids while the outgroup accessions are clustered in group B; however, a clear discrimination between the two parents and the four hybrids is clearly noted.

Our findings confirm the fact that microsatellite markers are a reliable molecular tool for identifying and distinctiveness of the parental lines from their hybrids. They own their efficiency from their co-dominance inheritance allowing the best monitoring of alleles' transfer from generation to the followed. Besides, it has been reported on the suitability of

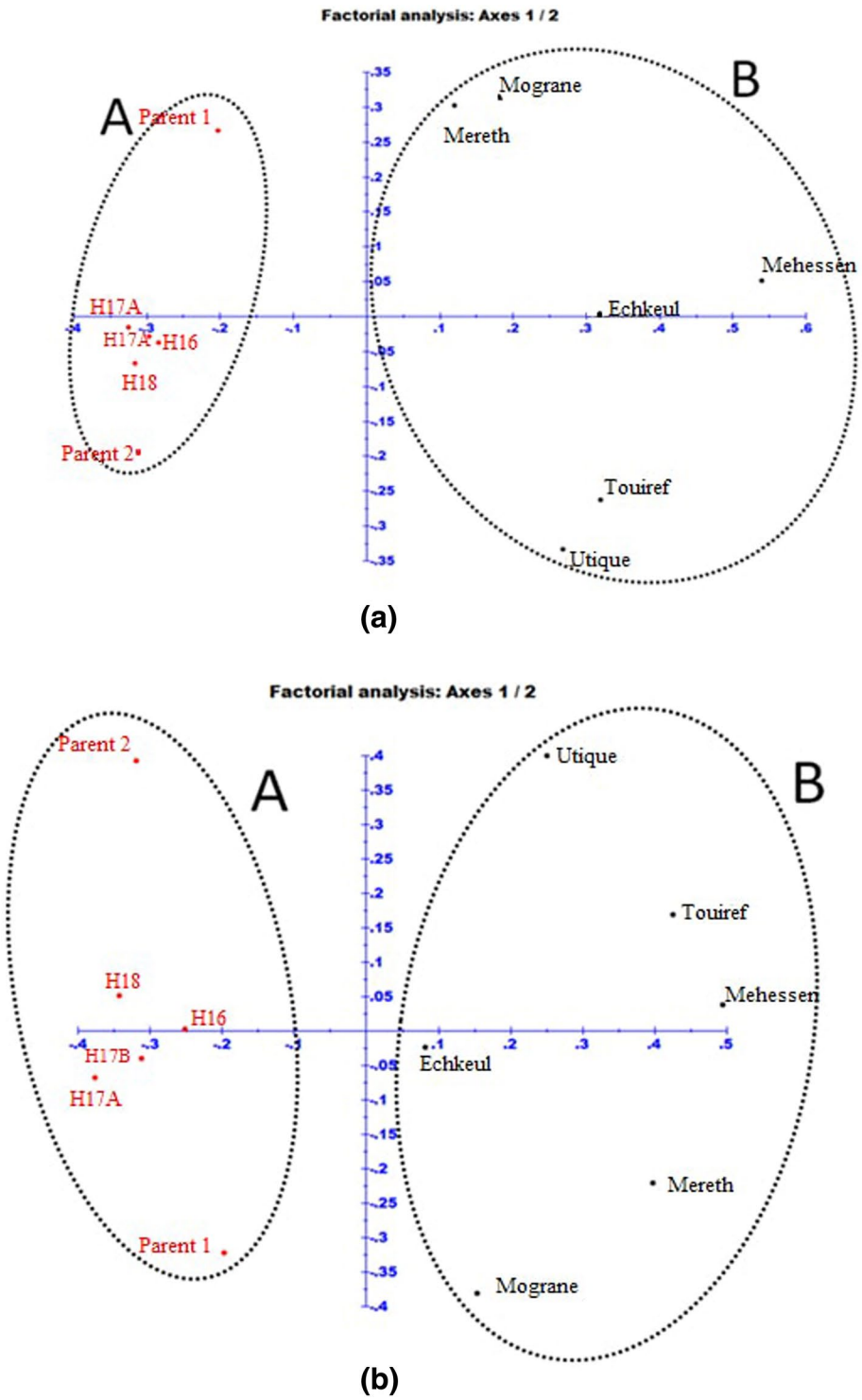

Fig. 3 Factorial analysis of the 12 barley genotypes based on 11 SSR (**a**) and five chosen set (**b**) of SSR markers

SSRs in hybrid purity testing in rice (Gimhani et al. 2014), sorghum (Arya et al. 2014), pearl millet (Kumar et al. 2015, Nagawade et al. 2016), maize (Wang et al. 2002; Hipi et al. 2013), flax (Pali et al. 2014) and sunflower (Pallavi et al. 2011).

Thus, these five can be used as referral markers for the unambiguous identification of these hybrids when the use of the set of eleven SSR is not allowed. These five can ascertains the crossing between barley parents and could ensure accurately the control of the genetic purity of the studied hybrids and thereby used as referential markers in hybrid certification programs of *Hordeum vulgare*. The SSR markers are not only useful for confirmation of hybrid purity but also for marker-assisted selection applications in barley.

Conclusion

In this study, molecular identification and characterization of four barley hybrids along with their two respective parental genotypes was assed based on informative nSSR. On the other hand, the hybrid genetic purity was investigated using microsatellite polymorphisms. Ours findings highlight the usefulness of the used set of nSSR for genetic purity test and parentage analysis in barley. Due to their high information content, a set of five nSSR (*HVM33*, *WMC1E8*, *BMAC0154*, *BMAC0040* and *BMAG0007*) showed their ability to discriminate and characterize relatively high close barley genetic material. Thus, the minimal set of SSR identified in the study could be used in further routine applications in molecular fingerprinting, hybrid purity assays and the certification of crossings in barley breeding programs.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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