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Short Communication

Molecular characterization of new oat crown rust resistant sources

I Hammami*, Y Sliti, and M El-Gazzah

Université de Tunis El Manar, Faculté de Science de Tunis, Laboratoire de Biodiversité, Biotechnologie et Changement Climatique, Tunis, Campus Universitaire, El Manar, 2094, Tunisie

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Crown rust, caused by the fungus *Puccinia coronata* f. *sp. avenae*, is a widespread and damaging disease of oat. Spontaneous oat species are an important and abundant source of new rust resistance genes. Two Tunisian resistant spontaneous oat accession JT_0 and JT_5 (*Avena sterilis*) was characterized using 21 RAPD primers and compared to the differential lines *Pc38*, *Pc39* and *Pc68* (*Avena sativa* L.), which showed previously a high resistance level to the local population of crown rust. The effectiveness of molecular markers could be evaluated through such parameters as the polymorphic information content (PIC), marker index (MI), heterozygosity (H) and the resolving power (Rp). Banding patterns obtained by RAPD analysis revealed polymorphism between accessions & RAPD profiles showed 33 common bands, 4 markers specific to *A. sterilis* accessions and 3 markers specific to *A. sativa* species. JT_0 showed the lowest RAPDs number (61) among oat accessions. The Neighbor-Joining dendrogram inferred from the Slatkin's pair wise distances (F_{ST}) showed that JT_0 accession was clearly separated from the other accessions. JT_5, *Pc68*, *Pc38* and *Pc39* were clustered in one group. It's interesting to state that an *A. sterilis* line (JT_5) would be more closely related to 3 *A. sativa* lines than a fellow an *A. sterlis* line. JT_0 and JT_5 could be an interesting provider of a genetic control of the resistance to oat crown rust. A crossing between the spontaneous accessions and the differential lines needs to be done to conclude about the originality of the resistance genetic control in JT_0 and JT_5.

Keywords: Crown rust resistance, oat differential lines, RAPD

Introduction

Oat (*Avena sativa* L.) is a cereal crop that is used all over the world for human nourishment and animal forage[1]. Oat *(Avena sativa* and *Avena byzantina*) are the main livestock fodder in Tunisia[2]. Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is deemed as the most pervasive and destructive disease of oat in Tunisia[3]. National and world oat production, declined in favour of other crops during the last century[4], it is often limited by the disease caused by rust. Genetic resistance has been the main method used to control this disease. Several major genes for resistance to crown rust (*pc* genes) have been identified in cultivated oat and its wild relatives[5,6]. Sources of resistance to oat crown rust used elsewhere are essentially based on a set of 16 lines "*Pc*-genes" having a specific resistance[7], with the majority considered as dominant genes[8]. Nevertheless, because of its extreme genetic diversity, the *P. coronata* population has swiftly adapted to most major gene resistance in a short period of time[9]. Survey done previously in Tunisia over a period of seven years, showed that *Pc38*, *Pc39* and *Pc68* differential lines showed a high level of resistance to the natural population of *P. coronata*[10]. Studies made in Tunisia on the screening of more than 90 spontaneous oats accessions locally collected enabled us to select two accessions: JT_0 and JT_5. These two accessions showed a high level of resistance to crown rust after being subjected to artificial and natural inoculations[5]. Molecular markers have been demonstrated to be important and effective tool in the characterization and assessment of genetic resources. Between molecular practices, RAPD is rapid and the easiest. Williams *et al*[11] uphold this technique for genetic analysis of populations that are used in breeding programs. This work aimed to characterize new sources of resistance to oat crown rust (JT_0 and JT_5) by RAPD analysis and comparing them with the differential lines *Pc38*, *Pc39* and *Pc68*.

DNA extraction was done using dithiothreitol (DTT) method[12]. The RAPD analysis[11] was conducted as described by Drassou *et al*[13] with minor modifications. Twenty three different decamer primers were tested. The oligonucleotides used are marketed as lyophilized by the company "Operon" (California, USA) University and British Columbia (Canada). The length of the analysed fragments was determined using Gel-Pro analyser (Media Cybernetics) software. Performance analysis for all markers was carried out. Polymorphic information content (PIC) and heterozygosity (H), were calculated online[14]. Marker Index (MI) was assessed based on Varshney *et al*[15] method. The resolving power (Rp) was calculated followed the formula of Prevost & Wilkinson[16]. To test the distribution of the variance of RAPD profiles among accessions, analysis of molecular variance (AMOVA)[17]

*Author for correspondence:
Fax: +216-71-317-349
Imran.hammami@fst.rnu.tn

was applied using Arlequin 3.1 software (http:// cmpg. unibe.ch/software/arlequin3/)[25]. Pairwise F_{ST} distances[18] between RAPD profiles computed with Arlequin were used to infer a Neighbor-Joining dendrogram with the software MEGA5[19].

On total RAPD fragments, 144 (41%) were polymorphic, 165 (47%) were monomorphic at the level p = 0.05, and 44 (12%) were unique. The highest percentage values of polymorphic fragment across a primer were observed on OPB-06 (100%), UBC263 (91.7%) and OPG-12 (85.7%). The set of RAPD primers used in this survey engendered 134 usable loci. The average PIC for the particular marker ranged from 0.1504 (UBC167) to 0.3648 (OPB-06) with the mean 0.2192. Mean H values varied from 0.160 (UBC254) to 0.480 (OPB-06). Only 14% of RAPD primers showed high H values (≥ 0.400). The Rp values varied between 0.8 for primer UBC189 and 7.2 for primer OPD-16 with a mean value of 2.64 among primers. The highest MI value was obtained for OPG-12 primer (5.77), and the lowest for UBC167 (0.08) (data not shown).

Common RAPD markers (33) were scored between accessions. Four markers (OPAN "550 bp"; OPD "525 bp"; OPG12 "620 bp" and UBC109 "680 bp") present in JT_0 and JT_5 were absent in all the three differential lines tested. In other level, 3 markers (OPD "500 bp"; OPD "700 bp" and OPG12 "600 bp") in the differential lines were absent in the spontaneous accessions (data not shown). JT_0, JT_5, *Pc68*, *Pc38* and *Pc39* exhibited specific bands which were 7, 5, 5, 16 and 12 respectively (Fig. 1a). JT_0 showed the lowest RAPDs number (61) comparing to the remaining accessions. However, JT_5, *Pc68*, *Pc38* and *Pc39* showed approximately the same level of RAPDs richness (71, 69, 78 and 74 respectively) (Fig. 1b).

The Neighbor-Joining dendrogram inferred from the Slatkin's pairwise distances (F_{ST}) showed that JT_0 accession was clearly separated from JT_5 and the differential lines which were clustered together indicating the close relationship between them (Fig. 2). Pairwise comparisons showed high F_{ST} value (0.97119) (Table 1). The percentage of variance among accessions was significantly high (97.12%), in contrast it was restricted within accessions (2.88%) (Table 1). Locus-by-locus AMOVA for polymorphic loci only sorted out 129 RAPDs (89.58% of NPF) with statistically significant among accessions variance component (data not shown) and consequently mostly contributed to the differentiation of oat accessions.

The improvement of long-lasting crown rust resistance is a main goal given the quick failure of crown rust resistance established upon race-specific seedling resistance genes. Numerous molecular markers described wide-reaching pools of oats. The oat accession had received only 41% of polymorphic RAPD fragments which is very low comparing to

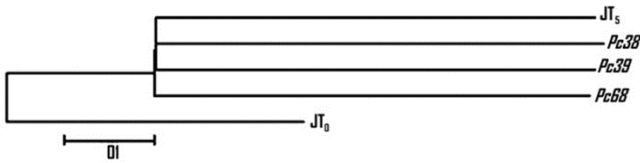

Fig. 1 — Schematic representation of reproducible RAPD profiles of the five accessions; (a) Number of common and specific markers among accessions; numbers in black box represents the number of specific markers and (b) Distribution of total markers among accession; black colours: presence of RAPD fragments; white colour: absence of RAPD fragments. This representation is not to scale.

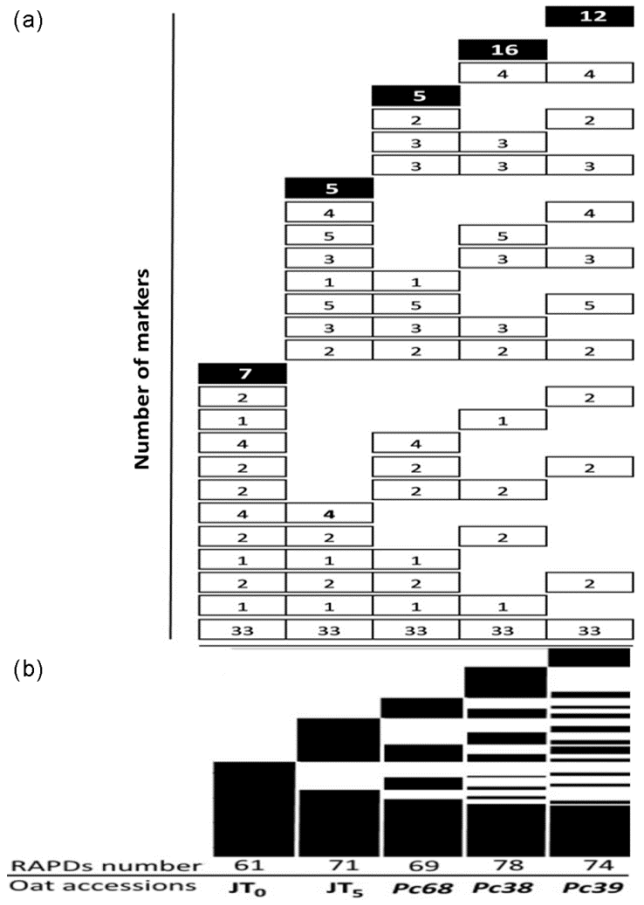

Fig. 2 — Neighbor-joining dendrogram of Slatkin's pairwise F_{ST} distance. Scale bar indicates pairwise F_{ST} values.

Table 1 — Analysis of molecular variance (AMOVA) for RAPD diversity of the five different accessions by using a total of 353 RAPD markers.

Sources of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value*
Among accessions	4	892.800	24.718	97.12	0.000000
Within accessions	40	29.333	0.7333	2.88	0.000000
Total	44	922.133	25.451		
Fixation Index	F_{ST}: 0.97119				

*Significance tests (1023 permutations)

early Polish oat cultivars[20] and Polish landrace of common oats[21]. However, in this study, a suitable level of polymorphism was detected, which was higher than the results scored for *Avena sativa* cultivars[22] and wheat varieties[23]. That confirm the appropriateness efficiency of this technique to show genetic variation of oat and to engender molecular markers for traits in a crop species with a very large genome and a high percentage content of repetitive DNA[22]. On the other hand, an important number of specific fragments was detected that it could be used in the identification between each oat accession. The effectiveness of molecular markers could be evaluated through such parameters as PIC, MI, H and Rp. The results obtained were compared with results of these coefficients scored by ISSR for Iran oat genotype[24], AFLP and RAPD detected for Polish oat cultivars[20] and for Greek *Aegilops* accessions[26] and SSR markers for mays inbred lines[25]. Recorded coefficients in this survey proved that used RAPD primers significantly discriminated between the tested oat accessions. RAPDs, either specific to the species or specific to the accession itself could be related to the gene for resistance to oat crown rust. This statement couldn't be affirmed just by using RAPD markers, since they might be amplified in a non-coding region of the genome. The precise locus of rust resistance genes on oat chromosomes has not yet been defined because unlike the other cereal grains, there has not yet been any specific chromosome numbers allocated. Many genes might be alleles or the similar gene since in most cases no full genetic surveys exist[27]. RAPD marker (UBC269) has been linked to *Pc68*[22]. Two restriction fragment length polymorphism (RFLP) markers, cdo673 and wg420, were shown to be linked to *Pc38*, and one RFLP marker (cdo666) was defined to be linked to Pc39[28].

Neighbour joining dendrogram and the AMOVA analysis showed that JT_0 was genetically distinct to the remained accessions even to the spontaneous one JT_5. Thus, the high F_{ST} value could be the result of the distance of JT_0 to this group (Fig. 2). An interesting finding that an *A. sterilis* line would be more closely related to *A. sativa* lines than a fellow an *A. sterlis* line. JT_0 and JT_5 which were characterized as a resistant accession to oat crown rust and showed approximately the same resistance reaction as *Pc38*, *Pc39* and *Pc68*[29,2,10] could probably host an interesting genetic control of the resistance to crown rust disease. The improved sources of genes for resistance to crown rust can be acquired among spontaneous oat lineages in the regions of its origin[27]. Among the spontaneous relatives, *A. sterilis* is the most important source of resistance genes[5] and can be easily crossed with cultivated oat.

The selected spontaneous accession JT_0 and JT_5 could be a remarkable provider of an interesting genetic control of the resistance to oat crown rust. Genetic study on the inheritance of resistance to crown rust in the selected spontaneous oats needed to be completed to confirm this conclusion. The experiments are at an initial phase and need to be followed through in terms of genetic studies of the resistance in order to make conclusions concerning the originality of the genetic control of the selected spontaneous oat resistance. The best way is to cross the spontaneous accessions with the differential lines and to analyse the reaction of the individuals F_2 to crown rust infection.

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