Use of interspecific hybridization for the transfer of hessian fly resistance from *Triticum araraticum* to durum wheat.

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Abstract

Hessian fly, Mayetiola destructor Say, is the most destructive insect pest of cereals in the arid and semi-arid areas of the Mediterranean region. The use of wild relatives from the primary and secondary gene pools allows the transfer of new Hessian fly resistance genes to cultivated wheat. The objective of this research was to study the hybridization between durum wheat and Triticum araraticum and the usefulness of this interspecific cross in transferring Hessian fly resistance to the cultivated species.

Four accessions of T. araraticum, were crossed with 7 durum wheat cultivars (ACSAD65; Cocorit; Kebir; Jori; Marzak; Oum rabi; Sebou) in order to produce interspecific F1 hybrid plants. The use of hybrid embryo rescue was necessary for the success of these crosses. Among the 337 saved embryos saed, 170 were able to germinate and 126 grew into plants. The genetic analysis of the crossability of T. araraticum with durum wheat showed the presence of genetic variability for this character, and allowed the identification of the best genotypes best for eventual further hybridization. Thirty-seven hybrids of the first backcrosses were fertile. Testing of their reaction to Hessian fly revealed the presence of resistance in 5 of them. Further backcrosses are needed to obtain the some morphology as close as possible to that of durum wheat before the introduction into the crossing blocs of the current breeding program.

Key words : interspecific hybridization, Hessian fly, *Mayetiola destructor*, backcross.

ملخص

تعتبر ذبابة هس *Maytiola destructor* من الأفات الحشرية الأكثر تسببا فى تلف محصول الحبوب في المناطق الجافة و الشبه الجافة بالمغرب وبعض البلدان المتوسطية.
إن استعمال بعض المصادر الوراثية المقاومة لهذه الآفة والموجودة في بعض السلالات الوراثية *Triticum araraticum* يمكن نقلها عن طريق التهجين نحو القمح الصلب.
أربع سلالات من *Triticum araraticum* وسبعة أصناف من القمح الصلب استعملوا في في التهجين بين الأجناس.
كان لابد من زرع الأجنة الهجينة الغير الناضجة في بيئة مصطنعة لإنجاح هذا النوع من التزاوج. ثلاث مئة وسبعة وثلاثون أجنت استعملت للإنقاذ. من أصل 170 هجين غير ناضج تم إنقاذها في بيئة مصطنعة 126 استطاعت أن تعطي نبات قمح غير خصب.
التحاليل الوراثية للنزعة نحو التزاوج مع جنس *Triticum araraticum* أمكنت من انتقاء بعض أصناف القمح الصلب للتزاوج في المستقبل.
أمكن تخصيب الهجين الأول عن طريق Bakc-cross من الحصول على 37 هجين Bakc-cross1 والإصابة المصطنعة بذبابة هس داخل البيوت الزجاجية أظهرت وجود مقاومة لخمسة منها.

الكلمات المفتاحية: تهجين بين الأجناس *Triticum araraticum* قمح صلب، ذبابة هس

Résumé

La cécidomyie ou mouche de Hesse (Mayetiola destructor Say), est l'insecte ravageur le plus destructif des céréales dans les zones arides et semi-arides du Maroc et de certains pays méditerranéens. L'utilisation des espèces sauvages apparentées aux blés du groupe génétique primaire et secondaire, permet de transférer de nouvelles sources de résistance à la cécidomyie sans trop modifier leur fond génétique. L'objectif de cette étude est d'étudier l'hybridation du Triticum araraticum avec le blé dur et son utilité pour le transfert de gènes d'intérêt comme la résistance à la cécidomyie.

Quatre accessions de T. araraticum et sept cultivars de blé dur ont été utilisés en hybridation interspécifique. Le recours au sauvetage de l'embryon hybride a été nécessaire pour la réussite de cette hybridation. Trois cent trente sept embryons ont été soumis au sauvetage de l'embryon. Parmi les 170 embryons sauvés, 126 ont pu germer et donner des plantes hybrides mâle stériles. L'analyse génétique de l'aptitude au croisement avec le T. araraticum, a montré l'existence d'une variabilité génétique pour cette aptitude et a permis d'identifier des génotypes pour des hybridations éventuelles. A partir du premier rétrocroisement, 37 hybrides ont été rendus fertiles et l'infestation artificielle contrôlée par la mouche de Hesse, a révélé la présence de la résistance chez cinq d'entre eux. D'autres rétrocroisements sont nécessaires pour s'approcher au maximum de la morphologie du blé dur avant que ces hybrides n'intègrent les blocs de croisement de blé dur.

Mots clés : hybridation interspécifique, mouche de Hesse *Mayetiola destructor*, étrocroisement.

Introduction

The objectives of interspecific and intergeneric hybridization are generally the transfer of rusticity and disease resistance genes present in wild relative species to cultivated species. According to the extent of their genetic affinity with the cultivated species to be improved, the wild species are classified in primary, secondary or tertiary genetic pools. In wheat improvement, allopolyploid species having some completely homologous genomes like *T. monococcum, Ae. squarrosa* constitute the primary genetic group and are the most used in interspecific hybridization. Species with genomes partially homologous to those of wheat, like *T. araraticum*, constitute the secondary genetic group, and contribute withabit more difficulty to wheat improvement. In this case, new gene introduction is more complex without the presence of genomic counterpart. Indeed, rather than having a simple crossing-over between homologous chromosomes and hence the transfer of small genome portions, it is the totality of the homoeologous chromosome that may be introduced after the hybridization (Mujeeb-kazi, 1998).

In durum wheat, where the A and B genomes have cohabited during millions of years, interspecific crosses concerning the B genome drag some deleterious effects such as the loss of vigour, and the result of several generations of backcrossing is generally weak. This was been the case in the hybridization of wheat with *Ae. speltoïdes* (Knott and Dvorak, 1981). The wild species *T. araraticum* is of the secondary genetic group and presents a good genetic affinity with durum wheat giving easily interspecific hybrids. Such hybridization has already permitted the transfer of leaf rust, stem rust, and powdery mildew resistance to durum wheat (Allard and Shands, 1954).

Hessian fly (*Mayetiola destructor* (Say), (Diptera: Cecidomyiidae) is the most damaging insect pest to both bread and durum wheat (Hatchett and Gallun, 1970). Its life cycle comprises an adult stage represented by a small fly, 4 mm long. The female fly lays up to 450 eggs on wheat leaves after fertilisation. These eggs produce larvae that penetrate between the leaf sheet and the stem and migrate to the first node level where it get settled and start feeding. The feeding process involves the injection of an enzymatic juice that causes tiller degeneration (Lhaloui, 1986). Symptoms are characterised by a dark green coloration of the leaves, the stunting and the degeneration of the tillers (Lhaloui, 1986). Yield losses are estimated at 22 to 32% (Amri et al., 1992) and are more severe under delayed plantings and drought (Lhaloui et al., 1992). Cultural practices such as stubble burying and incineration, crop rotation (Ouchaou, 1972), insecticide use (Carbofuran) and the introduction of insect-natural predators (Lhaloui, 1992); contribuate to an efficient and economically profitable protection of wheat crop in the case of rainfed agriculture. Plant genetic resistance or tolerance to the insect is considerat as the most appropiate means of control. Plant tolerance to insect attacks could be achieved through the activation of tillering, the non-preference of females for plants as sites of egg laying (morphological characters not desirable by the fly such as leaves pubescence). The most important genetic control mechanism is resistance by antibiosis that kills larvae of the first stage as soon as they start feeding on the plant. The antibiotic resistance of the plant and virulence of the fly biotype are under genetic control with a gene for gene relation. Twenty six resistance genes named H1 to H26, have been identified in the USA (El Bouhsini et al., 1996). Most of these genes are not effective on the Moroccan populations of the fly. The relatively high frequency of the insect biotype require a continuous search for new genes and the availability to the breeder of a large genetic base for resistance.

In durum wheat no sources of resistance to Hessian fly were identified for Morocco until recently. One accession from the international durum wheat collection was recently characterised as resistant but had poor agronomic characters prevating a rapid improvement. The necessity to move toward the use of genes present in bread wheat and its related wild species is obvious. The majority of the wild accessions of *T. araraticum*, whose genome is partially homologous to those of durum wheat, produce interspecific hybrids with durum wheat male sterile hybrids. Several backcrosses are necessary to transfer the resistance to durum wheat.

The objective of this experimentation is to transfer Hessian fly resistance identified in *T. araraticum* to durum wheat and to study the ability to interspecific hybridization of durum wheat varieties with some selected wild accessions.

Materials and methods.

Seven Durum wheat varieties (*T. turgidum* ssp. *Durum*, genome AB, 2n = 4x = 28 chromosomes) : 'ACSAD 65', 'Cocorit' , 'Kebir', , 'Jori','Marzak','Oumrabi' and 'Sebou', were crossed to four accessions of *T. araraticum* (TA28; TA36; TA925 and TA927). The TA accessions were introduced from the Centre of Genetic Resources at the Kansas State University, USA, and have been selected for resistance to Hessian fly in Morocco (Amri, 1989). Seeds of durum wheat varieties were put in gradual germination, in order to match crossing times of material differing in flowering dates. The accessions of *T. araraticum* were of winter type; they were vernalized during 42 days at 5°C then transferred to the greenhouse. Pollen freshly collected from *T.araraticum*, was brought on stigmas of castrated spikes two days after durum wheat emasculation. Injection of 100 ppm of the dichlorophénoxyacétique acid (2.4 DS) solution was made in the upper internodes cavity. One day after pollination, a second injection of DCPA and a pulverisation of 75ppm of gibberellic acid solution (GA3) on the ears were made (Laurie and Bennett, 1986). Two weeks after pollination, embryos were transferred on 'MS' regeneration synthetic medium (Murashige and Skoog, 1962). After one week of acclimatisation, the regenerated plants were placed in pots containing a soil-peat-sand mixture. Interspecific hybrid plants were tested for the resistance to the Hessian fly. The test consisted in infesting plants at the three leaf stage with female flies during two days. Resistance through antibiosis was to be confirmed by the presence of dead larvae of Hessian fly.

The results were collected and expressed in relation to the number of pollinated flowers in order to appreciate interspecific hybridization success per genotypes. The following parameters were used in data collection and analysis:

- Seed formation percentage: number of seeds formed per pollinated flowers.
- Number of embryos obtained per 100 pollinated flowers.
- Number of hybrid plants per 100 pollinated flowers.

The analysis of variance was made using a completely randomised design model based on female plants and male pollen (Ms-Stat-C software, version 3.0 statistical programs. Michigan State University East Lansing, USA.). The experiment was entirely repeated in two sets. For the comparison of means, the Least Significant Difference (LSD) at the 5% probability statistic was used.

Results and discussion :

The number and the percentage of seed formation, embryos and hybrid plants are shown in table I. Among 840 pollinated flowers, 337 were successfully fertilized by pollen of *T. araraticum*, representing an overall mean ratio of 40.12 %. This result indicates a good genetic affinity between the two species. Almost all genotypes produced seeds with varying percentages of seed set per durum wheat genotype. These percentages varied between 16.25 and 65% (maximum with the cross 'Cocorit' x TA36). Using these seeds, 170 embryos have been rescued by transfer on synthetic medium and 126 hybrid plants have been regenerated, thus making more than 74% of embryos germinate in plants.

Table I. Seed set, embryos and hybrid plants obtained from crosses between durum wheat and *Triticum araraticum.*

Hybrid	Flowers	Seed set		Embryos		Hybrid plants	
	pollinated	Number	%*	Number	%*	Number	%*
ACSAD 65 x TA28	40	14	35	4	10	0	0
Cocorit x TA28	40	26	65	6	15	1	25
Kebir x TA28	120	72	60	29	24.2	22	18.3
ACSAD 65 x TA36	60	32	53.3	4	6.7	2	3:3
Cocorit x TA36	80	29	36.3	12	15	6	7.5
Sebou x TA36	80	13	16.3	12	15	10	12.5
Jori x TA36	100	37	37	30	30	27	27
Oum rabi x TA36	60	18	30	14	23.3	11	18.33
Kebir x TA925	40	17	42.5	3	7.5	1	2.5
Jori x TA925	40	13	32.5	16	40	13	32.5
Marzak x TA925	40	15	37/5	14	35	13	32.5
Oum rabi x TA925	40	18	45	10	25	8	2
Kebir x TA927	40	17	42.5	3	7.5	1	2.5
Oum rabi x TA927	60	16	26.6	13	21.,7	11	18.3
Total	840	337		170		126	

Seed formation

The analysis of variance (Table II), did not reveal any statistically significant effect of the male and female parents on the seed formation percentage. This may be related to the even genetic affinity between the two species across genotypes.

Embryos formation and rescue

Significant differences between genotypes of durum wheat have been observed for percentages of obtained embryos (Table II). These results permitted the selection of genotypes such as 'Kebir', 'Jori' and 'OumRabia' for possible future crossegs with *T. araraticum*.

Table II. Analysis of variance for seed formation, number of embryos and hybrid plants obtained from the cross between durum wheat and *Triticum araraticum*.

Source de variation	d. f.	F ratio		
		% seed set	% Embryos	% Hybrid plants
Replicate	1	-	-	-
Female parent	6	1,87NS	17,56 ***	22,2***
Male parent	3	0,73NS	4,14 NS	1,46 NS

Hybrid plant number

Differences among genotypes for the total number of regenerated hybrid plants were observed. Genotypes like 'Sebou', 'Kebir' and 'Jori', have produced the largest number of hybrids.

Interspecific Hybrids derived from durum wheat and *T. araraticum* are male sterile. Their backcross with the recurrent female parent permits the restoration of fertility and the production of seeds set with variable levels of success. Among 171 regenerated plants, 37 produce seeds in the first backcross. The number of fertile plants per durum wheat genotype after backcrossing is shown in table III. The highest numbers of fertile plants are obtained with the durum cultivars 'Kebir', 'Cocorit', 'Sebou' and 'Jori'. The backcrossing of the male sterile hybrid constitutes the most difficult stage and requires more time to get of the first hybrid. Indeed, if there is a genetic ability to interspecific hybridization, there may be also genes that are suspected to control the ability of backcrossing to the recurrent parent. It is therefore clear that the prior choice of the intended parents to interspecific hybridization must not only take in account their good ability to succeed in the first interspecific hybridization but also in the backcrosses that follow. Kebir, Cocorit and Jori, are considered as good parents for interspecific crossing with *T. araraticum*.

The plants obtained from the backcrosses in this experiment were submitted to artificial infestation by Hessian fly. The results of the Hessian fly resistance test are shown in table III. Five fertile hybrid plants were found to be resistant to Hessian fly. These plants are derived from the crosses: Kebir / TA28 (3 plants), Cocorit / TA36 (1 plant), and Jori / TA36 (1 plant).

Table III. Number of regenerated plants, fertile plants at first back cross and reaction to Hessian fly of progenies from crosses between durum wheat and *Triticum araraticum.*

Hybrid	Number of regenerated plants	Number of fertile plants at first Back cross	Reaction to Hessian fly	
			Number of susceptible plants	Number of resistant plants
Cocorit x TA28	1	0	//	//
Kebir x TA28	22	16	13	3
ACSAD 65 x TA36	2	1	1	0
Cocorit x TA36	6	5	4	1
Sebou x TA36	10	3	3	0
Jori x TA36	27	3	2	1
Oum rabi x TA36	11	2	2	0
Kebir x TA925	1	1	1	0
Jori x TA925	13	2	2	0
Marzak x TA925	13	3	3	0
Oum rabi x TA925	8	0	//	//
Kebir x TA927	1	1	1	0
Oum rabi x TA927	11	0	//	//

Conclusions

Interspecific hybridization between durum wheat and *T. araraticum* coupled to embryo rescue and backcrossing produced viable fertile hybrids.

Variable rates of success of the different and subsequent operations were observed according to the genotypes of the parents, No effect of the pollen was detected while maternal plant effect was significant on the percent. of embryos and the percent of hybrid plants obtained.

Hessian fly resistance was effectively transferred to five plants of durum wheat.

The new resistance in durum wheat should be submitted to genetic studies and to further back-crosses (four to five) in order to be integrated into the durum wheat crossing block.

This study shows the potential for interspecific crosses and embryo rescue in enhancing breeding programs.

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