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Study of genetic basis of resistance to leaf rust isolates in durum wheat

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SUMMARY – In the present work a phenotipical and molecular marker-based approach is described, in an attempt to unravel the genetic basis of the postulated durable resistance to leaf rust in durum wheat cv. Creso. A recombinant inbred line (RIL) population ($F_{6:7}$) was developed by crossing durum wheat cvs Creso (resistant) and Pedroso (susceptible). Resistance evaluation of lines suggests that durable resistance of Creso could be due to the combination of different mechanisms with distinct genetic basis. A molecular mapping approach is in course in order to individuate chromosomic regions involved in control of these mechanisms: more than 300 microsatellite PCR-based markers have been tested on parental lines, all with a known position on the wheat genome. About 30% of them were found polimorfic between Creso and Pedroso.

Introduction

Leaf rust caused by *Puccinia triticina* is an important disease of wheat that in recent times it turning to be particularly severe on durum wheat (Cátedra *et al.*, 2003) where less resistance is available than in bread wheat (Martínez *et al.*, 2006). Creso is a durum wheat cultivar whose resistance has been postulated to be durable (Pasquini and Casulli, 1993). There is evidence that its durability is based on a combination of hypersensitive resistance and partial resistance (Martínez and Rubiales, 2002). The combination of different mechanisms of resistance in a cultivar can be very effective in increasing and protecting the resistance (Rubiales and Niks, 2000).

Materials and methods

123 $F_{6:7}$ lines from a cross Creso x Pedroso were studied for resistance to *P. triticina* in first and fourth-fifth leaf stage. Isolate PS-03 (monosporic derived from a population collected at Puerto Serrano, Cádiz, Spain, in 2003) was selected out of 60 *P. triticina* isolates as the most virulent one on Creso (IT – Infection Type – X) in 4-5th leaf stage. All other isolates were avirulent on Creso (IT ranging from 0 to 4). Plants were inoculated with five milligrams of spores of PS-03 and incubated during 24 hours in humidity at saturation and darkness at 20℃. Plants were then transferred to a growth chamber at 20℃ in a daily regi me of 14 h light (112 µmol photons/m^2/s). Latency period (LP) was calculated by interpolation as the time period from the beginning of incubation until 50% of the total number of pustules appeared (Rubiales and Niks, 1995). LP was calculated on all RILs, even on those with IT X, but in this case, only well formed pustules, no associated with host-cell necrosis were considered. LP vuales were expressed as relative LP values (rLP) to the cultivar Pedroso (=100%). Twelve days after incubation, infection types were recorded following a 0-9 scale (McNeal *et al*., 1971).

Genomic DNA was extracted by the DNAzol method starting from young leaves of parental varieties and segregating lines in order to perform their molecular characterisation by means of microsatellite markers with a known position on the wheat genome. PCR reactions were performed in a thermalcycler in a volume of 15 µl. The PCR products were separated on 3% agarose gels and, when the polymorfism was not detectable on agarose gel, the samples were loaded also on the higher resolution polyacrylamide gels stained with ethidium bromide.

Results and discussion

In the seedling test, the 123 RILs segregated for infection type with 63 RILs showing IT X

(resistant infection) and 60 showing IT 8 or 9 (susceptible reaction). A similar segregation was obtained in the 4th-5th leaves stage with 62 RILs with IT X and 61 RILs with IT 8 or 9. These observed ratios do not deviate significantly from the 1:1 ratio, suggesting a single gene for hypersensitive resistance, as earlier suggested in F2 studies (Martínez and Rubiales, 2002).

There was a continuous segregation for rLP in the RILs, shifted towards susceptibility in 1st leaf (Fig. 1) but not in 4-5th leaf (Fig. 2). LP expanding effect (partial resistance) derived from Creso was more marked in 4-5th leaf (Fig. 2) than in 1st leaf stage (Fig. 1).

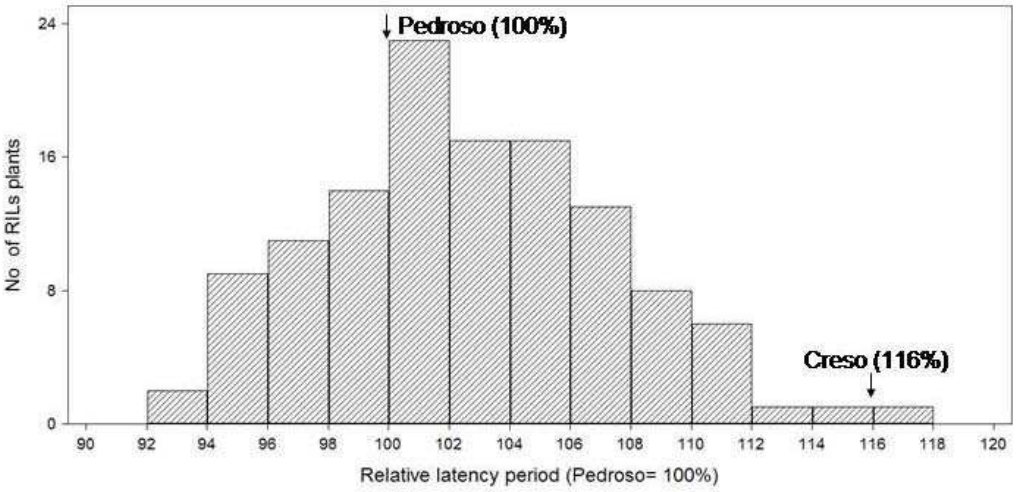

Fig. 1. Distribution of relative latency period in the RILs plants in 1st leaf stage inoculated with *P. triticina* isolate PS03.

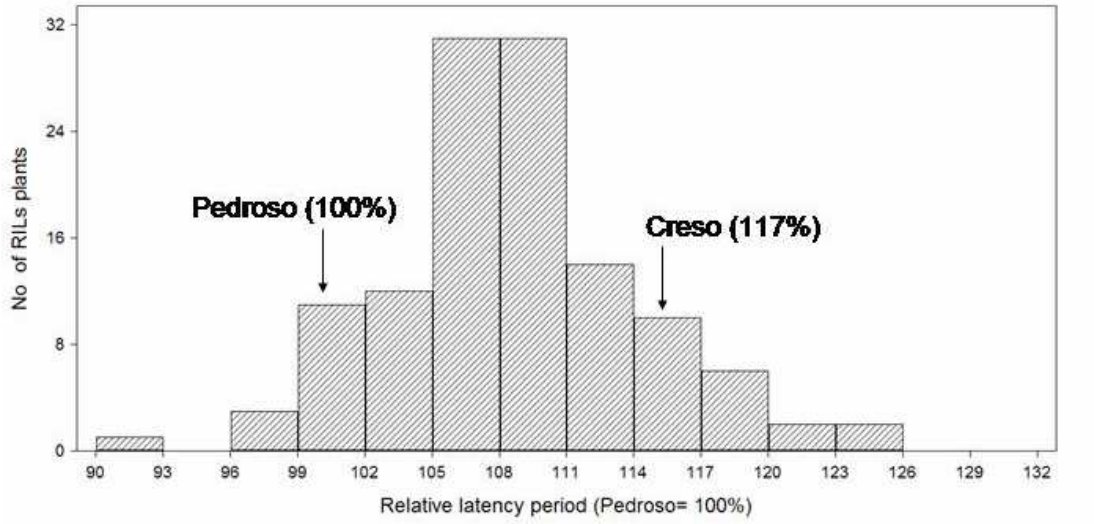

Fig. 2. Distribution of relative latency period in the RILs plants in 4th-5th leaf stage inoculated with *P. triticina* isolate PS03.

More than 300 microsatellite markers with known position and well distributed on the whole genome, were tested on parents of the segregant population, in order to construct a basic map. More than one hundred markers were found polimorfic between Creso and Pedroso (about 30%, according to levels of polymorphism reported for intraspecific crosses), with a good distribution among nearly all

chromosome arms (a mean of four markers per chromosome arm). The screening with additional molecular markers is in course on Creso and Pedroso DNA, as well as the segregation analisys of polymorfic markers on all recombinant inbred lines in order to individuate genomic regions involved in control of multiple mechanisms underlying the durable resistance observed in Creso variety.

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