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BACKGROUND

Fungal diseases can cause severe yield losses in wheat, together with downgrading in quality. The wheat *Lr67* gene (*Yr46/Sr55/Pm46*), also indicated as "magic gene", isolated from a common wheat landrace, is known to confer partial resistance to all three wheat rust pathogen species (stem, leaf and stripe rusts) and powdery mildew. The aim of this study was to introduce the resistant allele of this gene by cisgenesis in the durum wheat variety "Svevo", a good Italian variety in terms of quality traits and partially susceptible to stem and yellow rusts, based on previous phenotypic evaluations.

- The cultivar Svevo, following Sanger sequencing, showed the susceptible allele at the *Lr67* locus on chromosomes 4A and 4B. Three biolistic transformation experiments were carried out using a "minimal gene cassette" consisting of linear DNA fragments containing the promoter, the coding sequence and the terminator of the *LR67* gene, and without any herbicide or antibiotic resistance marker genes.

Transformation experiment	1 (July 2019)	2 (December 2019)	3 (May 2020)
N° isolated embryos	7121	6700	8210
N° induced calli	2627	2545	1345
% calli induction	37	38	16.4
N° bombarded calli	2536	2495	1345
N° regenerated plants	1123	40	109
% regeneration	44	1.6	8.1

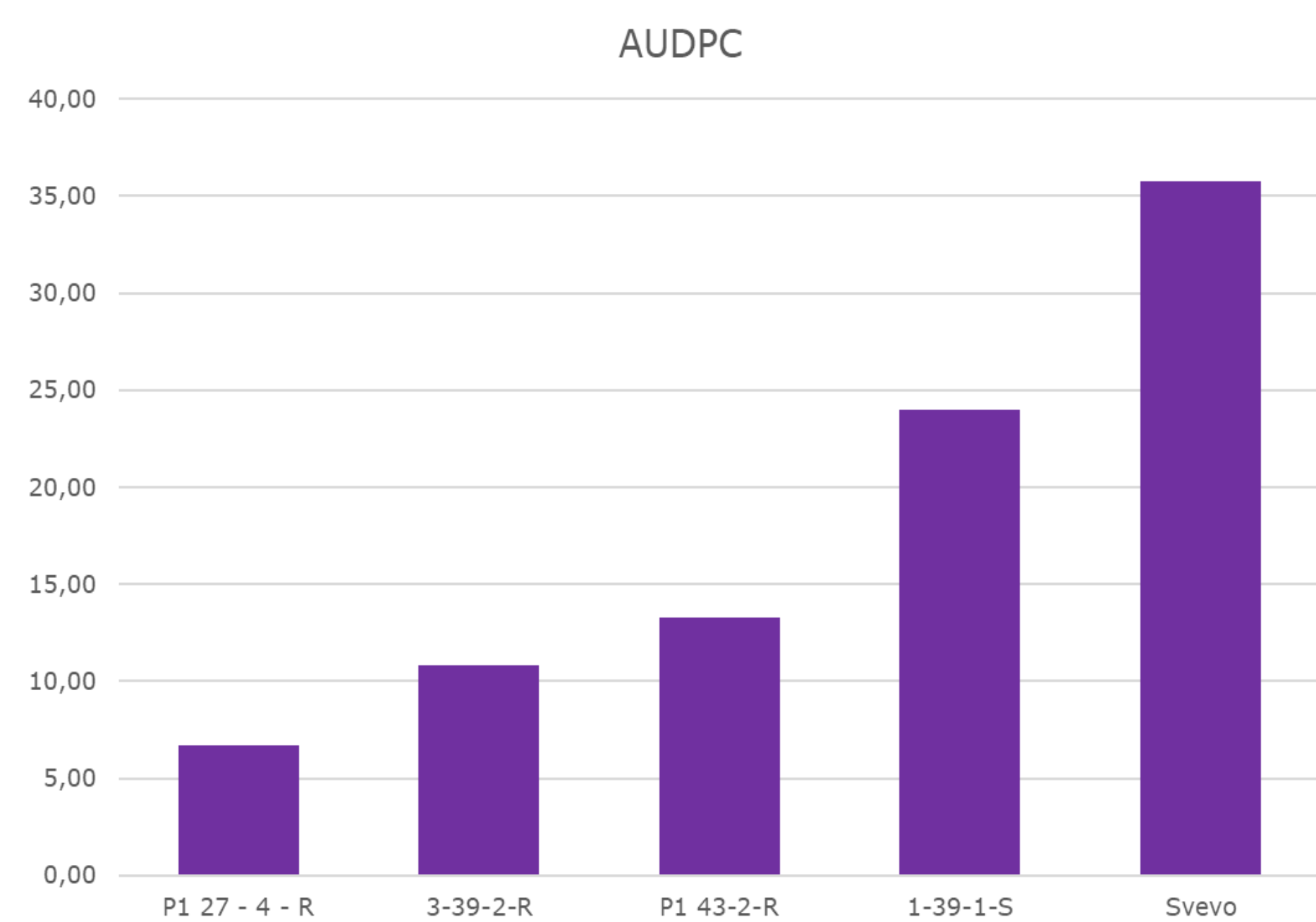


In the figure are reported the amplification patterns of the cisgene in some trasformants, and in particular the second lane showed the positive result of a plant successfully transformed.

A total of ten, eight and seven T0 plants were identified from each experiment respectively, as containing the *Lr67* gene by PCR assay with primers designed on the entire cassette (7133 bp).

These lines have been grown up to the next generations, and four of them (2 T2 and 2 T1, named P1-27-4, P1-43, 3-39-2 and 1-39-1) putatively transformed, together with the cv. Svevo, have been considered for a first round of phenotypic evaluation for response against powdery mildew by using natural inoculation, under controlled conditions in growth chamber at the University of Bari.

15-old days seedlings were inoculated by shaking conidia collected from infected plants over the seedling leaves of healthy plants. The inoculated seedlings were maintained in the growth chamber at 20 ± 2 °C, 70–90% relative humidity under 16 h photoperiod with light intensity of approximately 14,000 lux meter and grown for 7 days until evaluation of symptoms. Seedling reaction scoring was detected following to the modified version of Cobb's scale (0–9) as described by Peterson et al. 1948, where 0 means immune and 9 the 90% -100% of leaf surface area covered by mycelia. In particular, plants were considered as following, based on scoring values: Resistant (0-3), moderately resistant (4-6) and susceptible (7-9). The evaluation of symptoms was repeated three times every seven days from the symptom appearance.



In the graph the AUDPC, the area under the disease progress curve, is reported as useful quantitative summary of disease intensity over time, being higher in the wild type.



A total of fifteen plants showed to be resistant from this first phenotypic experiment, therefore grown up to seed production (T2 and T3).

A second phenotypic evaluation for reaction to powdery mildew under natural infection in growth chamber on the most promising lines T2 and T3 obtained during the first experiment was carried out. Fifteen plants for each line were inoculated, by using the same protocol as the first experiment. Data analysis is in course, whereas results on resistance registered on the tested plants based on scoring values are shown in the following table.

Scoring values	0-3	4-6	7-9
N. plants	21	30	19



Actually, the phenotypic evaluation for stem rust reaction (*Puccinia graminis*) by artificial inoculation with the JRCQC race is in course on 7 cisgenic plants and Svevo at the Minnesota University, to confirm the durable resistance also against the fungus *Puccinia graminis*.

CONCLUSIONS

Up to now, T2 and T3 cisgenic plants carrying the resistant allele of the *Lr67* gene conferring durable resistance against multiple fungal diseases are available, on which the resistance phenotype will be further verified. The work will proceed with the search for homozygotes containing and expressing the cisgene.