

**KARTIRANJE LOKUSA ZA KVANTITATIVNO SVOJSTVO (QTLs)  
OTPORNOSTI GRAŠKA (*Pisum sativum* L.) NA *Mycosphaerella pinodes***

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*Mycosphaerella pinodes* je uzročnik nekroze na svim nadzemnim organima graška koja rezultira znatnim gubicima prinosa kao i smanjenjem kakvoće sjemena. Bolest se javlja u većini proizvodnih regija i to gotovo svake godine. Tretiranje usjeva kemijskim sredstvima može smanjiti opseg zaraze i održati visoke prinose, no potrebno je višestruko tretiranje tijekom vegetacije. U svrhu smanjenja troškova proizvodnje i zaštite okoliša, oplemenjivanje otpornih kultivara je važna sastavnica integralne strategije kontrole ove bolesti.

Cilj ovog istraživanja bila je analiza genetskih čimbenika koji utječu na otpornost graška na *M. pinodes*. U tu je svrhu korištena populacija rekombinantnih inbred linija (*Recombinant Inbred Lines - RIL*) F<sub>6</sub> generacije dobivene metodom potomstva jedne sjemenke (*Single-Seed Descent - SSD*) F<sub>2</sub> generacije nastale križanjem linije P-665 *P. sativum* L. ssp. *syriacum* Berg (= *P. humile* Boiss and Nöe) djelomično otporne na *M. pinodes* s osjetljivim kultivarom *P. sativum* L. cv. Messire.

Ukupno 111 rekombinantnih inbred linija je analizirano pomoću 235 biljega RAPD, 1 izoenzimskog biljega, te 12 biljega STS (*Sequence-Tagged Site - STS*). Praćena su i tri morfološka biljega: *a* za spječavanje proizvodnje antocijanina, *d* za nedostatak aksilarnog obojenja i *Pl* za crnu boju hiluma. Otpornost biljaka na *M. pinodes* je analizirana u komorama za rast nakon inokulacije s monospornim izolatom iz Córdoba, Španjolska prikupljenim 2001. godine i ocijenjena na skladi od 0 do 5.

Od ukupno 251 biljega koji su razdvajali u populaciji RIL, njih 246 je kartirano u 9 skupina vezanih gena koje pokrivaju 1214 cM genoma graška. Prosječna udaljenost između susjednih biljega iznosila je 5.84 cM. Šest skupina vezanih gena (I, II, III, IV, VI, and VII) bilo je moguće pridružiti Međunarodnoj dogovornoj genetskoj karti graška (*International pea consensus genetic map*) koristeći devet biljega (3 morfološka i 6 biljega STS) zajedničkih prethodno izrađenim genetskim kartama graška. Nepridružene skupine su imenovane A, B i C.

Prosječni udio genoma majčinskog roditelja (*P. sativum* L. ssp. *syriacum* Berg) u populaciji RIL bio je nešto manji (43%) od udjela očinskog roditelja *P. sativum* L. cv. Messire. Od 251 analizirana biljega, 98 (39%) je pokazalo

odstupajuće razdvajanje na razini  $p < 0.05$ . Čak 95% odstupajućih biljega pokazalo je veći udio alela cv. Messire, a samo njih 5% je imalo veći udio alela ssp. *syriacum*. Odstupajući su biljezi uočeni u svim skupinama vezanih gena osim u skupini C. No, znatno grupiranje odstupajućih biljega primijećeno je u skupinama III i VI.

Jednostavno intervalno kartiranje (*Simple Interval Mapping - SIM*) i Sastavljeno intervalno kartiranje (*Composite Interval Mapping - CIM*) je provedeno pomoću programa Windows QTL Cartographer. Četiri QTL-a (*mp1*, *mp2*, *mp3*, and *mp4*) povezana s otpornošću na *M. pinodes* je lokalizirano u skupinama A, III, II i III. Ovi QTL-i zajedno objašnjavaju 68% fenotipske varijance ovog svojstva. Aleli koji povećavaju otpornost na *M. pinodes* naslijeđeni su od otpornog roditelja P-665 osim QTL-a *mp3* u skupini II u slučaju kojeg je donor alela koji povećavaju otpornost bio osjetljivi roditelj - kultivar Messire.

Ključne riječi: *Pisum sativum* L., *Mycosphaerella pinodes*, otpornost na bolesti, genetsko kartiranje, lokusi za kvantitativna svojstva

**MAPPING OF QUANTITATIVE TRAIT LOCI (QTLs) FOR CONTROLLING  
*Mycosphaerella pinodes* RESISTANCE IN PEA (*Pisum sativum* L.)**

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*Mycosphaerella pinodes* causes necrotic spots on all aerial parts of the pea plant and is responsible for important yield and seed quality losses. It is found in most of the pea-growing areas of the world almost every year. While chemical treatments can reduce disease severity and preserve pea yields, multiple sprays are often needed during the growing season. To decrease production costs and to better preserve the environment, the development of resistant cultivars is therefore an important component in an integrated strategy to control *M. pinodes*.

The objective of this study was to gain a better knowledge of the genetic factors controlling pea resistance to *M. pinodes*. The mapping population used was a F<sub>2</sub>-derived F<sub>6</sub> recombinant inbred lines (RIL) population developed by single-seed descent (SSD) from the cross between *P. sativum* L. ssp. *syriacum* Berg (= *P. humile* Boiss and Nöe) line P-665 partially resistant to *M. pinodes* and the susceptible cultivar *P. sativum* L. cv. Messire.

A total of 111 F<sub>2:6</sub>-derived RILs were analysed using 235 RAPD markers, 1 isozyme, and 12 sequence-tagged site (STS) markers. Three morphological markers were scored: *a* for absence of anthocyanin production, *d* for disappearance of axil colour, and *Pl* for black hilum colour. Resistance scoring was carried out in the growth chamber after inoculation with a single monospore isolate from Córdoba, Spain collected in 2001. Disease severity was scored on a 0-5 scale.

Of the 251 marker loci segregating in the RIL population, 246 could be mapped into 9 linkage groups covering 1214 cM of the pea genome. Mean intermarker distance was 5.84 cM. Six (I, II, III, IV, VI, and VII) out of nine linkage groups (LG) were assigned to the international pea consensus genetic map using nine markers (3 morphological and 6 STS markers) common to other pea genetic maps previously described in the literature. Unassigned groups were named A, B, and C.

The average genome proportion of female parent (*P. sativum* L. ssp. *syriacum* Berg) in the RIL population was slightly lower (43 %) than that of the male parent *P. sativum* L. cv. Messire. Among the 251 markers, 98 (39%)

showed distorted segregation at  $p < 0.05$  level. Up to 95% of the distorted segregation markers were toward cv. Messire alleles, and only 5% of them were toward ssp. *syriacum* alleles. Markers showing segregation distortion were found on all linkage groups, except LG C. Nevertheless, clusters of distorted markers were identified especially on linkage groups III and VI.

Simple Interval Mapping (*SIM*) and Composite Interval Mapping (*CIM*) were performed using Windows QTL Cartographer. Four QTLs (*mp1*, *mp2*, *mp3*, and *mp4*) associated with *M. pinodes* resistance were localized on linkage groups A, III, II, and III, respectively. Altogether, the QTLs accounted for 68% of the total phenotypic variance of the resistance score. Resistance alleles originated from the partially resistance parent P-665, except for the QTL *mp3* on LG II in case of which the resistance alleles were donated by the susceptible parent, cv. Messire.

Key words: *Pisum sativum* L., *Mycosphaerella pinodes*, disease resistance, genetic mapping, quantitative trait loci