Molekulaarsete ja koekultuurimeetodite rakendamine sordiaretuses ja taimse materjali analüüsis

EV Põllumajandusministeeriumi rakendusuuring

Koondaruanne

Projekti algus: 04.09.2003
Projekti lõpp: 31.12.2007

Projekti juhid:

Erkki Truve, professor, Tallinna Tehnikaülikooli geenitehnoloogia instituudi geenitehnoloogia õppetooli juhataja

Kadri Järve, bioloogiakandidaat, Tallinna Tehnikaülikooli geenitehnoloogia instituudi geenitehnoloogia õppetooli vanemteadur

Tallinn 2008

Sisukord

Tabelid:

Joonised:

Lisad:

Lisa 1. Irena Jakobson, Hilma Peusha, Ljudmilla Timofejeva, Kadri Järve. 2006. Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum / Triticum militinae* hybrid line. *Theor Appl Genet* 112: 760–769.

Lisa 2. Jelena Tsõmbalova, Merlin Haljak, Anne Ingver, Kadri Järve. 2008. Genetic diversity of Estonian-grown spring wheat varieties assessed by microsatellite and morphological analyses. *Acta Agriculturae Scandinavi ca Section B - Soil and Plant Science*: 58: 97-104.

Lisa 3. Hilma Peusha, Tamara Enno, Irena Jakobson, Jelena Tsõmbalova, Anne Ingver, Kadri Järve. Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia. Acta Agriculturae Scandinavica Section *B – Soil and Plant Science*, avaldamiseks vastu võetud 26.09.2007

Lisa 4. Topelthaploidsete nisutaimede saamine. Katseprotokoll

Lisa 5. Mikrosatelliitjärjestuste amplifitseerimine nisu genoomist. Protokoll

Sissejuhatus

Viimasel aastakümnel on rahvusvaheliselt aksepteeritaval tasemel toimuvas põllukultuuride aretuses kasutusele võetud molekulaarbioloogilised ja koekultuuri-meetodid. Seejuures on metoodiline areng olnud väga kiire ja nimetatud meetodite rakendused on viimasel 5-10 aastal muutunud maailma selektsioonilaborites rutiinseteks. TTÜ geenitehnoloogia instituudi geenitehnoloogia õppetooli (kuni 2005. a. – EPMÜ EBI taimegeneetika osakonna) plaanilises taimegeneetikaalases teadustöös olid projekti alustamise ajal ja on ka praegu kasutusel mitmed tänapäevases selektsioonitöös kasutatavad meetodid ning seega olid olemas võimalused nende rakendamiseks Jõgeva Sordiaretuse Instituudis. Jõgeva Sordiaretuse Instituut oli oma aretustegevuses nii käesoleva projekti täitja kui ka projekti tulemuste lõpp-tarbija.

Selektsioonialase töö kaasajastamisel omavad otsustavat tähtsust, esiteks, gameetilise embrüogeneesi meetodid, mis võimaldavad geneetiliselt ühetaolise materjali kiiret saamist (nn. topelthaploidide saamise meetod) ja teiseks, määrava tähtsusega komponendiks tänapäevastes selektsioonisüsteemides on molekulaarsete markerite kasutamine (molekulaarsed meetodid selektsioonis).

Molekulaarsete markerite kasutamisel kiireneb oluliselt selektsioonimaterjali valik ning on garanteeritud selektsioonikäigu kontroll. Aretuses rakendatava markeranalüüsi eelduseks on ristlusvanemaid iseloomustavate polümorfsete (vanematel erinevate) alleelidega markerite olemasolu. Kõikide põllumajanduslikult oluliste liikide genoomikaartidele lisatakse igal aastal sadu DNA-markereid, mis on kasutatavad fenotüübi erinevate omaduste (saagi kvaliteet, morfoloogilised tunnused, haiguskindlus) kiireks määramiseks. Kuigi fenotüübilise tunnusega seotud markeri tuvastamine on keeruka ja pikaajalise geneetilise analüüsi lõpptulemus, on need markerid tavaliselt vabalt kasutatavad (st ei ole seotud patendipiiranguga).

Molekulaarsed markerid on kasutatavad ka sortide ja liinide identifitseerimiseks. Piisav arv polümorfsete molekulaarsete markerite alleelide kirjeldusi iseloomustab üheselt antud sorti või aretusliini, st. taime

identifitseerimiseks tuleb tema genoomis määrata teatud arvu markerite alleelid (genotüpiseerimine). Näiteks, väga polümorfsete mikrosateliitmarkerite kasutamisel on Eesti sordilehe suvinisusortide identifitseerimiseks vaja määrata markerite alleelid 2-4 lookuses (vt Tabel 4). Genotüpiseerimine (e. taimse materjali genoomne kaardistamine) on laia rakendusulatusega meetod taimse materjali analüüsiks, mis lisaks selektsioonile on rakendatav ka sordiehtsuse kontrollis ning sortide sertifitseerimisel.

Fenotüübipõhine valik ja hindamine morfoloogiliste tunnuste põhjal, mis oli Eestis ainsa kriteeriumina kasutusel nii sordiaretuses kui ka sordipuhtuse ja -ehtsuse hindamisel, ei võimalda taimse materjali identifitseerimist tänapäeval nõutaval tasemel. Seni Eestis kasutusel olevatest fenotüübil põhinevatest sordi- ja taimekirjelduse meetoditest tuleb kaasaegseimaks lugeda teraviljade varu-valkude fraktsioneerimisel saadavate nö varuvalguspektrite põhjal koostatud sordiiseloomustusi. Dr. M. Tohver (EBI, hiljem GTI vanemteadur, praegu pensionär) on koostanud valguspektrid põhjamaades ja Eestis kultiveeritavate teraviljasortide identifitseerimiseks (123 tali- ja 106 suvinisusorti, publitseeritud *Genetic Resources and Crop Evolution* (2007) 54:67–81). Sordiomaduste hindamine varuvalkude elektroforeetiliste spektrite alusel on on rakendatud kõigis Euroopa Liidu maades ja lähiaastatel muutub varuvalkude spekter ilmselt EL piires kohustuslikuks teraviljade sordipassi koostisosaks. Samas ei saa pidada soovitavaks selle meetodi rakendamist teraviljade sordipuhtuse määramisel ning sortide identifitseerimisel, sest saadavat identifitseerimistäpsust ei saa hinnata kõrgemaks kui 60-70% .

UPOVi (Union for the Protection of New Varieties of Plants) bioloogiliste ja molekulaarsete tehnoloogiate töörühmas on välja töötatud genotüübi analüüsil põhinev sortide identifitseerimise süsteem. DNA järjestuses leiduvate erinevuste määramine võimaldab taimse materjali (ühe taime või identsete taimede populatsiooni - sordi) identifitseerimist praktiliselt 100%-lise täpsusega (genotüübi identifitseerimise tõenäosus läheneb 100%-le uuritavate lookuste arvu kasvades). Analüüsitavate markerite (tunnuste) arv ei ole piiratud, analüüsi

lõpptulemuseks võib olla ka taime (populatsiooni) enam või vähem täpne genoomikaart. Viimaste aastate arengus on suhteliselt aeganõudvatele RFLP ja AFLP analüüsimeetoditele lisandunud DNA mikrosatelliitjärjestuste polümorfismi analüüsi meetod, mis on kiire, suhteliselt odav ning võimaldab kasutada genotüübi analüüsi praktikas rakendavates testides.

Projekti „Molekulaarsete ja koekultuurimeetodite rakendamine sordiaretuses ja taimse materjali analüüsis“ eesmärgiks oli rakendada Eestis kaasaegsel tasemel meetodid taimse materjali geneetilise analüüsiks. Projekt oli koostatud neljaks aastaks, projektis osalesid Tallinna Tehnikaülikooli Geenitehnoloogia Instituut (edasi GTI), Eesti Põllumajandusülikooli Eksperimentaalbioloogia Instituut (edasi EBI) ja Jõgeva Sordiaretuse Instituut (edasi SAI).

Projekti rakendamisega alustati 2004. a. septembris.

Projekti täitmisel saadud tulemused on publitseeritud kolmes rahvusvahelises eelretsenseeritavas ISI poolt refereeritavas ajakirjas avaldatud artklis:

1. Irena Jakobson, Hilma Peusha, Ljudmilla Timofejeva, Kadri Järve. 2006. Adult plant and seedling resistance to powdery mildew in a Triticum aestivum · Triticum militinae hybrid line. *Theor Appl Genet* 112: 760–769.
2. Jelena Tsõmbalova, Merlin Haljak, Anne Ingver, Kadri Järve. 2008. Genetic diversity of Estonian-grown spring wheat varieties assessed by microsatellite and morphological analyses. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*: 58: 97-104.
3. Hilma Peusha, Tamara Enno, Irena Jakobson, Jelena Tsõmbalova, Anne Ingver, Kadri Järve. Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia. Acta Agriculturae Scandinavica Section *B - Soil and Plant Science*, avaldamiseks vastu võetud 26.09.2007.

Kõikides publikatsioonides on viidatud Põllumajandusministeeriumi finantsosalusele. Publikatsioonid on käesolevale aruandele lisatud (Lisad 1-3).

Järgnevas esitatakse projekti täitmisel saadud tulemused.

1. Koekultuurimeetodi rakendamine aretuses (topelthaploidsete taimede saamine)

Ristamiste esimeste põlvkondade heterosügootsetest taimedest homosügootsete järglaste saamine koekultuurimeetodil kiirendab oluliselt aretustööd, võimaldades uue sordi väljatöötamist 5-6 aastaga. Koekultuurimeetodi rakendamine on eriti oluline juhul, kui ristamise tulemusena tahetakse ühelt sordilt (genotüübilt) teisele üle kanda mitut geeni, st kui soovitav fenotüüp tekib mitme erineva geeni koostoimel. Sellisel juhul ei ole homosügootse materjali saamine klassikaliste meetodite abil mitte ainult aegavõttev, vaid sageli ka praktiliselt võimatu.

Topelthaploidide meetodi kohaselt saadakse esimese põlvkonna taimede viljastamata mikrospoore indutseerides haploidne kallus, mille regenereerimisel saadavaid haploidsed taimed spontaanselt diploidiseeruvad - tulemuseks on homosügootne diploidne taim. Kirjeldatud metoodika on liigi- ja sordispetsiifiline, ning vajab objektile vastavat tehniliste tingimuste kohandamist.

Käesoleva projekti käigus asutati meetodi suuremahuliseks kasutamiseks EBI koekultuuri labori baasil JSIs koekultuuri labor (2003-2004), koolitati JSI töötajaid meetodi kasutamise alal (2004-2006), modifitseeriti meetodit vastavalt selektsioonis kasutatavatele nisu genotüüpidele (2003-2007). Meetodi rakendamisel oli abiks osalemine Euroopa komisjoni COST programmis 851 "*Gametic cells and molecular improvement for crop improvement*" (2003-2006).

Projekti alustamise ajal oli EBI-s rakendatud töötav metoodika suvinisu Tähti topelthaploidide regenereerimiseks mikrospooride meetodil. Topelthaploidsete taimede saamise metoodikat kirjeldab aruandele lisatud katseprotokoll (vt Lisa 4). Metoodikat modifitseeriti nii, et ta sobiks enamikule aretuses olevatest nisu genotüüpidest. Lisas 4 esitatud embrügeneesi + regeneratsiooni metoodika võimaldas Jõgeva aretiste 524 F_4 T.*timopheevii* x *Täht*i x *T.timopheevii* // *Meri*, 478 F_4 *Raisa/Mahti* ja 404 F_4 *Boj10103/N226* puhul suure saagisega indutseerida embrüogenees ning samuti kõrge sagedusega regenereerida embrüod taimedeks. Hilisemal rakendamisel JSAIs osutus metoodika sobivaks suure valiku suvinisugenotüüpide puhul.

Paralleelselt tegeldi topelthaploidide saamiseks vajalike taimede optimaalsete ettekasvatusreziimide väljatöötamisega Jõgeva SAI-s ning topelthaploidide masstootmiseks vajaliku laboratooriumi käivitamisega. 2003-2004. a. oli EBIs ligikaudu 3-kuulisel väljaõppel Irina Puzõrjova, Tartu Ülikooli bakalaureuseõppe lõpetanud geenitehnoloog, kelle edasiseks tööülesandeks oli meetodi praktiline rakendamine SAIs. Jõgeva SAI topelthaploidide tootmise labori jaoks koostati vajalike tehniliste tingimuste ja laboratoorse varustuse nimekiri, katseprotokoll koos vajalike koekultuurisöötmete retseptidega, vajalike reagentide nimekiri (koos neile esitatavate kvaliteedinõuetega ja nende kulu kalkulatsiooniga). Eelpoonimetatud materjalid esitatati koos 2004.a. aruandega. 2007. a. viibis GTIs väljaõppel uus SAI töötaja Terje Põvvat (TÜ bioloogia bakalaureus), kes asendab nüüd SAIst lahkunud I. Puzõrjovat.

Topelthaploidide meetod on kasutatav enamiku põllumajanduskultuuride aretuses: projektikohaselt alustati rakendust suvinisu topelthaploidide saamisega, praegu rakendatakse Jõgeval topelthaploidide meetodit odrale.

Kõikide suvinisugenotüüpide puhul on probleemiks väga suur albinootiliste taimede osakaal. COST 851 töökoosolekul Palermos 2004.a. novembris (meilt osalesid Järve ja Tiidema), oli albinootiliste taimede protsendi vähendamine üks olulisemaid arutlusobjekte, kahjuks on probleemi lahendamine endiselt empiirika tasemel ning metoodikate varieerimise katseid jätkatakse.

2. Molekulaarsete markerite kasutamine Eesti suvi- ja talinisusortide ja aretiste analüüsis

1. 2004. aastal analüüsiti 12 Eesti Sordilehele kantud **suvinisu**sorti: *Baldus, Helle, Mahti, Manu, Meri, Munk, Satu, SWEstrad, Zebra, Tjalve, Triso* ja *Vinjett* (vt Tabel 1). Analüüsitavate sortide seemned saadi Jõgeva Sordiaretuse Instituudist.

Analüüsi läbiviimiseks valiti eelnevatalt uuritud mikrosatelliitmarkerid. Kuna nisu polümorfsuse tase ei ole eriti kõrge, siis võeti käesolevassse analüüsi need markerid, mis meie laboris saadud andmetel olid polümorfsed genotüüpides *Chinese Spring, Opata, Tähti* ja nisuliinis 146-155. Valitud markerite nimekiri on

toodud Tabelis 2, markerid on välja töötatud kas dr M.Röderi poolt Gaterslebenis Saksamaal (tähistuses *gwm*) või dr P.Stephensoni poolt Norwich'is, UK (tähistuses *PSP*). Kõikide mikrosatelliitjärjestuste amplifitseerimisel kasutatud praimerite järjestused on publitseeritud ja oligonukleotiidid telliti firmalt *DNA-Technology* A/S (Taani).

Esialgu väljavalitud praimeritest osutusid 5 praimeripaari monomorfseteks, nende produktide edasine analüüs katkestati. Kokku jäi analüüsi 37 praimeripaari, mis amplifitseerisid 44 lookuses kaardistatud mikrosatelliitjärjestusi. Pärast veelkordset vähepolümersete lookuste eemaldamist jäi analüüsi 39 lookust.

Taimed kasvatati kasvuhoones pottides, igast sordist 5 taime. 10-päevastelt idanditelt lõigati osa lehti jahukastekindluse määramiseks ning taimi kasvatati edasi kuni ühe kuu vanuseni (ainult DNA eraldamiseks oleks piisanud ka 10 päevast). Igast sordi viie taime lehtedest (ca 1,5 grammi) eraldati summaarne genoomne DNA (vt Lsa 5) ning PCR-amplifitseeriti eraldi kõigi praimeripaaridega (multipleks-PCR võimalusi ei proovitud). Saadavad produktid märgistati ^{32}P-dCTP lisamisega reaktsioonisegusse ning lahutati polüakrüülamiidgeelil. Autograafiliselt visualiseeritud produktid registreeriti arvutis programmi *CrossChecker* vers. 2.91 (J.Buntjer, Wageningen'i Ülikool, Holland) abil. Fülogeneetilised seosed arvutati programmi *Phylip* (J. Felsenstein, Washingtoni Ülikool) abil.

Kõiki 12 Sordilehe suvinisugenotüüpi on nende segus võimalik omavahel eristada kasutades maksimaalselt 4 valitud markerit Seitsme sordi jaoks (*Baldus, Mahti, Manu, Munk, Satu, Zebra* ja *Triso*) leiti sordispetsiifiline markerialleel, mis võimaldab konkreetse sordi taimi identifitseerida ühe markeri abil. Viie sordi puhul on vaja kahte markerit (*Helle, Meri, SWEstrad, Tjalve* ja *Vinjett,* vt Tabel 4).

Markerite valik sõltub konkreetsest ülesandest: kas on vaja eristada (tõestada) ühe konkreetse sordi olemasolu (puudumist) uuritavate taimede hulgas, või on vaja identifitseerida kõigi taimede genotüübid. Igal juhul on leitud markereid võimalik kasutada suuremastaabilises analüüsis, näiteks sordiehtsuse või –puhtuse kontrollis. Meetodi võib rakendada Põllumajandusuuringute Keskuses.

Metoodiliselt võiks kaaluda multipleks-PCR (mitu reaktsiooni koos ühes tuubis) väljatöötamist – aga seda juhul, kui tekib vajadus suure arvu analüüside järele.

Eesti Sordilehe markeranalüüsi tulemused on kokkuvõtvalt esitatud Joonistel 1-3.

Eesti Sordilehel olevate suvinisusortidest kuulub enamus nn Põhjamaade sordigruppi (vt Tabel 3), mille sordid on saadud suhteliselt piiratud hulga lähtesortide erinevate ristluskombinatsioonide tulemusena – need sordid on geneetiliselt sarnased. Enamiku Eesti Sordilehe sortide sugupuu on teada.

Võrdlemaks sortide identifitseerimiseks ja sertifitseerimiseks kasutatavat UPOVi 24 morfoloogilise tunnuse süsteemi ja meie poolt analüüsitud molekulaarsete markerite abil saadud tulemuste vastavust juba teadaolevate sugupuu-andmetega, koostasime me nn. fülogeneetilised puud nii molekulaarse markeranalüüsi (Joonis 4A) kui ka morfoloogiliste andmete (Joonis 4B) põhjal. Morfoloogilised tunnused määras Jõgeval magistrant Merlin Haljak.

Jooniselt 4 on näha, et markeranalüüsi tulemused korreleeluvad sortide sugupuude andmestikuga tunduvalt paremini kui morfoloogilise analüüsi tulemused. (’Helle’ on saadud sordist ’Satu’, ’Estrad’ on saadud sordist ’Vinjett’ jne, võrdle Tabeliga 3).

2. Lisaks Sordilehe genotüüpidele analüüsiti samu lookusi Jõgeva Sordiaretuse Instituudi **aretusliinides** (vt Tabel 1). Määrati seitsme töös oleva suvinisuaretise (F_8–F_{10}) alleelne koostis 41 lookuses. Aretiste alleelset koostist võrreldi vanemate alleelse koostisega (vt. Joonis 5). Näidati, et ka sellisel juhul, kui aretise mõlemaks vanemaks on suhteliselt sarnased Põhjamaade päritoluga sordid, on 50,9 % markeritest siiski informatiivsed, st on aretise vanematel erinevad. Kui üks vanematest oli Saksamaal aretatud sort ’Munk’, siis olid informatiivsed 68,5% analüüsitud markeritest. Markeranalüüsi abil demonstreeriti, et isegi aretiste F_8–F_{10} põlvkondades on veel kuni 40% geneetilisest materjalist heterogeenses seisundis – mis demonstreerib seda, et klassikaliste aretusmeetodite rakendamisel kulub mittelahkneva, stabiilse sordi samiseks vähemalt 12 põlvkonna valikut ja paljundamist. Aretiste markeranalüüsi oota-

matuks tulemuseks oli mittevanemlike alleelide leidmine kahes uuritud liinis. Kõik analüüsitud liinid olid saadud nn *"bulk selection method"*i abil, kus kaks korda mindi tagasi ühe nisupeani (F_1 ja F_4). Kuna nisupead ei olnud ristamisele järgnevates põlvkondades õitsemise ajal isoleeritud, siis tuleb järeldada, et katselappidel on toimunud nisu jaoks harvaesinev risttolmlemine ja „võõrlookused" kinnistati genoomis järgneva valikuga nendele lookustele lähedaste „positiivsete" tunnuste järgi. Nisu risttolmlemist on kirjanduses varem kirjeldatud kui nähtust, mis sõltub erinevate sortide (liinide) kasvukohtade vahelisest kaugusest (mitte rohkem kui 30 m). Tulenevalt geneetiliselt modifitseeritud nisu sortide võimalikust kommertsiaalsest levikust lähitulevikus, tuleks nisu risttolmlemise võimalikkust arvatavasti täpsemalt uurida.

Suvinisusortide ja –aretiste markeranalüüsi tulemused on publitseeritud (vt. Lisa 2).

3. 2007. a. analüüsiti 20 mikrosatelliitmarkeri abil 17 Eesti Sordilehe **talinisu**sorti ja täiendavalt kaheksat suvinisusorti (vt. Tabel 1), seega oli lõplikult analüüsis 40 sorti (12+8 suvinisusorti + 17 talinisusorti + 3 kontrollgenotüüpi). Analüüsiks kasutatud 20 markerit valiti 2006. a. analüüsitud suvinisugenotüüpide valimis polümorfsete markerite seast. Analüüsitud 20 markeri seast valiti omakorda 14 uues valimis (40 sorti) polümorfsemat markerit, mis eristasid kõiki 40 sorti nagu on näha sortide omavaheliste geneetiliste kauguste dendrogrammil (vt Joonis 6). Seejärel valiti analüüsitud markerite seast minimaalne kombinatsioon, mis võimaldab eristada uuritavaid 40 sorti. Selleks on vaja, sõltuvalt püstitatud ülesandest, kolme kuni nelja järjestikust PCR-reaktsiooni.

Töötati välja strateegia identifitseerivate alleelide odavamaks ja kiiremaks määramiseks, seda strateegiat on võimalik rakendada ka teiste kultuuride puhul. Põllumajandusuuringute Keskuse jaoks valmib 2008. aasta esimesel poolel metoodiline juhend nisusortide identifitseerimiseks molekulaarsete markerite abil, kus on toodud täpsed juhendid kasutatavate markerite, reaktsioonitingimuste ja reaktsiooniproduktide analüüsi jaoks. Praktikas kasutamiseks ettenähtud metoodikas ei kasutata analüüsil radioaktiivse P^{32} –ga märgistamist, samuti ei ole Põllumajandusuuringute Keskusel vaja täiendavat aparatuuri.

4. Markeranalüüsi kasutamine **jahukastekindlusgeeni *Pm3* alleelid**e identifitseerimiseks Eesti Sordilehe suvi- ja talinisusortides.

2006. aastal publitseeriti esimesed nö funktsionaalsed markerid nisu resistentsusgeeni määramiseks (Yahiaoui et al.,The Plant Journal (2006) 47, 85–98; Tommasini et al., Theor Appl Genet (2006) 114:165–175). Need markerid võimaldavad geeni järjestuses leiduvate erisuste järgi identifitseerida geeni *Pm3* seitset alleeli. Me analüüsisime projektis uuritavaid 40 nisu genotüüpi ja tuvastasime alleeli *Pm3d* olemasolu viies suvinisusordis (*Munk, Estrad, Zebra, Vinjett, Tjalve*; vt tabel 5) ja kahes talinisusordis (*Vergas, Portal*). Lisaks leidsime alleeli *Pm3f* suvinisusortides *Helle, Satu, Polkka* ja *Laari*. *Pm3d* alleel on Euroopa nisusortides küllalt levinud, ja seda alleeli peetakse veel mõningal määral toimivaks Euroopas leviva jahukastetekitaja populatsioonide suhtes. Üllatuseks oli alleeli *Pm3f* esinemine Soome päritolu sortides – see alleel on Euroopa sortides haruldane. Jahukastetekitaja Eesti populatsiooni suhtes on mõlemad leitud *Pm3* geeni alleelid väheefektiivsed (vt Tabel 11).

5. Projekti raames võeti töörühmas kasutusele identifitseerimata genotüüpide võrdlemisel rakendatav **AFLP** meetod (amplicication fragment length polymorphism- amplifitseeritud fragmentide polümorfismi analüüs), mis lisatuna juba rakendatud mikrosatelliitanalüüsi ja RFLP meetodile laiendab taimese materjali genotüpiseerimise baasi. Meetodi rakendamisega olid seotud Olga Romantsova (2005) ja Helle Sadama (2006) magistritööd (juhendaja biol. kand. K.Järve)

6. Projekti raames kaasajastati seni äärmiselt piiratud võimalusega arvutustehnikat ning rakendati genotüpiseerimisel **arvutianalüüs**iks vajalikud tarkvaraprogrammid *Mapmaker, MapmakerQTL*, (tasuta teaduslik tarkvara).

Jahukastekindla hübriidnisu 8/1 geneetiline analüüs

Pehme nisu sordi Tähti ja tetraploidse timofejevi rühma nisu *Triticum militinae* ristamisel saadud järglakonnast valiti hübriidliinid, mida iseloomustas täiskasvanud taime suurenenud resistentsus jahukaste suhtes. Parimal neist

liinidest (liin 8/1) on jahukastekindlus on viis kuni seitse korda suurem kui lähtesordil (Joonis 7). Mikrosatelliitmarkerite abil määrati hübriidliini 8/1 genoomis translokatsioonid *T. militinae*'lt, mis moodustavad ligikaudu 20% hübriidliini genoomist. Jahukastekindluse kui kvantitatiivse tunnuse (QTL) lahknemise suhtes analüüsiti ühest seemnest lähtuvat F_2 kaardistamispopulatsiooni, mis oli saadud liini 8/1 tagasiristamisel sordiga Tähti (Joonis 8). 4A kromosoomi pikemal õlal leiti QTL, mis vastutab kuni 54% ulatuses täiskasvanud taime resistentsuse eest ja mis on seotud *Xgwm160* lookusega *Triticum militinae'* translokatsioonil (Joonised 9, 10). Sama translokatsioon mõjutab idandi jahukastekindlust pärast taime inokuleerimist jahukastetekitaja *Blumeria graminis* DC. f. sp. *tritici* sünteetilise populatsiooniga ja määrab 28-33% sellest fenotüübilisest tunnusest. Hübriiliini 8/1 analüüsi senikirjeldatud osa on publitseeritud (vt Lisa 1).

Koekultuurimeetodi abil saadi 2005-2006. a. kaardistamispopulatsiooni haigus-kindlatest järglastest 121 erineva genotüübiga topelthaploidset (DH) taime. Eesmärgiks oli 1) valida homosügootsed suvinisuliinid, mille genoom sisaldab jahukastekindlust tagavaid lookuseid minimaalse (või erineva) koguse resistentsusdoonorilt ülekantud geneetilise materjali taustal – selleks, et kasutada neid liine molekulaarse markeriga kontrollitavas (marker-assisted) haiguskindla nisu selektsioonis, 2) kasutada DH populatsiooni resistentsuse edasiseks geneetiliseks analüüsiks ja 3) saada lähtematerjal 4A kromosoomil lokaliseeritud, resistentsuse tekkes otsustavat rolli omava geeni kloneerimiseks. 2007. a. lõpuks oli DH populatsiooni kaardistamine mikrosatelliitmarkerite abil lõpetatud (vt Tabel 6).

Jõgeva Sordiaretuse Instituudis hinnati 2007. a. põldkatsetes DH populatsiooni taimede valiku (63 erinevat genotüüpi) resistentsust jahukastetekitaja (*Blumeria graminis ssp. tritici*), roostetekitajate (*Puccinia graminis, Puccinia recondita*), septooriate (*Septoria nodorum, Septoria tritici*) ja DTR (*Drechslera tritici-repen-tis,* sünonüüm*: Pyrenophora tritici-repentis*) suhtes. Populatsioonis leiti lisaks jahukastekindlatele genotüüpidele ka leherooste ja DTR-resistentseid taimi, kõik taimed olid kõrreroostekindlad (Tabel 7).

Laborikatsetes hinnati kaheksa erineva jahukastetekitajaseene isolaadi mõju DH populatsiooni taimede idanditele (Tabel 8) ja arvutianalüüsi (*QTL mapping*) abil tuvastati, et sõltumatult isolaadist osaleb analüüsitavas jahukastekindlas hübriidliinis reistentsuse tekkes peamiselt kolm lookust. Põhiosa tunnusest oli määratud sama lookuse poolt 4A kromosoomil, mille roll oli suurim ka täiskasvanud taimede jahukastekindluse tagamises.

Ettevalmistamisel on käsikiri hübriidliini 8/1 jahukastekindlusest ontogeneesi varases staadiumis ja selle sõltumisest patogeeni isolaadist (avaldamiseks ajakirjas *Theoretical and Applied Genetics*).

2006. aastal alustati haiguskindlate homosügootsete liinide lülitamist praktilisse aretusse (ristamised sortidega Vinjett ja Manu, 2008. a. ka Luja), kusjuures aretuse praktikas rakendatakse esmakordselt Eestis markeranalüüsi ja koekultuurimeetodit topelthaploidide saamiseks.

Eesti sordilehe suvinisusortide jahukastekindlus

Andmeid Eestis kasvatatavate suvinisusortides esinevate jahukastekindlusgeeni-de kohta analüüsiti viimati 1997. aastal.

Jõgeva Sordiaretuse Instituudis hinnati jahukastekindlust põldkatsetes aastatel 2000- 2007 (vt Tabel 9). TTÜ taimegeneetika töögrupp hindas Eesti Sordilehe suvinisusortide jahukastekindlust patogeeni testisolaatide suhtes (vt Tabel 10) , määras molekulaarsete markerite abil geeni *Pm3* alleelide esinemise neis sortides (eelpool kirjeldatud) ning identifitseeris monosoomanalüüsi abil jahukastekindlust tagavad kromosoomid sordis ’Vinjett’ (Tabel 11).

Tuvastati, et kaks geneetiliselt väga sarnast Sordilehe sorti, ’Vinjett’ ja SWEstrad’, on jahukastetekitaja suhtes peaagu immuunsed. Samuti ületavad jahukastekindluse poolest teisi Eestis kasvatatavaid sorte Eesti päritolu sordid ’Helle’ ja ’Meri’. 2007.a. saadi sordi ’Meri’ ristamisel kontrollsordiga ’Opata’ F_1 ja F_2 kaardistamispopulatsioonid, mille abil me kavatseme identifitseerida varem meie poolt 1B kromosoomil lokaliseeritud dominantse resistensusgeeni sordis ’Meri’. Eesmärgiks on leida aretuses kasutatav molekulaarne marker.

Eestis kasvatatavate suvinisusortide jahukastekindlust tagavate geenide kohta saadud andmed summeerib Tabel 12.

Ajakirjale *Acta Agriculturae Scandinavica, Section B* esitati käsikiri Eestis kasvatatavate suvinisusortide jahukastekindlusest, mis võeti avaldamiseks vastu 26.09.2007. (vt käsikiri Lisa 3).

Tabel 1 **Markeranalüüsis uuritud nisugenotüübid**

Jrk		Genotüüp	Päritolu	Seeme saadud
1	suvinisu	Baldus	Holland	Kollektsioon 2002
2	suvinisu	Helle	Soome-Eesti	Kollektsioon 2002
3	suvinisu	Mahti	Soomei	Kollektsioon 2002
4	suvinisu	Manu	Soome	Kollektsioon 2002
5	suvinisu	Meri	Soome-Eesti	Kollektsioon 2002
6	suvinisu	Munk	Saksa	Kollektsioon 2002
7	suvinisu	Satu	Rootsi	Kollektsioon 2002
8	suvinisu	SWEstrad	Rootsi	Kollektsioon 2002
9	suvinisu	Zebra	Rootsi	Kollektsioon 2002
10	suvinisu	Tjalve	Rootsi	Kollektsioon 2002
11	suvinisu	Triso	Saksa	Kollektsioon 2002
12	suvinisu	Vinjett	Rootsi	Kollektsioon 2002
13	suvinisu	6.1.10.3	Eesti	PV2003
14	suvinisu	91060202	Eesti	EV1 2003
15	suvinisu	670101	Eesti	EV2 2003
16	suvinisu	127.1.6	Eesti	JF10 2003
17	suvinisu	143	Eesti	JF10 2003
18	suvinisu	213	Eesti	JF8 2003
19	suvinisu	214	Eesti	JF8 2003
20	suvinisu	231	Eesti	JF8 2003
21	suvinisu	232	Eesti	JF8 2003
22	suvinisu	Opata	Mehhiko	EPMÜ EBI
23	suvinisu	Chinese Spring	Hiina	EPMÜ EBI
24	suvinisu	Tähti	Soome	EPMÜ EBI
25		*Triticum militinae*	Venemaa	K-59942, EPMÜ EBI
26		*Triticum timopheevii*	Venemaa	K-38555, EPMÜ EBI
27	suvinisu	Troll	Rootsi	kollektsioon 2005
28	suvinisu	Dragon	Rootsi	kollektsioon 2005
29	suvinisu	Luja	Soome	kollektsioon 2005
30	suvinisu	Ulla	Soome	kollektsioon 2005
31	suvinisu	Polkka	Rootsi	kollektsioon 2005
32	suvinisu	Monsun	Saksa	kollektsioon 2005
33	suvinisu	Kadrilj	Rootsi	kollektsioon 2005
34	suvinisu	Piccolo	Saksa	kollektsioon 2005
35	talinisu	Ada	Leedu	kollektsioon 2005
36	talinisu	Anthus	Saksa	kollektsioon 2005
37	talinisu	Bill	Saksa	kollektsioon 2005
38	talinisu	Bjorke	Rootsi	kollektsioon 2005
39	talinisu	Compliment	Saksa	kollektsioon 2005
40	talinisu	Flair	Saksa	kollektsioon 2005
41	talinisu	Gunbo	Rootsi	kollektsioon 2005
42	talinisu	Korweta	Poola	kollektsioon 2005
43	talinisu	Lars	Saksa	kollektsioon 2005
44	talinisu	Olivin	Saksa	kollektsioon 2005
45	talinisu	Portal	Saksa	kollektsioon 2005
46	talinisu	Ramiro	Saksa	kollektsioon 2005
47	talinisu	Residence	Holland	kollektsioon 2005
48	talinisu	Sani	Eesti	kollektsioon 2005
49	talinisu	Sirvinta	Leedu	kollektsioon 2005
50	talinisu	Tarso	Saksa	kollektsioon 2005
51	talinisu	Vergas	Saksa	kollektsioon 2005

Vanemad

SatuxTjalve
SatxTjalve
SatuxMunk
ManuxTjalve
SatuxMunk
MunkxMahti
MunkxManu
TjalvexManu
TjalvexMunk

Tabel 2 Kasutatud markerid

gwm337	PSP3030
gwm14	PSP3000
gwm232	PSP3131
gwm635	PSP3027
gwm508	PSP3103
gwm274	PSP3028
gwm33	PSP3058
gwm99	
gwm205	
gwm410	
gwm71	
gwm311	
gwm210	
gwm526	
gwm604	
gwm162	
gwm382	
gwm383	
gwm493	
gwm624	
gwm160	
gwm296	
gwm358	
gwm47	
gwm459	
gwm66	
gwm368	
gmw814	

Tabel 3. Eesti Sordilehe suvinisusortide sugupuud

Sort	Tootja	Aasta	Sugupuu[1]
‘Baldus’	Cebeco Seeds, The Netherlands	1992	‘SICCO’/4/(SEL.)’SICCO’/3/’N-66’/’MGH-653’//’KOLIBRI’ (‘PEKO’)
‘Helle’	Boreal PB/ JPBI; Finland/Estonia	1999	‘SATU’/’POLKKA’;
‘Mahti’ [1]	Boreal PB/Finland	1994	‘CEBECO-1036’ (Neth)/’HJA-20519’ (Fin)
‘Manu’	Boreal PB	1992	‘RUSO’/’RUNAR’
‘Meri’	Boreal PB/ JPBI	1999	‘WW 21220’ x ‘HJA 22058’
‘Munk’	Lochow Petkus, Germany	1993	’RALLE’//(’VO-9-P-78’) ’KOLIBRI’/’SOMARA’/3/’STAR’
‘Satu’	Svalöf Weibull AB, Sweden	1990	‘SNABBE’/’DRABANT’/’T-106’/ ‘SNABBE’;
‘SWEstrad’	Svalöf Weibull AB	2002	‘HANNO’/’HUGIN’/’VINJETT’
‘Zebra’	Svalöf Weibull AB	2001	‘RALLE’/’DRAGON’
‘Tjalve’	Svalöf Weibull AB	1987	‘RENO’ / ‘WW16679’[5] // ‘WW15432’
‘Triso’	Deutsche Saatver-edelung AG Germany	1996	‘KADETT’/’WEIHENSTEPHANER-STAMM’
‘Vinjett’	Svalöf Weibull AB	1998	‘Tjalve M14’/’Tjalve M15’/’Canon’

[1] vastavalt *Wheat Pedigree and Identified Alleles of Genes,* http://genbank.vurv.cz/wheat/pedigree/default.htm

Tabel 4. Mikrosatelliitmarkerite alleelid Eestis kasvatatavate suvinisusortides ja alleelid, mille abil on võimalik neid sorte identifiteerida.

Marker	**Esinevad alleelid (bp)**	**Identifitseeriv alleel**
PSP3027	167, 164, 161	Munk (161), Mahti(167)
gwm382	159, 134, 122, null	Baldus (159), Munk (null)
gwm296	164, 140, 138	Manu (164)
gwm210-2D	190, null	Munk (190)
gwm71-1	120,121,124	
gwm71.2	110	Satu (110)
gwm71-2B	102, null	
gwm99-1A	132(121), 116, 113	
gwm160	218(230), 206(218), 184(197)	
gwm162	216,208,203	
PSP3028	165, 161, 153,143, 133	Zebra (133), Munk (143)
gwm186	132, 126, (102)	
gwm205-5A	160, 170, 158, 150	Munk (150)
gwm459	124, 158, 166, 168, 190	Baldus (190)
gwm570	148, 142, 13, 132	
gwm276	135, 128, 89	Vinjett (128+135)
gwm635	113, 109, 97	Meri (113), Mahti (97)
gwm274-1B	198, 182	
PSP3000	246,232,218,210	
gwm410	342, 335	
gwm493	179, 142	
PSP3030	208, 198	
gwm604	136, 133, 113, 107	Munk (107)
gwm68.2	176,169, 165, 143, 138	Mahti (176), Manu (138)
gwm814	164, 154, 152 146	Baldus(164), Triso (154)
gwm508	140, 142	
gwm274.2	165, 162	
gwm 68.1	212, 203, 184	
gwm232	146, 144, 140	Manu (146)
gwm296.1	189, 182	
PSP3103	188, 182, 178, 166	Zebra (166)
gwm624	153, 148	Zebra (153),
gwm205-5D	147, 144, 142, 140	Triso (140)
PSP3058	189, 186	
PSP3200	170, 166	
33-1A	130, 126, 116	Baldus 126
33.2	181, 175, 169	Meri (169)
gwm526.1	169, 167, 165, 154, 151	Satu (169), Helle (165)
gwm526.2	141+148, 136+148	Zebra (136+148)
gwm526.3	138, 130, 127, na	Zebra (127), Mahti (138)
PSP3131	144, 147	Zebra (147)

Tabel 5 Geeni *Pm3* esinemine Eestis kasvatatavates nisusortides (määratud Yahiaoui et al., 2006 ja Tommasini et al., 2006 järgi)

	UP3B/UP1A	Pm3a	Pm3b/Pm3j	Pm3c/Pm3i	Pm3d/Pm3h	Pm3e	Pm3g	Pm3f
suvinisu								
Baldus	B	-	-	-	-	-	-	-
Helle	AB	-	-	-	-	-	-	+
Manu	-	-	-	-	-	-	-	-
Meri	B	-	-	-	-	-	-	-
Munk*	AB	-	-	-	+	-	-	-
Satu	AB	-	-	-	-	-	-	+
SWEstrad	AB	-	-	-	+	-	-	-
Zebra	AB	-	-	-	+	-	-	-
Triso	B	-	-	-	-	-	-	-
Troll	B	-	-	-	-	-	-	-
Dragon	B	-	-	-	-	-	-	-
Luja	B	-	-	-	-	-	-	-
Ulla	B	-	-	-	-	-	-	-
Polkka	AB	-	-	-	-	-	-	+
Laari	AB	-	-	-	-	-	-	+
Reno	AB	-	-	-	-	-	-	-
Trappe	B	-	-	-	-	-	-	-
Fagott	B	-	-	-	-	-	-	-
Runar	B	-	-	-	-	-	-	-
Cannon	B	-	-	-	-	-	-	-
Tjalve	AB	-	-	-	+	-	-	-
Vinjett	AB	-	-	-	+	-	-	-
Tähti	-	-	-	-	-	-	-	-
CS	AB	-	-	-	-	-	-	-
talinisu								
Servinta	AB	-	-	-	-	-	-	-
Residence	B	-	-	-	-	-	-	-
Vergas	AB	-	-	-	+	-	-	-
Flair	B	-	-	-	-	-	-	-
Anthus	B	-	-	-	-	-	-	-
Gunbo	AB	-	-	-	-	-	-	-
Ada	-	-	-	-	-	-	-	-
Bjorke	AB	-	-	-	-	-	-	-
Complement	B	-	-	-	-	-	-	-
Bill	AB	-	-	-	-	-	-	-
Ramiro	B	-	-	-	-	-	-	-
Lars	AB	-	-	-	-	-	-	-
Olivin	AB	-	-	-	-	-	-	-
Raisa	AB	-	-	-	-	-	-	-
Portal	AB	-	-	-	+	-	-	-
Korweta	B	-	-	-	-	-	-	-
Sani	AB	-	-	-	-	-	-	-
Tarso	AB	-	-	-	-	-	-	-
Opata	A	-	-	-	-	-	-	-
Pm3a	AB	+	-	-	-	-	-	-
Pm3b	AB	-	+	-	-	-	-	-
Pm3c	AB	-	-	+	-	-	-	-
Pm3d	AB	-	-	-	+	-	-	-

Tabel 6. DH populatsiooni (121 genotüüpi) T. aestivum/T. militinae alleelide kaardistamine
(A- T. aestivumi alleel, B – T. militinae alleel)

		1A							1B				2A			4A								5B						
		Xpsp2999	Xgwm33	Xpsp3151	Xwmc95	Xwmc24	CFD2.1	Xgwm610.ti	Xpsp3000	TaglgaB	up3B/up1a	XBars153	Xgwm359	Xgwm71,1	Xgwm382	Xwmc232	Xgwm160	Xbarc78	Xbarc70.mi1	Xgwm855	Xgwm832	Xpsp3119	3. 1. 1	Xgwm234.1	Xgwm112	Xgwm234.2	Xgwm205.3ta-4ti	Xgwm133.1ta-6mi	Xgwm358.mil	Xgwm213.
1	DH 3	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
2	DH 5	B	B	B	B	B	B	B	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
3	DH 6	B	B	B	B	B	B	B	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
4	DH 7	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
5	DH 8	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
6	DH 9	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
7	DH 10	B	B	B	B	B	B	B	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
8	DH 11	A	A	A	A	A	A	A	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
9	DH 16	A	A	A	A	A	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
10	DH 17	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
11	DH 18	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
12	DH 19	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
13	DH 20	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
14	DH 21	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
15	DH 22	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
16	DH 23	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
17	DH 25	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
18	DH 26	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
19	DH 30	B	B	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
20	DH 32	B	B	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
21	DH 39	A	B	B	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
22	DH 40	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
23	DH 42	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
24	DH 43	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
25	DH 44	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
26	DH 45	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
27	DH 46	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
28	DH 47	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

		1A							1B				2A			4A								5B						
		Xpsp2999	Xgwm33	Xpsp3151	Xwmc95	Xwmc24	CFD2.1	Xgwm610.ti	Xpsp3000	TaglgaB	up3B/up1a	XBars153	Xgwm359	Xgwm71,1	Xgwm382	Xwmc232	Xgwm160	Xbarc78	Xbarc70.mi1	Xgwm855	Xgwm832	Xpsp3119	3. 1. 1	Xgwm234.1	Xgwm112	Xgwm234.2	Xgwm205.3ta-4ti	Xgwm133.1ta-6mi	Xgwm358.mil	Xgwm213.
29	DH 48	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
30	DH 49	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
31	DH 50	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
32	DH 51	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
33	DH 54	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
34	DH 55	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
35	DH 57	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
36	DH 59	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
37	DH 64	A	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
38	DH 68	A	A	B	B	B	B	B	A	A	A	A	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B
39	DH 72	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
40	DH 73	A	A	A	A	A	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
41	DH 76	B	B	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
42	DH 77	B	B	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
43	DH 82	B	B	B	B	B	A	A	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
44	DH 84	A	A	A	A	A	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
45	DH 86	B	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
46	DH 87	A	A	A	A	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
47	DH 88	A	A	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
48	DH 90	A	A	B	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
49	DH 91	A	A	B	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
50	DH 92	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
51	DH 94	B	B	A	A	A	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
52	DH 95	A	A	A	B	B	A	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	B	B	A	A	A	A
53	DH 96	B	B	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
54	DH 97	B	B	A	A	A	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
55	DH 99	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
56	DH 101	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	B	A	B	B	B	B
57	DH 102	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
58	DH 104	A	A	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
59	DH 105	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
60	DH 107	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
61	DH 109	B	B	B	A	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B

		1A							1B				2A			4A								5B						
		Xpsp2999	Xgwm33	Xpsp3151	Xwmc95	Xwmc24	CFD2.1	Xgwm610.ti	Xpsp3000	TaglgaB	up3B/up1a	XBars153	Xgwm359	Xgwm71,1	Xgwm382	Xwmc232	Xgwm160	Xbarc78	Xbarc70.mi1	Xgwm855	Xgwm832	Xpsp3119	3. 1. 1	Xgwm234.1	Xgwm112	Xgwm234.2	Xgwm205.3ta-4ti	Xgwm133.1ta-6mi	Xgwm358.mil	Xgwm213.
62	DH 110	B	B	B	B	B	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B
63	DH 111	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B
64	DH 112	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B
65	DH 119	B	B	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
66	DH 124	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
67	DH 125	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
68	DH 126	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
69	DH 128	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
70	DH 129	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B
71	DH 131	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
72	DH 132	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
73	DH 133	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
74	DH 134	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B
75	DH 138	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
76	DH 146	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B	B
77	DH 148	A	A	A	A	A	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
78	DH 155	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
79	DH 157	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
80	DH 158	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
81	DH 159	B	B	B	B	B	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
82	DH 161	B	A	A	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
83	DH 163	B	A	A	A	A	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
84	DH 166	B	A	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
85	DH 167	B	B	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
86	DH 168	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
87	DH 169	B	B	B	B	B	A	A	A	A	A	B	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	B	A	A
88	DH 174	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
89	DH 175	A	A	A	A	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
90	DH 176	A	A	A	A	A	B	B	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
91	DH 203	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B
92	DH 207	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B
93	DH 211	B	B	A	A	A	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
94	DH 214	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A

		1A							1B				2A			4A								5B						
		Xpsp2999	Xgwm33	Xpsp3151	Xwmc95	Xwmc24	CFD2.1	Xgwm610.ti	Xpsp3000	TaglgaB	up3B/up1a	XBars153	Xgwm359	Xgwm71.1	Xgwm382	Xwmc232	Xgwm160	Xbarc78	Xbarc70.mi1	Xgwm855	Xgwm832	Xpsp3119	3. 1. 1	Xgwm234.1	Xgwm112	Xgwm234.2	Xgwm205.3ta-4ti	Xgwm133.1ta-6mi	Xgwm358.mil	Xgwm213.
95	DH 216	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
96	DH 220	A	A	A	A	B	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
97	DH 221	A	A	A	A	B	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
98	DH 222	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
99	DH 223	B	B	B	A	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B
100	DH 224	A	A	A	A	A?	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
101	DH 226	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
102	DH 228	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
103	DH 229	B	B	B	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
104	DH 233	B	B	B	B	B	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
105	DH 235	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
106	DH 236	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
107	DH 237	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
108	DH 258	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
109	DH 264	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
110	DH 265	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
111	DH 266	B	B	B	B	B	A	A	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
112	DH 272	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
113	DH 274	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
114	DH 279	B	B	B	B	B	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
115	DH 280	B	B	B	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
116	DH 282	B	B	B	B	B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
117	DH 283	B	B	B	B	B	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
118	DH 285	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B
119	DH 288	B	B	B	B	B	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B
120	DH 303	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
121	DH 309	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A

		5A																		7A									
		Xgwm205.5	Xgwm205.1	Xgwm293.2ta	Xgwm415	Xbarc180	Xgwm186	CFD2.2	Xgwm617	Xwmc415	Xgwm639	Xbarc151	Xgwm666.1/2	CFD30	CFA2163	Xpsp3001.1mi	Xgwm6	Xgwm126	Xgwm410.240	Xgwm635	Xbarc70.4MI	Xbarc70.5TA	Waxy1.ta	Waxy1.mi	Xwmc283	Xgwm60	Xpsp3001.2ta	Xpsp3050
1	DH 3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
2	DH 5	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
3	DH 6	B	B	A	A	A	A	B	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
4	DH 7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
5	DH 8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
6	DH 9	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
7	DH 10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A
8	DH 11	B	B	B	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
9	DH 16	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	A	A	A	A	A	A	A	A	B
10	DH 17	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
11	DH 18	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
12	DH 19	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
13	DH 20	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
14	DH 21	B	B	B	B	B	B	A	A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A
15	DH 22	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
16	DH 23	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B
17	DH 25	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A
18	DH 26	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A
19	DH 30	A	A	B	B	B	B	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	B	A	A
20	DH 32	A	A	B	B	B	B	A	A	A	A	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	A
21	DH 39	A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
22	DH 40	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
23	DH 42	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
24	DH 43	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
25	DH 44	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A	A	A	B
26	DH 45	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A	A	A	B
27	DH 46	B	B	B	B	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
28	DH 47	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

		5A																		7A								
		Xgwm205.5	Xgwm205.1	Xgwm293.2ta	Xgwm415	Xbarc180	Xgwm186	CFD2.2	Xgwm617	Xwmc415	Xgwm639	Xbarc151	Xgwm666.1/2	CFD30	CFA2163	Xpsp3001.1mi	Xgwm6	Xgwm126	Xgwm410.240	Xgwm635	Xbarc70.4MI	Xbarc70.5TA	Waxy1.ta	Waxy1.mi	Xwmc283	Xgwm60	Xpsp3001.2ta	Xpsp3050
29	DH 48	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
30	DH 49	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	-	-	B	B	B?	B?	B	A
31	DH 50	B	B	B	B	A	B	B	B	B	B	A	A	A	A	A	A	A	A	A	-	-	B	A	B?	B	B	A
32	DH 51	B	B	B	B	B	A	A?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
33	DH 54	B	B	B	B	B	A	A?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
34	DH 55	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A
35	DH 57	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
36	DH 59	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
37	DH 64	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
38	DH 68	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
39	DH 72	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B
40	DH 73	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B
41	DH 76	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B
42	DH 77	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A	B	B	B	A	A	B	B	B	B
43	DH 82	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	B	B	B	B
44	DH 84	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	B	B	B	B
45	DH 86	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	A	A	B	B	B	B
46	DH 87	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B
47	DH 88	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B
48	DH 90	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
49	DH 91	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
50	DH 92	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
51	DH 94	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
52	DH 95	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B
53	DH 96	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B
54	DH 97	B	B	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B
55	DH 99	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B
56	DH 101	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B
57	DH 102	A	B	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	B	B	B	B
58	DH 104	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	A	A	A	A	A	B
59	DH 105	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	B
60	DH 107	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	B
61	DH 109	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	A

		5A																		7A									
		Xgwm205.5	Xgwm205.1	Xgwm293.2ta	Xgwm415	Xbarc180	Xgwm186	CFD2.2	Xgwm617	Xwmc415	Xgwm639	Xbarc151	Xgwm666.1/2	CFD30	CFA2163	Xpsp3001.1mi	Xgwm6	Xgwm126	Xgwm410.240	Xgwm635	Xbarc70.4MI	Xbarc70.5TA	Waxy1.ta	Waxy1.mi	Xwmc283	Xgwm60	Xpsp3001.2ta	Xpsp3050
62	DH 110	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	A
63	DH 111	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	A
64	DH 112	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	A
65	DH 119	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
66	DH 124	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
67	DH 125	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
68	DH 126	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
69	DH 128	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
70	DH 129	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
71	DH 131	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
72	DH 132	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
73	DH 133	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
74	DH 134	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
75	DH 138	B	B	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	B	B	B	B
76	DH 146	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
77	DH 148	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
78	DH 155	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
79	DH 157	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	A	B
80	DH 158	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B
81	DH 159	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B
82	DH 161	A	A	A	A	B	B	B	A	A	A	A	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
83	DH 163	B	B	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
84	DH 166	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B
85	DH 167	A	A	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A	B	B	B	B	B	A	B	A	B
86	DH 168	B	B	A	A	A	B	B	A	A	A	B	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
87	DH 169	A	A	B	B	B	B	B	A	A	A	A	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	B
88	DH 174	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B
89	DH 175	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B
90	DH 176	A	A	A	A	A	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A
91	DH 203	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B
92	DH 207	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
93	DH 211	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
94	DH 214	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A

		5A																		7A									
		Xgwm205.5	Xgwm205.1	Xgwm293.2ta	Xgwm415	Xbarc180	Xgwm186	CFD2.2	Xgwm617	Xwmc415	Xgwm639	Xbarc151	Xgwm666.1/2	CFD30	CFA2163	Xpsp3001.1mi	Xgwm6	Xgwm126	Xgwm410.240	Xgwm635	Xbarc70.4MI	Xbarc70.5TA	Waxy1.ta	Waxy1.mi	Xwmc283	Xgwm60	Xpsp3001.2ta	Xpsp3050
95	DH 216	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
96	DH 220	B	B	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B
97	DH 221	B	B	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B
98	DH 222	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B
99	DH 223	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B	B	B	B	A
100	DH 224	B	B	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B
101	DH 226	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
102	DH 228	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
103	DH 229	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
104	DH 233	A	A	A	A	A	B	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
105	DH 235	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
106	DH 236	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
107	DH 237	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
108	DH 258	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
109	DH 264	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
110	DH 265	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
111	DH 266	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
112	DH 272	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
113	DH 274	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
114	DH 279	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
115	DH 280	A	A	A	A	A	B	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
116	DH 282	A	A	A	A	A	B	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
117	DH 283	A	A	A	A	A	B	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
118	DH 285	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
119	DH 288	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
120	DH 303	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
121	DH 309	A	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Tabel 7 DH populatsiooni haiguskindluse hindamine põldkatsetes Jõgeval 2007. a.

	nimi	*S.nodorum*	*S.tritici*	*E.graminis*	*P.recondita*	*D.tritici-repentis*	*P.graminis*
1	F5 90/1-4	4	4	1	6	4	1
2	Tähti	6	5	5	6	4	1
3	control 8/1-3-3/1	4	4	2	1	4	1
4	DH3	4	4	4	5	4	1
5	DH5	5	5	1	7	1	1
6	DH9	5	5	4	6	2	1
7	DH11	5	4	3	6	2	1
8	DH15	4	4	3	3	3	1
9	DH21	4	5	1	6	4	1
10	DH22	4	5	4	7	4	1
11	DH36	5	5	1	6	3	1
12	DH38	5	5	3	6	4	1
13	DH42	5	4	1	7	1	1
14	DH44	5	6	5	3	3	1
15	DH46	4	5	1	6	3	1
16	DH49	4	6	1	7	3	1
17	DH51	5	5	4	6	3	1
18	DH59	5	4	1	6	3	1
19	DH68	4	5	4	7	2	1
20	DH73	4	4	1	4	2	1
21	DH76	4	3	2	4	1	1
22	DH77	4	4	1	5	3	1
23	DH81	4	4	1	6	4	1
24	DH82	4	4	3	6	3	1
25	DH86	5	6	3	4	3	1
26	DH88	5	4	3	4	5	1
27	DH90	4	5	1	6	4	1
28	DH94	4	4	1	3	3	1
29	DH95	4	4	3	5	3	1
30	DH96	4	4	3	6	3	1
31	DH97	4	4	1	4	3	1
32	DH99	5	5	1	5	3	1
33	DH100	4	5	4	1	4	1
34	DH101	5	4	1	6	2	1
35	DH102	4	5	3	6	2	1
36	DH104	5	4	4	6	4	1
37	DH105	4	5	4	5	4	1
38	DH109	5	5	3	4	4	1
39	DH112	5	4	4	7	1	3
40	DH124	4	3	1	3	3	1
41	DH126	4	5	4	6	4	1
42	DH129	4	4	4	4	5	1
43	DH131	4	4	4	5	3	1
44	DH132	4	4	4	7	3	1
45	DH134	4	4	1	5	3	1
46	DH163	4	4	1	7	3	1
47	DH167	4	4	4	5	2	1
48	DH168	4	4	1	5	2	1
49	DH169	4	4	1	5	4	1
50	DH175	3	4	1	4	3	1
51	DH218	3	4	3	4	4	1
52	DH222	4	5	1	6	3	1
53	DH223	4	6	4	4	4	1
54	DH224	4	4	4	5	5	1
55	DH228	4	4	2	4	3	1
56	DH255	4	5	1	6	3	1
57	DH258	4	4	3	4	3	1
58	DH264	4	4	3	5	3	1
59	DH266	4	3	2	4	2	1
60	DH272	4	3	3	4	3	1
61	DH319	3	4	1	4	2	1
62	DH321	4	5	2	5	4	1
63	DH324	4	4	5	6	3	1

Tabel 8

DH populatsiooni jahukastekindlus kaheksa erineva Blumeria graminis'e isolaadi suhtes laboritestis

			9.8		9.21		2.1		2.7		10.1		10.12		15	16
			I	II	I	II	I	II	I	II	I	II	I	II	I	I
1	DH	5	5,0	4,8	5,3	5,8	4,9	5,0	4,4	6,0	3,8	3,3	4,5	4,5	4,5	4,8
2	DH	6	4,5	3,0	5,0	4,3	3,0	4,0	4,8	5,8	1,9	4,3	3,8	3,8	4,5	5,8
3	DH	7	6,0	3,1	5,0	4,5	1,3	4,3	4,3	4,8	1,5	4,9	3,8	3,8	5,3	3,8
4	DH	8	2,1	2,4	5,8	3,5	3,8	3,4	3,0	5,3	2,8	4,5	2,0	3,5	5,3	3,3
5	DH	9	6,0	3,3	6,0	5,8	1,8	3,1	5,0	5,5	3,0	3,5	4,0	4,0	5,3	4,8
6	DH	10	3,0	3,9	5,8	4,5	3,0	2,7	5,3	5,8	4,3	3,0	4,0	4,3	4,7	4,7
7	DH	11	1,3	1,0	4,0	2,0	2,3	2,8	4,0	4,0	3,0	2,5	4,5	2,0	2,1	1,5
8	DH	16	3,0	1,0	5,0	3,3	3,4	4,3	5,0	3,0	3,3	3,3	4,9	3,0	4,3	4,5
9	DH	17	1,3	0,3	3,9	1,8	3,4	2,4	4,0	3,3	3,9	1,8	4,5	1,5	1,6	1,3
10	DH	18	0,0	0,0	2,3	3,0	2,5	1,3	3,3	1,5	3,3	1,3	3,5	1,8	1,3	1,8
11	DH	21	2,0	0,0	4,0	1,3	2,3	2,3	3,3	1,5	2,5	1,3	4,5	1,0	2,0	2,0
12	DH	22	1,0	0,0	2,5	1,0	2,5	2,9	3,3	3,0	3,4	2,8	4,5	2,0	2,0	2,3
13	DH	23	0,5	0,0	2,0	1,0	3,3	2,3	4,3	1,8	4,5	3,4	4,0	1,5	1,3	1,3
14	DH	25	1,0	0,3	4,0	1,8	2,0	3,0	2,0	2,3	1,9	2,8	3,4	2,0	1,6	2,5
15	DH	30	1,3	0,0	3,3	3,0	3,0	2,6	5,5	3,3	3,3	3,8	4,8	1,5	3,1	3,8
16	DH	32	2,0	0,0	4,0	1,8	1,5	2,0	3,3	2,3	2,0	3,5	3,3	1,5	1,6	1,5
17	DH	37	1,0	0,0	2,3	1,0	1,0	1,1	2,5	1,3	1,5	1,6	1,6	1,3	1,0	1,0
18	DH	40	1,0	0,3	2,0	1,5	1,0	1,3	1,3	3,3	1,0	2,0	1,5	1,0	2,8	1,5
19	DH	42	0,5	0,0	1,8	1,0	1,0	2,5	2,3	2,5	1,5	2,5	2,0	1,0	1,8	3,0
20	DH	43	2,1	0,0	4,0	2,7	2,1	2,0	2,3	2,3	2,3	2,6	3,0	2,3	1,3	1,8
21	DH	45	4,3	5,0	6,0	6,0	5,3	4,9	6,0	6,0	6,0	4,1	5,8	4,3	4,9	4,1
22	DH	46	5,0	5,0	6,0	5,7	4,8	4,3	5,8	5,7	5,3	4,7	6,0	5,3	5,0	5,3
23	DH	47	5,0	4,8	6,0	5,5	4,4	3,9	4,0	5,8	4,3	4,5	4,5	3,5	5,3	5,0
24	DH	48	5,3	5,5	6,0	6,0	4,0	3,8	5,5	6,0	5,0	4,0	6,0	5,3	4,9	4,8

			9.8		9.21		2.1		2.7		10.1		10.12		15	16
			I	II	I	II	I	II	I	II	I	II	I	II	I	I
25	DH	51	5,5	4,5	6,0	6,0	4,0	5,0	5,8	5,3	3,0	4,3	4,0	4,0	5,1	5,3
26	DH	54	3,8	5,0	6,0	5,8	3,3	5,0	5,0	5,5	4,3	4,4	6,0	5,3	5,3	5,3
27	DH	55	3,3	5,0	5,0	5,8	4,3	6,3	5,8	5,8	6,0	4,3	6,0	4,5	5,4	5,0
28	DH	57	6,0	4,0	6,0	5,8	5,4	5,0	5,5	6,0	6,0	4,5	6,0	5,3	5,5	5,5
29	DH	59	6,0	3,1	6,0	6,0	-	4,8	5,5	5,5	-	4,3	5,8	5,0	5,3	6,0
30	DH	64	2,0	0,3	4,0	4,0	3,0	2,1	3,6	4,0	5,3	3,8	4,8	3,0	3,4	2,0
31	DH	66	4,0	1,9	6,0	2,5	1,9	4,3	5,5	3,3	2,0	2,8	5,5	2,8	3,8	2,5
32	DH	67	2,8	-	5,5	-	1,9	-	1,5	-	2,1	-	3,4	-	-	-
33	DH	68	2,0	0,8	5,0	2,0	1,5	1,1	4,5	3,3	1,8	3,5	3,5	1,3	1,5	1,0
34	DH	72	1,3	1,3	3,5	4,0	1,3	2,3	1,8	5,0	3,0	2,5	2,5	4,0	2,3	2,0
35	DH	73	1,8	0,0	4,5	2,8	2,1	2,9	4,0	3,0	2,3	3,0	3,8	2,5	3,1	3,0
36	DH	76	0,5	0,0	3,0	1,0	1,0	1,0	2,3	1,5	1,7	1,0	3,9	1,0	1,5	1,0
37	DH	77	1,1	0,0	2,5	1,5	0,8	1,0	1,8	2,0	1,0	1,8	2,7	1,0	1,0	1,5
38	DH	81	0,0	-	2,5	-	2,3	-	0,8	-	3,6	-	0,8	-	-	-
39	DH	82	1,0	0,0	5,0	1,8	2,1	1,8	3,4	2,3	3,3	3,0	4,6	1,0	2,0	3,4
40	DH	84	1,3	0,0	4,8	1,5	3,8	3,1	3,5	1,8	5,3	2,9	4,1	1,3	2,8	2,0
41	DH	86	1,0	1,3	5,0	4,0	-	4,0	4,5	3,3	-	3,0	5,0	3,0	3,4	3,3
42	DH	87	-	0,9	-	3,3	2,3	2,0	-	4,0	4,1	2,0	-	2,3	1,8	2,5
43	DH	88	0,0	0,0	4,0	1,5	2,6	2,0	3,3	2,8	4,3	2,8	4,0	2,0	2,0	3,5
44	DH	90	1,3	0,0	4,0	0,3	1,8	3,1	4,4	2,5	1,5	2,8	4,3	1,5	2,3	3,1
45	DH	91	0,8	0,8	3,0	2,8	1,0	1,0	3,3	2,3	2,8	2,8	3,4	1,0	2,0	1,8
46	DH	92	1,0	0,0	5,0	1,8	1,3	1,9	1,8	3,3	4,3	2,8	2,5	2,0	2,5	1,0
47	DH	94	1,8	0,0	4,3	1,3	2,5	1,6	4,5	1,8	4,0	2,8	3,3	1,8	2,4	1,0
48	DH	95	1,0	0,8	5,0	3,5	2,1	1,0	3,5	3,3	2,6	3,0	4,0	1,8	1,7	1,0
49	DH	96	1,1	0,3	4,0	2,3	2,3	3,6	4,0	3,3	3,3	3,0	3,3	1,5	3,0	2,8
50	DH	97	0,8	0,5	3,0	2,5	1,0	2,5	3,1	4,3	2,1	2,1	3,3	2,3	2,3	1,0

			9.8		9.21		2.1		2.7		10.1		10.12		15	16
			I	II	I	II	I	II	I	II	I	II	I	II	I	I
51	DH	99	1,3	0,0	4,3	1,3	1,0	1,5	3,6	1,3	1,3	2,0	3,0	1,3	2,0	1,5
52	DH	100	1,0	-	3,0	-	1,0	-	2,0	-	1,3	-	1,3	-	-	-
53	DH	101	1,0	0,0	3,0	1,0	1,3	1,5	1,5	1,5	2,4	2,3	2,9	1,5	1,6	1,8
54	DH	102	1,0	0,0	4,0	1,3	1,8	1,3	2,4	1,3	2,0	2,3	2,9	1,0	2,0	1,3
55	DH	104	1,0	0,5	5,0	3,0	-	1,5	2,0	3,3	-	3,4	3,3	1,3	1,4	1,5
56	DH	105	-	4,5	-	5,8	4,5	5,3	-	5,5	5,8	6,0	-	4,8	6,3	6,0
57	DH	107	5,8	3,8	6,0	6,0	1,5	4,0	5,5	6,0	1,8	4,1	6,0	4,8	5,8	5,5
58	DH	109	1,0	0,0	3,0	1,0	1,3	1,8	3,3	2,0	2,3	2,0	3,0	1,0	1,5	1,0
59	DH	110	1,0	0,0	4,0	2,3	2,4	3,1	1,6	2,8	4,4	3,0	3,6	1,5	2,3	2,3
60	DH	111	1,3	0,0	4,0	3,0	1,0	1,0	4,0	3,8	2,6	2,1	4,5	3,3	1,5	1,8
61	DH	112	1,0	0,8	5,0	3,0	-	2,8	2,5	3,3	-	2,5	4,0	2,0	2,8	3,6
62	DH	119	-	0,0	-	1,0	2,8	3,0	-	2,0	3,0	2,5	-	1,0	1,8	2,5
63	DH	124	0,8	0,8	4,0	2,0	2,9	3,1	3,0	3,0	4,5	3,0	3,5	2,0	2,6	1,0
64	DH	125	1,0	0,5	3,3	3,8	4,3	3,4	5,3	3,5	4,5	3,5	4,5	1,5	3,6	2,9
65	DH	126	5,0	4,9	6,0	5,0	4,0	4,5	4,4	5,3	4,8	3,8	5,0	4,5	5,0	5,3
66	DH	128	3,8	3,3	4,6	4,5	4,9	3,3	5,0	4,0	4,0	4,0	5,0	3,3	5,3	3,3
67	DH	129	3,0	3,8	6,0	5,0	-	4,5	4,8	5,5	-	4,3	4,8	4,5	4,8	4,8
68	DH	131	-	3,8	-	5,5	3,5	5,0	-	6,0	5,0	4,3	-	1,0	4,8	5,0
69	DH	132	4,1	3,9	6,0	5,8	3,0	4,8	5,5	5,5	4,0	4,8	6,0	5,0	4,8	4,6
70	DH	133	5,0	3,5	6,0	4,5	3,3	3,8	3,5	5,5	4,8	3,0	6,0	4,5	4,3	5,0
71	DH	134	4,4	3,5	6,0	6,0	2,8	1,1	5,3	4,0	5,5	3,3	4,3	2,0	4,5	3,0
72	DH	138	5,8	4,3	6,0	5,8	4,8	4,0	5,0	5,3	6,0	4,1	6,0	3,5	5,3	5,0
74	DH	146	3,0	2,1	6,0	5,3	1,8	4,3	5,0	6,0	4,0	4,5	5,5	4,0	5,8	5,0
75	DH	148	2,0	0,0	5,0	2,3	-	1,5	3,5	2,3	-	2,3	4,5	1,0	1,3	1,9
76	DH	159	-	0,5	-	5,0	1,0	3,6	-	3,8	4,0	2,4	-	4,0	3,5	4,0
77	DH	161	2,0	0,3	4,5	2,3	2,4	2,5	3,0	3,0	4,0	2,5	3,9	1,8	2,3	1,0
78	DH	163	3,0	0,0	5,0	1,3	3,0	1,5	4,0	2,0	3,3	1,8	4,5	1,0	1,9	1,0

			9.8		9.21		2.1		2.7		10.1		10.12		15	16
			I	II	I	II	I	II	I	II	I	II	I	II	I	I
79	DH	166	2,1	0,0	4,3	1,0	1,9	2,1	4,0	1,5	3,3	2,9	4,0	1,3	1,8	1,0
80	DH	167	2,0	0,0	5,0	1,0	2,1	0,5	3,8	2,3	4,8	1,3	3,8	1,0	1,3	2,0
81	DH	168	2,0	0,0	4,0	2,0	-	2,8	3,3	1,8	-	2,8	4,0	2,5	2,0	2,8
82	DH	169	-	0,5	-	3,0	2,8	2,1	-	1,5	2,6	1,5	-	1,8	1,3	1,5
83	DH	174	3,0	0,0	5,0	4,0	-	3,3	5,0	4,3	-	3,4	4,5	3,8	2,3	3,9
84	DH	175	-	0,0	-	4,3	4,5	4,5	-	5,3	3,0	4,0	-	3,5	4,1	4,0
85	DH	176	3,8	1,4	5,8	4,8	2,3	4,5	4,0	4,5	2,8	3,0	4,0	3,3	4,3	5,0
86	DH	203	5,5	0,0	6,0	5,8	2,5	3,0	4,3	5,8	4,8	4,0	5,0	5,3	4,8	4,3
87	DH	207	3,6	4,6	5,5	5,5	3,3	4,8	4,0	5,3	4,3	4,1	3,8	5,0	4,3	5,3
88	DH	211	1,0	-	2,1	-	3,0	-	5,0	-	3,5	-	4,0	-	-	-
89	DH	214	1,0	0,3	4,0	1,3	3,3	1,9	3,3	3,0	4,8	4,1	4,0	1,3	3,3	1,3
90	DH	216	1,3	-	4,5	-	2,0	-	4,5	-	2,0	-	5,0	-	-	-
91	DH	220	1,0	-	3,0	-	2,1	-	1,0	-	2,4	-	2,0	-	-	-
92	DH	221	1,0	0,0	4,0	1,3	1,0	2,3	1,9	1,5	3,3	2,5	3,8	1,0	2,0	1,5
93	DH	222	1,3	0,0	4,3	2,0	1,8	1,0	1,5	2,5	3,5	1,3	3,8	2,0	2,0	1,0
94	DH	223	1,0	0,0	3,0	2,0	-	3,9	3,3	2,3	-	3,0	4,0	1,5	3,8	2,5
95	DH	224	-	1,0	-	4,0	4,0	2,3	-	5,0	4,3	1,1	-	3,0	1,6	1,4
96	DH	226	1,0	0,0	4,0	1,5	2,0	3,0	4,3	3,3	1,9	3,8	3,3	1,5	3,8	3,3
97	DH	228	0,5	0,8	1,3	1,5	1,4	2,0	3,8	3,5	2,4	3,0	3,0	1,3	2,0	2,5
98	DH	229	0,8	0,0	3,1	2,3	4,0	1,9	4,5	3,8	4,3	4,1	3,3	2,0	1,3	0,8
99	DH	233	1,0	0,3	4,0	1,0	3,1	2,9	4,8	3,0	6,0	3,0	4,0	1,0	1,8	3,5
100	DH	235	2,5	1,0	5,0	4,0	1,8	3,4	4,5	2,3	3,5	2,0	5,5	1,8	2,9	3,8
101	DH	236	1,0	0,0	2,8	0,3	1,0	1,3	2,9	1,8	2,0	2,1	3,0	0,5	1,3	2,3
102	DH	237	1,0	0,0	2,0	1,8	1,9	4,3	1,5	3,3	2,1	2,5	2,1	2,3	3,1	3,5
103	DH	244	2,0	-	4,0	-	4,0	-	3,0	-	4,5	-	2,0	-	-	-
104	DH	246	2,3	-	5,0	-	2,0	-	4,3	-	2,8	-	5,0	-	-	-
105	DH	249	0,8	-	3,0	-	2,1	-	2,5	-	2,3	-	2,6	-	-	-

			9.8		9.21		2.1		2.7		10.1		10.12		15	16
			I	II	I	II	I	II	I	II	I	II	I	II	I	I
106	DH	258	0,0	0,0	2,8	1,0	1,5	1,0	2,9	1,0	2,4	1,0	3,5	0,3	1,0	0,8
107	DH	264	0,0	0,0	2,3	4,0	2,0	1,5	3,5	4,3	2,8	2,5	2,8	2,3	1,8	1,5
108	DH	265	0,0	0,3	3,0	1,8	1,8	3,0	2,8	3,0	3,5	2,9	3,5	1,8	1,8	2,5
109	DH	266	0,8	0,0	4,0	0,3	2,0	4,4	4,0	1,8	2,8	2,9	4,1	0,5	2,1	4,3
110	DH	271	0,8	-	2,3	-	1,0	-	3,4	-	1,8	-	3,3	-	-	-
111	DH	272	1,0	0,0	2,0	1,8	1,9	2,0	3,0	2,3	3,0	2,8	2,1	1,5	1,0	1,0
112	DH	279	-	0,0	-	3,0	-	-	-	3,5	-	-	-	2,5	-	-
113	DH	280	-	0,5	-	0,8	-	2,0	-	3,0	-	2,0	-	1,5	1,8	1,3
114	DH	282	-	0,5	-	1,5	0,8	3,5	-	2,3	2,8	3,0	-	2,5	2,3	3,0
115	DH	283	0,8	0,0	2,3	1,8	-	3,4	2,1	3,5	-	1,8	1,0	0,5	3,4	3,0
116	DH	285	-	0,5	-	1,8	-	4,0	-	2,5	-	3,4	-	3,0	1,0	2,5
117	DH	288	-	0,0	-	0,8	3,5	0,5	-	2,0	4,3	1,8	-	1,0	0,8	1,0
118	DH	303	1,0	0,3	3,0	2,5	1,8	2,8	4,0	3,5	2,8	1,8	3,6	2,3	2,5	3,6
119	DH	309	1,0	-	2,3	-	2,8	-	2,6	-	4,0	-	2,5	-	-	-
120	DH	311	3,0	5,7	6,0	6,0	1,5	3,8	5,3	6,0	1,5	3,9	4,5	5,0	4,3	4,5
121	DH	312	0,3	1,0	2,8	3,0	4,5		2,0	4,0	5,5		2,0	1,3	-	-

Tabel 9. Eestis kasvatatavate suvinisusortide jahukastekindlus põldkatsetes Jõgeval 1996-2006

Sort	Resistentsus *Blumeria graminis* f. sp. *tritici* suhtes									
	1996*	1997	1999	2000	2001	2002	2004	2005	2006	Keskmine
'Vinjett'	0***	0	0	1	1	1	0	0	**1*****	0.4
'SWEstrad'	-****	-	-	-	0	**2**	0	0	1	0.6
'Zebra'	-	-	-	-	0	**2**	1	0.7	0	0.7
'Meri'	0.3	0	0	1	0.7	**2**	1	1.7	0	0.7
'Satu'	1	0	0.5	**2.7**	0	2	0	0	0	0.7
'Helle'	0.8	0	0	1.7	0	**3**	1	2	0	0.9
'Baldus'	0	0	0.5	**4.3**	1.3	1	2	2.3	1	1.4
'Triso'	0.7	1.5	0	3.7	**4.3**	2	2	0	0	1.6
'Munk'	2	0	0	**4.3**	3	3	4	3.7	1	2.3
'Manu'	2	2	0	5.3	4.7	3	2	**6.3**	1	2.9
'Mahti'	2.5	0	0	5.3	5.3	4	4	**5.3**	2	3.2
'Tjalve'	1	0	1	4.3	5.3	4	**6**	5.7	3	3.4
'Tähti'	5	-	5	4	4	5	**7**	6	6	5.3
'Luja'	6	**6**	-	-	-	**-**	**-**	-	-	(6)

* andmeid aastate 1998 ja 2003 ei esitata kuna jahukastetekitaja nendel aastatel looduses puudus
** sordid on järjestatud vastavalt nende keskmisele ja maksimaalsele vastuvõtlikkusele jahukastetekitaja suhtes
*** kolme korduse keskmine (igaüks 10m^2)
**** - ei hinnatud
***** üheksa aasta maksimaalne vastuvõtlikkus on tumedas trükis

Tabel 10. Suvinisusortide resistentsus *Blumeria graminis* f. sp. *tritici* loodusliku populatsiooni ja üksikute testisolaatide suhtes taimekasvu varases (idandite) staadiumis

Sort	Idandite resistentsus *Blumeria graminis* f. sp. *tritici* suhtes											
	diferentseerivad testisolaadid (No)											looduslik
	2	5	6	9	10	12	13	14	15	16	17	populatsioon
'Vinjett'	r	r	r	r	r	r	r	r	r	r	r	0
'SWEstrad'	r	r	r	r	r	r	r	r	r	r	r	0
'Zebra'	r,i	r	r	r	r	r	r	r	r	r	r	0
'Meri'	r	r	r	r,i	r	r,i	r	r	r	s	s	1
'Satu'	i	r	r	r	r	s	r	s	r	s	s	1
'Helle'	r	r	r	r	r	r	r	r	r	s	s	1
'Baldus'	r	r	r	r	r	s	r	i/r	r,i	i	r	1
'Triso'	r	r	r	r	r	r	r	r	r	r	r	3.5
'Munk'	s	r	s	r,i	i	i	r	r	r	s	r	4
'Manu'	s	r	s	r	r	r	s	s	i	s	s	4
'Mahti'**	r,i	r	s	r	r	s	r,i	r,i	r	s	s	4
'Tjalve'	s	r	r	r	r	r	r	r	r	r	r	6
'Tähti'	s	s	s	s	s	s	s	s	s	s	s	4.5
'Luja'	s	s	s	s	s	s	s	s	s	s	s	6
'Kolibri'(Pm3d)	-	-	-	-	-	-	-	-	-	-	-	5.9
'Michigan Amber' (Pm3f)	-	-	-	-	-	-	-	-	-	-	-	1

* sordid on järjestatud vastavalt nende keskmisele ja maksimaalsele vastuvõtlikkusele jahukastetekitaja suhtes põldkatsetes (Tabel 8)

** taimne materjal heterogeenne

- ei hinnatud

r – resistentne, s - vastuvõtlik

Tabel 11. Sordi 'Chinese Spring' (CS) ja 21 CS monosoomse taime ristamisel suvinisusordiga 'Vinjett' saadud F_2 põlvkonna lahknemine idandite resistentsuse suhtes *Blumeria graminis* f. sp*. tritici* isolaadiga No.12 nakatamisel

Chromosome involved	Observed segregation		
	resistant	susceptible	χ^2 **
1A	249	0	**12.260***
2A	297	14	0.023
3A	166	7	0.158
4A	343	15	0.197
5A	313	11	0.207
6A	147	7	0.006
7A	126	4	0.752
1B	319	17	0.103
2B	189	7	0.542
3B	210	7	0.037
4B	166	7	0.156
5B	145	5	0.615
6B	223	8	0.770
7B	305	18	0.566
1D	92	3	0.495
2D	105	4	0.248
3D	226	12	0.067
4D	312	16	0.026
5D	300	2	**10.945***
6D	214	9	0.211
7D	314	3	**9.921***
CS xVinjett	356	15	0.344

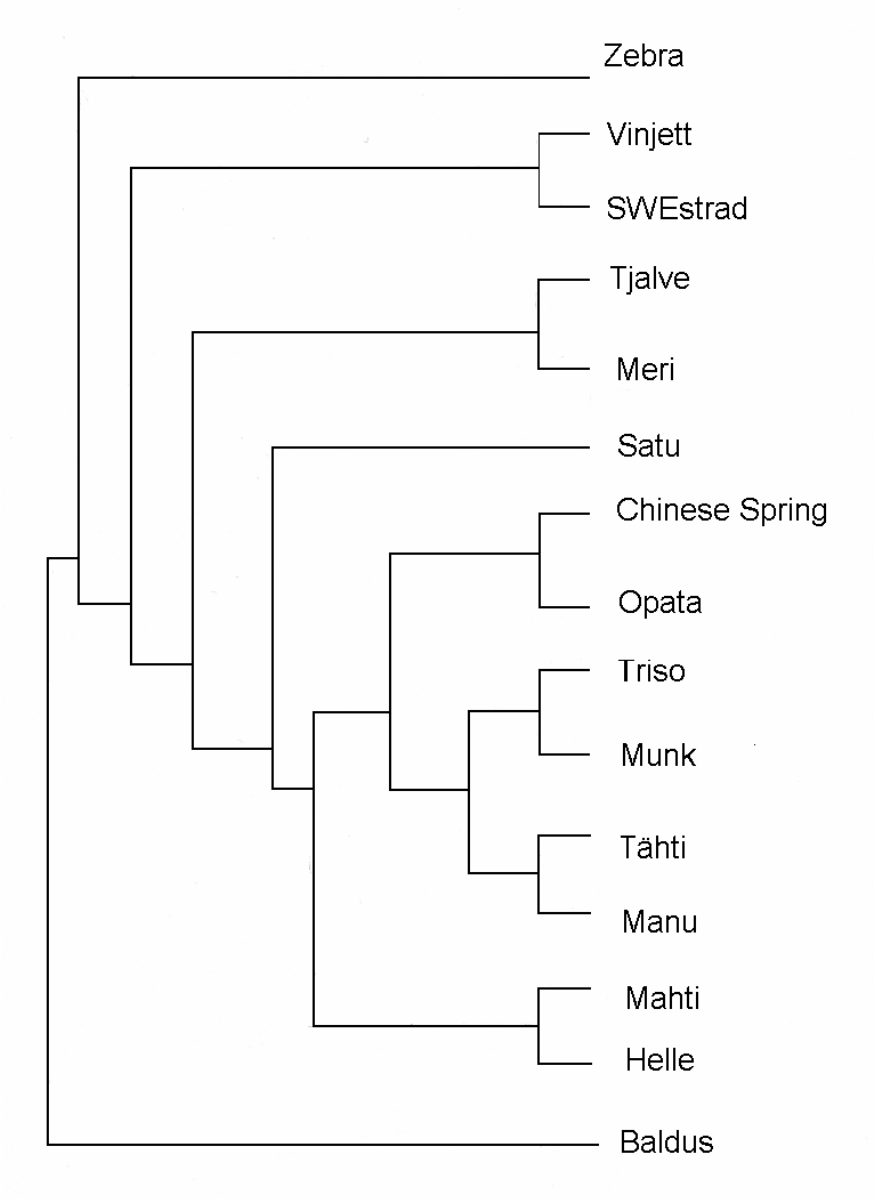
* P>0.01

** lahknemise kõrvalekalle teoreetiliselt oodatavast lahknemisest 63:1

Tabel 12. Monosoomanalüüsi ja funktsionaalsete markerite abil tuvastatud jahukastekindlusgeenid Eestis kasvatatavates suvinisusortides

Sort	Idandite resistentsust tagavad kromosoomid monosoomanalüüsi andmetel (sulgudes testisolaadi number)	*Pm3* alleelid (markeranalüüs)
'Vinjett'	1A+5D+7D (No.12)	*Pm3d*
'SW Estrad'		*Pm3d*
'Zebra'		*Pm3d*
'Meri'	1B (No. 2, 9; Peusha et al, 2000a)	-
'Satu'		*Pm3f*
'Helle'	3D (No. 6; Peusha et al. 2005)	*Pm3f*
'Baldus'		-
'Triso'		-
'Munk'		*Pm3d***
'Manu'		-
'Mahti'		*Pm3f*
'Tjalve'	1A+3B (No. 9, 10; Peusha et al., 2000b)	*Pm3d*
'Tähti'		-
'Luja'		-
'Canon'		-
'Polkka'		*Pm3f*

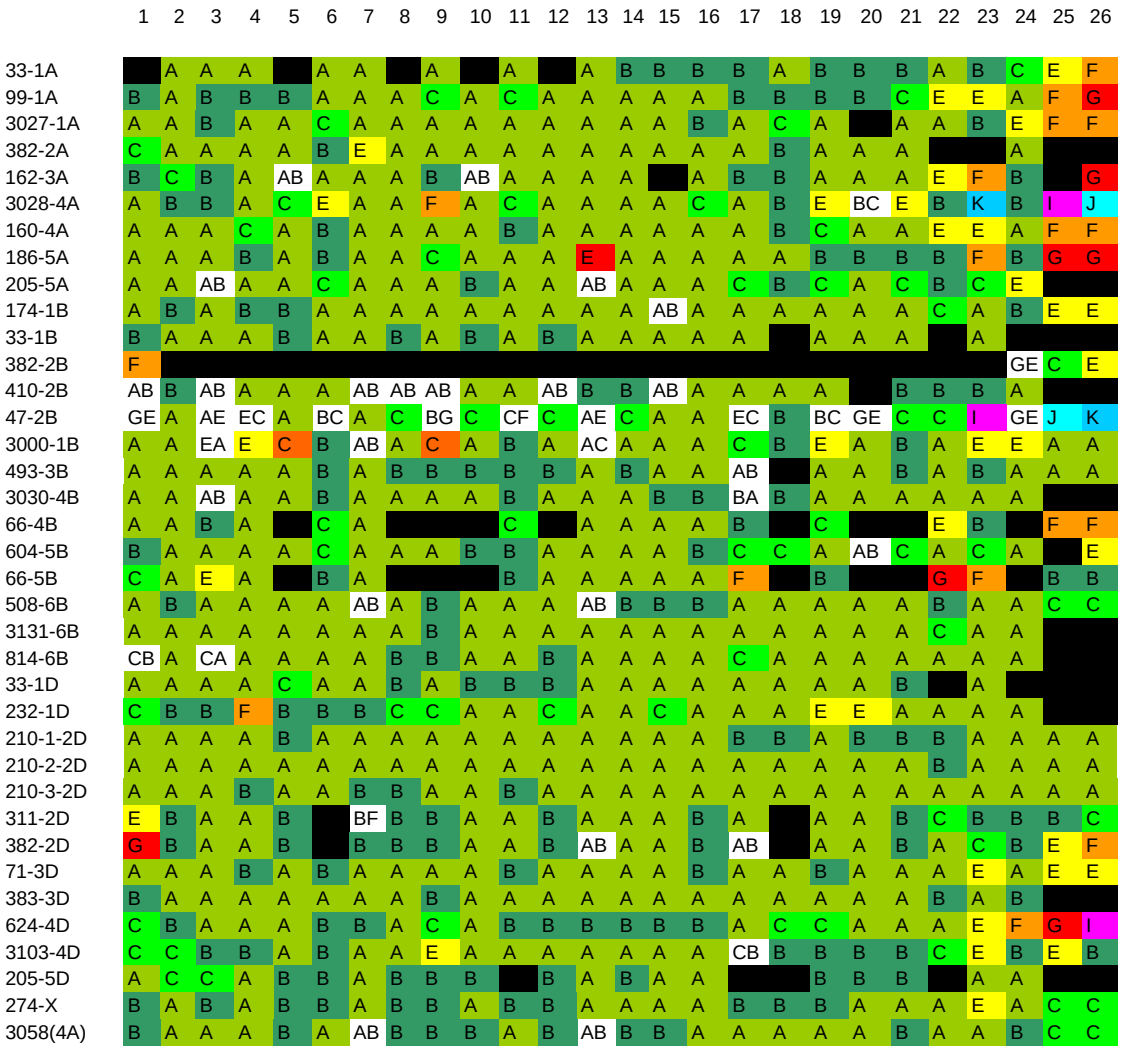
* sordid on järjestatud vastavalt nende keskmisele ja maksimaalsele vastuvõtlikkusele jahukastetekitaja suhtes põldkatsetes (Tabel 8)
** esmalt tuvastatud Tommasini et al., 2006 poolt



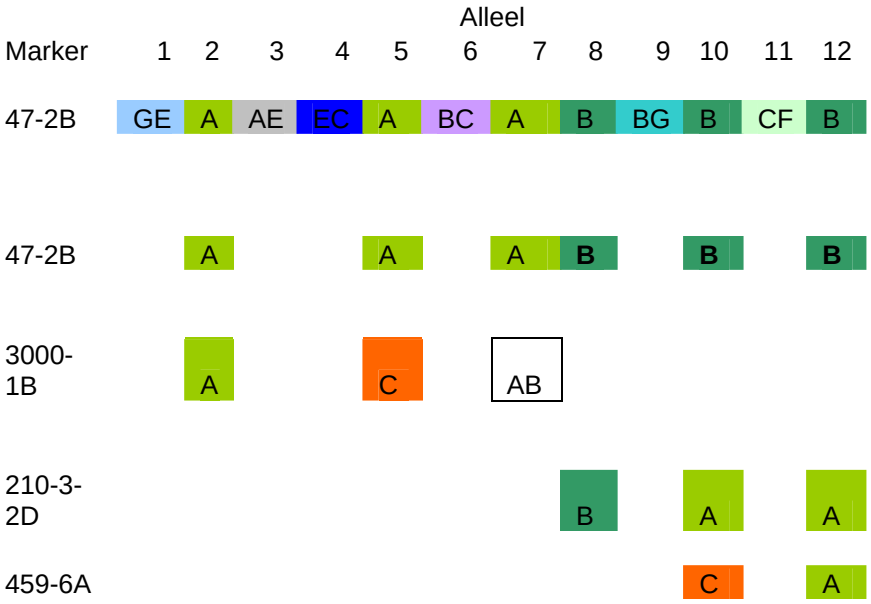
JOONIS 1

Eesti Sordilehe suvinisusortide fülogeneetiline puu (võrdluseks on lisatud sordid Cninese Spring, Opata ja Tähti)

JOONIS 2 Eesti Sordilehe suvinisusortide ja nisuaretiste mikrosatelliitanalüüsi tulemused

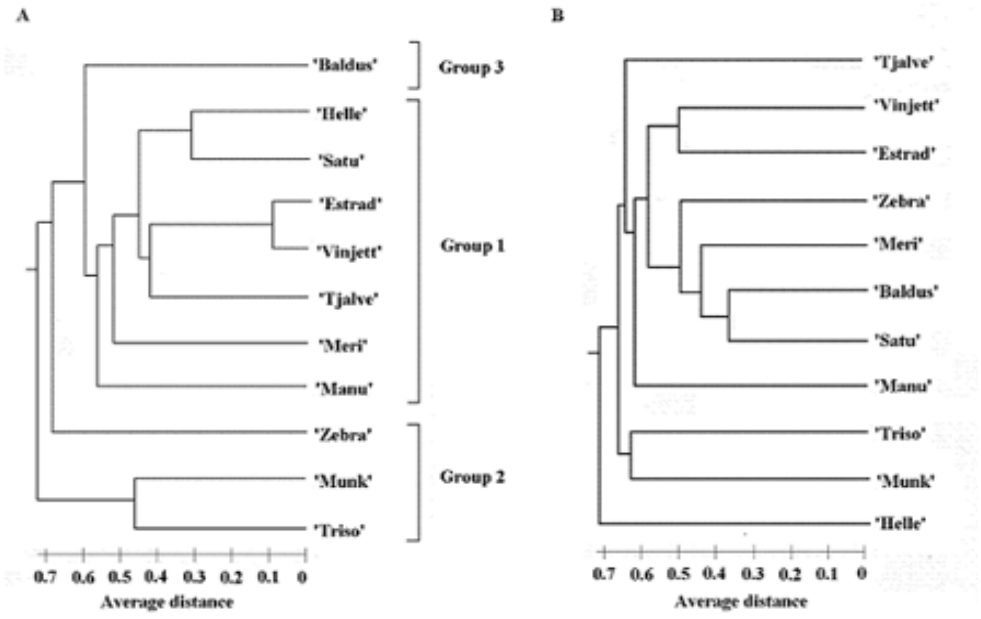


Baldus	1	
Helle	2	
Mahti	3	
Manu	4	
Meri	5	
Munk	6	
Satu	7	
SWEstrad	8	
Zebra	9	
Tjalve	10	
Friso	11	
Vinjett	12	
6.1.10.3	13	SatuxTjalve
91060202	14	SatxTjalve
670101	15	SatuxMunk
127.1.6	16	ManuxTjalve
143	17	SatuxMunk
213	18	MunkxMahti
214	19	MunkxManu
231	20	TjalvexManu
232	21	TjalvexMunk
Opata	22	
Chinese Spring	23	
Tähti	24	
T.militinae	25	
T.timopheevii	26	



JOONIS 3

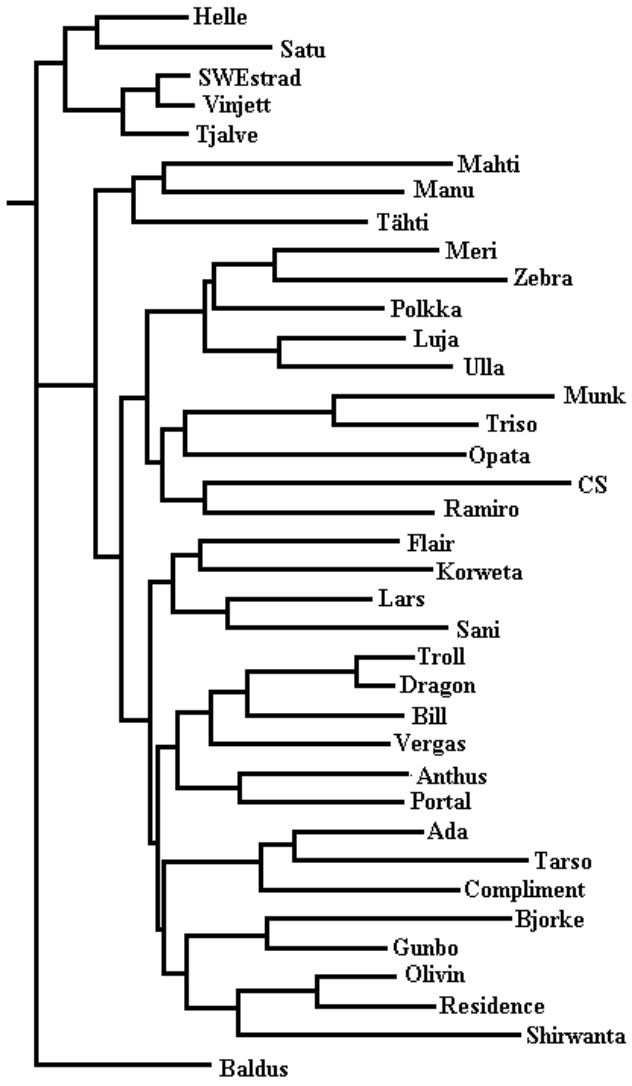
Üks võimalikest skeemidest Eesti Sordilehe suvinisusortide eristamiseks mikrosateliitjärjestuste PCR amplifitseerimise teel.
1- Baldus, 2- Helle, 3- Mahti, 4- Manu, 5- Meri, 6- Munk, 7- Satu, 8- SWEstrad, 9- Zebra, 10- Tjalve, 11- Triso ja 12- Vinjett



Joonis 4. Molekulaarse markeranalüüsi (A) ja morfoloogiliste andmete (B) põhjal koostatud fülogeneetilised puud.

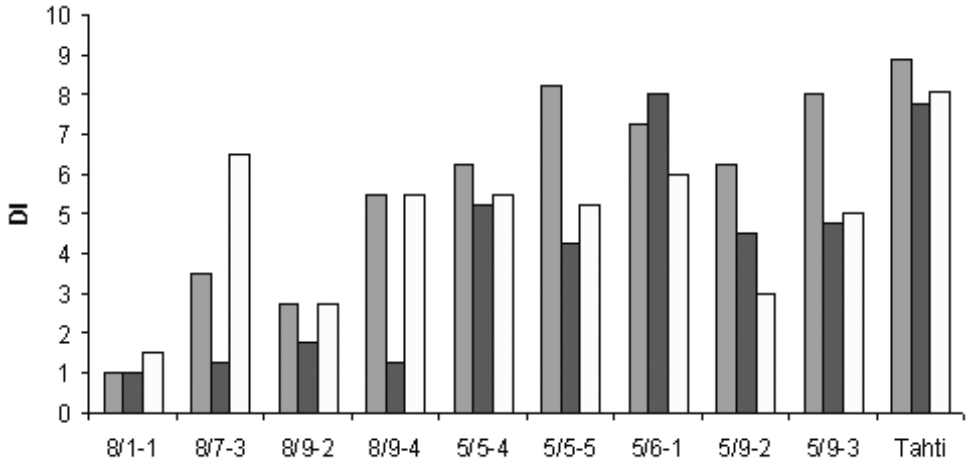
JOONIS 5 Näide aretise ja tema vanemate genotüüpide võrdlemisest

Näide võimalikust analüüsist. Aretise 6.1.10.3 vanemate identifitseerimine 40 mikrosatelliitmarkeriga tehtud amplifitseerimiste alusel. Võimalikeks vanemakandidaatideks osutusid Satu, Helle ja Tjalve (tegelikud vanemad Satu ja Tjalve). Aretise genotüübis leiti 3 alleeli, mida ei esinenud kumbalgi vanemal, ühte neist, markeri 186-5A alleeli E, ei leitud ühelgi sordilehe genotüübil.

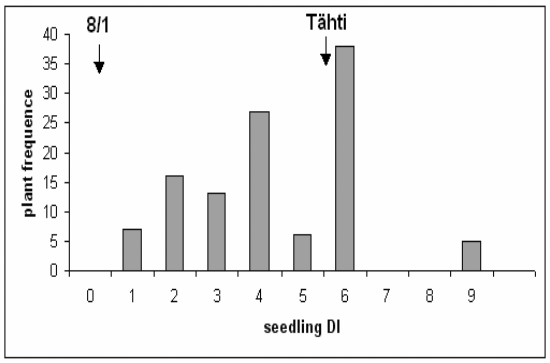


Joonis 6

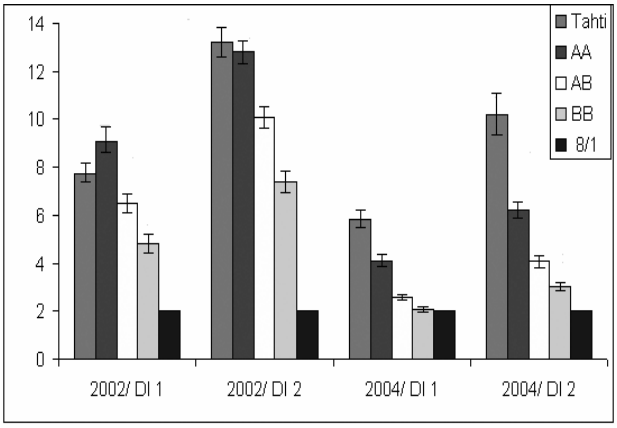
Eesti Sordilehe suvi- ja talinisusortide geneetiliste kauguste põhjal koostatud dendrogramm. Dendrogrammi koostamiseks on kasutatud on 14 polümorfse mikrosatelliitjärjestse alleelset varieeruvust



Joonis 7. Üheksa Tähti x *T. militinae* ristamisel saadud hübriidliini täiskasvanud taime jahukastekindluse fenotüübiline jaotus kolme aasta jooksul

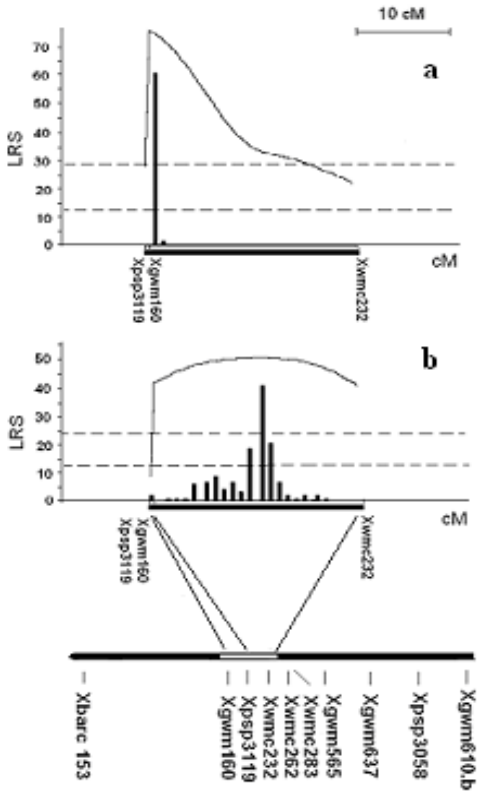


Joonis 8. F_2 kaardistamispopulatsiooni taimede jaotus idandi jahukastekindluse järgi (0 – ei ole nähtavaid sümptoome, 9 – väga tugev nakkus). Nooltega on näidatud vanemate reaktsioon.



Joonis 9. Kromosoomi 4A markeri *Xgwm160* erinevate alleelide mõju kaardistamispopulatsiooni taimede jahukastekindlusele täiskasvanud taime staadiumis 2002.a. ja 2004.a., kahe hindamise (DI1 - pea loomisel ja DI2 - piimküpsuse staadiumis) andmed.

AA – homosügootne Tähti alleel, AB – heterosügootne, BB – homosügootne *T. militinae*'lt pärinev alleel



Joonis 10. Täiskasvanud taime (a, 2002.a. DI) ja idandi (b, 2002.a. DI) jahukastekindluse QTLi seotus kromosoomiga 4A.

Theor Appl Genet (2006) 112: 760–769
DOI 10.1007/s00122-005-0181-2

ORIGINAL PAPER

Irena Jakobson · Hilma Peusha
Ljudmilla Timofejeva · Kadri Järve

Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line

Received: 18 July 2005 / Accepted: 30 November 2005 / Published online: 17 December 2005
© Springer-Verlag 2005

Abstract In the progeny of a cross between the common wheat cultivar Tähti and *Triticum militinae*, a member of the timopheevii group of tetraploid wheats, several hybrid lines were selected that are characterized by improved seedling and adult plant resistance (APR) to powdery mildew. An F_2 single-seed descendant mapping population segregating for seedling resistance and APR to powdery mildew was analysed for the identification of quantitative trait loci (QTL). The main QTL responsible for APR was detected on the long arm of chromosome 4A tightly linked to the *Xgwm160* locus on a *T. militinae* translocation explaining up to 54% of phenotypic variance. The same translocation influenced seedling resistance to powdery mildew upon inoculation of plants with a synthetic population of *Blumeria graminis* DC. f. sp. *tritici*, and explained 28–33% of the phenotypic variance.

Introduction

The obligate fungus *Blumeria graminis* DC. f. sp. *tritici* can infect plants from the first leaf stage until senescence. Resistance to the infection of powdery mildew fungus may be based on a race-specific gene-for-gene interaction of resistance gene(s) (*Pm*-genes) in wheat and avirulence gene(s) in the infecting fungus isolate. This type of plant–pathogen interaction is associated with the hypersensitive response and may not be durable. Most of the powdery mildew resistance genes identified in different wheat genotypes (*Pm1* – *Pm31*, McIntosh et al. 2003) confer complete resistance to different sets of fungus isolates in the seedling stage of the host plant (Hsam and Zeller 2002).

Some wheat genotypes exhibit a different type of resistance, which is non-isolate-specific and partial, retarding infection, growth and reproduction of the powdery mildew fungus. This resistance is generally observed in adult plants. Adult plant or durable resistance (APR) is a quantitative trait and can be resolved into discrete genetic loci (quantitative trait loci, QTL; Paterson et al. 1988).

Several sets of QTLs for adult plant powdery mildew resistance have been detected and mapped in different segregating wheat populations (Huang and Röder 2004). In the heritance of adult plant powdery mildew resistance, the additive effects of the detected QTLs prevail (Griffey and Das 1994; Keller et al. 1999; Chantret et al. 2001; Mingeot et al. 2002; Liu et al. 2001).

Pm-genes conferring powdery mildew resistance have been transferred to *Triticum aestivum* ($2n = 42$, AABBDD) from different species of the genus *Triticum*, including the timopheevii group of wheats (*Pm6, Pm27* from *Triticum timopheevii* (Zhuk.) Zhuk. ssp. *timopheevii*) (Shands 1941; Allard and Shands 1954; Järve et al. 2000). To our knowledge, APR to powdery mildew has not been transferred into *T. aestivum* from the timopheevii group of wheats.

The timopheevi group of tetraploid wheats ($2n = 28$) with the genome formula A^tA^tGG includes the wild form of timopheevi wheat known as *T. timopheevii* (Zhuk.) Zhuk. ssp. *armeniacum* (Jakubz.) van Slageren, the domesticated form *T. timopheevii* (Zhuk.) Zhuk. ssp. *timopheevii* (later abbreviated as *T. timopheevii*), and also a third, free-threshing form of wheat discovered by Zhukovsky in 1950, *Triticum militinae* Zhuk. et Migusch. (Zhukovsky and Migushova 1969). *T. militinae* is considered to be a spontaneous mutant of *T. timopheevii* (Dorofeev 1987); however, it has also been supposed to originate from an introgressive hybridization between *T. timopheevii* and *T. persicum* (Navruzbekov 1981; Järve et al. 2002).

Communicated by B. Keller

I. Jakobson (✉) · H. Peusha · L. Timofejeva · K. Järve
Department of Gene Technology,
Tallinn University of Technology,
Akadeemia tee 15, 19086 Tallinn, Estonia
E-mail: irena.jakobson@ttu.ee
Tel.: +372-6-204427

Using microsatellites derived from bread wheat, the genomic organization of *T. timopheevii* and *T. militinae* has been compared in a comparative mapping with that of hexaploid wheat (Salina et al. 2005). According to the results of this study, polymorphism between *T. militinae* and *T. timop*heevii (accession no. K-38555) is approximately at the same level as polymorphism between two accessions of *T. timopheevii* (*T. timopheevii* var. *timopheevii* and *T. timopheevii* var. *typica*).

Immunity to powdery mildew, leaf and yellow rusts, as well as high resistance to stem rust, loose and dwarf smuts have been reported as useful traits of *T. militinae* (Dorofeev et al. 1987). In three different climatic regions, *T. militinae* has been found to be unsusceptible to fungal diseases; mere traces of stem rust were detected (Migushova 1975). No resistance genes originating from *T. militinae* have been identified in common wheat.

The aims of this study were to evaluate powdery mildew resistance of hybrid wheat lines selected from the progeny of a cross between *T. aestivum* and *T. militinae*, and to estimate the number and genomic positions of the QTLs with significant effects on seedling resistance and APR.

Materials and methods

Plant material

As the female parent, the Finnish wheat cultivar Tähti was crossed with *T. militinae* Zhuk. et Migush. (accession no. K-46007 from the N.I.Vavilov Institute of Plant Industry, St.Petersburg, Russia). The self-sterile F_1 plants were backcrossed to Tähti once. BC_1F_2 plants were self-pollinated and hybrid population was advanced to BC_1F_4.

Random seeds from the hybrid population were planted. The somatic chromosome numbers of the selected plants were determined in root-tip cells using standard Feulgen staining procedures. One ear of each plant was pollinated with the pollen of the susceptible mother cultivar, the others were self-fertilized. Self-pollinated F_1 plants were advanced as hybrid lines.

From an F_1 plant of a backcross (hybrid plant 8/1 × Tähti), an F_2 population was derived and further used for microsatellite screening and QTL mapping. F_2 plants were selfed to produce F_2-derived F_3 families. The mapping population consisted of 134 F_2 plants and 130 F_2-derived F_3 families.

Parental plants of the studied hybrid lines were resistant to the synthetic population of *B. graminis* at the seedling stage (score 0–2).

Disease resistance

During the first 15 days, the plants were grown in the greenhouse. On the tenth day of growth, the reaction to 11 test-isolates of *B. graminis* (DC. et Marat*) speer* f. sp. *tritici* (kindly provided by Dr. F. Felsenstein, Freising-Weihenstephan, Germany) and to a synthetic population of mixed test-isolates was estimated on detached leaves and scored (0, no visible symptoms; 9, heavy sporulation, Lutz et al. 1992). Five days later, the plants were planted in the field in a completely randomized design, ten plants in a 1 m row. Strips of plants of the susceptible cultivar Saratovskaya-29 surrounded every fifth row.

For the estimation of APR under natural infection with the native population of the pathogen, the mildew was assessed on the upper two leaves as a visually estimated percentage of leaves covered with mildew. If the leaves became senescent, the previous score for that leaf was used in calculating the cumulative mildew cover for the upper two leaves. Disease severity was expressed as a disease index (DI) on a 0–9 scale (Yu et al. 2001). The plants were scored twice, at first in the ear emergence stage (DI 1) and the second time 14 days later (DI 2) in the milky ripe stage.

In the mapping population, APR was tested in 2 years (in 2002 and 2004). In 2004, F_2-derived F_3 families were grown in two replications (five plants in a block) using a completely randomized block design. In all the 130 F_3 families, adult resistance was scored for four individual F_3 plants per replication (1,040 F_3 plants were tested altogether).

The parental lines were included as controls in all experiments.

Microsatellite marker analysis

A total of 129 simple sequence repeats (SSR) were used to screen the parental lines: *gwm* markers (Röder et al. 1998), *psp* markers, kindly provided by Dr. P. Stephenson (Norwich, UK), *wmc* markers (Gupta et al. 2002), and *BARC* marker, developed by P. Cregan, Q. Song and associates (http://www.wheat.pw.usda.gov/) (Table 1).

Total genomic DNA was extracted from young leaf tissue (approximately 300 mg) frozen in liquid nitrogen, according to the method described by Huang et al. (2000) with minor modifications. Radioactive PCR amplifications of microsatellite fragments were performed as described by Röder et al. (1998) or Bryan et al. (1997), the annealing temperature depending on the type of the primer. Amplified DNA fragments were separated on a 5 or 6% polyacrylamide denaturing gel and autoradiographed.

QTL mapping

The linkage groups were established with the Map Manager QTX Version b16 software for genetic mapping of Mendelian markers and QTLs (Meer et al. 2002; Chmielewicz and Manly 2002) with a minimal LOD score of 3 and a maximum genetic distance of 30 cM.

Table 1 List of microsatellite markers used in the screening of hybrid lines

Chromosome	Markers
1A	Xpsp2999, Xpsp3027, Xpsp3151, Xgwm33, Xgwm99, **Xgwm136**
2A	Xpsp3029, Xpsp3039, Xpsp3088, **Xpsp3142**, Xpsp3153, Xgwm47, Xgwm71, Xgwm294, Xgwm296, Xgwm311, Xgwm356, Xgwm359, Xgwm372, Xgwm382, Xgwm512
3A	Xpsp3047, Xgwm2, Xgwm155, Xgwm162, Xgwm369, Xgwm480, Xgwm666
4A	Xpsp3028, Xpsp3058, Xpsp3119, **Xpsp3142**, Xgwm160, Xgwm610, Xgwm637, Xbars153, Xbarc52, Xbarc184, **Xwmc219,** Xwmc232, Xwmc283, **Xwmc313, Xwmc497**
5A	Xgwm126, **Xgwm156**, Xgwm186, Xgwm205, Xgwm293, **Xgwm304**, Xgwm410, Xgwm415, Xgwm595, Xgwm617, Xgwm639, Xgwm666, Xwmc415, Xwmc492
6A	Xpsp3029, Xpsp3071, Xpsp3152, Xgwm427, Xgwm459, Xgwm570, Xgwm617
7A	Xpsp3050, Xpsp3094, Xpsp3114, Xgwm60, Xgwm130, Xgwm260, Xgwm276, Xgwm350, Xgwm573, Xgwm635, Xgwm666
1B	Xpsp3000, Xpsp3100**, Xgwm33**, Xgwm153, Xgwm264, Xgwm274, Xgwm550
2B	Xpsp3030, Xgwm16, Xgwm47, Xgwm71, Xgwm210, Xgwm382, Xgwm410, Xgwm501, Xgwm526
3B	Xpsp3003, Xpsp3030, Xpsp3035, Xpsp3078, Xpsp3081, Xpsp3112, Xpsp3144, Xgwm112, Xgwm247, Xgwm264, Xgwm285, Xgwm493
4B	Xpsp3030, Xpsp3078, Xgwm66, Xgwm368
5B	Xpsp3037, Xpsp3065, Xgwm66, Xgwm68, Xgwm604, Xgwm639, Xgwm213, Xgwm159
6B	Xpsp3009, Xpsp3079, Xpsp3112, Xpsp3131, Xpsp3139, Xgwm132, Xgwm133, Xgwm508, Xgwm518, Xgwm613, Xgwm626, Xgwm921
7B	Xpsp3033, Xpsp3081, Xgwm16, Xgwm46, Xgwm68, Xgwm112, Xgwm274, Xgwm302, Xgwm333, Xgwm573, Xgwm611
1D	Xpsp3037, Xpsp3137, **Xgwm33**, Xgwm147, Xgwm232, Xgwm337, Xgwm458
2D	Xpsp3058, Xgwm71, Xgwm311, Xgwm210, Xgwm296, Xgwm382
3D	Xpsp3019, Xgwm2, Xgwm71, Xgwm383
4D	Xpsp3007, Xpsp3079, Xpsp3103, Xpsp3112, Xgwm624
5D	Xgwm16, Xgwm174, Xgwm192, Xgwm205, Xgwm358, Xgwm565, Xgwm639
6D	Xpsp3058, Xpsp3200
7D	Xpsp3035, Xpsp3079, Xpsp3094, Xpsp3113, Xpsp3123, Xgwm350, Xgwm635

The primers printed in bold were not polymorphic between Tähti and *Triticum militinae*

Genetic distances between markers were estimated using the mapping function of Kosambi (1944). The chromosomal alignment of linkage groups was deduced from the published wheat maps (Röder et al. 1998; Gale et al. 1995; Somers et al. 2004) and from the *GrainGenes* database (http://www.wheat.pw.usda.gov).

The association between phenotype and marker genotype was investigated using single marker regression. The positions of the detected QTLs were determined using simple interval mapping (SIM) and composite interval mapping (CIM). The free-regression model was applied. The likelihood ratio statistic (LRS) threshold for declaring the statistical significance of association was calculated empirically for each experiment using the permutation test, at 1,000 iterations. Confidence interval was estimated by bootstrap analysis using the same software.

QTL effects were estimated as the percentage of phenotypic variation explained by QTL.

Statistical analysis

Log_{10}-transformed data was used in all statistical and QTL analyses. Chi-square analyses were performed to test the significance of deviations of observed segregation ratio from theoretical expectations. The ANOVA (analysis of variance) was performed to determine the significance of differences between the genotypes. Components of variance were computed considering the effects of the environment (year) and the genotype as random. Estimates of variance components δ^2_G (genetic variance), δ^2_E (environmental variance), δ^2_{GxE} (genotype × environment interaction variance) and δ^2_{Err} (error variance) were calculated.

As the ANOVA showed no significant effect of replications (blocks) for the disease resistance in F_3, we have further used the mean of the data from two blocks (eight plants) for each family to search for the QTLs. The phenotypic correlation coefficient of adult plant powdery mildew resistance between F_2 and F_3 progenies was calculated.

Results

Hybrid lines

In 1995, the Finnish cultivar of spring wheat Tähti was crossed as a female parent with *T. militinae;* approximately 5% of pollinated florets gave a seed. The F_1 hybrids between the common wheat and *T. militinae* are self-sterile and, usually, two to three backcrosses are needed to fully restore the self-fertility. In this study, the F_1 hybrids were maintained by backcrossing them once as females with *T. aestivum*. The fertile F_2 hybrids were

grown as a population without isolating the ears. The derived F_4 hybrid population consisted of phenotypically heterogeneous but cytogenetically stable plant material and showed improved resistance to powdery mildew in field tests (data not shown). The four hybrid lines (8/1, 8/4, 8/7 and 8/9) derived in this study were single-seed descendant lines advanced from plants randomly selected from the hybrid population.

Disease resistance in hybrid lines

The self-pollinated F_2 families were divided into three groups and tested for APR in the field conditions during 3 years (2002–2004). In general, hybrid lines expressed higher levels of APR than the parent cultivar Tähti (Fig. 1), the average DI of lines derived from the hybrid population 4.8 ± 1.8 being significantly lower as compared to the DI of Tähti 11.9 ± 0.1.

Significant differences both between the lines and between the years ($P < 0.0001$), as well as a highly significant line-by-year (genotype × environment) interaction were detected ($P < 0.0001$) by ANOVA. Genotypic differences between the lines explained 32% of the phenotypic variation for APR in the hybrid lines and the line-by-year interaction explained 29% of the variance.

APR in the mapping population

Hybrid line 8/1 showed a high and stable level of APR and for further marker analysis and QTL detection, a single-plant descendant F_2 mapping population was derived from a cross of this line with Tähti.

Two adult plant disease assessments corresponding to the beginning of ear emergence (DI 1) and milky ripe (DI 2) development stages were carried out in the mapping population, both in 2002 and in 2004. The distribution of the DI assessments is presented in Fig. 2. For all four assessments, DIs showed a continuous variation with one peak and with a distribution slightly deviated from normal. Transgressive segregation towards the susceptible parent was observed. The average of DI of F_2 plants and F_3 families from the cross (8/1 × Tähti) was approximately the same as the mean of parental DIs, indicating an additive inheritance for APR in the line 8/1.

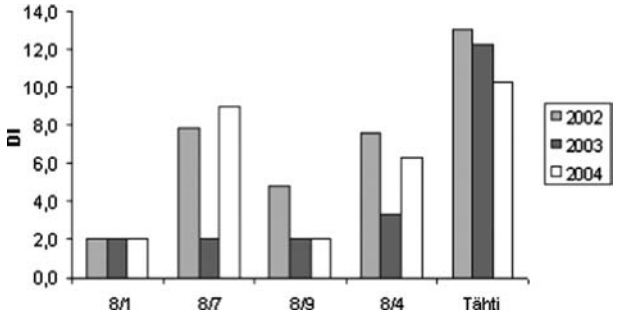


Fig. 1 Phenotypic distribution of four hybrid lines from the cross Tähti × *T. militinae* for APR across 3 years

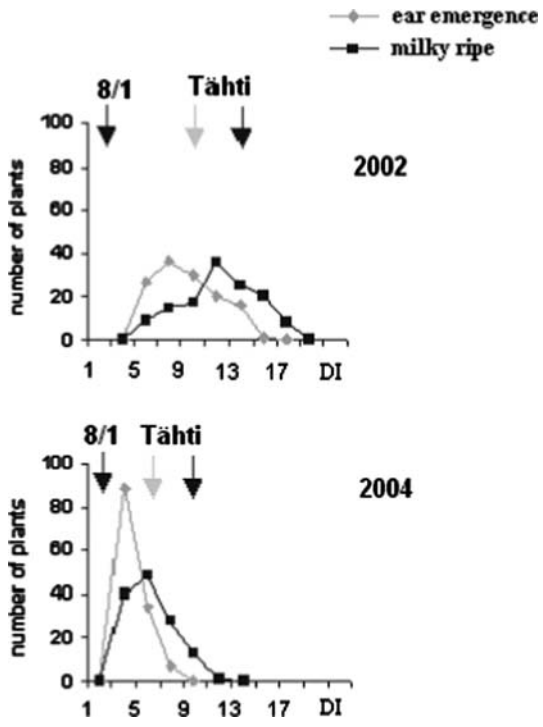


Fig. 2 Distribution of the adult plant powdery mildew DI of F_2 plants (2002) and $F_{2:3}$ families (2004) derived from the cross 8/1 × Tähti in the first and second assessment. The adult plant powdery mildew DI of two parents (8/1 and Tähti) is indicated

The contribution of the two variance components (genotype of the F_2 plant or F_3 family and time of the assessment) to the DI variability was calculated from ANOVA, separately for either of the years. Both variance components were found to be significant ($P < 0.0001$). The genotype effect explained about 43 and 47% of the total trait variance in 2002 and 2004, respectively. As the effect of the time of assessment explained about 30% of the phenotypic variance, both DI 1 and DI 2 scorings were further separately used for QTL mapping.

Both the genotype and the year had a significant effect on the total DI variance over 2 years ($P < 0.0001$), the effect of genotype × environment (year) interactions was not significant ($P = 0.138$). The significant correlation of the corresponding DIs estimated in different years ($r = 0.46$ and 0.49 for DI 1 and DI 2, respectively; $P < 0.001$) indicated that the same genetic factor was acting in different environments.

Microsatellite marker analysis in hybrid lines

Totally, 93% of the 129 microsatellite markers analysed showed a polymorphism between the *T. militinae* and Tähti genotypes. The 120 polymorphic microsatellites revealed 174 loci in the genome of hybrid wheat, on average 1, 45 loci per marker.

Table 2 Translocations in the hybrid lines

Chromosome	8/1	8/7	8/9	8/4
1A	+	+	–	–
2A	+	–	–	–
3A	–	+/–	+	+
4A	+	+/–	+/–	+/–
5A	+	+	+/–	+/–
7A	+/–	+/–	+/–	+–
1B	+/–	+/–	–	–
3B	–	+	+	+
5B	+	–	–	–
6B	–	+/–	+/–	+/–

+ *T. militinae* translocation; – no translocation; +/– heterozygote

In the four hybrid lines, *T. militinae*/*T. aestivum* replacements were detected in 73 loci (42%). The number of translocations per hybrid line varied from 6 to 8 (Table 2). According to the C-banding data, in *T. timopheevii*- and *T. militinae*-derived introgressive lines, substitutions of the whole chromosome were far more frequent than translocations (Badaeva et al. 1991, 2000). On the contrary, our molecular study identified intercalary translocations while no whole chromosome substitutions were detected. The translocations ranged from a few centimorgans to the almost complete chromosome arm substitutions. In our hybrid lines, altogether six chromosomes of the A genome and four chromosomes of the B genome were involved in translocations (Table 2), while no translocations were found in the D genome. However, according to Badaeva et al. (1991, 2000), the G/D chromosome substitutions have been detected in *T. timopheevii*- and *T. militinae*-derived introgressive lines.

Microsatellite marker analysis in the mapping population

A total of 37 markers showing polymorphism between 8/1 and Tähti produced 42 segregating fragments in the mapping population derived from the cross 8/1 × Tähti. 40% of markers were scored as codominant, 45% as null alleles in *T. militinae* and 15% amplified independently segregating fragments from Tähti and *T. militinae* genomes.

The mapping of 42 loci resulted in 9 linkage groups (LOD >3). Colinearity of markers with the published maps was observed and seven linkage groups could be assigned to a chromosome of hexaploid wheat (Fig. 3).

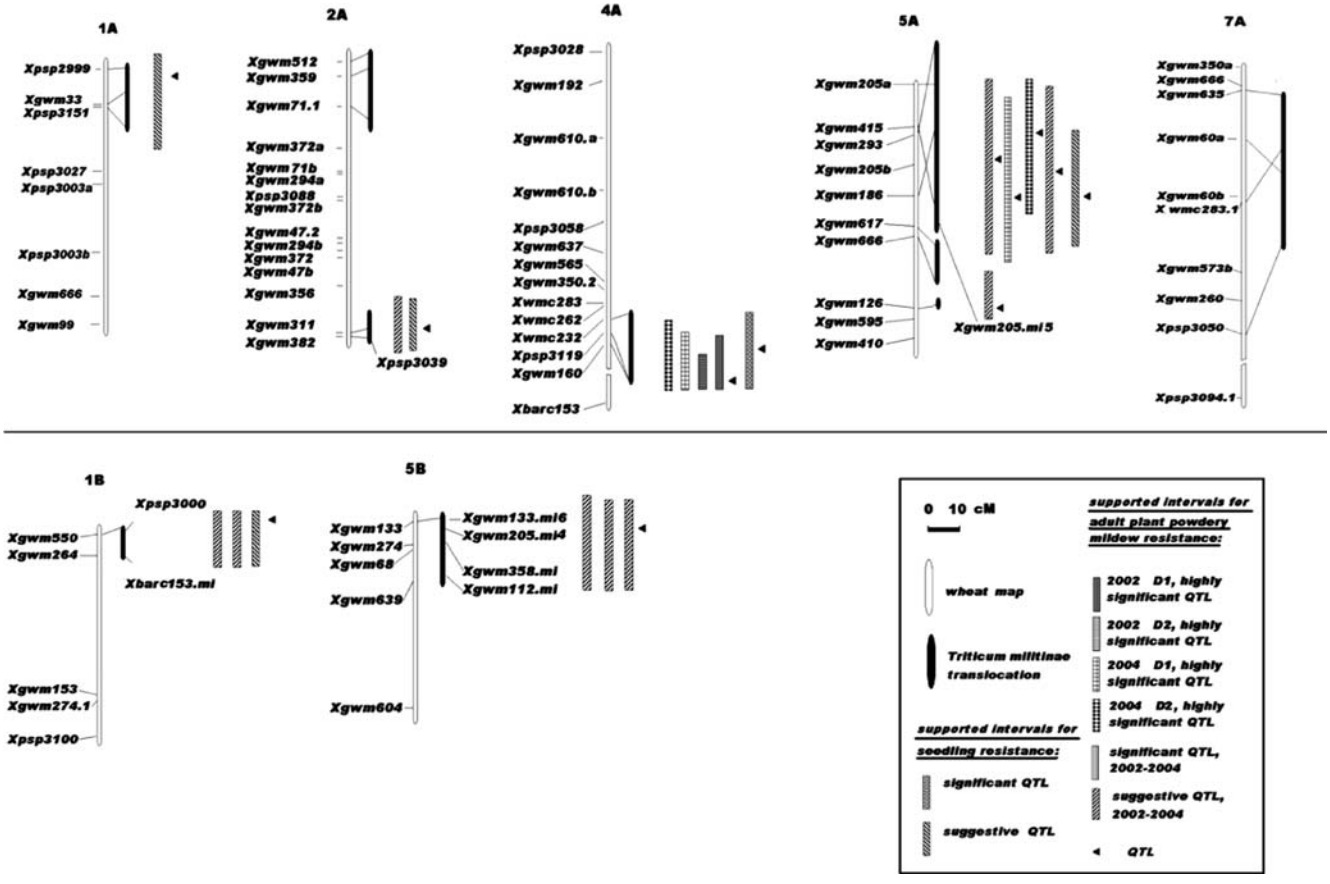


Fig. 3 The map of *T. militinae*-derived translocations and powdery mildew resistance QTLs in the mapping population derived from a cross between the hybrid line 8/1 and cultivar Tähti. Only chromosomes with *T. militinae* translocations are represented. Multiple loci detected by single markers have a suffix (1–6) added following the marker name. Suffix mi designates a *T. militinae* allele

Table 3 Powdery mildew resistance QTLs detected at the different plant stage for F2 plants or for F3 families issued from cross between 8/1 and Tähti

Chromosome	Marker interval	Adult plant resistance												Seedling plant resistance		
		2002						2004								
		DI 1			DI 2			DI 1			DI 2					
		R^2	LRS	Add Dom	R^2	LRS	Add Dom	R^2	LRS	Add Dom	R^2	LRS	Add Dom	R^2	LRS	Add Dom
SIM																
4A	Xgwm232–Xgwm160	27	36.6***	−0.330.01	35	51.4***	−0.290.03	54	90.4***	−0.32−0.11	41	61.0***	−0.35−0.04	28	35.7***	−0.290.18
5A	Xgwm186–Xgwm415	5	6.3 *	−0.14−0.08				7	8.7*	−0.100.10						
	Xgwm666–Xgwm126	5	9.6*	−0.32−0.01												
2A	Xgwm311–Xgwm382										5	6.2*	−0.13−0.01	6	6.5*	−0.12−0.20
1A	Xpsp2999–Xpsp3151													6	6.7*	0.010.08
CIM																
4A	Xgwm232–Xgwm160	25	36.2***	−0.320.01	34	53.0***	−0.28−0.02	49	84.8***	−0.32−0.11	40	55.5***	−0.33−0.03	33	42.9***	−0.290.22
5A	Xgwm186–Xgwm415	6	8.4*	−0.48−0.56	4	13.9**	−0.05−0.13	6	14.3**	−0.100.08	6	8.2*	0.210.68	5	7.6*	0.47−0.64
	Xgwm666–Xgwm126	7	12.1*	3.487.58												
2A	Xgwm311–Xgwm382										5	6.4*	−0.13−0.02	6	6.9*	−0.12−0.09
1A	Xpsp2999–Xpsp3151													5	7.4*	0.06−0.16
1B	Xgwm3000	5	7.7*	−0.130.04	4	5.6*	−0.070.08							4	6.4*	0.00−0.20
5B	Xgwm133.mi6–Xgwm205.mi1				4	5.6*	0.74−1.8	5	9.8*	−0.931.56	6	6.4*	0.771.68			

R^2 percentage of variance explained by individual QTL; *LRS* likelihood ratio statistic; *add.* additive effect due to a substitution of a Tähti allele by a corresponding *T. militinae* allele in the line 8/1; *dom.* dominance effect due to a substitution of a Tähti allele by a corresponding *T. militinae* allele in the line 8/1

P level of significancy: $*P < 0.05$; $**P < 0.01$; $***P < 0.0001$

The genetic map covered the introgressive part of the genome of line 8/1, comprising 38 loci and spanning 251 cM. Four amplification products remained unlinked, three of them originated from the genome of *T. militinae*. Four markers showed a distorted segregation favouring the Tähti allele, three of them were clustered in a tightly linked group (LOD >15) assigned to chromosome 5B; one marker with distorted segregation remained unlinked to any other marker.

Compared to the published genetic maps of wheat (Röder et al 1998; Gale et al. 1995), the genetic distance of the detected translocations was significantly reduced on some chromosomes (chromosome 7A). This may indicate regions with reduced recombination in some of the translocated segments. *T. timopheevii* translocations showing no or extremely low levels of recombination in crosses with hexaploid wheat have been referred to earlier (Devos et al. 1993; Järve et al. 2000; Kammholz et al. 2001).

QTL analysis

The microsatellite map and DI assessments were used to identify the genetic associations. The data of two assessments in different years were used separately for QTL mapping.

A QTL with a LRS score greater than the threshold required for declaring a highly significant QTL linkage to a locus was detected by SIM and CIM analyses (Table 3). The highly significant QTL for powdery mildew resistance on chromosome 4A had the highest LRS score ($P < 0.0001$) both in 2002 and in 2004. The QTL peaked at the microsatellite marker *Xgwm160* in an *Xwmc232 – Xpsp3119* introgressive translocation interval (Fig. 5).

Four LRS curves (DI1 and DI2 in 2002 and in 2004) for chromosome 4A showed identical peaks despite the marked difference in the distribution of the two DI scores in a year and the differences between the results obtained for F_2 and F_3 progenies, indicating that the genetic factors contributing to APR are not environmentally sensitive. The major QTL explained up to 35% of the variance in 2002, and up to 54% in 2004. The allele for improved resistance originated from *T. militinae* and acted in a nearly additive fashion (Table 3). The additivity of the main QTL is consistent with the results from the field studies based on classical quantitative genetic analysis.

An additional QTL with significant LRS score was detected by CIM analysis (considering *Xgwm160* as a cofactor; Table 3). The QTL was located on chromosome 5A; however, its precise location depended on the year of the experiment. The minor QTL explained 4–6% of trait phenotypic variance.

On chromosomes 1B, 2A, 5A and 5B, suggestive minor QTLs for APR were detected, either in 2002 or in 2004 (Table 3). The non-reproducible QTL effect on these chromosomes may be a false positive effect rather than that of an environment-specific gene. However, reporting suggestive linkages may be useful, taking into account that non-significance in statistical terms might not denote insignificance in biological terms (Freymark et al. 1993).

Thus, two QTLs located on two different chromosomes (4A and 5A) control the quantitative resistance to powdery mildew in the mapping population at the adult plant stage and explain up to 38 and 55% of total trait variance in F_2 and F_3, respectively. If the suggestive QTLs on 5B, 1B, 2A and 5A are included, up to 69% of total trait variance can be explained.

To demonstrate the selective power of *Xgwm160* in the selection for APR to powdery mildew, F_2 plants and F_3 families were grouped according to the genotypes in this locus (Fig. 4). At the adult plant stage, the average disease severity in plants with the Tähti alleles was twice as high as in plants with the *T. militinae* alleles. However, the DI score for plants with homozygous *T. militinae* alleles in *Xgwm160* locus remained higher than the score for line 8/1, indicating, that not only the main QTL on chromosome 4A is responsible for the high and stable level of APR in this hybrid line.

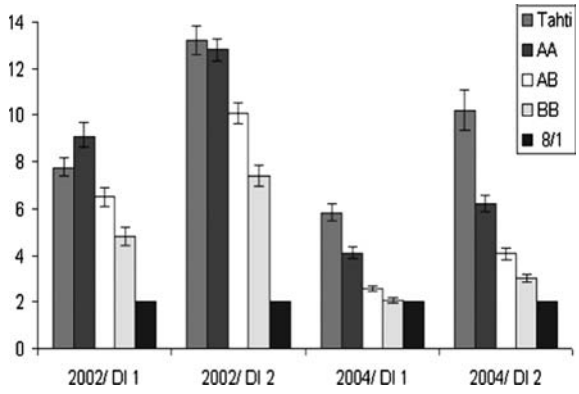


Fig. 4 The effect of alternative alleles in the QTL region of chromosome 4A (*Xgwm160*) on APR in the mapping population for the two assessments, in 2002 and in 2004. *AA* homozygous Tähti allele, *AB* heterozygous, *BB* homozygous *T. militinae*-derived allele

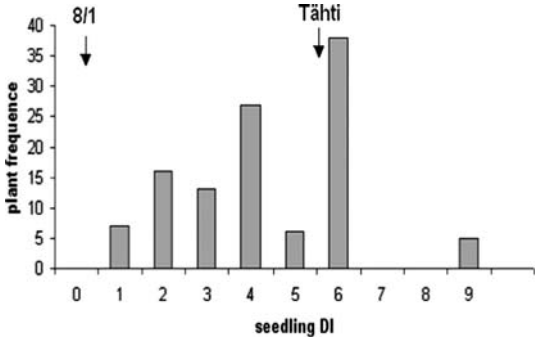


Fig. 5 Distribution of the seedling plant powdery mildew DI for F_2 plants in the mapping population (*0* no visible symptoms; *9* heavy sporulation). The scores for the parents are indicated by *arrows*

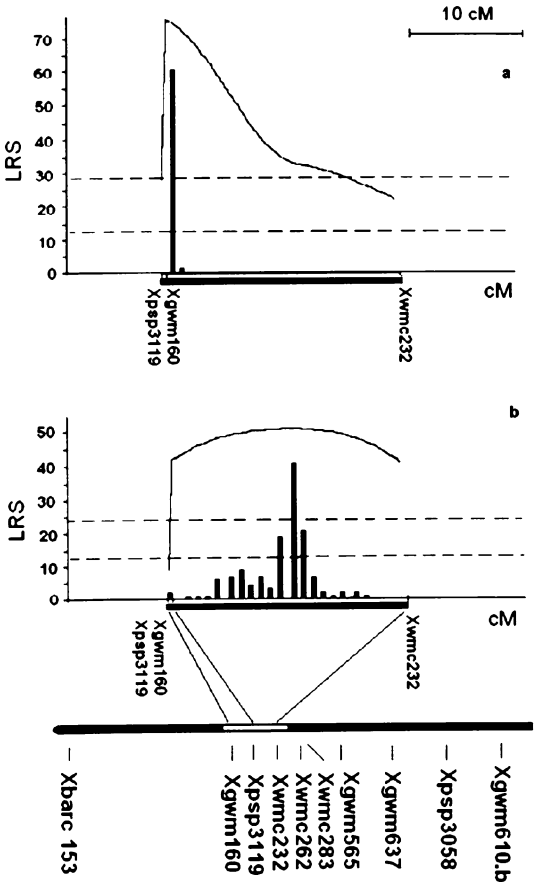


Fig. 6 The likelihood plots of QTLs associated with the adult plant (**a** 2002, DI 1) and seedling resistance (**b** 2002) to powdery mildew on chromosome 4A in the 8/1 × Tähti mapping population. The estimations of confidence intervals by bootstrap resampling are plotted as histograms. The *horizontal dashed lines* represent the significant and the highly significant LRSs

Seedling resistance

Hybrid line 8/1 was resistant to the synthetic population of *B. graminis* DC. f. sp. *tritici* at the seedling stage (score 0–2).

The seedling resistance score in the mapping population showed a continuous distribution (Fig. 5). To avoid the necessity for a qualitative distinction between the resistant/susceptible plants, a quantitative interpretation of the seedling tests of F_2 plants was conducted (Chantret et al. 2000).

A QTL with a significant effect on the seedling resistance to powdery mildew was detected on chromosome 4A by both SIM and CIM analyses. LRS values above the highly significant threshold (Table 3) were registered over the entire translocation on this chromosome. The peak for the LRS score was 9–12 cM away from the *Xgwm232* locus, between loci *Xgwm232* and *Xgwm160* (Fig. 6). The allele conferring resistance originated from *T. militinae*.

Suggestive QTLs for seedling resistance were detected on chromosomes 1A, 1B, 5A (all of them in Tähti alleles) and 2A (*T. militinae* allele) (Table 3). A suggestive QTL for seedling resistance originating from the Tähti genome may explain the transgressive segregation of seedling resistance in the mapping population (Fig. 5).

The main QTL for seedling resistance explained about 33% of phenotypic variance for the trait, each of the suggestive QTLs added about 4–6% (Table 3). Thus, up to 53% of total trait variance could be explained by the QTLs.

Discussion

The objective of this study was to elucidate the genetic basis of adult plant powdery mildew resistance transferred from *T. militinae* into the Finnish spring wheat cultivar Tähti. Simultaneously, hybrid lines were advanced from seeds randomly selected from a heterogeneous hybrid population, powdery mildew resistance levels were assessed and chromosomal segments introgressed from *T. militinae* were mapped in the selected hybrid lines.

In the F_2 backcross-mapping population derived from the hybrid plant 8/1, a *T. militinae*-origin genomic region on chromosome 4A was responsible for up to 54% of the APR variance. Despite the differences in environmental conditions, the results of the QTL mapping were remarkably consistent for F_2 plants and F_3 families in 2002 and 2004, respectively.

Thus far, three QTLs for adult plant resistance (APR) to powdery mildew have been mapped on chromosome 4A (Huang XQ, Röder MS 2004). Two QTLs mapped on chromosome 4AS in the regions *Xgwm111–Xpsp934* and *Xglk128–Xcdo475* in a segregating wheat/spelt population explained 7–14% of the phenotypic variance (Keller et al. 1999). One QTL for adult powdery mildew resistance in the winter wheat line RE174 was detected on chromosome 4A (*XgbxG036–XgbxG542*) in the study by Chantret et al. (2001). This QTL explained 5–6% of the genetic variability over 2 years. However, the position of the QTL for APR in the line 8/1 does not coincide with the abovementioned QTLs.

The QTL for powdery mildew resistance at the seedling stage shows a highly significant LRS score at the same introgressive translocational region on the chromosome 4A (*Xwmc232–Xgwm160*, Table 3). This chromosomal region is probably responsible for the correlation found between the DIs for resistance at the seedling and adult plant stages (0.29 and 0.38, for adult plant data set in 2002 and 2004, $P < 0.001$). The exact positions of the highest LRS values for the adult and seedling stage resistance differ somewhat, although the confidence intervals for both peaks overlap (Fig. 6), permitting either a single gene or a cluster of genes to be involved in the resistance at different stages of plant growth. A cluster of genes related to resistance seems to be the case, taking into account that the resistance allele for APR on chromosome 4A acted in a near additive

fashion and the QTL for seedling resistance was inherited as a recessive locus (Table 3).

It has been repeatedly observed that disease resistance genes may be located in a complex region containing different race specifics (Hammond-Kosack and Jones 1997; Hsam and Zeller 2002). A number of loci for resistance to different pathogens (*H25, Lr28* and *Lr30, Sr7*, *YrMin* and *YrND, Stb7)* have been located on chromosome 4A (McIntosh et al. 2003). The gene for race-specific resistance to *Mycosphaerella graminicola* in the spring wheat, *Stb7*, has been mapped precisely at the distal end of chromosome 4AL in a region closely linked to *Xgwm160* (McCartney et al. 2003). Since homologous relationships have been found between disease resistance loci (Hammond-Kosack and Jones 1997), it is possible that the genome of the line 8/1 includes a cluster of *T. militinae*-derived genes on chromosome 4A, responsible for the non-race-specific powdery mildew resistance in the seedling and adult plant stages of plant growth. Further analysis of recombinant plants will allow us to map this region precisely.

It has been suggested that the same loci could be responsible for qualitative and quantitative traits (Robertson 1985). A race-specific gene for resistance to powdery mildew, *Pm 16*, derived from wild emmer (*Triticum dicoccoides*) has been mapped on chromosome 4AL (Reader and Miller 1991; McIntosh et al. 2003). However, Chen et al. (2005) showed that a SSR marker located on the short arm of chromosome 5B, *Xgwm159*, is closely linked to *Pm16* (genetic distance 5.3 cM), and suggested that *Pm16* might be located on a translocated 4A.5BS chromosome. In our mapping population, the amplification product of *Xgwm159* primers was not segregating, however, the hybrid line 8/1 carries a *T. militinae* translocation in the region of *Xgwm213–Xgwm68–Xgwm66* close to the marker *Xgwm159* on chromosome 5B. Markers *Xgwm66, Xgwm68* and *Xgwm213* have been mapped on chromosome 5B in the ITMI mapping population (Röder et al. 1998), and, in our mapping population, they form a linkage group with no linkage to the *Xgwm160–Xwmc232* translocation on chromosome 4A. Therefore we suggest that rather than *Pm16*, some unknown gene(s) located on the *Xgwm160-Xwmc232* translocation on chromosome 4A is involved in the detected QTL for APR in the line 8/1.

In 8/1, the suggestive QTLs ($P < 0.05$) were detected for APR and for seedling resistance, all of them explaining 4–6% of the respective phenotypic variance. It has been indicated that the minor QTLs for non-race-specific powdery mildew resistance could be less effective or "defeated" alleles of *Pm* genes (Nass et al. 1981; Chantret et al. 1999). For some of the minor QTLs detected in the line 8/1, this may be the case. A residual effect on APR has been demonstrated for the resistance gene allele *Pm4b* (Chantret et al. 1999). *Pm4b* originates from *T. carthlicum* (synonym *Triticum persicum)* (McIntosh et al. 2003). Since it has been assumed that *T. militinae* has arisen as a result of an introgressive hybridization between *T. timopheevii* and *T. persicum* (Navruzbekov 1981), the introgressed into 8/1 chromosomal segment on chromosome 2A with minor QTLs for APR and seedling resistance may include a homologous to resistance gene *Pm4* region. Further analysis is required to prove this suggestion.

In the hybrid line 8/1, 54% of APR and 33% of variance in seedling resistance can be explained by the major QTLs detected on chromosome 4A. Suggestive QTLs on different chromosomes add up to 69 and 53%, respectively, of the phenotypic variance, which is a slightly higher percentage than Kearsey and Farquhar (1998) have found to be an average (46%). Even if the detected effect of the *T. militinae* translocation on chromosome 4A is one of several clustered genes, it may be considered as a new genetic factor for marker-assisted selection for *T. militinae*-derived resistance to powdery mildew (Fig. 4).

Acknowledgements The authors gratefully acknowledge Prof. A. Veske and Prof. E. Truve for their constructive comments to improve this paper. This work was supported by Estonian Science Foundation (grant No 4720) and Estonian Ministry of Agriculture.

References

Allard RW, Shands RG (1954) Inheritance to resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevii*. Phytopathology 44:266–274

Badaeva ED, Budashkina EB, Badaev NS, Kalinina NP, Shkutina FM (1991) General features of chromosome substitutions in *Triticum aestivum* × *T. timopheevii* hybrids. Theor Appl Genet 82:227–232

Badaeva ED, Prokofieva ZD, Bilinskaya EN, Obolenkova LA, Solomatin DA, Zelenin AV, Pukhalskyi VA (2000) Cytogenetic analysis on hybrids resistant to yellow rust and powdery mildew obtained by crossing common wheat (*Triticum aestivum* L., AABBDD) with wheats of Timopheevi group (A^tA^tGG) (in Russian). Genetika 36:1663–1673

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Chantret N, Pavoine MT, Doussinault G. (1999) The race-specific resistance gene to powdery mildew, *MlRE*, has a residual effect on adult plant resistance of winter wheat line RE714. Phytopathology 89:533–539

Chantret N, Mingeot D, Sourdille P, Bernard M, Jacquemin JM, Doussinault G (2001) A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. Theor Appl Genet 103:962–971

Chantret N, Sourdille P, Röder M, Tavaud M, Bernard M, Doussinault G (2000) Location and mapping of the powdery mildew resistance gene MIRE and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor Appl Genet 100:1217–1224

Chen XM, Luo YH, Xia XC, Xia LQ, Chen X, Ren ZL, He ZH, Jia JZ (2005) Chromosomal location of powdery mildew gene *Pm16* in wheat using SSR marker analysis. Plant Breed 124:225–228

Chmielewicz KM, Manly KF (2002) User manual for QTX. Roswell Park Cancer Institute

Devos KM, Millan T, Gale MD (1993) Comparative RFLP maps of homoeologous group 2 chromosomes of wheat, rye, and barley. Theor Appl Genet 85:784–792

Dorofeev VF, Udaczin RA, Semenova LV, Novikova MV, Gradchaninova OD, Shitova IP, Merezhko AF, Filatenko AA (1987) World wheats. Agropromizdat, Leningrad, pp 75–87

Freymark PJ, Lee M, Woodman WL, Martinson CA (1993) Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). Theor Appl Genet 87:537–544

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Proceedings of the 8th international wheat genetics symposium, vol 1. China Agricultural Scientech Press, Beijing, pp 29–40

Griffey CA, Das MK (1994) Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Sci 34: 641–646

Gupta PK, Balyan H, Edwards K, Isaac P, Korzun V, Röder M, Gautier M-F, Joudrier P, Schlatter A, Dubcovsky J, De la Pena R, Khairallah M, Penner G, Hayden M, Sharp P, Keller B, Wang R, Hardouin J, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Hsam SLK and Zeller FJ (2002) Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In: Belanger RR et al (eds) The powdery mildews. A comprehensive treatise, pp 219–238

Huang X, Zeller FJ, Hsam SLK, Wenzel G, Mohler V (2000) Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome 43:298–305

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137:203–223

Järve K, Peusha HO, Tsymbalova J, Tamm S, Devos KM, Enno TM (2000) Chromosomal location of a *T. timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome 43:377–381

Järve K, Jakobson I, Enno T (2002) Tetraploid wheat species *Triticum timopheevii* and *Triticum militinae* in common wheat improvement. Acta Agron Hung 50(4):463–477

Kammholz SJ, Campbell AW, Sutherland MW, Hollamby GJ, Martin PJ, Eastwood RF, Barclay I, Wilson RE, Brennan PS, Sheppard JA (2001) Establishment and characterisation of wheat genetic mapping populations. Aust J Agric Res 52:1079–1088

Kearsey MJ, Farquhar GL (1998) QTL analysis in plants; where are we now? Heredity 80:137–142

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. Theor Appl Genet 98:903–912

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Liu SX, Griffey CA, Saghai-Maroof MA (2001) Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci 41:1268–1275

Lutz J, Limpert E, Bartoa P, Zeller FJ (1992) Identification of powddery mildew resistance genes in common wheat (*Triticum aestivum* L.). I. Czechoslovakian cultivars. Plant Breed 108:33–39

McCartney CA, Brüle-Babel AL, Lamari L, Somers DJ (2003) Chromosomal location of race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6. Theor Appl Genet 107:1181–1186

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat http://www.wheat.pw.usda.gov/ggpages/wgc/ 2003/)

Meer JM, Manly KF, Cudmore RH (2002) Software for genetic mapping of Mendelian markers and quantitative trait loci. Roswell Park Cancer Institute

Migushova EF (1975) About the origin of wheat genomes (in Russian). Bull Appl Bot Genet Breed 55(3):16

Mingeot D, Chantret N, Baret PV, Dekeyser A, Boukhatem N, Sourdille P, Doussinault G, Jacquemin JM (2002) Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. Plant Breed 121:133–140

Nass HA, Pedersen WL, MacKenzie DR, Nelson RR (1981) The residual effects of some ''defeated'' powdery mildew resistance genes in isolines of winter wheat. Phytopathology 71: 1315–1318

Navruzbekov NA (1981) Toward the origin of *T. militinae* Zhuk. Et Migush. In: Botanical and genetic resources of dagestan flora. Makhachkala (in Russian)

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Reader SM, Miller TE (1991) The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat. Euphytica 53:57–60

Robertson DS (1985) A possible technique for isolating genic DNA for quantitative trait in plants. J Theor Biol 117:1–10

Röder MS, Korzun V, Wendehake K, Plasche J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149: 2007–2023

Salina EA, Leonova IN, Efremova TT, Röder MS (2005) Wheat genome structure: translocations during the course of polyploidization. Funct Integr Genom 6:71–80

Shands RG (1941) Disease resistance of *Triticum timopheevii* transferred to common winter wheat. Amer Soc Agron J33:709–712

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Zhukovsky PM, Migushova EF (1969) A highly immune endemic gene pool for breeding of resistant cultivars of common wheat *via* distant hybridization. Proc Agric Sci 2:9–20

Yu DZ, Yang XJ, Yang LJ, Jeger MJ, Brown JKM (2001) Assessment of partial resistance to powdery mildew in Chinese wheat varieties. Plant Breed 12: 279–284

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

Acta Agriculturae Scandinavica Section B – Soil and Plant Science, 2008; 58: 97–104

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Genetic diversity of Estonian-grown spring wheat varieties assessed by microsatellite and morphological analyses

JELENA TSÕMBALOVA[1], MERLIN HALJAK[2], ANNE INGVER[2] & KADRI JÄRVE[1]

[1]*Department of Gene Technology, Tallinn University of Technology, Tallinn, and* [2]*Jõgeva Plant Breeding Institute, Jõgeva, Estonia*

Abstract

In order to select molecular markers suitable for the seed control practice, 12 regionally cultivated spring wheat varieties were analysed by 41 microsatellite markers (SSRs). The analysed group included a pair ('Satu'-'Helle') and a triplet ('Tjalve'-'Vinjett'-'SWEstrad') of varieties derived from each other. A dendrogram resulting from analysis of the matrix of dissimilarities discriminated all varieties, and in full agreement with the pedigree data, the varieties were divided into three groups consisting of Nordic, German and Dutch varieties, respectively. In the genetically close Nordic subgroup, Swedish varieties 'Vinjett' and 'SWEstrad' could be distinguished only by three of the analysed 41 SSR markers. An attempt was made to analyse genetic diversity in the group on the basis of morphological characteristics evaluated according to the UPOV guidelines. The clustering of morphological data resulted in a dendrogram which agreed neither with the known pedigree data nor with the conducted SSR analysis. Multilocus SSR analysis revealed heterogeneity in the analysed plant material. In order to monitor inheritance of the detected heterogeneous or null-alleles, six breeding lines derived from the studied varieties were analysed. In two of the breeding lines, non-parental SSR alleles were detected. Outcrossing is suggested as a possible source of the inconsistent alleles. A minimal set of molecular markers needed to identify/verify a variety in a group can be composed of markers amplifying fragments unique in the group length. In this study, identifying markers were found for seven varieties of the group; the remaining five varieties could be identified in the group by combinations of two or three microsatellite markers.

Keywords: *Genetic diversity, Nordic spring wheat, Triticum aestivum.*

Introduction

A large collection of microsatellite markers (simple sequence repeats, SSRs) is available for the genome of *Triticum aestivum* (http://wheat.pw.usda.gov), and numerous diversity studies in wheat accessions have been conducted using microsatellites. Depending on the number of accessions studied and the set of microsatellites analysed, different authors have found 3.6 to 18.1 alleles per microsatellite locus on average (Plaschke et al., 1995; Stachel et al., 2000; Röder et al., 2002; Christiansen et al., 2002; Huang et al., 2002; Roussel et al., 2005).

The number of detected alleles has been lower for varieties originating from the same region, and it has been shown repeatedly that alleles of microsatellite markers in wheat cluster in accordance with the origin of the variety. For example, as a result of the analysis of 47 microstellite markers, Christiansen et al. have grouped 75 Nordic varieties in accordance with their origin from Norway, Sweden and Finland (Christiansen et al., 2002).

Microsatellite analysis has been suggested as an alternative to the evaluation of morphological characteristics in the identification of new plant varieties (Röder et al., 2002). However, it is not clear which microsatellite markers and how many of them should be included in the databases in order to distinguish the world wheat genotypes. Due to the regional agro-ecological and economic conditions, only a small number of recently released local varieties is generally cultivated in a region. If a representative database of microsatellite alleles is created for this local set of varieties, microsatellite markers can, for

Correspondence: K. Järve, Department of Gene Technology, Tallinn University of Technology, Akadeemia 15, 12618 Tallinn, Estonia. E-mail: kadri.jarve@ttu.ee

(Received 13 October 2006; accepted 19 January 2007)

ISSN 0906-4710 print/ISSN 1651-1913 online © 2008 Taylor & Francis
DOI: 10.1080/09064710701228296

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

example, provide an alternative method for seed genetic purity determination in the region. Traditionally, genetic purity has been determined by the evaluation of morphological or physiological traits expressed by seeds, seedlings or mature plants. Some of these characters interact with the environment in which the plants are grown and thus make the process of variety identification subjective. Microsatellite markers, in contrast, being based on DNA sequence variation, provide an unbiased means of identifying varieties. In seed control practice, the number of microsatellite markers to be analysed should be minimal, and a subset of markers capable of distinguishing the analysed set of varieties should be determined.

Genetically close varieties are often used as genitors in regional breeding programmes, and genetic diversity of wheat cultivated in a region may be relatively small. As a result of a strong selection pressure in favour of one parent in the breeding process, relatively small fractions from the genome of the other parent may be included into the offspring. Consequently, it is not clear how many microsatellite loci must be analysed to create a subset of the database, allowing discrimination of varieties in a group of genetically close genotypes.

In this study, polymorphism of microsatellite markers was analysed in a group of 12 spring wheat varieties registered in Estonia. The aim of the study was to select markers suitable for practical identification and verification of varieties in this small group of presumably genetically close genotypes.

The power of microsatellite analysis in estimating genetic relations in a small group of wheat genotypes was compared with that of the morphological characteristics evaluated according to the guidelines established by the International Union for the Protection of New Varieties of Plants (UPOV) to examine distinctness (D), uniformity (U) and stability (S) of the new varieties of wheat (UPOV document TG/3/11, Correction, 1996).

Material and methods

Plant material

Table I presents pedigree data for the analysed 12 spring wheat varieties of the Estonian Variety List according to the European Wheat Database and/or European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR). Plant material was obtained from a sample of the variety submitted to be examined for official registration in Estonia and was multiplied at the Jõgeva Plant Breeding Institute.

Varieties 'Opata 85' ('Opata') and 'Chinese Spring' ('CS') were included in the study of SSR markers as reference standards.

Six breeding lines (F_8–F_{13}, Table V) derived from the Variety List genotypes by the bulk selection method with two (F_2 and F_4) separations onto one-spike level (Jensen, 1988), were included in the microsatellite analysis in order to study the inheritance of the detected alleles.

Morphological characteristics

Morphological characterization was performed according to the 'Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability' (UPOV TG/3/11 for wheat (*Triticum aestivum* L. emend. Fiori et Paol. (with corrections)). The 24 evaluated characteristics are listed in Table II.

DNA isolation and microsatellite analysis

Total genomic DNA was extracted from a young leaf tissue frozen in liquid nitrogen, according to the method described by Huang et al. (2000) with minor modifications. DNA was extracted from a bulk of five plants, or, if indicated, from single plants.

Radioactive PCR amplifications of gwm, PSP and taglgap microsatellite markers were performed as described by Röder et al. (1998), Stephenson et al. (1998) and Devos et al. (1995), respectively. Amplified DNA fragments were separated on a 5% or 6% denaturing polyacrylamide gel (PAGE). Gels were dehydrated and autoradiographed.

Data analysis

SSR markers. Fragments amplified by microsatellite primers were scored by size and counted on 0/1 basis using the Cross Checker version 2.91 (J.B. Buntjer, Laboratory of Plant Breeding, Wageningen Agricultural University, The Netherlands) programme. Polymorphic Information Content (PIC) index and the power of discrimination of markers were calculated for each locus, using PowerStatsV12 progamme (A. Tereba). The genetic distance matrix was computed by PhylTools version 1.32 software (J.B. Buntjer, Laboratory of Plant Breeding, Wageningen Agricultural University, The Netherlands) using Nei's index (Nei & Li, 1979).

A dendrogram of 11 varieties was constructed by clustering the matrix using the unweighted pair-group method with arithmetic average (UPGMA) by use of Phylip Phylogeny Inference Package version 3.6 (J. Felsenstein, 2004). Gene diversity and pairwise differences (F_{ST}) for the pairs and clusters

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

Table I. Estonian-grown spring wheat varieties analysed.

Variety	Producer	Year	Pedigree[2]	No of identifying SSR alleles[3]	No of identifying morphological characteristics[4]
'Baldus'	Cebeco Seeds, The Netherlands	1992	'SICCO'/4/(SEL.)'SICCO'/3/'N-66'/ 'MGH-653'//'KOLIBRI' ('Peko')	4	0
'Helle'	Boreal PB/ JPBI; Finland/Estonia	1999	'SATU'/'POLKKA';	2	3
'Mahti'[1]	Boreal PB/Finland	1994	'CEBECO-1036' (Neth)/'HJA-20519' (Fin)	ND	ND
'Manu'	Boreal PB	1992	'RUSO'/'RUNAR'	3	3
'Meri'	Boreal PB/ JPBI	1999	'WW 21220' × 'Hja 22058'	2	2
'Munk'	Lochow Petkus, Germany	1993	'RALLE'//('VO-9-P-78') 'KOLIBRI'/ 'SOMARA'/3/'STAR'	6	3
'Satu'	Svalöf Weibull AB, Sweden	1990	'SNABBE'/'DRABANT'/'T-106'/'SNABBE';	2	0
'SWEstrad'	Svalöf Weibull AB	2002	'HANNO'/'HUGIN'/'VINJETT'	0	2
'Zebra'	Svalöf Weibull AB	2001	'RALLE'/'DRAGON'	7	4
'Tjalve'	Svalöf Weibull AB	1987	'Reno'/'WW16679'[5] // 'WW15432'	0	4
'Triso'	Deutsche Saatveredelung AG Germany	1996	'KADETT'/'WEIHENSTEPHANER-STAMM'	2	1
'Vinjett'	Svalöf Weibull AB	1998	'Tjalve M14'/'Tjalve M15'/'Canon'	0	1

[1]: plant material heterogenous.
[2]: data from the website *Wheat Pedigree and Identified Alleles of Genes*, http://genbank.vurv.cz/wheat/pedigree/default.htm.
[3]: number of unique microsatellite alleles in the studied set of 12 Estonian-grown spring wheat varieties analysed by 35 microsatellite markers.
[4]: number of unique variants of morphological characeristics in the studied set of 12 Estonian-grown spring wheat varieties.
[5]: 'WW16679' is a backcross line with the variety 'Kolibri' as the recurrent parent and 'WW15432' is a backcross line with the variety 'Drabant' as recurrent parent (J.Ö. Jönsson, Cereal Breeding Department, Svalöf Weibull AB, Sweden; personal communication).

Table II. Morphological characteristics evaluated in 12 spring wheat varieties grown in Estonia.

No.	Characteristics	No. of detected variants
1	anthocyanin coloration of auricles of flag leaf	3
2	frequency of plants with recurved flag leaves	4
3	time of ear emergence	3
4	glaucosity of sheath of flag leaf	4
5	glaucosity of ear	5
6	glaucosity of neck of culm	3
7	plant length	2
8	pith in cross section of straw	1
9	ear shape	5
10	ear density	3
11	ear length	3
12	presence of awns or scurs	1
13	length of awns or scurs at tip of ear	4
14	ear colour	2
15	hairiness of convex surface of apical rachis segment	5
16	width of shoulder of lower glume	5
17	shoulder shape of lower glume	5
18	beak length of lower glume	5
19	beak shape of lower glume	3
20	extent of internal hairiness of lower glume	4
21	beak shape of lowest lemma	3
22	grain colour	1
23	grain coloration with phenol	4
24	seasonal type	1

detected on the dendrogram were computed by Arlequin software (Schneider et al., 2000).

Varieties were grouped according to the dendrogram and total variance between the 11 varieties was partitioned into hierarchical covariance components of intravarietal covariance (heterogeneity), covariance between groups of the analysed varieties and covariance between the varieties in groups (ANOVA analysis by Arlequin).

Morphological data. Numerical values for morphological characteristics were converted into the binary system and the genetic distance matrix was computed by PhylTools version 1.32 software. A dendrogram was constructed by clustering the matrix using the UPGMA method by use of Phylip version 3.6 software.

Results

SSR analysis

Overall, 41 polymorphic products amplified by 35 microsatellite primers were scored (Table III). According to the published wheat maps (Röder et al., 1998; Gale et al., 1995; Somers et al., 2004) and the GrainGenes database (http://www.wheat.pw.usda.

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

Table III. Microsatellite marker alleles in a group of 12 Estonian-grown spring wheat varieties.

Marker	Chromosomal location	PIC	Alleles (bp)	Identifying alleles (bp)
gwm33.1	1(A,B,D)	0.46	181, 175, 169	'Meri' (169)
gwm33.2	1(A,B,D)	0.37	139, 125	
gwm47	2(A, B)	0.59	202, 192, 164, 146	
gwm 68.1	7B	0.46	212, 203, 184	
gwm 68.2	5B	0.62	169, 165, 143, 138	'Manu' (138)
gwm71.1	2A	0.36	120, 118, 116	
gwm71.2	3D	0.32	111, 106, 103	
gwm99	1A	0.35	132, 110	
gwm160	4A	0.40	188, 184, 182	
gwm162	3A	0.35	208, 204	
gwm186	5AL	0.30	138, 126	
gwm205.1	5A	0.32	168, 158, 150	'Munk' (150)
gwm205.2	5D	0.60	147, 144, 142, 140	'Triso' (140)
gwm210	2D	0.14	190, na	'Munk' (190)
gwm232	1D	0.46	146, 144, 140	'Manu' (146)
gwm274.1	1B	0.30	198, 182	
gwm274.2	7B	0.35	165, 162	
gwm276	7A	0.57	135, 128, 89	
gwm382	2(A,B,D)	0.27	179, 134, 122, 90	'Baldus' (179), 'Munk' (90)
gwm410	2B	0.35	342, 335	
gwm459	6A	0.75	190, 168, 166, 156, 119	'Baldus' (190)
gwm493	3B	0.38	179, 142	
gwm508	6B	0.28	174, 142	
gwm526.1	2(A,B)	0.38	161, 158	
gwm526.2	2(A,B)	0.46	130, 125, na	'Zebra' (125)
gwm570	6A	0.64	148, 142, 138, 132	
gwm604	5B	0.57	138, 133, 113, 107	'Munk' (107)
gwm624	4D	0.14	143, 138	'Zebra' (143),
gwm635	7D	0.27	109, 107, 97	'Meri' (109), 'Mahti' (97)
gwm814	6B	0.53	164, 154, 152, 146	'Baldus'(164), 'Triso' (154)
PSP3000	1B	0.64	252, 236, 222, 214	
PSP3027	1A	0.36	167, 164, 161	'Munk' (161), 'Mahti'(167)
PSP3028	4A	0.60	162, 156, 149, 147, 140, 132	'Zebra' (132), 'Munk' (140) 'Mahti' (162)
PSP3030	4B	0.28	208, 198	
PSP3058	6D	0.37	189, 186	
Psp3094.1	7A	0.55	170, 163, 152	Groups 1, 2, 3
Psp3094.2	7D	0.42	230, 190, 175	
PSP3103	4D	0.60	188, 182, 178, 166	'Zebra' (166)
PSP3131	6B	0.14	144, 148	'Zebra' (148)
PSP3200	6D	0.50	170, 164	
taglgap	1B	0.68	263, 234, 220, 213	

na: null-allele.

gov), the analysed microsatellites covered all the 21 chromosomes of the bread wheat genome.

In the studied 12 varieties, the primer pairs amplified from two to six alleles; the PIC value of the analysed microsatellite markers ranged from 0.14 to 0.75. Twenty-four products were amplified as unique for a variety of alleles in the analysed group. Seven of the studied varieties can be identified in the group by one marker and, in most cases, a second marker is available to verify the results (Table III). For example, the 190 bp fragment amplified in 'Munk' by gwm210, which was absent in all other studied genotypes and was inherited in the breeding lines derived from 'Munk', appears to be an excellent marker for the identification of 'Munk' in the set of the studied varieties.

In eight of the studied varieties, two alleles of a marker were detected. Six heterogeneous loci were detected in 'Mahti', two in 'Meri' and one locus was heterogeneous in 'Satu', 'Helle', 'Vinjett', 'Zebra', 'Triso' and 'Tjalve'. Both the SSR analysis of individual DNA preparations from five single plants, and the morphological examination, demonstrated a high level of heterogeneity in the 'Mahti' plant material and the data for this variety were excluded from further genetic analyses.

In spite of the comparatively high mean value of average gene diversity for the 12 genotypes studied (0.52), the detected differences between the individual varieties (F_{ST}) were insignificant.

All the studied varieties were discriminated in the cluster analysis of the genetic distance matrix derived

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

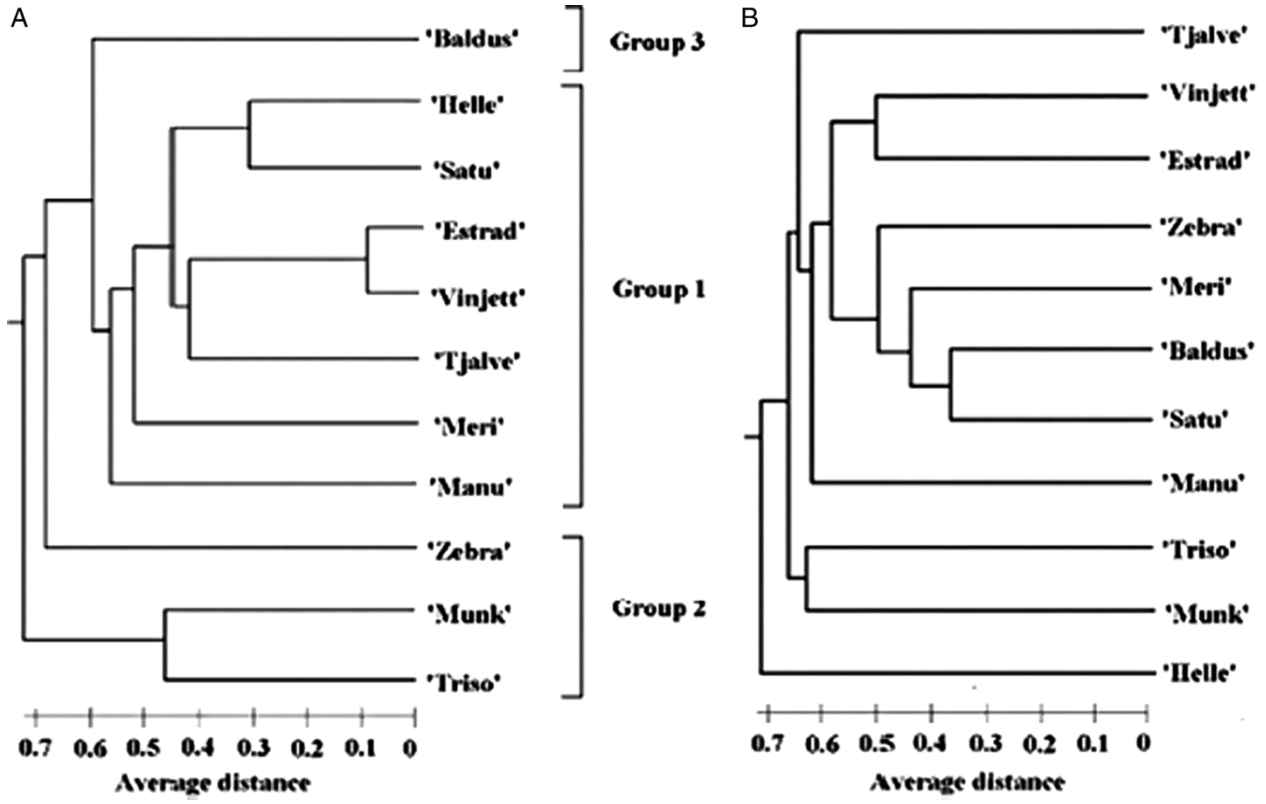


Figure 1. Dendrograms (UPGMA) drawn from the distance matrices based on the SSR analysis of 43 loci (A) and analysis of 24 morphological traits (B) in 11 Estonian Variety List spring wheat varieties.

from the SSR analysis data, and three major groups were distinguished on the dendrogram (Figure 1A).

The main group (group 1) included all Nordic varieties of the list with the exception of 'Zebra'. The second group (group 2) included two varieties released in Germany, 'Triso' and 'Munk', and the Swedish variety 'Zebra'. The linkage of the Swedish variety 'Zebra' to the group of 'Triso'+'Munk' can be explained by a common parent of 'Zebra' and 'Munk' (variety 'Ralle' from Germany). The third group consisted of the variety from the Netherlands, 'Baldus'. Primer pair PSP3094 amplified group-specific alleles in each of the studied varieties.

Pairwise differences between groups 1 and 2, 1 and 3, 2 and 3 were found to be significant (Fst = 0.26344, 0.32179 and 0.42820, respectively, $p < 0.05$). The Nordic group is genetically closer to the 'Triso'/'Munk'/'Zebra' group than to the Netherlands' variety 'Baldus'. According to ANOVA, 14.97% ($p = 0.011$) of the total variance in the 11 varieties over all the analysed loci results from covariance among the three main groups.

The main group of Nordic varieties was genetically heterogeneous. Average gene diversity in the Nordic group was 0.44, being close to the average gene diversity in the whole set of the 12 studied varieties (0.52). However, some closely related subgroups were formed. In the subgroup of 'Tjalve'-'Vinjett'-'SWEstrad', 55.8% of alleles were shared, and varieties 'Vinjett' and 'SWEstrad' could be distiguished only by alleles of three markers: gwm66 and PSP3200, both common in 'SWEstrad' and 'Tjalve', and by alleles of PSP3094 which were common in 'Tjalve' and 'Vinjett'. The number of polymorphic loci in the subgroups is presented in Table IV.

Six breeding lines (F_8–F_{10}) derived from the EVL varieties were analysed in order to verify the inheritance of heterogeneous and/or unique alleles. Depending on the polymorphism between the parents, on average 50.9% of the loci analysed were informative in the breeding lines derived from the Nordic varieties. In the breeding lines with the 'non-Nordic' parent 'Munk', 68.5% of loci on average were informative (Table V).

Three of the breeding lines were homogenous in all the analysed loci; however, 40% of the informative loci were in a heterogeneous state in the breeding line 6.1.10.3 ('Satu' × 'Tjalve'). In two breeding lines, inconsistent non-parental alleles were detected. The presence of illegitimate alleles was verified by repeated amplifications. The illegitimate alleles were detected in different loci; in line 6.1.10.3 all three non-parental alleles were in a heterogeneous state.

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

Table IV. Polymorphism in subgroups of Estonian-grown spring wheat varieties analysed by 35 microsatellite primers and by 24 morphological characteristics.

Cluster of varieties	Number of polymorphic SSR loci	Percentage of polymorphic SSR loci	Percentage of polymorphic morphological characteristics
'Satu'/'Helle'	14	32.6	54.2
'SWEstrad'/'Vinjett'/'Tjalve'	19	44.2	66.7
'SWEstrad'/'Vinjett'	3	7.3	41.7
'Vinjett'/'Tjalve'	18	41.8	50.0
'Triso'/'Munk'	19	44.2	50.0
'Triso'/'Munk'/'Zebra'	32	74.4	70.8

Morphological analysis

The results of the morphological evaluation (Table II) were in full agreement with the producers' descriptions of the varieties and with the results of the examination conducted by Estonian national authorities.

The dendrogram resulting from the clustering of the distance matrix based on morphological evaluation (Figure 1B) and the dendrogram drawn from the distance matrix based on the SSR analysis (Figure 1A) appear to have marked dissimilarities. Although all varieties could be distinguished on the dendrogram, no distinctive groups of Nordic, German and Dutch varieties were formed. Varieties 'Baldus' and 'Satu', which, according to their pedigree data should not be genetically closely related, formed a subcluster in the morphological data dendrogram.

For variety subgroups, the genetic distance values were considerably higher when based on morphological data (Figure 1A, B). For example, variety 'SWEstrad' is derived from variety 'Vinjett' and their genetic distance according to the SSR analysis is 0.077. Morphological trait analysis resulted in a genetic distance value of 0.435 between these two varieties.

Discussion

The 12 spring wheat genotypes of the Estonian Variety List present a good model to be used in the analysis of the power of microsatellite markers in fingerprinting and parentage analysis in small groups of wheat genotypes. According to the known pedigree data, it was presumed that three genotypes of spring wheat (varieties 'Baldus', 'Triso' and 'Munk') in the Estonian Variety List would genetically be distinct from the main group of Nordic varieties. The group of Nordic varieties included a pair ('Satu'-'Helle') and a triplet ('Tjalve'-'Vinjett'-'SWEstrad'), both consisting of varieties derived from each other.

In the studied group, all varieties were discriminated both by the SSR and the morphological analyses and the average gene diversity (0.52) detected by the SSR analysis over 41 loci was very high. In the subgroup of eight Nordic varieties ('SWEstrad', 'Vinjett', 'Meri', 'Tjalve', 'Satu', 'Helle', 'Mahti', 'Manu'), the average gene diversity of 0.44 is in agreement with the gene diversity for the Swedish and Finnish varieties (0.52 and 0.45, respectively) observed by Christiansen et al. (2002).

In the analysed group, identifying SSR markers could be selected for seven varieties; the remaining five varieties were identified by two or three markers. Even an extreme lack of polymorphism which may occur between some closely related genotypes (in our case, 'Vinjett'-'SWEstrad') does not increase the number of markers needed for the identification beyond the limits of practical usability. In practical use, the identification power of each marker must be verified if additional varieties are included in the group to be analysed.

Multilocus SSR analysis of this study revealed heterogeneity in most of the analysed plant material.

Table V. Microsatellite analysis of breeding lines derived from EVL spring wheat varieties.

Identification number	Parents	Generation	Scorable alleles	M:P alleles	Heterogeneous alleles	Inconsistent alleles
231	'Tjalve' × 'Manu'	F_8	18 (50%)	8:10	1	0
6.1.10.3	'Satu' × 'Tjalve'	F_{13}	20 (55.6%)	15:1	8	2
91060202	'Satu' × 'Tjalve'	F_{11}	17 (47.2%)	8:9	0	0
670101	'Satu' × 'Munk'	F_{10}	29 (80.6%)	25:3	2	3
213	'Munk' × 'Mahti'	F_8	24 (66.7%)	16:7	0	0
214	'Munk' × 'Manu'	F_8	21 (58.3%)	8:13	2	0

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

Six heterogeneous SSR loci were detected in 'Mahti'; the heterogeneity of this plant material was also demonstrated by morphological analysis (data not shown).

Analysis of the inheritance of alleles from their immediate parents was included in this study as a means to verify the allelic content of the varieties, especially for unique and/or heterogeneous alleles. Although inheritance of the predominant part of the alleles could be proved, a relatively high number of inconsistent alleles absent in both of the parents was detected. Detection of inconsistent alleles has been reported in different species (Sjakste et al., 2003; Yazdani et al., 2003). In the simplest case, an inconsistent allele may appear as a result of an experimental mistake, or an allele may be falsely classified as inconsistent, if the decision is based on incomplete pedigree data. However, in *Picea abies*, inconsistent alleles in the SSR inheritance pattern were detected in three of the seven families of the studied controlled crosses (Yazdani et al., 2003).

In this study, inconsistent alleles were detected in two of the six breeding lines analysed. Inconsistent alleles may result from the breeding system used for the development of these lines. The lines were developed by a 'bulk selection method' with two one-spike separations in F_1 and F_4, respectively. Starting from the F_1 generation, the spikes were not covered during the flowering. Although wheat is a predominantly self-pollinating species, outcrossing rates up to 6–9%, depending on population density, genotype and environmental conditions, have been detected in wheat (Jain, 1975; Hucl, 1996). Isolation distances for preventing the gene flow may reach 30 metres (Hucl & Matus-Cadiz, 2001). The inconsistent alleles in our breeding lines may originate from outcrossing events during the first generation(s) after the initial cross, on condition that the fixed 'wrong' allele(s) are cosegregating with some advantageous trait(s) for which the selection of the breeding lines was held.

Corresponding to the UPOV rule of distinctness, the studied varieties were distinguished by the used set of morphological characteristics, and an attempt was made to compare the genetic distances between the studied varieties revealed by the SSR and morphological analyses. However, the comparison of clustering analyses shows that the use of morphological charateristics for that purpose may lead to erroneous conclusions.

Microsatellite markers reflect the non-coding regions of the genome, and their superiority to morphological markers is supposed to be based on their less limited variation. Morphological characteristics may be based on the coordinated expression of several genes. Therefore, it should be assume that, in contrast to the phenotypical characteristics, the results of the SSR analysis should overestimate the overall genetic distance between the two genotypes. However, in this study, larger genetic distances between the pairs of varieties were detected in the morphological analysis.

One of the reasons for the contradiction may lie in the overestimatation of phenotypical differences. For example, if besides 'absent' and 'very weak', a characteristic is evaluated as 'absent or very weak', most probably we have to accept the genotype 'very weak', which due to the environmental effects is phenotypically not expressed in the whole set of the analysed plants.

Acknowledgements

This work was supported by the Estonian Ministry of Agriculture.

References

Christiansen, M. J., Andersen, S. B., & Ortiz, R. (2002). Diversity changes in intensively bred wheat germplasm during the 20th century. *Molecular Breeding*, *9*, 1–11.

Devos, K. M., Bryan, G. J., Collins, A. J., Stephenson, P., & Gale, M. D. (1995). Application of two microsatellite sequences in wheat storage proteins as molecular markers. *Theoretical and Applied Genetics*, *90*, 247–252.

Felsenstein, J. (2004). PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. *Department of Genome Sciences, University of Washington, Seattle.*

Gale, M.D., Atkinson, M.D., Chinoy, C.N., Harcourt, R.L., Jia, J., Li, Q.Y. & Devos, K.M. (1995). Genetic maps of hexaploid wheat. In *Proceedings of the 8th International Wheat Genetics Symposium*, vol. 1 (pp. 29–40). China Agricultural Scientech Press, Beijng.

Huang, X. Q., Börner, A., Röder, M. S., & Ganal, M. W. (2002). Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*, *105*, 699–707.

Huang, X., Zeller, F. J., Hsam, S. L. K., Wenzel, G., & Mohler, V. (2000). Chromosomal location of AFLP markers in common wheat utilizing null-tetrasomic stocks. *Genome*, *43*, 298–305.

Hucl, P. (1996). Out-crossing rates for ten Canadian spring wheat cultivars. *Canadian Journal of Plant Sciences*, *76*, 423–427.

Hucl, P., & Matus-Cadiz, M. (2001). Isolation distances for minimizing out-crossing in spring wheat. *Crop Science*, *41*, 1348–1351.

Jain, S. K. (1975). Population structure and the effects of breeding system. In O. H. Frankel, & J. G. Hawkes (Eds.), *Crop Genetic Resources for Today and Tomorrow* (pp. 15–36). Cambridge University Press.

Jensen N. F. (1988). Breeding and selection methods.*Plant breeding methodology*(pp. 15–301). John Wiley & Sons Inc, USA.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, *76*, 5269–5273.

Plaschke, J., Ganal, M. W., & Röder, M. S. (1995). Detection of genetic diversity in closely related bread wheat using

Downloaded By: [ELNET Estonian Libraries Network Consortium] At: 15:20 20 December 20(

microsatellite markers. *Theoretical and Applied Genetics*, *91*, 1001–1007.

Roussel, V., Leisova, L., Exbrayat, F., Stehno, Z., & Balfourier, F. (2005). SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. *Theoretical and Applied Genetics*, *111*, 162–170.

Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M. H., Leroy, P., & Ganal, M. W. (1998). A microsatellite map of wheat. *Genetics*, *149*, 2007–2023.

Röder, M. S., Wendehake, K., Korzun, V., Bredemeijer, G., Laborie, D., Bertrand, L., et al. (2002). Construction and analysis of a microsatellite-based database of European wheat varieties. *Theoretical and Applied Genetics*, *106*, 67–73.

Schneider, S., Kneffer, J. M., Roessle, D. & Excoffier, L. (2000). ARLEQUIN version 2.000. A software for population genetics data analysis. Genetics and Biometry Laboratory, Department of Anthropology University of Geneva, Switzerland.

Sjakste, T. G., Rashal, I., & Röder, M. S. (2003). Inheritance of microsatellite alleles in pedigrees of Latvian barley varieties and related European ancestors. *Theoretical and Applied Genetics*, *106*, 539–549.

Somers, D. J., Isaac, P., & Edwards, K. (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *109*, 1105–1114.

Stachel, M., Lelley, T., Grausgruber, H., & Vollmann, J. (2000). Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. *Theoretical and Applied Genetics*, *100*, 242–248.

Stephenson, P., Bryan, G., Kirby, J., Collins, A., Devos, K., Busso, C., & Gale, M. (1998). Fifty new microsatellite loci for the wheat genetic map. *Theoretical and Applied Genetics*, *97*, 946–949.

Yazdani, R., Scotti, I., Jansson, G., Plomion, C., & Mathur, G. (2003). Inheritance and diversity of simple sequence repeat (SSR) microsatellite markers in various families of *Picea abies*. *Hereditas*, *138*, 219–227.

Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2007; 00: 0–00

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia

HILMA PEUSHA[1], TAMARA ENNO[1], IRENA JAKOBSON[1], JELENA TSÕMBALOVA[1], ANNE INGVER[2] & KADRI JÄRVE[1,2]

[1]*Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia,* [2]*Jõgeva Plant Breeding Institute, Aamisepa 1, 48309 Jõgeva, Estonia*

Abstract

A group of spring wheat cultivars originating from Sweden, Finland, Norway, Germany, and the Netherlands was analysed for powdery mildew resistance. Using functional molecular markers, two alleles of the major resistance gene *Pm3* were detected among the cultivars under the study. One of the alleles, *Pm3d*, was detected in the resistant cultivars 'Vinjett', 'SW Estrad', and 'Zebra', and in 'Tjalve', a cultivar of earlier release susceptible to the local population of powdery mildew. The second allele of *Pm3* detected in the analysed group of cultivars was the allele *Pm3f,* rarely used in Europe. It was identified in the resistant cultivars Satu', 'Helle', and in the moderatively resistant cultivar 'Mahti'. *Pm3f* was found to be effective against the local population of powdery mildew in Estonia, while *Pm3d* provided no protective effect. Besides the *Pm3d* allele on chromosome 1A, monosomic analysis of the cultivar 'Vinjett',which is almost immune to powdery mildew, identified two additional loci on chromosomes 5D and 7D, respectively, presumably responsible for the high resistance in this cultivar.In contrast to the earlier cultivars, six recently released cultivars ('Vinjett', 'SW Estrad', 'Zebra', 'Satu', 'Helle', 'Meri') demonstrated a high resistance to the powdery mildew fungus *Blumeria graminis* DC. f. sp. *tritici* both in the field and seedling tests, showing that the genetic basis of powdery mildew resistance in Nordic spring wheat has been improved noticeably in the last ten years.

Keywords: *Nordic spring wheat, powdery mildew resistance,* Triticum aestivum.

Introduction

The obligate fungus *Blumeria graminis* DC. f. sp. *tritici* can infect plants from the first leaf stage until senescence. Resistance to the infection of the powdery mildew fungus may be based on a race-specific gene-for-gene interaction of resistance gene(s) (*Pm*-genes) in wheat and avirulence gene(s) in the infecting fungus isolate (Flor, 1971). Race-specific *Pm*-genes are effective against some powdery mildew isolates but ineffective against others; the cultivars carrying these gene can be clearly differentiated into susceptible or resistant ones. This type of a plant–pathogen interaction may not be durable, being overcome by pathogen races with matching virulence alleles. Up to now, thirty four powdery mildew resistance genes have been identified in different wheat genotypes (*Pm1–Pm34*, Huang & Röder, 2004, Zhu et al., 2005, Miranda et al., 2006).

In the 1990s, nine race-specific resistance genes/alleles (*Pm1, Pm2, Pm3c, Pm3d, Pm4b, Pm5, Pm6, Pm8, Pm9*) prevailed in the European spring wheat cultivars (Zeller et al., 1993b). *Pm3d* is considered to be the most effective among those named above and has so far not been overcome in Germany (Felsenstein & Jaser, 2005).

The powdery mildew resistance gene *Pm3b* located on chromosome 1A is the first functional *Pm*-gene conferring disease resistance in hexaploid wheat to have been finely mapped and isolated (Yahiaoui et al., 2004). Recently, a PCR-based marker was developed for a conservative promotor region of *Pm3* genes (Yahiaoui et al., 2006), and

Correspondence: K. Jarve, Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia. E-mail: kadri.jarve@ttu.ee

(Received 13 April 2007; accepted 23 September 2007)

ISSN 0906-4710 print/ISSN 1651-1913 online © 2007 Taylor & Francis
DOI: 10.1080/09064710701706242

UNCORRECTED PROOF

seven PCR-based markers distinguishing the allelic series *Pm3a–Pm3g* have been reported (Tommasini et al., 2006).

At the adult stage of plant growth, some wheat genotypes exhibit a different type of resistance, which is non-isolate-specific and partial, retarding infection, growth, and reproduction of the powdery mildew fungus. Adult plant or durable resistance (APR) is a quantitative trait and can be resolved into discrete genetic loci (quantitative trait loci, QTL) (Paterson et al., 1988). Several sets of QTLs for adult plant powdery mildew resistance have been detected and mapped in different segregating wheat populations (Huang and Röder, 2004). In the heritance of adult plant powdery mildew resistance, the additive effects of the detected QTLs prevail (Griffey & Das, 1994; Keller et al., 1999; Chantret et al., 2001; Mingeot et al., 2002; Liu et al., 2001; Jakobson et al., 2006). Some of the detected QTLs for adult plant resistance have been mapped in the same genome regions as those of some major *Pm*-genes (Bougot et al., 2006).

This study analyses the powdery mildew resistance in a group of 14 spring wheat cultivars grown in Estonia, originating from Sweden, Finland, Norway, Germany, and the Netherlands. Data for the field resistance are compared with the seedling resistance to a natural powdery mildew population collected in Estonia and to 11 test-isolates of the powdery mildew fungus. The occurrence of *Pm3* alleles in the analysed cultivars was established. Monosomic analysis of the cultivar 'Vinjett', highly resistant to powdery mildew, was carried out.

Materials and methods

Plant material

The analysed set included cultivars from the Estonian Variety Lists 2003–2007 ('Vinjett', 'SW Estrad', 'Zebra', 'Meri', 'Baldus', 'Triso', 'Munk', 'Mahti', 'Helle', 'Tjalve', 'Manu', 'Satu'). The cultivars 'Tähti' and 'Luja' of earlier release were included in the study as plant material of comparatively low resistance to powdery mildew (Peusha et al., 1996; our unpublished data). In addition, seedling resistance of the cultivar 'Canon' and the isogenic lines carrying different major *Pm*-genes (*Pm1, Pm2, Pm3a, Pm3b, Pm3c, Pm3d, Pm3e, Pm3f, Pm4a, Pm4b, Pm5, Pm6, Pm8, Pm12, Pm16, Pm17, Pm18, Pm19*) was analysed.

Pedigree data for the analysed spring wheat cultivars according to the website Wheat *Pedigree* and *Identified Alleles of Genes (http://genbank.vurv.cz/wheat/pedigree/default.htm)* were collected in a previous publication on the diversity of the Estonian Variety List 2003 spring wheat genotypes (Tsõmbalova et al., 2007).

Plant material was obtained from the samples submitted for official registration in Estonia and was multiplied at the Jõgeva Plant Breeding Institute. Seeds of 'Canon', 'W150' (*Pm3e*) and 'Michigan Amber/8*Chancellor' (*Pm3f*) were kindly supplied by the John Innes Centre Norwich Research Park, Norwich, United Kingdom.

Assessment of powdery mildew in the field

14 Spring wheat cultivars were tested for the powdery mildew resistance in the years 1996–2006 (Table I). Tests were carried out in Jõgeva, Estonia on 10 m^2 plots in three replications for each cultivar. Average powdery mildew severity under natural disease pressure was rated two weeks after the ear emergence on a 0–9 scale. A score of '0' was given to immune plants with no signs of infection. If a few plants in the plot showed occasional necrotic lesions from hypersensitive response to infection on the lower leaves, the plot was scored '1' (very resistant). The plants were rated as '2' when a few to a moderate number of necrotic lesions were formed on the lower leaves with no or very little mycelium found (resistant). A score of '3' was given to cultivars when the lower leaves were slightly to moderately covered with the fungus mycelium with a few colonies formed (moderately resistant). A score of '5' was given to plants with moderate to abundant mycelial development with moderate sporulation on the lower leaves (moderately susceptible). The plants were rated as '7' (susceptible) when abundant mycelial development with heavy sporulation was detected on the lower leaves, and the plants were rated '9' (very susceptible) when abundant mycelial development and abundant sporulation were detected. The values of 4, 6, and 8 were used where ratings could not be clearly allocated to the values described above.

Seedling resistance to powdery mildew

The *Blumeria graminis* f. sp. *tritici* test-isolates used for the inoculation were kindly provided by Drs Hsam and Felsenstein. The isolates were collected from different regions of Europe and selected from single spore progenies (Felsenstein et al., 1991). The Estonian natural populations of *Blumeria graminis* f. sp. *tritici* was collected in 2006 from Jõgeva, Estonia. The test-isolates and populations were maintained on susceptible cv. 'Kanzler' with propagation at 3-week intervals.

Disease resistance was assessed on primary leaf segments of 12 ten-day-old seedlings. The expression

AQ1

UNCORRECTED PROOF

of resistance was scored 10 days after inoculation on a 0–9 scale where 0 was immune and 9 was very susceptible, as described by Heun & Fischbeck (1987).

Monosomic analysis

The 'Chinese Spring' (CS) monosomic lines were kindly supplied by Prof. F.J. Zeller. Cultivar CS and CS monosomic lines were crossed as female parents with cv. 'Vinjett'. Cytologically confirmed monosomic F_1 plants ($2n = 41$) were raised to maturity in the greenhouse and selfed to determine the disease segregation ratios for the F_2 generation. Somatic chromosome counts at mitosis were performed on root tips as described by Peusha et al. (2000b).

Powdery mildew isolate No.12, avirulent to 'Vinjett', was used to test disease segregation in F_2 populations. Chi-square tests were used to test for deviation of the observed data from the theoretically expected segregation.

Detection of Pm3 alleles

Total genomic DNA was extracted from young leaf tissue frozen in liquid nitrogen, according to the method described by Huang et al. (2000) with minor modifications. DNA was extracted from a bulk of five plants.

The presence or absence of a *Pm3*-type gene in the studied cultivars was determined by PCR amplification with the primer pair UP3B/UP1A according to Yahiaoui et al. (2006). Functional markers for seven *Pm3* alleles (*Pm3a–Pm3g*) developed by Tommasini et al. (2006) were used for PCR amplification in the cultivars where a *Pm3* type gene was detected.

Results

Field resistance to powdery mildew

The results of field tests for the powdery mildew resistance are presented in Table I. The year-by-year variance in disease severity depended strongly on the level of powdery mildew pressure, and no general trends of resistance declination during the evaluation years were detected. Six evaluated cultivars ('Vinjett', 'SW Estrad', 'Satu', 'Helle', 'Meri' and 'Zebra') were highly resistant to the natural populations of the fungus. For 'Vinjett', traces of the fungus were detected on the lower leaves of some plants only in the years of high powdery mildew pressure. 'SW Estrad' was evaluated in five years starting from the year 2000, and in 2002 disease level on the lower leaves of this cultivar was slightly higher than in 'Vinjett'. If 'Vinjett' could be considered as almost fully resistant to powdery mildew, cultivars 'SW Estrad', 'Satu', 'Meri', 'Helle', and 'Zebra' were still showing low levels of mildewing. In the years of high mildew pressure, lower leaves of the cultivars 'Baldus', 'Triso', 'Munk', and 'Mahti' were moderately covered with the fungus. Cultivars 'Tähti' and 'Luja' were susceptible to powdery mildew.

Table I. Field resistance to powdery mildew (1996–2006) of 14 spring wheat cultivars evaluated in Jõgeva, Estonia.

	Field resistance[2] to *Blumeria graminis* f. sp. *tritici*									
Cultivar[1]	1996[3]	1997	1999	2000	2001	2002	2004	2005	2006	Average
'Vinjett'	0[4]	0	0	1	1	1	0	0	**1**[5]	0.4
'SW Estrad'	–[6]	–	–	–	0	**2**	0	0	1	0.6
'Zebra'	–	–	–	–	0	**2**	1	0.7	0	0.7
'Meri'	0.3	0	0	1	0.7	**2**	1	1.7	0	0.7
'Satu'	1	0	0.5	**2.7**	0	2	0	0	0	0.7
'Helle'	0.8	0	0	1.7	0	**3**	1	2	0	0.9
'Baldus'	0	0	0.5	**4.3**	1.3	1	2	2.3	1	1.4
'Triso'	0.7	1.5	0	3.7	**4.3**	2	2	0	0	1.6
'Munk'	2	0	0	**4.3**	3	3	4	3.7	1	2.3
'Manu'	2	2	0	5.3	4.7	3	2	**6.3**	1	2.9
'Mahti'	2.5	0	0	5.3	5.3	4	4	**5.3**	2	3.2
'Tjalve'	1	0	1	4.3	5.3	4	**6**	5.7	3	3.4
'Tähti'	5	–	5	4	4	5	**7**	6	6	5.3
'Luja'	6	**6**	–	–	–	–	–	–	–	(6)

[1] Cultivars are arranged according to their average and maximal susceptibility to powdery mildew during the years of testing.
[2] 0 – immune; 1,2 – resistant; 3,4 – intermediate; 5,6,7,8,9 – susceptible.
[3] Data for the years 1998 and 2003 are exluded as no powdery mildew was detected in these years.
[4] Mean value for three replications (each 10 m^2).
[5] The maximal value over nine years is given in bold text.
[6] Not evaluated.

UNCORRECTED PROOF

Seedling resistance

Test-isolates of *Blumeria graminis* f. sp. *tritici* used in the study were characterized by differential reactions of wheat cultivars/lines possessing powdery mildew resistance genes after inoculation with the 11 test-isolates (Table IIA).

Estonian natural population of *Blumeria graminis* f. sp. *tritici* collected in 2006 was characterised by virulence/avirulence to wheat isogenic lines carrying different powdery mildew resistance genes/alleles. The local population was virulent to *Pm1, Pm2, Pm3d, Pm4a, Pm4b, Pm6, Pm17*, and avirulent to *Pm3a, Pm3b, Pm3c, Pm3e, Pm3f, Pm5, Pm8, Pm12, Pm16, Pm18, Pm19 (Table IIA)*.

Seedling resistance of the cultivars under study to the Estonian natural population of *Blumeria graminis* was in full correlation with the data of field resistance (Table IIB). In general, the same correlation could be observed between the field resistance and the seedling resistance to the differentiating test-isolates of *Blumeria graminis* f. sp. *tritici*. Cultivars resistant to powdery mildew in the field tests (Table I) were resistant to all or most of the test-isolates in the seedling stage. Cultivars 'Tähti' and 'Luja', susceptible to powdery mildew in the field, are susceptible to the differentiating test-isolates. Cultivar 'Tjalve' represents an exception from this overall tendency, being resistant to all test-isolates but one, and showing a very low level of resistance in the field and in the seedling test after inoculation with the natural population.

Monosomic analysis of the cultivar 'Vinjett'

According to the results of the field tests, the highest level of powdery mildew resistance was detected in the cultivar 'Vinjett', which was also resistant to the natural population and differentiating isolates of *Blumeria graminis* in the seedling stage. Monosomic analysis of 'Vinjett' was conducted to detect the chromosomal location of genes responsible for the seedling resistance in this cultivar.

F_2 plants from crosses of CS and 21 CS monosomic lines with 'Vinjett' were inoculated with the powdery mildew isolate No.12 (avirulence/virulence: *Pm3b, Pm3d, Pm4a, Pm4b, Pm8, Pm16/Pm1, Pm2, Pm3a, Pm3c, Pm3f, Pm5, Pm6*). Eighteen of the F_2 populations representing different hybrid combinations segregated in the ratio of 63 resistant to 1 susceptible plant. The combinations 'CSmono-1A'/'Vinjett', CSmono-5D/'Vinjett', and CSmono-7D/'Vinjett' deviated significantly from the expected 63:1 ratio, showing that genes conferring resistance to powdery mildew in the cultivar 'Vinjett' are located on chromosomes 1A, 5D, and 7D (Table III).

Occurrence of Pm3 alleles

Using the PCR primers designed by Tommasini et al. (2006), the major gene on chromosome 1A in the cultivar 'Vinjett' was identified as the *Pm3d* allele of the major gene *Pm3* (Table IV).

Altogether, the *Pm3d* allele was detected in five of the analysed cultivars (Table IV). In 'SW Estrad' ('Hanno'/'Hugin'/'Vinjett'), this allele most probably originates from 'Vinjett'. 'SW Estrad' and 'Vinjett' are genetically very close. In the microsatellite analysis, 'Vinjett' and 'SW Estrad' showed identical alleles in 44 of the 47 analysed loci (Tsõmbalova et al., 2007).

Pm3d was also detected in the cultivar 'Tjalve'. 'Vinjett' has been derived from the cultivars 'Tjalve' and 'Canon', and as no *Pm3* allele was detected in 'Canon', we can conclude that *Pm3d* in 'Vinjett' originates from 'Tjalve'. Published data on the pedigree of 'Tjalve' are contradictory, and in different variants of 'Tjalve' pedigree the wheat database (http://genbank.vurv.cz/wheat/pedigree/default.htm) refers to cultivars 'Reno', 'Kolibri-M', 'Benno', and some breeding lines not identified. 'Kolibri' is a well known source of the *Pm3d* allele.

In 'Zebra' ('Ralle'/'Dragon') and 'Munk' ['Ralle'//('VO-9-P-78') 'Kolibri'/'Somara'/3/'Star'], the *Pm3d* allele should originate from the cultivar 'Ralle', which is known to carry this gene (Zeller et al., 1993a).

Although *Pm3d* allele was detected in three cultivars of the highest resistance level, this gene is not responsible for the field resistance of these cultivars in Estonia. Inoculation of the isogenic cultivar 'Kolibri' carrying *Pm3d* as a single major gene with the local population (2006) of the fungus resulted in a high-level infection (Table IIA).

The second *Pm3* allele detected in the cultivars under study, *Pm3f*, was found in 'Mahti', 'Satu', and 'Helle' (pedigree: 'Satu' × 'Polkka'). Unfortunately, no pedigree data are available to trace the origin of this allele in 'Mahti' and 'Satu'. *Pm3f* allele in 'Helle' ('Satu'/'Polkka') may originate either from 'Satu' or from 'Polkka' as both of these cultivars carry the *Pm3f* allele.

In the seedling test, the isogenic line carrying *Pm3f* ('Michigan Amber') was found to be resistant to the natural population of powdery mildew (Table IIA), and the resistance of the cultivars 'Satu' and 'Helle' may be explained by the occurrence of *Pm3f* in these cultivars. 'Mahti' demonstrated a lower level of seedling and field resistance. However, the plant material of 'Mahti' available to us was highly heterogeneous, affecting the exact estimations both of the seedling and field resistance levels.

UNCORRECTED PROOF

Table II. Seedling resistance to *Blumeria graminis* f. sp. *tritici* (*Bgt*) assessed on primary leaf segments.

A

Cultivars and differentiating lines carrying known genes for powdery mildew resistance: reaction to the analysed differentiating *Bgt* test-isolates and to the natural population of *Bgt* collected in Jõgeva, Estonia, in 2006.

Cultivar/line	Resistance gene	Seedling resistance[1] to *Bgt* differentiating test-isolates (No.)											Seedling resistance to the *Bgt* natural population
		2	5	6	9	10	12	13	14	15	16	17	
Axminister/Cc8[2]	Pm1	0	0	0	3 (4)[3]	0	9	4	0	6	7	5	9
Ulka/Cc8	Pm2	8	0	5	5	0	9	5	4	5	5	8	5
Asosan/Cc8	Pm3a	0	0	0	0	0	8	0	0	9	8	6	0
Chul/Cc	Pm3b	0	0	0	0	0	0	0	0	0	0	0	0
Sonora/Cc8	Pm3c	0	0	0	3	0	5	1	0	4	4	4	0
Kolibri	Pm3d	9	7	9	2 (3)	8	0	4	0	5	5	1	6
W150	Pm3e	0	4	5	3	0	8	2	4	5	9	4	1
Michigan Amber/Cc8	Pm3f	0	5	4	4	0	7	1	3	6	6	4	0
Khapli/Cc8	Pm4a	5	2	5	0	2	0	5	4	4	4	0	3 (4)
Armada	Pm4b	5	0	4	0	0	0	5	4	4	4	0	5
Hope	Pm5	0	0	0	2 (3)	0	5	4	0	5	5	5	0
TP114/St.2[4]	Pm6	5	0	4	5	0	6	4	4	8	6	7	4 (5)
Disponent	Pm8	1	8	1	3	7	2 (3)	9	0	4 (3)	4	1	1
Normandie	Pm1+2+9	0	0	0	0	0	6	2 (3)	0	5	5	6	5
	Pm12	0	0	0	0	0	0	0	0	0	0	0	0
BRG 3N[5]	Pm16	0	0	0	0	0	0	0	0	0	0	0	0
Amigo	Pm17	4	4	3	2 (3)	4	7	2	0	3	3	4	4
	Pm18	0	0	0	r	0	0	0	0	0	0	0	1
	Pm19	5	1	4	0	0	1	0	0	5	5	4	0

[1] Disease resistance of 12 ten-day-old seedlings was assessed on primary leaf segments 10 days after inoculation on a 0–9 scale: 0 – immune; 1,2 – resistant; 3,4 – intermediate; 5,6,7,8,9 – susceptible [Heun & Fischbeck (1987)].
[2] Cc8 is a 8-fold backcross to 'Chancellor'.
[3] If the resistance of the analysed 12 seedlings was different, the less frequent reaction is given in parentheses.
[4] St.2 is a double backcross to 'Starke'.
[5] BRG 3N is a derivative of *T. turgidum* var. *dicoccoides*.

B.

Spring wheat cultivars grown in Estonia: reaction to the analysed differentiating *Bgt* test-isolates and to the natural population of *Bgt* collected in Jõgeva, Estonia, in 2006.

Cultivar[1]	Seedling resistance[2] to *Bgt* differentiating test-isolates (No)											Seedling resistance to the *Bgt* natural population
	2	5	6	9	10	12	13	14	15	16	17	
'Vinjett'	0	0	0	0	0	0	0	0	0	0	0	0
'SW Estrad'	0	0	0	0	0	1	0	0	0	1	1	0
'Zebra'	2	0	0	0	0	1	1	0	0	0	0	0
'Meri'	0	0	0	2	0	2 (3)[3]	0	0	0	5	4	1
'Satu'	4	0	0	0	0	2	1	2	3	3	3	1
'Helle'	0	0	2	0	0	0	0	2	0	6	5	1
'Baldus'	1	0	0	0	0	5 (4)	2	4	3	4	0	1
'Triso'	4	2	5	4	0	4	2	3	4	8	2	4
'Munk'	5	3	4	3 (4)	4	3	1	2 (3)	1	4	0	5
'Manu'	8	3	5	0	0	2	6	4	3	5	5	4
'Mahti'[4]	3	0	4	1	0	6	2 (3)	2	2	6	4	4
'Tjalve'	6	0	0	0	0	2	1	2	1	2	2	6
'Tähti'	9	8	8	9	4	9	6	9	7	7	9	4 (5)
'Luja'	6	7	6	5	7	9	6	8	6	9	9	9

[1] Cultivars are arranged according to the average and maximal susceptibility to powdery mildew in the field tests (Table I).
[2] Disease resistance of 12 ten-day-old seedlings was assessed on primary leaf segments 10 days after inoculation on a 0–9 scale: 0 – immune; 1,2 – resistant; 3,4 – intermediate; 5,6,7,8,9 – susceptible [Heun & Fischbeck (1987].
[3] If the resistance of the analysed 12 seedlings was different, the less frequent reaction is given in parentheses.
[4] Plant material heterogeneous.

UNCORRECTED PROOF

Table III. F_2 segregation for seedling reaction to *Blumeria graminis* f. sp. *tritici* isolate No. 12 in the progenies of monosomic F_1 plants from crosses of 'Chinese Spring' (CS) and 21 CS monosomics with the spring wheat cultivar 'Vinjett'.

	Observed segregation		
Chromosome involved	Resistant	Susceptible	χ^2 [1]
1A	249	0	12.260[2]
2A	297	14	0.023
3A	166	7	0.158
4A	343	15	0.197
5A	313	11	0.207
6A	147	7	0.006
7A	126	4	0.752
1B	319	17	0.103
2B	189	7	0.542
3B	210	7	0.037
4B	166	7	0.156
5B	145	5	0.615
6B	223	8	0.770
7B	305	18	0.566
1D	92	3	0.495
2D	105	4	0.248
3D	226	12	0.067
4D	312	16	0.026
5D	300	2	10.945*
6D	214	9	0.211
7D	314	3	9.921*
CS × Vinjett	356	15	0.344

[1] Deviation of the observed data from the theoretically expected segregation 63:1.
[2] $P > 0.01$.

Table IV. Detection, by monosomic and functional marker analyses, of major genes conferring seedling resistance.

Cultivar[1]	Monosomic analysis data on seedling resistance (test-isolate No.)	*Pm3* allele (marker analysis)
'Vinjett'	1A+5D+7D (No. 12)	*Pm3d*
'SW Estrad'		*Pm3d*
'Zebra'		*Pm3d*
'Meri'	1B (No. 2, 9; Peusha et al., 2000a)	
'Satu'		*Pm3f*
'Helle'	3D (No. 6; Peusha et al., 2005)	*Pm3f*
'Baldus'		
'Triso'		
'Munk'		*Pm3d*[2]
'Manu'		
'Mahti'		*Pm3f*
'Tjalve'	1A+3B (No. 9, 10; Peusha et al., (2000b)	*Pm3d*
'Tähti'		
'Luja'		
'Canon'		
'Polkka'		*Pm3f*

[1] Cultivars are arranged according to the average and maximal susceptibility to powdery mildew in the field tests (Table I).
[2] First detected by Tommasini et al. (2006).

Discussion

Five recently released cultivars ('Vinjett', 'SW Estrad', 'Zebra', 'Helle', 'Meri') of this study demonstrated a high resistance to powdery mildew both in the field and seedling tests.

An overall correlation between the levels of field and seedling resistance to the Estonian natural population of powdery mildew could be observed in all spring wheat cultivars under study. This result is in agreement with the hypothesis of the participation of major *Pm* genes for seedling resistance in the formation of adult plant (field) resistance (Martin & Ellingboe, 1976; Nass et.al., 1981; Parlevliet & Zadoks, 1977; Paillard et al., 2000). Assessments of field resistance depend strongly on the environmental conditions, and reliable results for field resistance to the natural populations of pathogen can be obtained on the basis of data covering 5–10 years. The phenotypic assessment of seedling resistance on leaf segments provides faster results, and, in general, natural populations of the fungus can be used to predict the field resistance of breeding lines.

Field resistance to powdery mildew is a quantitative trait caused by the additive effect of several loci, and so far little is known about the gene combinations involved in this trait. A residual effect of (defeated) race-specific resistance genes has been hypothesised, as wheat lines accumulating at least two specific *Pm* genes have been found to be more resistant in the adult stage (Paillard et al., 2000). It has been shown that the residual effects of the defeated race-specific genes *MlRE* and *Pm4b* are components of adult plant resistance in the wheat line RE714 (Chantret et al., 1999; Mingeot et al., 2002).

In isogenic wheat lines or cultivars, the eleven *Blumeria graminis* f. sp. *tritici* test-isolates used for inoculation in this study can differentiate at least 16 known major resistance genes or gene alleles (*Pm*-genes), and some combinations of these genes (Lutz et al., 1992; Huang & Röder, 2004). However, when a cultivar carries several major genes and is resistant to (almost) all test-isolates, phenotypic resistance gene analysis is hindered.

In this study, the monosomic analysis of the cultivar 'Vinjett', highly resistant in all stages of plant growth, was conducted to identify the number and chromsomal location of *Pm* genes. Three loci involved in the seedling resistance were detected. The locus detected on chromosome 1A was identified as the *Pm3d* allele of the resistance gene *Pm3*; the other two genes remained unknown. As the local population of powdery mildew was found to be virulent to *Pm3d*, the high seedling resistance of 'Vinjett' presumably depends on the unknown genes on chromosomes 5D and 7D.

UNCORRECTED PROOF

C:/3B2WIN/temp files/SAGB270517_S100.3d[x] Friday, 23rd November 2007 23:3:8

To identify the alleles of the first cloned powdery mildew resistance gene *Pm3*, the functional markers published recently (Yahiaoui et al., 2006; Tommasini et al., 2006) were used. Two alleles, *Pm3d* and *Pm3f*, of the resistance gene were detected in the analysed spring wheat cultivars.

The occurrence of the *Pm3d* ineffective in Estonia was detected in five cultivars ('Vinjett', 'SW Estrad', 'Zebra', 'Munk', 'Tjalve'). Surprisingly, the second allele detected in the analysed cultivars was *Pm3f*, which is supposed to be used rarely in European breeding programmes (Tommasini et al., 2006). In contrast to *Pm3d*, the allele *Pm3f* is effective against the local population of powdery mildew and, in Estonia, it gives a moderate protection against the fungus.

Pm3f was detected in the cultivars 'Satu', 'Helle', and 'Mahti'. According to an earlier microsatellite analysis of genetic diversity in Northern spring wheat cultivars (Tsõmbalova et al., 2007) and our unpublished data, cultivars 'Mahti' and 'Satu' are genetically close and, presumably, have a common ancestor as the donor of *Pm3f*.

In addition to the named, *Pm3f* was also identified in an earlier cultivar 'Polkka', and it should be found in the popular Finnish cultivar 'Anniina' derived from the same parents ('Satu × 'Polkka') as 'Helle'.

In 1996, a phenotypic analysis of powdery mildew resistance genes in spring wheat cultivars demonstrated that, except for the unknown but effective resistance in the cultivar 'Heta', *Pm4b* was the only resistance gene detected in the commercial cultivars grown in Scandinavia (Peusha et al., 1997). A need for the improvement of genetic diversity in wheat cultivars was obvious.

In this study, we have shown that the genetic basis of powdery mildew resistance in Nordic spring wheat has been improved noticeably in the last ten years, and cultivars of recent release demonstrate a high level of field resistance to powdery mildew. Precise localisation of the detected unknown genes on chromosomes 5D and 7D, responsible for the high resistance to powdery mildew in the cultivar 'Vinjett', would give valuable markers for marker-assisted breeding.

Acknowledgements

This work was supported by the Estonian Ministry of Agriculture.

References

Bougot, Y., Lemoine, J., Pavoine, M.T., Guyomarch, H., Gautier, V., Muranty, H., & Barloy, D. (2006). A major QTL effect controlling resistance to powdery mildew in winter wheat at the adult plant stage. *Plant Breeding*, *125*(6), 550–556.

Chantret, N., Mingeot, D., Sourdille, P., Bernard, M., Jacquemin, J.M., & Doussinault, G. (2001). A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. *Theoretical and Applied Genetics*, *103*, 962–971.

Chantret, N., Pavoine, M.T., & Doussinault, G. (1999). The race-specific resistance gene to powdery mildew, MlRE, has a residual effect on adult plant resistance of winter wheat line RE714. *Phytopathology*, 89, 533–539.

Felsenstein, F.G. (1991). Virulenz und Fungizidsensivität des Weizenmehltaus, *Erysiphe graminis* DC. f. sp. *tritici* Marchal, in Europa. Dissertation an der TU München, 179S

Felsenstein, F.G., & Jaser, B. (2005). Situationsbericht 2005. Fungizidresistenz bei pilzlichen Getreidepathogenen und Wirksamkeit der vertikalen (qualitativen) Mehltauresistenz bei Weizen und Gerste. EpiLogic GmbH Agrarbiol. Forschung und Beratung. *www.epilogic.de*

Flor, H.H. (1971). Current status of gene-for-gene concept. *Annual Review of Phytopathology*, *9*, 275–296.

Griffey, C.A., & Das, M.K. (1994). Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. *Crop Science*, *34*, 641–646.

Heun, M., & Fischbeck, G. (1987). Genes for powdery mildew resistance in cultivars of spring wheat. *Plant Breeding*, *99*, 282–288.

Hsam, S.L.K., & Zeller, F.J. (1997). Evidence of allelism between genes Pm8 and Pm17 and chromosomal location of powdery mildew and leaf rust resistance genes in the common wheat cultivar Amigo. *Plant Breeding*, *116*, 110–122.Ref.

Huang, X., Zeller, F.J., Hsam, S.L.K., Wenzel, G. & Mohler, V. (2000). Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. *Genome*, *43*, 298–305.

Huang, X.-Q., & Röder, M.S. (2004). Molecular mapping of powdery mildew resistance genes in wheat: a review. *Euphytica*, *137*, 203–223.

Jakobson, I., Peusha, H., Timofejeva, L., & Järve, K. (2006). Adult plant and seedling resistance to powdery mildew in a *Triticum aestivum* × *Triticum militinae* hybrid line. *Theoretical and Applied Genetics*, *112*, 760–769.

Keller, M., Keller, B., Schachermayr, G., Winzeler, M., Schmid, J.E., Stamp, P., & Messmer, M.M. (1999). Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. *Theoretical and Applied Genetics*, *98*, 903–912.

Liu, S.X., Griffey, C.A., & Saghai-Maroof, M.A. (2001). Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. *Crop Science*, *41*, 1268–1275.

Lutz, J., Limpert, E., Bartoa, P., & Zeller, F.J. (1992). Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). I. Czechoslovakian cultivars. *Plant Breeding*, *108*, 33–39.

Martin, T., & Ellingboe, A. (1976). Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the corresponding gene in *Erysiphe graminis* f. sp. *tritici*. *Genetics*, *66*, 1435–1438.

Mingeot, D., Chantret, N., Baret, P.V., Dekeyser, A., Boukhatem, N., Sourdille, P., Doussinault, G., & Jacquemin, J.M. (2002). Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. *Plant Breeding*, *121*, 133–140.

Miranda, L.M., Murphy, J.P., Marshall, D., & Leath, S. (2006). *Pm34*: a new powdery mildew resistance gene transferred from *Aegilops tauschii* Coss. to common wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *113*, 1497–1504.

UNCORRECTED PROOF

Nass, H.A., Pedersen, W.L., MacKenzie, D.R., & Nelson, R.R. (1981). The residual effects of some "defeated" powdery mildew resistance genes in isolines of winter wheat. *Phytopathology*, *71*, 1315–1318.

Paillard, S., Goldringer, I., Enjalbert, J., Doussinault, G., de Vallavieille-Pope, C., &,Brabant, P. (2000). Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. II – Adult plant resistance *Theoretical and Applied Genetics, 101*, 457–462.

Parlevliet, J.E., & Zadoks, J.C. (1977). The integrated concept of disease resistance: a new view including horizontal and vertical resistance in plants. *Euphytica*, *26*, 5–21.

Paterson, A., Lander, E., Lincoln, S., Hewitt, J., Peterson, S., & Tanksley, S. (1988). Resolution of quantitative traits into Mendelian factors using a complete linkage map of restriction fragment length polymorphism. *Nature*, *335*, 721–726.

Peusha, H., Enno, T., & Priilinn, O. (2000a). Chromosomal location of powdery mildew resistance genes and cytogenetic analysis of meiosis in common wheat cultivar 'Meri'. *Hereditas*, *132*, 29–34.

Peusha, H., Enno. T., & Priilinn, O. (2000b). Chromosome location of genes for resistance to powdery mildew in common wheat cultivar 'Tjalve'. *Acta Phytopathologica et Entomologica Hungarica*, *35*, 51–57.

Peusha, H., Hsam, S.L.K., Enno, T., & Zeller, F.J. (1996). Identification of powdery mildew resistance genes in common wheat (Triticum aestivum L. em. Thell.). VIII. Cultivars and advanced breeding lines grown in Finland. *Hereditas*, *124*, 91–93

Peusha, H., Ingver, A., & Priilinn, O. (1997). Screening for powdery mildew resistance in common wheat cultivars and breeding lines. *Annual Wheat Newsletter*, *43*, 96–100.

Peusha, H., Sadam, H., & Enno, T. (2005). Chromosome location of powdery mildew resistance in common wheat cultivar 'Helle'. *Annual Wheat Newsletter*, *51*, 29–31.

Tommasini, L., Yahiaoui, N., Srichumpa, P., & Keller, B. (2006). Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. *Theoretical and Applied Genetics, 114*, 165–175.

Tsõmbalova, J., Haljak, M., Ingver, A., & Järve, K. (2007). Genetic diversity of Estonian-grown spring wheat varieties assessed by microsatellite and morphological analyses. *Acta Agriculturae Scandinavica, Section B – Plant & Soil Science*, in press.

Yahiaoui, N., Brunner, S., & Keller, B. (2006). Rapid generation of new powdery mildew resistance genes after wheat domestication. *Plant Journal*, *47*, 85–98.

Yahiaoui, N., Srichumpa, P., Dudler, R., & Keller, B. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistant gene *Pm3b* from hexaploid wheat. *Plant Journal*, *37*, 528–538.

Zeller, F.J., Lutz, J., & Stephan, U. (1993a). Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). 1. *Mlk* and other alleles at the *Pm3* locus. *Euphytica, 68*(3), 223–229.

Zeller, F.J., Stephan, U., & Lutz, J. (1993b). Present status of wheat powdery mildew resistance genetics. *Proceedings of the 8th International Wheat Genetics Symposium*. Beijing, China, pp. 929–931.

Zhu, Z., Zhou, R., Kong, X., Dong, Y., & Jia, J. (2005). Microsatellite markers linked to *2* powdery mildew resistance genes introgressed from *Triticum carthlicum* accession PS5 into common wheat. *Genome, 48*, 585–590.

Wheat Pedigree and Identified Alleles of Genes. http://genbank.vurv.cz/wheat/pedigree/default.htm

UNCORRECTED PROOF

Lisa 4

Topelthaploidsete nisutaimede saamine. Katseprotokoll

Emataimede kasvatamine

Taimed kasvatatakse pottides täisväetisega mullas. Soovitav on mulla ja pottide eelnev autoklaaavimine.

Kasvurežiim: temperatuur 15-20 ^{o}C (optimaalne päevane temperatuur 18 ^{o}C, oluline on säilitada suhteliselt madal kasvutemperatuur), valgus/pimedus 16/8 tundi, valgustugevus 250-300 µmool/m^{2}s, spektraalne koostis maksimaalselt sarnane välisvalgusele (kõrgsurve Na-lambid (LUCALOX, Ungari) ja kõrgsurve Hg-lambid (POLAMP, Poola)).

Kasvuaeg sõltuvalt liigist (*T.aestivum , T.timofeevii*) ja sordist 1,5 kuni 3 kolm kuud.

Mikrospooride eraldamiseks sobiv kasvustaadium: võrsed, milles pea on 4/5 ulatuses kaetud ülemise lehega või on lehed veel ülalt avanemata (pea on kaetud), mõne sordi puhul ka keskelt veidi avanenud leht. Sobivas staadiumis võrsed lõigatakse, asetatakse steriilsesse vette ning hoitakse temperatuuril 4 ^{o}C 7-14 päeva.

Mikrospooride isoleerimine ja mikrospoorikultuur

Pead eraldatakse lehest, steriliseeritakse 70%-lises etanoolis 15 sekundit, seejärel 20%-lises ACE (kommertsiaalne valgendaja naatriumhüpokloriti baasil) lahuses 10 minutit ja veelkord 30 sekundit 70%-lises etanoolis. Loputatakse 3 korda steriilse destilleeritud veega. Tolmukad eraldatakse steriilsetes tingimustes (laminaar), kasutades teravaotsalisi pintsette ning külvatakse 3,5 cm diameetriga Petri tassidele 2 ml söötmele B (vt. Lisa). Tassid paigutatakse suurtesse Petri tassidesse (diameeter 10-12 cm), kusjuures igas suures tassis on püsiva niiskustaseme tagamiseks üks väiksem tass steriilse veega. Suur tass suletakse õhukindlalt (Parafilm). Tasse inkubeeritakse pimedas temperatuuril 33 ^{o}C. Mikrospooride eraldumist söötmesse kontrollitakse 2 ja 4 päeva möödudes inkubeerimise algusest. Kultuuride sobivuse kriteeriumiks on eraldunud mikrospooride hulk ning suurte (elujõuliste) ning väikeste kokkutõmbunud

tsütoplasmaga (surnud) mikrospooride suhe. Vaatlusteks sobib binokulaar suurendusega 87,5x (minimaalselt 50x).

Mikrospooride ülekanne stressisöötmelt B embrügeneesisöötmele:

1) mikrospoore sisaldav sööde sõelutakse (augu diameeter sõelas 80µm) steriilsesse klaasnõusse. Tolmukatesse jäänud mikrospoorid suspendeeritakse 3 ml söötmesse magnetsegaja abil (30 sekundit, 500-600 rpm, aste 6, magnetsegaja MR1000, Heidolph, Saksamaa), kasutades *Nalgene* segajaid (pikkus 8 x 22 mm) katseklaasis sisemise diameetriga 24 mm. Suspensioon sõelutakse läbi sõela (augu diameeter sõelas 80µm). Protsessi korratakse 3 korda, lisades iga kord enne segamist tolmukatele 3 ml värsket söödet. Kogutud ja ühendatud mikrospooride suspensioonid tsentrifuugitakse steriilsetes läbipaistvates koonilistes plastiktuubides (firma Kabe) 5 minutit kiirusel 1300 rpm (tsentrifuug T23, firma Janetzki). Supernatant dekanteeritakse ning sademele lisatakse 2 ml söödet B.

Elujõuliste mikrospooride eraldamiseks surnud mikrospooridest ning nende fragmentidest kasutatakse tsentrifuugimist läbi 10%-lise *Percoll*™ lahuse söötmes 3,3B. 2 ml lahjendatud mikrospooride suspensiooni söötmes B kantakse ettevaatlikult 2 ml 10% *Percoll* + 3,3 B söötme lahusele ning tsentrifuugitakse 5 minutit 1800 rpm juures. *Percolli*-lahuse pinnale jäävad elujõulised mikrospoorid pipeteeritakse 1 ml automaatpipeti abil ettevaatlikult uude tuubi, vältides seejuures võimalikult *Percolli*-lahuse kaasahaaramist. Pesemiseks lisatakse mikrospooridele cá 2 mahtu söödet B ning tsentrifuugitakse 5 minutit 1400 rpm juures. Supernatant eemaldatakse võimalikult täielikult ning sadestatud rakkudele lisatakse 2 x 1 ml söödet A_2 või AMC. Kultuur pannakse kasvama 3,5 cm diameetriga Petri tassidele, tassi kohta lisatakse 6-10 emakat. Emakad eemaldatakse eelnevalt samadest viljapeadest ning neid kasvatatakse neli päeva A_2 või AMC söötmel (3,5 cm diameetriga tassid).

Kultuuri inkubeeritakse pimedas temperatuuril 25 ^{o}C Petri tassidel (samal viisil kui eelnevalt kasvatati stressisöötmel tolmukaid 33 ^{o}C juures). 4, 6 ja 15 päeva möödudes hinnatakse mikrospooride kasvu binokulaari abil, tuumade arvu

kasvavates mikrospoorides hinnatakse fluorestsentsmikroskoobi abil, värvides mikrospoorid DAPI-ga.

Taimede regeneratsioon.

Pärast 21-30-päevast inkubeerimist temperaruuril 25 ^{o}C võetakse 1-4 mm-se suurusega embrüod ettevaatlikult väikese spaatli ja prepareerimisnõela abil söötmest välja ning kantakse regenereerimiseks üle A_2R söötmele (25 ml 10 cm diameetriga tassides). Tasse inkubeeritakse järgmistes tingimustes: fotoperiood 16/8 tundi, valgustugevus 60-70 µE/m^{2}s (luminenstentsvalgustid), päevane temperatuur 25 ^{o}C ja öine temperatuur 20 ^{o}C.

Kui regenereetitud taimede pikkus saavutab ca 1 cm, siis istutatakse rohelised taimed samasse söötmesse klaasist kasvutuubidesse (Sigma), kasvatatakse neid samades tingimustes kui Petri tassidel ligikaudu kuu aega, kuni taimed kasvavad ligikaudu 10 cm pikkusteks ning istutatakse seejärel ringi mullapottidesse. 1 cm pikkused albinootilised taimed loetakse, et arvutada roheliste taimede regeneratsiooni osakaalu ja visatakse seejärel minema.

Lisa katseprotokollile**:**

Söötmete retseptid:

Kõik söötmetes kasutatud ained on firmalt *Sigma* ja on ette nähtud koekultuurialasteks töödeks.

B meedium (stressiks 33 ^{o}C juures, 4 päeva)

1 liiter: KCl (1,49 g), Mg SO_4 x $7H_2O$ (0,25 g), $CaCl_2$ (0,11 g), mannitool (54,63 g)

pH viia kuni väärtuseni 7,0 0,5M KOH-ga, lisada 1 ml 1,0M fosfaatpuhvrit.

3,3 x B meedium (läbi Percolli tsentrifuugimiseks)

1 liiter: KCl (4,1 g), Mg SO_4 x $7H_2O$ (0,8 g), $CaCl_2$ (0,5 g), KH_2PO_4 (0,45 g), mannitool (180,4 g). Lisatakse Percoll™ kuni 10%-lise sisalduseni.

A_2 meedium (embrüode arenguks ja emakate kasvuks esimesel neljal päeval)

1 liiter: KNO_3 (1,415 g), $(NH_4)_2SO_4$ (0,23 g), KH_2PO_4 (0,2 g), $CaCl_2$ x $2H_2O$ (0,083 g), $MgSO_4$ x $7H_2O$ (0,2 g), FeNaEDTA (0,032 g), MES (1,95 g), glutamiin

(1,26 g), maltoos (108 g), ½ kontsentr. B_5 mikroelemendid (Sigma valmissegu), ½ kontsentr. B_5 vitamiinid (Sigma valmissegu). pH viia KOH-ga kuni 6,3.

AMC meedium (embrüode arenguks)

1 liiter: $FeCl_3$ (0,027 g), Na_2EDTA (0,0373 g), KNO_3 (1,0 g), KH_2PO_4 (0,2 g), $(NH_4)_2SO_4$ (0,1 g), $MgSO_4 \times 7H_2O$ (0,125 g), KCl (0,035 g), tiamiinkloriid (1 mg), 2,4D (1,5 mg), kinetin (0,5 mg), maltoos (90 g), glutamiin (1 g), seriin (0,1 g).

Mikroelemendid: $MnSO_4 \times 4H_2O$ (4,4 mg), $ZnSO_4 \times 7H_2O$ (1,5 mg), H_3BO_3 (1,6 mg), KJ (0,8 mg). pH viia KOH lisamisega kuni 5,8.

A_2 regeneratsioonimeedium

Sama koostisega kui A_2 meedium embrüode induktsiooniks, kuid suhkru allikana on lisatud 2% sahharoosi ning tardsöötme saamiseks 0,3% *Gelrite*'i.

(Protokolli koostas: Anu Tiidema, MSc, teadur)

Lisa 5

Mikrosatelliitjärjestuste amplifitseerimine nisu genoomist
Protokoll

DNA eraldamine
1,g lehti (10-30-päevased taimed) pannakse vahetult peale lõikamist vedela lämmastikuga jahutatud portselanuhmrisse ja hõõrutakse vedelas lämmastikus peeneks pulbriks. Lisatakse 5 ml ekstraktsioonipuhvrit A.
Puhver A: 1,5 w/v% CTAB (tsetüültrimetüülammoniumbromiid), 100mMTris-HCl, pH8,0, 1,05MnaCl, 20 mM EDTA, 1,% ß-merkaptoetanooli.
Inkubeeritakse 30 minutit temperatuuril 60 ^{o}C, jahutatakse 5 minutit jääl.
Lisatakse 5 ml kloroform:isoamüülpiirituse segu 24:1.
Loksutatakse toatemperatuuril 30 minutit.
Tsentrifuugitakse 30 minutit 2100xG juures. Vesikiht pipeteeritakse ettevaatlikult puhtasse katsutisse ja lisatakse 20 ul RNAaas A (10mg/ml), inkubeeritakse 30 minutit toatemperatuuril. Lisatakse võrdne maht isopropanooli, hoitakse 30 minutit temperatuuril 4 ^{o}C ja püütakse peene klaaspulga abil segades sadenenud DNA pulga ümber. Sadet pestkse pulga peal paar korda 75%-lises etanoolis (võib jätta ööseks etanooli). Kuivatatakse, kraabitakse ettevaatlikult Eppendorfi tuubi ja lahustatakse TEs. Amplifitseerimiseks lahjendatakse kontsentratsioonini 100ng/ul.

PCR reaktsiooni tingimused:

DNA 100ng	3ul
Reaktsioonipuhver $(NH_4)_2SO_4$ sisaldav) 3ul	
25 mM $MgCl_2$	2,4 ul
25 mM dNTP	0,3 ul
Taq DNA polümeraas (Fermentas, 5 U/ul)	0,2 ul
2 ul praimerid, kumbki	3 ul
^{32}P-dCTP	0,05-0,1 ul
H_20 kuni	30 ul

PCR tsüklid:
1. 94 ^{o}C 5 minutit
2. 94 ^{o}C 1 minut
3. 0,5 ^{o}C/sek ramp
4. annealing sõltuvalt praimeritest 50-60 ^{o}C 1 minut
5. 0,5 ^{o}C/sek ramp
6. 72 ^{o}C 1 minut
2.-7. 45 tsüklit

PCR produktid lahutatakse 5%-lisel PAA geelil (60 ml: karbamiidi 25,2g; 29% akrüülamiidilahust 10 ml; 3% bis-akrüülamiidilahust 5,2 ml, 5x Tris-boraatpuhvrit 12 ml, 10% APS 350 ul; TEMED 50 ul.
Forees: 1,5 kuni 2 tundi 500V.
Autoradiograafia: Fuiju Medical X-ray film „Super RX“.