Exploitation and characterisation of resistance to the root-knot nematode *Meloidogyne incognita* in soybean

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Abstract

Meloidogyne incognita (Kofoid and White) is a major pest of soybean in South Africa and due to its high level of pathogenicity to the crop it is quintessential that research in this regard should receive priority. Root-knot nematode control has in the past mostly included the use of nematicides, while crop rotation and inclusion of cultivars with genetic host plant resistance (henceforth referred to as resistance only) to these pests were also used. Since no synthetically-derived and/or biological agents are registered locally as nematicides on soybean, the use of resistant cultivars represents one of the most viable and environmentally-friendly strategies to protect local soybean crops against damage resulting from parasitism by *M. incognita*.

Although numerous exotic soybean cultivars have been identified with resistance to *M. incognita*, only a few locally adapted ones have proved to exhibit resistance to the latter species. Moreover, at present Egret is the only cultivar still available for commercial use in South Africa. Little and fragmented information is, however, available on the use of plant enzymes, that are interrelated in biochemical pathways that are expressed in root-knot nematode resistant cultivars, for its use as an additional parameter to exploit such a trait. Therefore, the present study was undertaken to identify *M. incognita* resistance in selected, locally adapted soybean cultivars by quantifying and exploiting the latter trait by using enzyme activities as an additional parameter. In addition, resistance to *M. incognita* in selected resistant soybean cultivars was also verified by means of histopathological studies to identify cellular changes associated with the trait.

In the first part of the present study, 31 locally adapted soybean cultivars of which 23 were commercially available in the 2012 growing season were evaluated for resistance to *M. incognita*. The latter was done by means of traditional screening protocols for which *M. incognita*-gall rating, egg and second-stage juvenile as well as the reproductive factor data per root system for each cultivar screened were recorded. Two greenhouse experiments were subsequently conducted concurrently, one of which the abovementioned nematode parameters were recorded 30 and the other 56 days after inoculation. Reproduction factor values were used as the main criterium to identify *M. incognita* resistance in local soybean cultivars since it is considered as a more reliable parameter for this specific type of evaluations. Reproduction factor values equal to and lower than

one, indicating resistance to the *M. incognita* population used in this study, were recorded only for cultivar LS5995, as well as seven pre-released GCI cultivars. These eight cultivars also had very low egg, as well as egg and second-stage juvenile counts per root system, all of which differed significantly from the susceptible control, as well as a number of other cultivars. Root gall indices, on the other hand, did not show consistent results in terms of the identification of the host status of the 31 cultivar screened during this study. Using reproduction factor values, local farmers can thus be supplied with information on the resistance of commercially-available soybean cultivars. Eventually, such *M. incognita*-resistant cultivars can be used to reduce population levels of this nematode pest in fields of producers and also as valuable germplasm sources in breeding programs to introgress/stack this trait in newly-developed soybean cultivars.

The second part of the study aimed to verify and exploit *M. incognita*-resistance in soybean either identified as resistant or susceptible during the screenings experiments, using enzymatic activity as biochemical markers. Cultivar LS5995 was included as the resistant and Dundee as the susceptible standard. The activity of three enzymes, namely guaiacol peroxidase, lipoxygenase and catalase were recorded at different time intervals in roots and leaf samples of the latter cultivars, of both nematode-inoculated and nematode-free plants of each cultivar. Significant ($P \leq 0.05$) increases in guaiacol peroxidase activity in leaf and root samples of the *M. incognita*-resistant cultivars GCI7 and LS5995 (inoculated with J2) were recorded 24 hours (h) after onset of the experiment. Use of this enzyme thus emanated as a useful parameter to identify soybean cultivars that exhibit resistance against *M. incognita*, especially in leaves, which could substantially reduce the time needed to screen cultivars. In terms of lipoxygenase activity recorded, substantial variation existed between the cultivars tested. The *M. incognita*-susceptible cultivar Egret was the only cultivar for which a significant ($P \leq 0.05$) increase in lipoxygenase activity in the roots was evident 24 h after inoculation. However, during the 48 h sampling time, significant ($P \leq 0.05$) differences in lipoxygenase activity were also recorded for the two *M. incognita* resistant cultivars GCI7 and LS5995. Although the increase in lipoxygenase activity for the susceptible cultivar Egret was unexpected, it may indicate that some level of resistance is present in the latter cultivar, which has in previous studies been identified as resistant to *M. incognita*. Other factors such as a different *M. incognita* populations used and temperature differences in greenhouse conditions that applied in this study compared to that for an earlier study may, however, serve as explanations for the latter differences in host status identification of cultivar Egret. In terms of catalase activity recorded in

leaf samples of the *M. incognita*-resistant cultivar LS5995, substantial reductions of as much as 35.6 % were recorded for J2-inoculated plants compared to those of the J2-free control plants. In leaf samples of the susceptible cultivars, Egret and Dundee, catalase was also reduced, but to a lesser extent and ranged from 6 to 26 %. Conversely, catalase activity in the leaves of J2-inoculated plants of the highly susceptible cultivar LS6248R was substantially increased by as much as 29.3 %. Enzyme data obtained as a result of the current study thus generally complemented those of traditional screening assays in which resistance in locally adapted cultivars were identified to a certain degree. It is, however, recommended that enzyme activity, to be used as bio-markers, still needs further refinement and more investigation to optimise their use in identification, verification and exploitation of *M. incognita* resistance in soybean cultivars.

The third and final part of the study encompassed a comparison of cellular changes induced by *M. incognita* in resistant and susceptible soybean cultivars to verify the resistant reactions expressed in the enzyme data. According to light- and transmission electron microscope observations, distinct differences in the appearance and development of giant cells in roots of the *M. incognita*-resistant cultivars LS5995 and GCI7 existed when compared to those in roots of the susceptible cultivars Dundee and LS6248R. In the latter cultivars, giant cells that formed were characteristically large and contained a dense cytoplasm, with thick irregularly surfaced cell walls. Cell walls also displayed thick aggregations that appeared to be cell-wall ingrowths. These giant cells are optimal to facilitate *M. incognita* development and reproduction. In contrast, giant cells that were associated with the resistant cultivars LS5995 and GCI7 were small, irregularly shaped and contained increased amounts of deposited cell-wall material in the cytoplasm known as cell wall inclusions. Necrosis was also present in *M. incognita*-infected root cells of both cultivars. Such giant cells have been associated with retarded feeding, development and reproduction of the latter root-knot nematode species. However, it was evident that neither GCI7 nor LS5995 are immune to *M. incognita* since J2 survived and developed to third- and fourth and ultimately mature females that reproduced in their roots. Optimal giant cells that were formed in the roots of the *M. incognita*-susceptible cultivars Dundee and LS6248R thus supported the nutritional needs of the developing *M. incognita* individuals and led to significant increases in *M. incognita* populations 56 days after inoculation as was evident from the high reproduction factor values that were obtained for such cultivars during host status assessments that represented the first part of this study. The opposite was recorded the *M. incognita*-resistant cultivars LS5995 and GCI7 since sub-optimal giant cells in

their roots could not sustain high offspring from such mature females. The presence of necrotic root tissue adjacent to giant cells, furthermore, indicated that hypersensitive reactions occurred in the latter resistant cultivars. Enzyme data obtained in the second part of this study supported the presence of hypersensitive reactions in root cells of the latter resistant cultivars. Guaiacol peroxidase and lipoxygenase inductions in particular in plant tissues have been reported to play integral roles in hypersensitive reactions that are exhibited by cultivars that are resistant to pests and diseases.

Finally, results obtained from the different parts of this study complemented each other. It resulted in the resistance that was identified in the GCI7 pre-released cultivar being verified and exploited against that of the resistant standard LS5995. Research that was done during this study also represented the first investigations into the use of enzymes as biochemical markers of resistance against *M. incognita* in soybean in South Africa.

Keywords: *Meloidogyne incognita*, Lipoxygenase, Peroxidase, Catalase, Nematodes, Soybean.

Uittreksel

Meloidogyne incognita (Kofoid & White) is ŉ ekonomies belangrike plaag organisme van sojabone in Suid-Afrika. Dit is daarom belangrik dat navorsing op hierdie gebied prioriteit geniet. Die beheer van knopwortelaalwurms op sojabone in ander wêrelddele was en is steeds tot ŉ groot mate afhanklik van die gebruik van Klas 1, chemiese nematisiede. Die verbouing van eksotiese sojaboonkultivars wat genetiese weerstand (voortaan slegs verwys na as weerstand) teen hierdie plaagorganisme besit, asook in ŉ mindere mate wisselbou-praktyk word ook aangewend om *M. incognita* bevolking in sojaboonrotasiestelsels drasties te verlaag. Sodanige beheerstrategieë is egter nie van toepassing in Suid-Afrika nie aangesien geen sintetiese of biologiese middels as nematisiede op sojaboon geregistreer is nie. Wisselbou is ook meestal ŉ onsuksesvolle en beperkende aalwurmbeheerstrategie weens die wye gasheerreeks van *M. incognita*. Die gebruik van weerstandbiedende kultivars is dus ŉ omgewingsvriendelike strategie om plaaslike sojaboonoeste te beskerm teen skade as gevolg van parasitering deur *M. incognita*. Die probleem is egter dat slegs enkele plaaslik-aangepaste sojaboonkultivars reeds geïdentifiseer is met gasweerstand teen hierdie knopwortelaalwurmspesie. Voorts is daar tans slegs een kultivar wat vroeër geïdentifiseer is as *M. incognita*-weerstandbiedend, nl. Egret, en is kommersieël beskikbaar vir gebruik in Suid-Afrika.

Beperkte inligting bestaan in terme van die gebruik van interafhanklike ensieme wat integrale rolle in die biochemiese sisteme van gasheerplante vervul. Dus is laasgenoemde benadering in hierdie studie as 'n potensiële, addisionele parameter ondersoek om weerstandsmeganismes en –vlakke in geselekteerde *M. incognita* weerstandbiedende kultvars te probeer kwantifiseer en verifieër. Plaaslike kultivars is dus aanvanklik geëvalueer vir hul *M. incognita*-gasheerstatus deur gebruik te maak van konversionele, glashuis-siftingseksperimente. Vervolgens is die aktiwiteit van drie ensieme, wat bekend is vir hul betrokkenheid in gasheer/plaag/siekte-interaksies, vasgestel in blaar- en wortelmonsters van geselekteerde *M. incognita*-weerstandbiedende sojaboonkultivars. Laasgenoemde kultivars se gasheerstatus is vasgestel tydens voorafgaande, konvensionele siftingseksperimente. Die laaste faset van hierdie studie het behels dat die sellulêre veranderinge in

die geselekteerde weerstandbiedende sojaboonkultivars deur middel van histopatologiese studies ondersoek en geverifieer is.

In die eerste deel van hierdie studie is 31 plaaslik aangepaste sojaboonkultivars, waarvan 23 kommersieel beskikbaar en sewe voor-vrygestelde GCI-kultivars vir weerstand teen *M. incognita* geëvalueer. Laasgenoemde is gedoen deur middel van konvensionele siftingseksperimente waartydens *M. incognita*-wortelgalindekse, eier en tweede jeugstadiumgetalle (J2) asook voortplantingsdata per wortelstelsel vir elke kultivar, aangeteken is. Twee glashuiseksperimente is vervolgens gelyktydig uitgevoer waartydens die wortelgalindekse 30 en 56 dae na nematoodinokulasie aangeteken is. Bykomende voortplantingsdata is ook tydens die laasgenoemde monsternemingsinterval aangeteken. Laasgenoemde parameter is gebruik as die belangrikste kriterium om *M. incognita* weerstand te identifiseer in die plaaslike sojaboonkultivars. Die rede hiervoor is dat voortplantingsdata algemeen beskou word as 'n meer betroubare parameter om die gasheerstatus van knopwortelnematode te bepaal. 'n Waarde laer as een vir laasgenoemde parameter toon weerstand aan teen die spesifieke *M. incognita*-bevolking wat gebruik was in hierdie studie. Weerstand teen *M. incognita* is slegs aangeteken vir kultivar LS5995, wat die weerstandbiedende standaard verteenwoordig het, asook die sewe voor-vrygestelde GCI-kultivars. Egret het 'n relatiewe hoë reproduksie faktor gekry en is dus in hierdie studie as vatbaar geïdentifiseer. Dundee en LS6248R was hoogs vatbaar as gevolg van baie hoë reproduksie-faktore. Wortelgal-indeksdata vir die verskillende kultivars het min ooreenstemming getoon ten opsigte van hul gasheerstatus met betrekking tot *M. incognita* tydens die twee monsternemings-intevalle.

Die agt voor-vrygestelde kultivars het ook laer eier sowel as eier en tweede jeugstadiums van *M. incognita* getalle per wortelstelsel gehad en het beduidend verskil van die vatbare standaard asook 'n aantal ander kultivars. In teenstelling het wortelgalindekse nie konsekwente resultate getoon in terme van gasheerstatus-identifisering van die 31 kultivars tydens hierdie studie nie. Dus word voorgestel dat voortplantingsdata gebruik word om plaaslike boere te voorsien van inligting rakende *M. incognita*-weerstand wat teenwoordig is in kommersieel-beskikbare sojaboonkultivars. Gevolglik kan sodanige *M. incognita*-weerstandbiedende kultivars gebruik word om bevolkingsvlakke van hierdie nematoodplaag in die lande van produsente te beheer. Voorts kan sulke kultivars ook gebruik word as waardevolle kiemplasmabronne in teelprogramme om sodoende *M. incognita*-weerstand te integreer in nuut-ontwikkelde sojaboonkultivars.

Die tweede deel van die studie was daarop gerig om *M. incognita* weerstand te kwantifiseer en/of te verifieer in sojaboonkultivars wat geïdentifiseer as weerstandbiedend of vatbaar gedurende die siftingseksperimente. Vir hierdie doel is ensiemaktiwiteit as biochemiese merkers ingespan. Kultivar-LS5995 is ingesluit as die *M. incognita*-weerstandbiedende en Dundee as die vatbare standaard. Die aktiwiteit van drie ensieme, naamlik guaiakolperoksidase, lipoksigenase en katalase is aangeteken op verskillende tydsintervalle in wortel- en blaarmonsters van die verskeie kultivars. Beide *M. incognita*-geïnokuleerde en -vrye plante van elke kultivar is vir laasgenoemde doel gebruik. Beduidende ($P \leq 0.05$) verhogings in guaiakolperoksidase-aktiwiteit in blaar- en wortelmonsters van die *M. incognita*-weerstandbiedende kultivars GCI7 en LS5995 wat geïnokuleer is met tweede jeugstadiums van *M. incognita* is 24 uur na aanvang van die eksperiment aangeteken. Hierdie ensiem kan dus moontlik as 'n nuttige parameter ingespan word om sojaboonkultivars te identifiseer wat weerstand besit teen *M. incognita*. Dit geld veral vir data wat verkry is ten opsigte van die blare van die weerstandbiedende kultivars, wat dus die tyd wat nodig is kultivars te sif aansienlik kan verminder.

In terme van die lipoksigenase-aktiwiteit wat aangeteken is in blaar- en wortelmonsters van *M. incognita*-weerstandbiedende kultivars het dit geblyk dat aansienlike variasie bestaan het vir hierdie parameters ten opsigte van nematood-geïnokuleerde versus –vrye plante van alle kultivars wat getoets is. Die *M. incognita*-vatbare kultivar Egret was die enigste kultivar wat 'n beduidende ($P \leq 0.05$) toename in lipoksigenase-aktiwiteit in die wortels getoon het 24 uur na nematoodinokulasie. Betekenisvolle ($P \leq 0.05$) verskille is egter ook aangeteken vir lipoksigenase aktiwiteit vir die twee *M. incognita*-weerstandbiedende kultivars GCI7 en LS5995 48 uur na aanvang van die eksperiment. Hoewel die toename in lipoksigenase aktiwiteit vir die vatbare kultivar Egret verassend was, kan dit daarop dui dat 'n sekere vlak van weerstand teenwoordig is in hierdie kultivar. Dit bevestig data van 'n vorige studie waarin Egret met weerstand teen *M. incognita* geïdentifiseer is. Ander faktore, soos die gebruik van 'n ander *M. incognita* bevolkings en temperatuurverskille in die glashuis, in vergelyking met dié van 'n vorige studie kan egter dien as verklarings vir die laasgenoemde verskille in gasheerstatus van kultivar Egret.

In terme van katalase-aktiwiteit wat aangeteken is in blaarmonsters van die *M. incognita*-weerstandbiedende kultivar LS5995, is 'n aansienlike verlaging in aktiwiteit aangemeld van tot soveel as 35.6 % vir plante wat met J2 van *M. incognita* geïnokuleer was in vergelyking met dié

wat nematoodvry was. In blaarmonsters van die vatbare kultivars Egret en Dundee was katalase-aktiwiteit ook verlaag maar tot ’n mindere mate en het gewissel vanaf 6 tot 26 %. In teenstelling was katalase-aktiwiteit in die blare van die *M. incognita*-geïnokuleerde plante van die hoogs vatbare kultivar LS6248R met soveel as 29.3 % verhoog. Samevattend het dit geblyk dat ensiemdata wat verkry van *M. incognita*-weerstandbiedende en –vatbare kultivars in die huidige studie, resultate van die konvensionele siftingseksperimente aangevul en ondersteun het. Dit word egter aanbeveel dat indien ensiemaktiwiteit gebruik sou word as bio-merkers vir weerstand, protokolle vir ensiembepalings verder verfyn moet word om die gebruik daarvan te optimaliseer en sodoende *M. incognita* weerstand identifisering en verifikasie in sojaboon kultivars te fasiliteer.

Laastens is 'n vergelykende studie gedoen ten opsigte van sellulêre veranderinge veroorsaak deur *M. incognita*-parasitisme van die wortels in geselekteerde weerstandbiedende en vatbare sojaboonkultivars. Volgens lig- en transmissie-elektronmikroskopiese waarnemings was merkbare verskille in die voorkoms en ontwikkeling van reuseselle in die wortels van *M. incognita*-weerstandbiedende kultivars LS5995 en GCI7 sigbaar in vergelyking met die wat in wortels van vatbare kultivars Dundee en LS6248R teenwoordig was. In laasgenoemde kultivars is reuseselle van kenmerkende grote, digte sitoplasma en dik selwande met ingroeiings aangetref. Hierdie tipe reuseselle is optimaal om ontwikkeling en voortplanting van *M. incognita*-individue te fasiliteer. In teenstelling met laasgenoemde optimale reuseselle is selle wat met die *M. incognita*-weerstandbiedende kultivars LS5995 en GCI7 geassosieer was, beduidend kleiner as die wat teenwoordig was in wortels van die vatbare kultivars. Hierdie sub-optimale reuseselle het ‘n onreëlmatige voorkoms getoon en het groot hoeveelhede gedeponeerde selwandmateriaal in die sitoplasma bevat. Nekrose was ook teenwoordig in beide weerstandbiedende kultivars in die area rondom die reuseselle waar *M. incognita*-individue besig was om te voed. Sulke sub-optimale reuseselle was verantwoordelik vir vertraagde ontwikkeling van en 'n beduidende afname in voortplanting van knopwortelaalwurmwyfies van *M. incognita*. Dit het egter geblyk uit hierdie studie dat GCI7 of LS5995 nie immuun is teen *M. incognita* nie aangesien tweede jeugstadiums in staat was om te oorleef en te ontwikkel tot die derde en vierde jeustadiums en uiteindelik volwasse, eierproduserende wyfies.

Optimale reuseselle wat gevorm is in die wortels van die *M. incognita*-vatbare kultivars Dundee en LS6248R het dus voldoen aan die voedingsbehoeftes van die ontwikkelende

nematoodlewenstadiums. Uiteindelik het voeding van *M. incognita* op laasgenoemde reuseselle gelei tot 'n beduidende toename in hul bevolking 56 dae na nematoodinokulasie, soos aangedui is deur die hoë voortplantingsdata vir laasgenoemde kultivars gedurende die gasheerstatus-siftingseksperimente in die eerste deel van die studie. Die teenoorgestelde is aangeteken vir *M. incognita*-weerstandbiedende kultivars LS5995 en GCI7 aangesien sub-optimale reuseselle in hul wortels aangetref is en lae vlakke van nematoodvoortplanting verkry is. Die teenwoordigheid van nekrotiese weefsel langs reuseselle in wortels van laasgenoemde kultivars het verder dui op die teenwoordigheid van hipersensitiewe reaksies. Ensiemdata wat in die tweede deel van hierdie studie vervat is, ondersteun ook die teenwoordigheid van die hipersensitiewe reaksies in wortelselle van hierdie kultivars. Die induksie van guaiakolperoksidase en lipoksigenase in plantweefsel van kultivars wat weerstand het teen peste en siektes is in die verlede aangemeld en speel dus 'n integrale rol in die hipersensitiewe reaksie soos hierbo aangedui.

Ten slotte blyk dit dat die resultate verkry vir die verskillende studies wat in die huidige navorsing omvat is, mekaar aanvul. Weerstand teen *M. incognita* wat dus geïdentifiseer is in die voor-vrygestelde kultivar GCI7, is geverifieer teen dié van die weerstandbiedende standaard LS5995. Navorsing wat tydens hierdie studie gedoen is, dien ook as die eerste ondersoek na die gebruik van ensieme as biochemiese merkers van weerstand teen *M. incognita* in sojabone in Suid-Afrika.

Sleutelwoorde: *Meloidogyne incognita*, Lipoksigenase, Peroksidase, Katalase, Nematode, Sojaboon.