CHARACTERISATION OF *RHIZOCTONIA* IN CROPPING SYSTEMS IN THE WESTERN CAPE PROVINCE

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SUMMARY

The genus *Rhizoctonia* includes important soilborne plant pathogens that can cause severe economic losses on a wide range of crops including cereal, canola, pasture and grain legume crops. Limited information is available on specific anastomosis groups (AGs), nuclear status, pathogenicity and cross-pathogenicity of *Rhizoctonia* isolates associated with each of these crops in the Western Cape province. Therefore, these aspects were investigated in this study, since information in this regard is crucial for the development of *Rhizoctonia* disease management practices.

Rhizoctonia isolates were characterised within a four-year crop rotation trial containing barley, clover, lucerne, lupin, medic, and wheat. A monoculture of lucerne was also included in the trial. A total of 428 *Rhizoctonia* isolates were characterised with regard to nuclear status, AG and *in vitro* response to temperature. The anastomosis group of all isolates was determined through sequence analysis of ribosomal deoxyribonucleic acid (rDNA) internal transcribed spacer (ITS) regions (conducted by Dr M. Mazzola, USDA, Wenatchee, WA, USA). The AG of a subset of isolates was also determined by traditional hyphal fusion reactions. Nuclear staining of the 428 *Rhizoctonia* isolates revealed the presence of 104 (24%) multinucleate and 324 (76%) binucleate isolates. The incidence of binucleate isolates was significantly higher than multinucleate isolates in all four rotation trial years. Sequence analysis of the rDNA-ITS region revealed that multinucleate isolates were comprised of AG-4 HG-II (69%), AG-2-1(19%), AG-3(8%), AG-2-2(2%) and AG-11(2%). Among the binucleate *Rhizoctonia* species 53% were AG-K, 10% were AG-A, 5% were AG-I and 32% were unidentified binucleate *Rhizoctonia* AGs. Traditional hyphal fusion of representative isolates with tester isolates confirmed these identifications, except for the AG-A isolates that did not anastomose with the tester isolate. AG-K and AG-4 HG-II were isolated from all crops, except for the latter that was not present on lucerne. Temperature growth studies further showed that these two AGs and AG-2-2 had significantly higher optimum growth temperatures than the other AGs. AG-2-2 and AG-11 were only isolated from medic/clover and lupin respectively. AG-2-1 was isolated from canola, lupin, medic/clover and wheat; AG-3 from canola,

lupin and wheat; AG-A from barley, canola, lucerne, medic/clover and wheat; whereas AG-I was isolated from barley, canola, lupin and wheat. Sampling time analysis showed that in two of the three years, significantly more *Rhizoctonia* isolates were obtained when sampling at the flowering stage, rather than at the seedling or mid-season stages.

The pathogenicity and virulence of *Rhizoctonia* isolates, representing nine AGs present within the crop rotation trial, were determined on emerging and 14-day-old seedlings of seven rotation crops in a growth room. Inoculum concentration studies showed that an inoculum concentration of 0.05% mass inoculum/mass planting medium was appropriate for use in the emerging seedling pathogenicity trials. *Rhizoctonia solani* AG-2-2 and AG-4 HG-II were the most virulent AGs on both seedling stages of all crops. However, these two AGs and AG-2-1 were either non-pathogenic or less virulent on barley and wheat than on the other crops. AG-2-1 was highly virulent on both seedlings stages of canola, but only moderately virulent on medic and lupin and weakly virulent on lucerne. *Rhizoctonia solani* AG-11 caused significant damping-off of lupin and medic, root rot on five crops (canola, clover lucerne, lupin and medic), as well as coleoptile or hypocotyl rot on five crops (barley, clover, lucerne, lupin and medic). *Rhizoctonia solani* AG-3 was only weakly virulent on canola, lupin and medic. The binucleate AGs viz., AG-A, AG-I and unidentified binucleate isolates were not considered pathogenic, even though very low disease incidence was present in a few instances. The binucleate AG-K was the only weakly virulent binucleate group capable of causing significant disease on canola, lucerne, lupin and medic seedlings.

This is the first report of the specified *Rhizoctonia* AGs and their pathogenicity on the seven rotation crops in South Africa. *Rhizoctonia solani* AG-4 HG-II was the most important AG, since it was not only associated with all the rotation crops, except lucerne, but was also one of the most virulent AGs on all the rotation crops. The high *in vitro* optimum growth temperature of AG-4 HG-II could indicate that prevailing temperatures at sowing time (April and May) can favour its growth. Information obtained in this study not only contributes to the knowledge of this very important group of soilborne plant pathogens, but is essential for developing sustainable disease management strategies.

OPSOMMING

Die genus *Rhizoctonia* sluit belangrike grondgedraagde plantpatogene in wat ernstige ekonomiese verliese op 'n wye reeks gewasse, insluitend graangewasse, canola, weidings- en graanpeulgewasse, kan veroorsaak. Beperkte inligting is beskikbaar rakende spesifieke anastomose groepe (AGs), nukleêre status, patogenisiteit en kruispatogenisiteit van *Rhizoctonia* isolate wat met elk van die gewasse in the Wes-Kaap provinsie geassosieer word. Hierdie aspekte is gevolglik in hierdie studie ondersoek, aangesien inligting in die verband beslissend is vir die ontwikkeling van *Rhizoctonia* siektebestuurspraktyke.

Rhizoctonia isolate is in 'n vierjaar gewasrotasieproef, bevattende gars, klawer, lusern, lupine, medic en koring gekarakteriseer. 'n Monokultuur van lusern is ook in die proef ingesluit. 'n Totaal van 428 *Rhizoctonia* isolate is volgens nukleêre status, AG en *in vitro* reaksie teenoor temperatuur gekarakteriseer. Die anastomose groep van al die isolate is deur basispaarvolgorde bepaling van die ribosomale deoksiribonukleïensuur (rDNA) interne getranskribeerde spasieërder (ITS) area bepaal (uitgevoer deur Dr M. Mazzola, USDA, Wenatchee, WA, VSA). Die AG van 'n subgroep isolate is ook deur tradisionele hife-fusie reaksies bepaal. Kernkleuring van die 428 *Rhizoctonia* isolate het die teenwoordigheid van 104 (24%) multinukleêre en 324 (76%) binukleêre isolate aangedui. In al vier jare van die rotasieproef, was die voorkoms van binukleêre isolate betekenisvol hoër as die multinukleêre isolate. Basispaarvolgorde bepaling van die rDNA-ITS area het getoon dat multinukleus isolate saamgestel is uit AG-4 HG-II (69%), AG-2-1(19%), AG-3(8%), AG-2-2(2%) en AG-11(2%). Tussen die binukleêre *Rhizoctonia* spesies was 53% AG-K, 10% was AG-A, 5% was AG-I en 32% was ongeïdentifiseerde binukleêre *Rhizoctonia* AGs. Tradisionele hife-fusies van verteenwoordigende isolate met toets isolate het die identifikasies bevestig, behalwe vir die AG-A isolate wat nie met die toets isolaat geanastomoseer het nie. AG-K en AG-4 HGII was die oorheersende AGs en is uit al die gewasse geïsoleer, behalwe vir laasgenoemde wat nie op lusern teenwoordig was nie. Temperatuurgroeistudies het verder aangedui dat hierdie twee AGs en AG-2-2 betekenisvolle hoër optimum

groeitemperature in vergelyking met die ander AGs gehad het. AG-2-2 en AG-11 is slegs van onderskeidelik medic/klawer en lupien geïsoleer. AG-2-1 is van canola, lupien, medic/klawer en koring geïsoleer; AG-3 van canola, lupien en koring; AG-A van gars, canola, lusern, medic/klawer en koring; terwyl AG-I vanaf gars, canola, lupien en koring geïsoleer is. Analise van die effek van versameltyd het aangedui dat in twee van die drie jaar, betekenisvol meer *Rhizoctonia* isolate verkry is wanneer tydens die blomtyd versamel is, eerder as tydens die saailing- of mid-seisoen stadiums.

Die patogenisiteit en virulensie van die *Rhizoctonia* isolate, wat die nege AGs teenwoordig in die gewasrotasieproef verteenwoordig, is op opkomende en 14-dag-oue saailinge van sewe rotasiegewasse in 'n groeikamer bepaal. Inokulumkonsentrasie studies het aangedui dat 'n inokulumkonsentrasie van 0,05% massa inokulum/massa plantmedium voldoende was om patogenisiteit en virulensie van isolate op opkomende saailinge te evalueer. *Rhizoctonia solani* AG-2-2 en AG-4 HG-II was die mees virulente AGs op beide saailingstadia van al die gewasse. Dié twee AGs en AG-2-1 was egter óf nie-patogenies óf minder virulent op gars en koring in vergelyking op die ander gewasse. AG-2-1 was hoogs virulent op beide saailingstadia van canola, maar slegs matig virulent op medic en lupien, en swak virulent op lusern. *Rhizoctonia solani* AG-11 het betekenisvolle omvalsiekte van lupien en medic veroorsaak, wortelvrot op vyf gewasse (canola, klawer, lusern, lupien en medic), asook epikotiel- en hipokotielvrot op vyf gewasse (gars, klawer, lusern, lupien en medic). *Rhizoctonia solani* AG-3 was slegs swak virulent op canola, lupien en medic. Die binukleêre AGs nl. AG-A, AG-I en ongeïdentifiseerde binukleêre isolate is nie as patogenies beskou nie, selfs al was baie lae siektevoorkoms in 'n paar gevalle teenwoordig. Die binukleêre AG-K was die enigste swak virulente binukleêre groep in staat om betekenisvolle siekte op canola, lusern, lupien en medic saailinge te veroorsaak.

Dit is die eerste verslag van die gespesifiseerde *Rhizoctonia* AGs en hul patogenisiteit op die sewe rotasiegewasse in Suid-Afrika. *Rhizoctonia solani* AG-4-HG-II blyk die belangrikste AG te wees aangesien dit nie slegs met al die rotasiegewasse, uitgesonderd lusern, geassosieer is nie, maar ook een van die mees virulente AGs op al

die rotasiegewasse was. Die hoë *in vitro* optimum groeitemperatuur van AG-4-HG-II kan ook aandui dat temperature wat tydens die saai van rotasiegewasse (April en Mei) heers, groei van die AG kan bevorder. Die inligting wat tydens die studie bekom is, dra nie net by tot die kennis van hierdie baie belangrike groep grondgedraagde plantpatogene nie, maar is noodsaaklik vir die ontwikkeling van volhoubare siektebestuurstrategieë.