Molecular and Physiological Detection of Cyst Nematodes on Potato During Plant Nematode Interactions

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ABSTRACT

Cyst nematodes involve over than 90 known species into six genera, *G. rostochiensis* and *G. pallida* are most important pathogens of potato crops where their economically a yield losses. The aim of present investigation is characterization the cyst nematode species (*G. rostochiensis*) using molecular techniques during plant-nematode interaction. The molecular identification of cyst nematode was carried out using ITS PCR. The results of ITS-PCR showed two bands with different molecular sizes 238 bp and 370 bp. The metabolic activation of potato leaves infected with cyst nematodes was detected. The results confirmed that, *G.rostochiensis* significantly elevated the contents of shoots lipid peroxidation (MDA) as compared to the untreated healthy plants.

Key words: Cyst nematodes, ITS PCR, potato

INTRODUCTION

Potato (*Solanum tuberosum*) is the most important economical crop in Egypt belonged to family Solanacea. The third economically most important worldwide food crop is potato (Gavrilenko*et al*., 2013). It is considered one of the largest agricultural exports in Egypt. Nematodes are roundworms classified in the Phylum Nematoda of the Animal Kingdom, because they inhabit soil, freshwater and marine environments. Potato cyst nematode is the combined term for the two species *G. rostochiensis* and *G. pallida* that are restricted to infecting a few species of Solanaceous plants. It is a major pathogen of the potato plants where, the yield losses of potato up to 50% (Trudgill, 1986). The losses of yields detect with general infestation average approximately 9%, but left uncontrolled, they are able to causing 100% crop loss (Brodie 1984; Brodie and Mai, 1989). Lipid peroxidation is a major complex process occurs in both plants and animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a multiplicity of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers (Dianzani and Barrera, 2008). Under physiological or low lipid peroxidation rates (subtoxic conditions), the cells stimulate their maintenance and survival through constitutive antioxidants defense systems or signaling pathways activation that upregulate antioxidants proteins resulting in an adaptive stress response. In contrast, under medium or high lipid peroxidation rates (toxic conditions) the extent of oxidative damage overwhelms repair capacity, and the cells induce apoptosis or necrosis programmed cell death; both processes ultimately lead to molecular cell damage which may facilitate development of various pathological circumstances and speeded senescence (Ayala *et al*., 2014).

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation, which reacts with lipid, glucose, nucleic acid and protein which, alternatively called ‘malonaldehyde (Esterbauer *et al*., 1991; Marnett, 2002). It is formed as an end or final product of lipid peroxidation and the content of it are often adopted as an appropriate physiological index to reflect the degree of lipid peroxidation and stress tolerance of plants (Shi *et al*., 1995; Cheng *et al*., 2002 and Jiang *et al*., 2009). The fatty acids lipid peroxidation product Malondialdehyde (MDA) has protective and physiological function as signaling molecule stimulating gene expression and cell survival, but also its cytotoxic role inhibiting gene expression and promoting cell death (Ayala *et al*., 2014). The objectives of this investigation were; identify the molecular characteristic ofthe cyst nematode isolates and metabolic activation detection for potato plants infected with cyst nematode during plant-nematode interactions.

MATERIALS AND METHODS

Cyst nematode isolation and propagation

Approximately, 1.5 kg of sandy clay soil samples infected with cyst nematodes were collected from the soil layers at 20-40 cm depth and then placed in a plastic

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bag and was labelled with date and soil type. These samples were collected from El-Beheira Governorate (El-Nubaria). Cyst nematodes were extracted from the soil using a pair of sieves (250 and 840 mesh size) (Shepherd, 1985) and then stored in eppendorf tubes at room temperature. These cyst nematodes were propagated on the roots potato plants (Spunta cultivar under greenhouse conditions 27° C; 20-22 L: D photoperiod). Subsequently, the propagated cysts were isolated after 10 to 12 weeks' post inoculated the potato plant with the examined nematode according to (Cotton *et al*., 2014).

Nematode identification using specific PCR

Total genomic DNA was extracted from the cyst nematode samples using Genomic DNA extraction kit (iNtRoNBiotechnology, USA). The genomic DNA was subjected to PCR amplification using the universal primers (ITS1:5`-TCCGTAGGTGAACCTGCGG-`3 and ITS4:5`-TCCTCCGCTTATTGATATG`-3) (White *et al*., 1990). The PCR product was loaded into 2% agarose gel, stained with ethidium bromide and separated by electrophoresis in 0.5X TBE buffer at 120 volt for 20 min.

Nematode infection assay for potato plants

Healthy potato tubers of Spunta cultivar were selected, surface sterilized with 75% ethanol for 5 min, followed by 10 min in 2.5% sodium hypochlorite and finally, the tubers were rinsed three times with sterile water. The sterile tubers were cultivated singly in 25 cm plastic pots in (3:1 mixture of autoclaved loam and sand) without fertilizers. Potato plants cultivated at greenhouse under conditions (27° C; 20-22 L: D photoperiod) were inoculated four weeks after planting. The plants were irrigated every two days. Cysts were used as inoculation approximately, 60 cysts of *G. rostochiensis* per pot and pots were irrigated with tap water. Cyst insertion was performed into the soil near to the roots (Andreas *et al*., 1995). The leaves of inoculated potato plants were collected on intervals after the inoculation; 12 h, 24 h, 48 hr, 7d, 14d, 21d, 28d, 35d, 42d and 49 days post infection (dpi).

Detection of metabolic activation in potato leaves infected with cyst nematode

The leaves of healthy control and infected potato plants which collected on intervals after the inoculation; 12 h, 24 h, 48 hr, 7d, 14d, 21d, 28d, 35d, 42d and 49 days post infection were screened using Lipid peroxide MDA (Malondialdehyde) kit (Biodiagnostic Company, Egypt).

RESULTS AND DISCUSSION

Identification of cyst nematode using ITS specific PCR

PCR amplification of the ITS regions of the isolated nematode showed two different amplicons were 238 bp and 370 bp as shown in (Fig. 1). Similar result was performed by PCR using *G. rostochiensis* specific primer and succeeded to identify *G. rostochiensis* ribosomal DNA sequence matching the PCR primer was described by Fleming *et al*., (1998) and Lisnawita *et al*., (2012) while, they found, the PCR amplification product of DNA was 238 bp. At the investigation of Fleming *et al*., (1998), the PCR result amplification product of internal standard template DNA was 748 bp according to their use of potato cyst nematode universal primer and it contradicted our result which was 370 bp because our using of ITS only without universal primer of cyst nematode. According to Fleming *et al*., (1998) they used target artificial internal control template to assess the quantity of target nematode DNA in a sample and thus determine the viability and pest potential of the population. This could allow population levels of cyst nematodes to be measured along with species composition, so that was co-amplify with *G. rostochiensis* DNA.

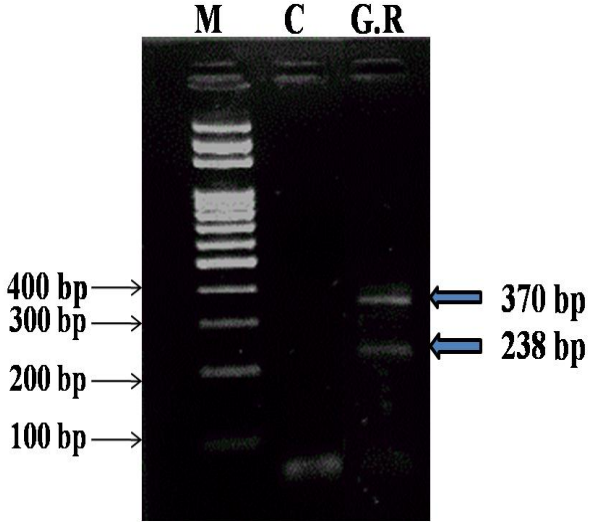

Fig. 1. Amplified PCR products from cyst nematode *G. rostochiensis* generated by the ITS primers; where M: 1.5 kbp DNA marker, lane C (negative control) and lane G.R (*G. rostochiensis*) PCR amplicons were 238 bp and 370 bp

Content of potato oxidative burstas well as enzyme activity by lipid peroxidation determination (MDA contents) in healthy and infected potato with cyst nematodes

Oxidative burst lipid peroxidation (MDA) was determined in shoots of healthy and infected potato plants with *G.rostochiensis*. Results in Figure (2) revealed that, *G.rostochiensis* significantly elevated the contents of shoots lipid peroxidation (MDA) as compared to the untreated healthy plants. Nematodes were increased of MDA in potato shoots during intervals beginning of 12 hr post infection until 49 days after infection as shown in (Figure 2). Virtually, similar results were confirmed that increasing of lipid peroxidation in infected tomato's shoots and roots with root-knot nematode, *Meloidogyne incognita* compared with healthy uninfected plants (Elbeltagi *et al*., 2012).

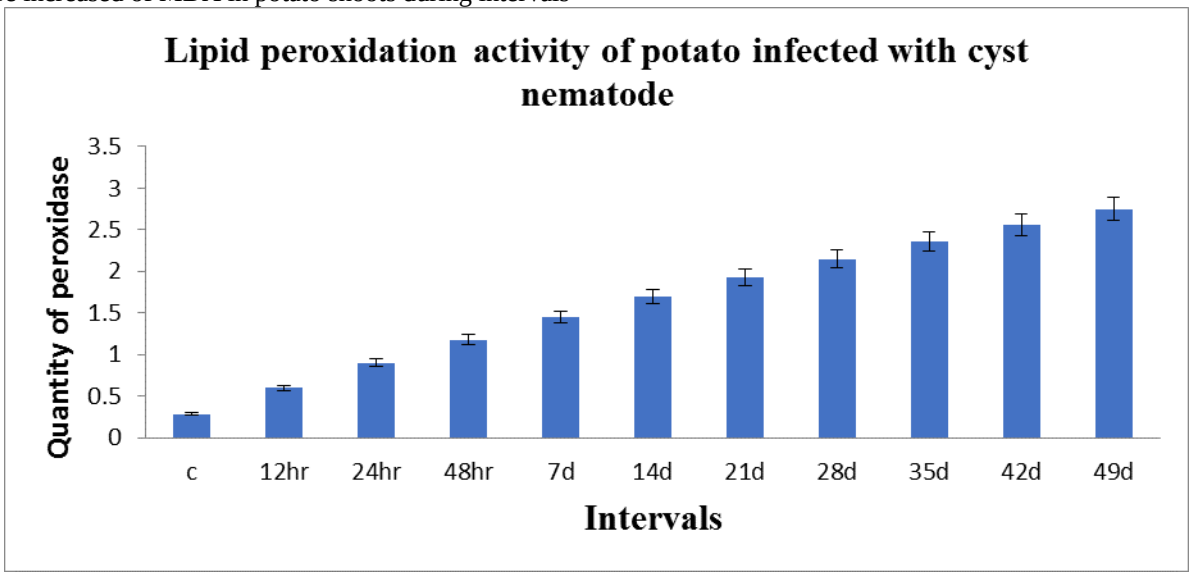

Fig. 2. Determination of lipid peroxidation by malondialdehyde (MDA) assay

CONCLUSION

Current investigation aims to identify the molecular characteristic of the cyst isolates during plant-nematode interactions. To achieve these objectives; soil samples infected with cyst nematodes were collected from El-Beheira Governorate (El-Nubaria) in the present study and some molecular and physiological techniques were applied. The DNA of cyst nematode was identified using specific PCR (polymerase chain reaction) technique with specific ITS primers indicated that, the PCR amplification of *Globodera rostochiensis* showed two different amplicons 238 bp and 370 bp. In addition, the metabolic activation of potato leaves infected with cyst nematodes was detected. The results confirmed that, cyst nematodes (*G. rostochiensis*) significantly elevated the contents of potato shoots lipid peroxidation (MDA) as compared to the untreated healthy plants.

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الملخص العربي

التقدير الجزيئي والفسيولوجي لنيماتودا الحوصلات على البطاطس أثناء التفاعلات بين النيماتودا والنبات

دينا حسن القبرصي ، داليا جميل أصيل ، نادر رجب عبد السلام ، محمد أنور الصعيدي ،سعد شمة و السيد السيد حافظ

الهدف الرئيسي لهذه الدراسة هو تعريف الخصائص الجزيئية لعزلة نيماتودا الحوصلات والتقدير الفسيولوجي لنبات البطاطس المصاب بهذا النوع من النيماتودا أثناء التفاعلات بين النيماتودا والنبات . لتحقيق هذا الغرض تم تجميع عينات تربة مصابة بنيماتودا الحوصلات من محافظة البحيرة (النوبارية) وتم تطبيق بعض التقنيات الجزيئية والفسيولوجية في هذه الدراسة. حيث أوضحت الدراسة وصف وتحديد جينات نيماتودا الحوصلات باستخدام تكنيك PCR (تفاعل البلمرة المتسلسل) المتخصص باستخدام بادئات متخصصة حيث كانت نتائج التفاعل جزيئين مختلفين الوزن الجزيئي للنوع الجلوبوديرا وهما ٢٣٨ زوج من القواعد و ٣٧٠ زوج من القواعد. أيضاً تم تحديد النشاط الميتابوليزمي لأوراق نباتات البطاطس المصابة بالنيماتودا وأكدت النتائج معنوية تأثير النيماتودا حيث ارتفعت محتويات الأوراق من فوق أكسدة اللبيدات (المالوندهايد) مقارنة بالنباتات السليمة الكونترول كنتيجة رد فعل النبات ضد الممرض .