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Comparative Analysis of Antifungal Activity of Total Phenolics from Different Date Palm Cultivars Against Five Phyto Pathogenic Fungi

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Introduction

Pathogen attacks impose natural selection on plants to evolve complex arrays of defensive strategies. Among the diverse defensive mechanisms evolved by plants to withstand pathogen attack, the ability to synthesize an arsenal of low-molecular weight volatile and non-volatile chemicals including phenolics helps them to prepare a robust defense response against pathogen entry. Systemic induction and accumulation of low molecular weight phenolics is observed in response to various diseases and thus are studied as markers for resistance to pathogens. Phenolics that exhibit anti-oxidant activity exert their inhibitory effects on pathogen colonization via protein precipitation and iron depletion.

Phytochemical analysis have been proved that date palm is rich source of phenol. Very little information is available on the inherent Date palm phenolic content that has been involved as resistance factors. All the studies are focused on phenolic content from date palm fruit and its property. Here we focusing on comparative analysis of phenolics from different cultivars leaf and how it affect the different pathogenic fungi.

Material and Methods

We conducted a genome mining analysis of date palm whole genome available in the NCBI site, to detect the presents of enzyme involved in the secondary metabolite pathway. Analyzed the presents of receptor protein specific for the recognition of fungal pathogen.

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Five date palm pathogens were isolated from the diseased date palm and surrounding soil from the date palm field located in northern region of Qatar. Leaf, shoot and root samples collected from the diseased date palm and rhizosphere soil collected from near the diseased date palm. Samples were stored at 40 C in aseptic condition until further use. Sterilized plant samples were plated in the potato dextrose agar (PDA) for the fungal isolation and the soil were plated on molten agar for fungal isolation. The plates were incubated at 250 C until single colony appeared. The isolated fungi were examined under microscope. Based on the microscopic and physical characteristics fungi were identified.

The pathogenicity were determined with detached leaf inoculation analysis and in vivo pathogenicity analysis with three date palm cultivar varieties. Detached leaf inoculation analysis performed in laboratory condition and the in vivo pathogenicity conducted in green house with controlled growth condition. The date palm varieties used in this current study are Khalas, Khneezi and Barhi. All the four pathogens, Fusarium solani, Fusarium oxysporum, Rhizectonia solani Fusarium sp and Ceratocystis radicicola were used for pathogenicity analysis.

Total phenolic were extracted from three date palm culvars through water extraction procedure. Extraction performed with different temperature range. Comparative analysis of antifungal property of total phenolics from different date palm cultivars such as Khalas, Khneezi and Barhi was carried out after optimizing extraction temperature. Antifungal activity is determined with disc diffusion analysis. 100 µl of extract impregnated filter disc (10 mm in diameter) placed on the PDA plate followed by fungal disc placed on the disc. Plates were incubated at 250 C and the fungal growth monitored. Experiment repeated in triplicate along with control.

Results and conclusion

The genome mining analysis of date palm result revealed 45 enzyme sequences from shikimate pathway, which is a support for the active synthesis of phenolic content in date palm. Plant phenolics synthesize via shikimate-phenylpropanoid-flavonoid pathways and include phenolic acids, flavanoids, tannins and less common stilbenes and lignins. Presents of chitin elicitor receptor kinase in date palm indicate the phytopathogenic fungal detection ability of date palm.

From the isolated fungi, the date palm pathogenic fungi were screened and subcultured. Five pathogenic fungi were isolated, Fusarium solani, Fusarium oxysporum, Rhizectonia solani, Fusarium sp and Ceratocystis radicicola. Pathogenicity of all the five isolated fungi were confirmed by analyzing necrosis caused on the date palm leaf (figure 1). The frequency of necrotic lesion and disease susceptibility found more in Khneezi than Khalas and Barhi.

Water extraction procedure conducted at 400 C for 24 hrs were accepted as standardized phenolic extract for antifungal activity. Growths of the fungi were measured after 3 day and 5 days of incubation to determine the antifungal activity of phenolic extract (table 1). Phenolic extract from the Khalas showed more antagonistic activity against Rhizectonia solani whereas phenolic extract from Barhi showed more inhibitory activity against Fusarium solani, Fusarium oxysporum and Ceratocystis radicicola. In all the experiment Khneezi showed week inhibitory activity this supports our previous susceptibility study (not published) in that Khneezi showed more susceptible to C.radicicola. This result is an evident for the disease resistant activity of date palm phenolics.