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Studies on the Chitinase Activity in Coffee (*Coffea Arabica* L.) Genetic Resources in India

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Abstract: Diseases and pests cause considerable crop losses and coffee is not an exception to this. In this context, evolving resistant planting stocks is an important breeding objective and finding germplasm carrying resistance is a very important forerunning exercise. Thus, in an exercise of characterizing coffee germplasm, chitinase enzyme activity in leaf and stem bark tissues of various wild and cultivated Arabica coffees is considered important to identify genetic resources carrying resistance to leaf rust disease and white stem borer pest of coffee. A preliminary study to optimize an efficient assay protocol was undertaken with fifteen accessions of Arabica coffee from Ethiopia. The enzyme activity was generally found to be higher in the bark than in the leaf tissues of all accessions studied. However, there are variations of activity within leaf and bark tissues indicating the possibility of selecting plants with higher enzyme activity associated with resistance to leaf rust and/or white stem borer in marker-assisted breeding against these adversaries.

Key words: Characterization, Chitinase, Descriptors, Enzyme assay, Germplasm, India.

INTRODUCTION

Coffee (*Coffea arabica* L.; family, Rubiaceae) is one of the oldest non-alcoholic stimulant beverages in the World. The mild stimulating property of coffee is attributed to the presence of caffeine, a purine alkaloid in the beverage. In India arabica and robusta are the two types of coffee cultivated on commercial scale in almost equal proportions, which contributes 4.54% of the world production. In India coffee yield and production is influenced by the outbreaks of diseases and pests. Most important adversaries are the fungus, leaf rust (*Hemileia vastatrix* Berk. & Br.) on leaves and the insect, white stem borer (*Xylothrechus quadripes* Chevrolat) on Arabica coffee stems. Control is generally achieved by spraying the fungicides for fungal attack and application of insecticides for pest control. The cost and the negative environmental impact associated with such plant protection measures led to the search for alternative strategies including breeding strategies involving wild genetic resources resistant to these important adversaries. During the course of fungal or insect attack, plants are observed to manifest higher activity of a number of enzymes to actively combat infection or infestation, one of such enzymes is the chitinase [16]. Since chitin is present in the cell walls of fungi and exoskeleton of insect pests, the study of chitinases is considered relevant when analyzing response of resistant coffee plants to the fungal and insect attacks.

Chitinase (EC 3.2.1.14) catalyzes the hydrolysis of chitin, a linear homopolymer of β-1,4-linked N-acetylglucosamine (GlcNAC) residues, and is the second most abundant biopolymer in nature next to cellulose. No substrates for this group of enzymes have been identified in plants, whereas chitin is commonly a component of fungal cell walls and exoskeleton of arthropods that include many important pathogens and pests [9]. Thus, chitinases in plants would have evolved as a mechanism of defense against these adversaries [9,10]. Most of the plant chitinases belong to endochtinase group that randomly hydrolyze chitin polymers.

The work presented in this paper was initiated in order to characterize the germplasm with morphological (unpublished data) and biochemical descriptors and to optimize an efficient protocol to detect the chitinase enzyme activity in coffee plant in order to understand the value of relative chitinase enzyme activity in leaf and bark samples in expressing resistance against the leaf rust disease and white stem borer. These results will eventually serve as baseline data for selecting wild coffee genetic resources in conventional and marker-assisted breeding.

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MATERIALS AND METHODS

Fifteen different coffee germplasm accessions enlisted as S.2440, S.2441, S.2600, S.2601, S.2602, S.2615, S.2604, S.2608, S.2642, S.2644, S.2649, S.2650, S.2707, S.2709 and S.2708 in the Gene Bank planting register of Plant Breeding and Genetics Division, Central Coffee Research Institute (CCRI), Balehonnur, India and growing in the Germplasm blocks of CCRI, constituted the experimental material. The materials S.2440 and S.2441 were collected from Abyssinia during 1955 and the rest were from different geographical locations of Ethiopia collected in the wild during an F.A.O. sponsored expedition to Ethiopia in 1964 for exploration of coffee germplasm and the establishment of World Coffee Collections [14]. Accessions S.2600 and S.2601 represent Harrar province; S.2602 and S.2615 represent Shoa province; S.2604 and S.2608 represent Sidamo province; S.2642 and S.2644 represent Kaffa province; S.2649 and S.2650 represent Illubabor province; S.2707 and S.2709 represent Gojjam province and S.2708 represents Eritrea province. The three cultivated types of Ethiopian arabica coffee *viz.,* Agaro, Cioccie and Tafarikela were included as standards.

Extraction of Chitinase: Fresh leaf samples of the fully expanded 2nd pair of leaves from the primary branches of the coffee plant and bark samples from the main stem about 15 cm above the ground level were collected. The collected samples were immediately wrapped in envelopes and placed in the ice bucket to avoid browning of the tissue. One gram each of leaf and bark samples was homogenized into a fine paste with the help of glass beads in a pre-cooled mortar and pestle with 2 ml sodium acetate buffer (SAB; 0.1 M), pH 5.0. The homogenate was centrifuged for 20 min at 12,000 rpm. Supernatant was used as enzyme source.

Chitinase Activity Determination: Colloidal chitin was prepared as described by Berger and Reynolds [1] and used as the substrate. Assay mixture consisted of 0.4 ml of enzyme extract, 0.4 ml of colloidal chitin. After 2 h of incubation at 37°C, the reaction was stopped by centrifugation. An aliquot of the supernatant (0.5 ml) was pipetted into a glass reagent tube containing 0.1 ml borate buffer (1 M), pH 9.8. The mixture was incubated in boiling water for 3 min and then rapidly cooled on ice. Three ml of *p*-dimethylaminobenzaldehyde (DMAB) reagent was added and the mixture was incubated for 20 min at 37°C. The absorbance was read at 585 nm in a spectrophotometer (Shimadzu, Japan). N-acetylglucosamine (GlcNac) was used as a standard. The specific activity of chitinase enzyme was calculated after estimating the amount of protein present in one gram of the material under study by Lowry's [12] method of protein estimation and expressed as nmol GlcNac min^{-1} mg^{-1} protein. For calculations enzyme control and substrate control were maintained with borate buffer (1 M), pH 9.8 in place of enzyme and substrate respectively in the reaction mixture.

RESULTS AND DISCUSSION

The specific chitinase activity expressed as (n mol min^{-1} mg $^{-1}$ protein) in different accessions of wild genetic resources of arabica coffee are presented in Fig. 2. From the results in Fig.2, it is clear that chitinase activities in leaf sample were generally higher than those of bark. Among the leaf samples, highest chitinase activity was found in the crude extracts of S.2601 and it was lowest in S.2440, S.2642 and S.2708 and this value is closer to that observed in Cioccie, one of the standards chosen for comparison. Among the studied leaf materials of wild coffee genetic resources, chitinase activity of S.2441, S.2644, S.2601 and S.2650 had the enzyme activity range nearer to Agaro and Tafarikela the plant materials chosen as standards for comparison. Enzyme activity of a slightly higher level than that in standards was observed in the samples of S.2608, S.2649 and S.2709. The enzyme activity is notably higher in the leaves of S.2600, S.2601, S.2602, S.2615, S.2604 and S.2607. For bark samples, high enzyme activity was noted for S.2642 and S.2600. Moderately high activity similar to the standards Agaro, Cioccie and Tafarikela was observed in S.2440, S.2441, S.2644 and S.2708. In all the remaining materials studied the enzyme activity is very low. The chitinase activities in the extracts of fresh leaf and bark samples of coffee are presented in Fig 1 for comparisons.

Discussion: Because no substrates are known for chitinase in plant tissues, so far, plant chitinases are believed to play a role in defense against fungal diseases and insect pests whose cell walls are mainly made up of chitin that is the substrate of this enzyme [3,5,7,8,11]. It has been demonstrated that enhanced chitinase activity in transgenic plants can indeed reduce the damage caused by pathogens and pests [6]. The induction of chitinases and other hydrolytic enzymes is perceived to be one of the coordinated, often complex and multifaceted defense mechanisms, which are triggered in response to pathogen attack. Chitinases are synergistically induced during attack by fungal pathogens and also by fungal elicitors. Their induction is generally considered to be part of a non-specific defense response initiated in plants after pathogen attack, but it can also be a consequence of various physical, chemical and environmental stresses [15].

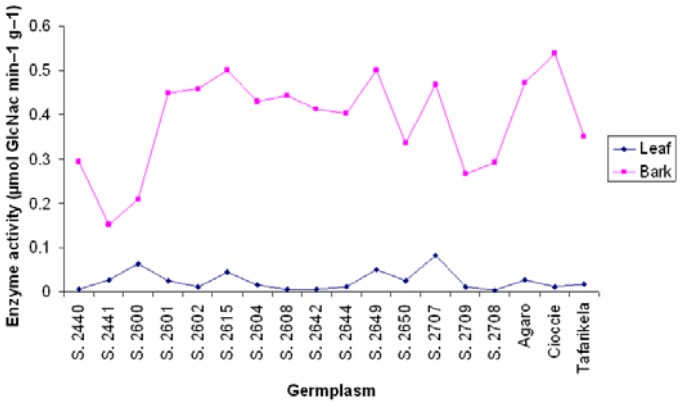

Fig. 1: Enzyme activity in crude extracts of leaf and bark samples of wild accessions of *Coffea*

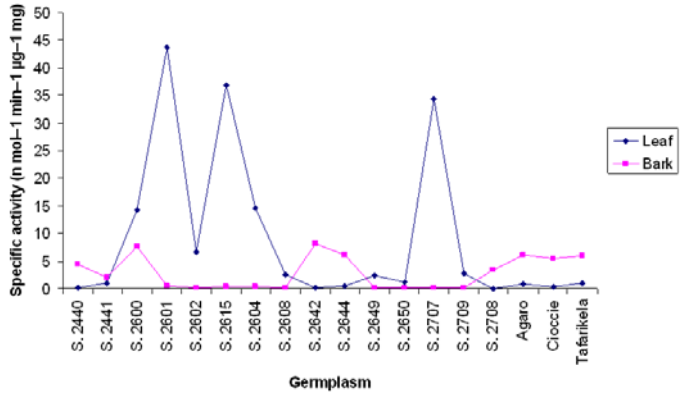

Fig. 2: Specific activity in crude extracts of leaf and bark samples of wild accessions of *Coffea.*

In a screening study for resistance against coffee leaf rust *H. vastartix* in the germplasm collections from Ethiopia, Sreenivasan [17], recorded 100% resistance in S.2604; 79.16% resistance in S.2600; 51.84% resistance in S.2601; 45.8% resistance in S.2642; 40% resistance in S.2650 and 17.4% resistance in S.2644. Maxemiuc-Naccache and associates [13], made a few observations on the early increase of chitinase and glucanase activity in coffee-leaf rust incompatible interactions using crude extracts of coffee leaves. Chitinase activity was reported in different plants and most of the enzymes belong to the group of endochitinases, which randomly hydrolyze chitin polymers. High chitinase activities have already been found to be induced by ethylene or pathogens in several plants including bean [4], pea [18], sweet potato [9] and rubber [2]. Thus, the higher activity observed in the leaves of many accessions and the bark samples in some accessions could be an indicator of resistance.

In summary, our preliminary results regarding chitinase in coffee indicate variability of the activity of this enzyme among the accessions of *C. arabica* and the possibility of selecting plants with higher activity that can be associated with resistance against leaf rust or white stem borer. This will be helpful for further investigations into the possible defense roles and the isozymes present in the coffee genetic resources and subsequently in marker assisted disease and pest resistance breeding.

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