Accumulation of protein in wild relatives of Pigeon pea leaves in response to wounding

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Abstract

Plant defense related proteins are induced during wounding and pathogen attack. These proteins are present in various parts of plant like seeds, fruits, leaves and flower. These proteins are also produced in pigeon pea in response to insect attack or mechanical wounding. Such proteins are responsible for defence system to protect plant from insect pastes. We have studied the induction of proteins in wild relatives of pigeon pea in response to artificial wounding. Seeds of five wild relatives of pigeon pea were sown under normal conditions and allowed to grow for three months. For induction of proteins, leaves were wounded by pointed needle. The protein concentration in leaves with and without wounded was estimated by Lowry method. Out of five wild relatives of pigeon pea, the induction of proteins was observed in three wild species. The maximum level of protein induction was found in leaves of *R. sublobata* (ICP-15868). There was no substantial protein induction found in leaves of *R. bracteata* (ICP-15815) and *C. scarabaeoides* (ICP-15712). This study may be beneficial if further biochemical and molecular characterization is done on the basis of inducible proteins in wild relative of pigeon pea against wounding and its role in the development of insect resistant pigeonpea.

Key words: *C. cyanofolius, C. scarabaeoides, R. bracteata, R. sublobata, R. minima,* Inducible proteins, Pigeonpea

1. Introduction

Pigeonpea (*Cajanus cajan* (L) Millsp.) is a hardy grain legume crop grown in the semi-arid tropics and subtropics by poor farmers in many developing countries. Especially it is cultivated as important source of protein in human diet as dhal and green vegetable to the vegetarian population[1].The genus *Cajanus* belong to 34 species, out of which 17 are found in Australia (15 endemic) and other species found on the Indian subcontinent[2]. The animal feed is prepared by crushing dried seeds of pigeon pea[3]. The loss of this crop is occurred due to attack of insect pests on developing seed[4]. Pigeon pea produces some biologically active components which are involved in defence mechanism against insect paste and microbial attacks. Some of these components are proteases inhibitors, amylase inhibitors, lectins and class of pathogenesis related proteins[5, 6]. Protease inhibitors are accumulated in various parts of plant and they protect to plant from insects by the inhibition of digestive proteinases in larval gut that retard the growth and development of larvae due to impairment of amino acids intake[7]. This type of defences is of two types: constitutive defences and inducible defences. The constitutive defences developed prior to insect attacks and inducible defences are produced when insect attacks[8]. The inducible defence can be produced by local or systemic. In case of local defence the protease inhibitors are accumulated in locally wounded tissue while protease inhibitors are accumulated all over the plant with wounded and without wounded tissue in case of systemic defence[8]. The synthesis of proteins and protease inhibitors after local induction by wounding in wild varieties of pigeon pea is not studied yet. In this study we have evaluated the level of protein concentration in wild varieties of pigeon pea leaves with and without mechanically wounding.

2. Material & Method

2.1 Chemicals and Sample collection

Copper sulphate, NaOH, sodium carbonate was supplied by Loba chemicals. The seeds of wild varieties such as *Cajanus cyanofolius* (ICP-15632), *Cajanus scarabaeoides* (ICP-15712), *Rhynchosia bracteata*

(ICP-15815), *Rynchosia sublobata* (ICP-15868), *Rhynchosia minima* (ICP15838) of pigeon pea were obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India.

2.2 Cultivation of pigeon pea varieties

Seeds of wild relatives of pigeon pea plants were sown on well aerated soil under controlled conditions in the campus of Ahmednagar College, Ahmednagar (M. S.) India. Cultivation of plant was done under net covering so that plants protect from pests. The plants were allowed to grow for about three months till all plants starts flowering.

2.3 Induction of protein in plant leaves

Induction of protein in plant leaves was done by wounding. The leaves were wounded by mechanical action like scratching the surface of leaves by pointed needle at the growing plant. Simultaneously some plant leaves were kept without wounding for control. The leaves of plants (wounded and without wounded) were collected after 96 hrs of wounding and preserved in cold acetone.

2.4 Sample preparation

Acetone from collected leaves was decanted and leaves were crushed by mortar and pestle to make fine pastes. Pigments from pastes were removed with washing of cold acetone and after washing pastes were kept in hexane for overnight so as to remove remaining fat. The hexane was decanted and pastes were allowed to dry completely. After drying fine pastes became fine powders. These powdered samples were soaked in distilled water (1: 10 W/V) and these suspensions were kept at 15^0C for two days. Thereafter, water extracts were centrifuged at 10,000 rpm at 4^0C for 15 min. The supernatants were collected and preserved at 4^0C in refrigerator.

2.5 Estimation of protein

Estimation of proteins in extracts was done by using Lowry's method[9].

3. Result and Discussion:

3.1 Protein content in Pigeon pea varieties

Five wild varieties such as *C. cyanofolius* (ICP-15632), *C. Scarabaeoides* (ICP-15712), *R. bracteata* (ICP-15815), *R. sublobata* (ICP-15868) and *R. minima* (ICP15838) of pigeon pea were used to study of induction of proteins in leaves. The protein was estimated from wounded and without wounded leaves by using Lowry method. Figure 1 shows that there was substantial protein concentration increased in wounded leaves as compared to without wounded leaves of wild relatives of pigeonpea. Out of five pigeon pea varieties, the maximum level of protein concentration was induced in *R. sublobata* leaf (Figure 1). There is no induction of protein was found in *R. bracteata* leaf. Remarkable increase in protein concentration was found in *C. scarabaeoides* leaves. In case of *C. Cyanofolius* the protein concentration was found to 750 and 1100µg/ml in without wounded and with wounded leaves respectively. Similarly, in case of *R. minima* the protein concentration was found to 550 and 950µg/mlin without wounded and with wounded leaves respectively as shown in Figure 1.

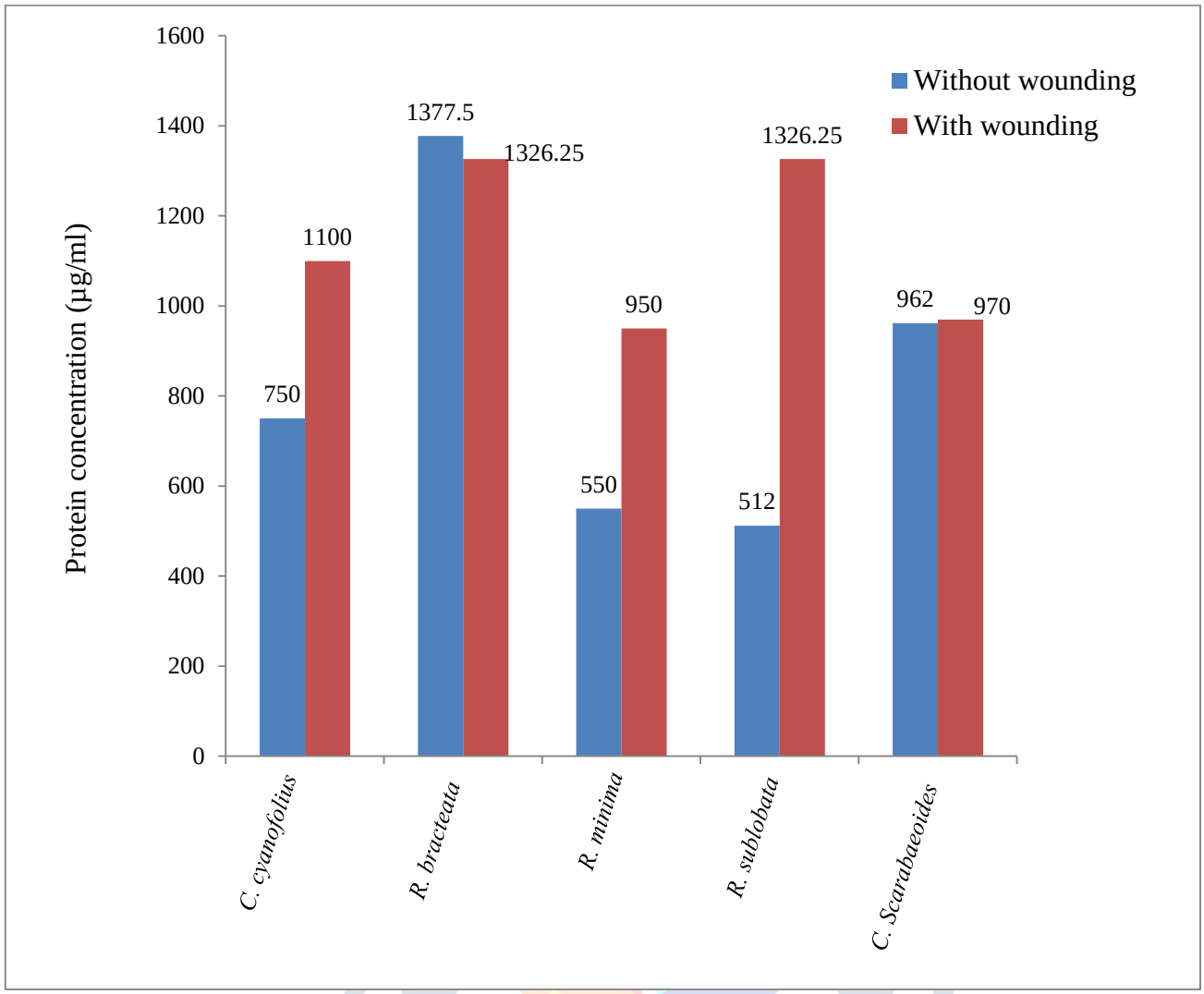

Figure 1: Comparative inducible protein in wild relatives of pigeon pea against wounding

In this study we have analyzed protein concentration in different varieties of pigeon pea with and without wounding. The protein concentration was estimated after 96 hrs of wounding. Increase in protein concentration was observed in three varieties out of five varieties of pigeon pea. It indicates some proteins may be expressed in pigeon pea varieties due to mechanical induction. Defensive proteins may be produced due to mechanical injury in pigeon pea to protect plant against paste. It has been reported that proteinaceous protease inhibitors are induced in leaves of pigeonpea by various mechanical actions such as wounding, insect chewing, fungal pathogenesis and application of salicylic acid[10]. It was possibility that protease inhibitors are induced and accumulated in all varieties of pigeon pea. Tamayo et al[11] have reported the protease inhibitors are expressed and accumulated in maize in response to mechanical wounding and insect feeding.

4. Conclusion

From the result of this study, wild varieties of pigeon pea contribute the response in the synthesis of protein due to mechanical wounding. The response of protein synthesis produced by pigeon pea varieties can be defence mechanism as like protease inhibitors accumulation. This study signifies that, if analysis of induced protein or protease inhibitors is beneficial for development of defence system in pigeon pea.

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