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Full Length Research Paper

Lectin status, protein contents and ammonium assimilating enzymatic activity of two indigenous cultivars of mulberry species, *Morus alba* and *Morus nigra*

Muhammad Zahoor*, Zakia Javaid, Muhammad Ali, Saleem Khan, Farhad Ali and Zahoor A. Swati

Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University Peshawar, Pakistan.

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The studies were carried out with plantlets of two indigenous cultivars of mulberry species *Morus alba* and *Morus nigra* grown in soil less cultures, where extracts from roots, shoots, leaves, and reproductive organs were determined for protein content, agglutination of human erythrocytes and the activity of the ammonium assimilatory enzyme glutamine synthetase. *M. nigra* and *M. alba* extracts contained potent phytoagglutinins in various tissues with highest contents in *M. nigra*. The leaves and roots of both species of mulberry were used to determine the glutamine synthetase activity and high level of activity was found in both tissues. The glutamine synthetase enzymatic activity was higher in roots (1.37 µmol.gfw^{-1}.min^{-1}). The extract from all tissues of both species were used to determine the total protein contents and proteins found higher in the leaves (2.129 µg/gfw in *M. nigra* and 0.973 µg/gfw in *M. alba*).

Key words: Lectins, GS Assay, ammonium assimilation, mulberry species, phytoagglutanins.

INTRODUCTION

Lectins are non-immunoglobulin-type carbohydrate recognition molecules that are involved in haemagglutination, lymphocyte transformation, inactivation of certain types of tumor cells and precipitation of certain polysaccharides and glycoproteins (Lis and Sharon, 1986; Goldstein and Hayes, 1978). These multivalent cell-agglutinating proteins are highly specific in binding with their carbohydrates moieties and due to this binding specificity; they are being used increasingly to probe the structure of carbohydrates on the surfaces of normal and malignant cells (Liener et al., 1986). Lectins attract many researchers for their unique biological activities and have been isolated from different plants species like *Phaseolus vulgaris* seeds (Itoh et al., 1980), *Viscum album* L (Franz et al., 1981), *Lathyrus sativus* seeds (Kolberg and Sletten, 1982) and *Vicia unijuga* leaves (Yanagi et al., 1990). Lectins have also been isolated from mulberry seed (Yeasmin et al., 2001).

Mulberry has key role in sericulture industry and its leaves are the only source of nutrition for silkworm. The growth and development of the larvae and subsequent cocoon production is much influenced by the nutritive value of mulberry leaves (Anonymous, 1975; Krishnaswami, 1978). It is reported that 70% of silk produced by silkworm is directly derived from proteins of mulberry leaves (Narayanan et al., 1967; Krishnaswami et al., 1970; Petkov and Dona, 1979; Fukuda et al., 1959).

Glutamine synthetase (GS) is the key enzyme involved in the assimilation of inorganic nitrogen in higher plants. It work together with glutamine-oxoglutarate aminotransferase (GOGAT); GS/GOGAT pair provide primary port of entry for nitrogen in whole plant metabolism (Lea and Miflin, 1974; Keys et al., 1978; Miflin and Lea, 1980; Stewart et al., 1980). Inorganic nitrogen, in the form of ammonia, is assimilated via this glutamate synthetase cycle into the organic nitrogen compounds glutamine and

*Corresponding author. E-mail: mzkhaans@yahoo.com.

Table 1. Agglutination of erythrocytes by extracts from different organs of *Morus alba* and *Morus nigra*.

Plant part	Blood Group A		Blood Group B		Blood Group AB		Blood Group O	
	M. alba	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*
Leaves	+	+++++	++++	+++++	++++	++++	+++++	+++++
Reproductive	+++++	+++++	-	+++++	+++	++++	+	++
Shoot	+++++	++++	-	+++++	+++	+++++	+++++	+++++
Root	+++++	+++++	+++++	+++++	+++	++++	++++	+++++

- = Nil, + = satisfactory, ++ = fair, +++ = good, ++++ = very good, and +++++ = excellent.

glutamate further used in amino acids, nucleic acids, and chlorophyll syntheses (Sechley et al., 1992). In addition to its major role in primary nitrogen assimilation, the GS/GOGAT cycle also plays a crucial role in reassimilating the large amount of ammonia released during photorespiration (Somerville and Ogren, 1980; Kendall et al., 1986). The present study was aimed to determine the lectins status, total protein contents and glutamine synthetase activity in the different tissues of mulberry two indigenous cultivars.

MATERIALS AND METHODS

Lectin extraction

2 g of roots, shoots, leaves and fruits from plantlets, grown in soil less culture were finely ground and stirred with 20 ml of 0.15 M NaCl. Suspensions were maintained for at least 3 h at room temperature or stored at 4°C for 1-2 days and then centrifuged at 10,000 g for 30 min at 10°C. The clear supernatants were used to determine the haemagglutinating activity.

Erythrocyte agglutination

The assay was carried out in glass test tubes. The clarified extract was serially diluted with 0.15 M NaCl and each dilution was tested for erythrocyte agglutination by mixing the extract 1:1 with a 2% erythrocyte suspension. The extent of hemagglutination was monitored visually after the tubes were allowed to stand at room temperature for 30 min. The results were recorded as haemagglutination titer (the reciprocal of the highest dilution given visible haemagglutination).

Detecting lectin molecules in protein fractions

A blood supply facility was established to obtain erythrocytes from humans for testing hemagglutinating activities in the isolated protein fractions. Hemagglutination of erythrocytes was carried out with human erythrocytes of different blood groups to test the potency of extracted lectins.

Protein content determination

Protein in the soluble fraction was determined by colorimetric method using Futura System Kit containing pyrogallol as the dye. The readings were obtained at 600 nm on a Smart Spec Plus UV-Visible spectrophotometer using bovine serum as standard.

Enzyme extraction

Plant material was homogenized in a mortal and pestle with 2 volumes of an extraction buffer containing 50 mM Tris-HCl, pH 7.6, 5 mM sodium Glu, 10 mM $MgSO_4$, 1 mM dithiothreitol, 600 mM sorbitol 4 and 1% glycine betain. The homogenates were centrifuged at 10,000 g for 10 min. The extracts were assayed for glutamine synthetase activity by the transferase assay.

Glutamine synthetase assay

The reaction is started in a 2 mL reaction mixture with 100 mM Tris-acetate buffer, pH 6.4, containing 100 mM glutamine, 30 mM hydroxylamine, 30 mM sodium arsenate, 1.5 mM $MnCl_2$, 0.2 mM ADP and 200 µl enzyme extract. The reaction is stopped after 30 min by the addition of 1 ml of 3-3% (w/v) FeCl, 8% (w/v) TCA in 2 N HCl and the glutamine synthetase activity is measured spectrophotometrically at 540 nm by comparing with blank as standard.

RESULTS

Agglutination of human erythrocytes as an indicator of lectin presence

Phytoagglutination of human erythrocytes with extracts from different organs of *Morus alba* (White mulberry) and *Morus nigra* (Black mulberry) means the presence of lectin protein that is presented in Tables 1 and 2. The extract of *M. alba* different tissues showed a strong ability to coagulate human blood, and the strength of their reaction was dependent on the plant organ and the type of blood group tested. In the leaf extract, the strongest reaction was observed with blood group O and with some decline in reaction with blood group B and AB. Agglutination with blood group A was found positive. The reproductive tissues extract showed the most potent reaction with blood group A and a strong reaction with blood group AB. A detectable reaction was observed with blood group O while reproductive tissues did not show any reaction with blood group B. The reaction of shoot extract was very strong with blood groups A and O with a decline in reaction with blood AB. In the root extract, the strength of the coagulating reaction was highest with blood groups A and B and positive coagulation was found in reaction with blood group AB and O (Table 1).

In *M. nigra*, extracts of all tissues reacted strongly with the four blood groups (Table 1). Its leaf extract agglutina-

Table 2. Agglutination by *M. alba* and *M. nigra* extracts following x10 dilution.

Plant part	Blood Group A		Blood Group B		Blood Group AB		Blood Group O	
	M. alba	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*
Leaves	-	+++	++	+++	++	+	++	++
Reproductive	+++	+++	-	++	-	+	-	-
Shoot	++	+	-	+++	-	++	+++	+++
Root	+++	+++	++	++	++	+	++	+++

- = Nil, + = satisfactory, ++ = fair, +++ = good, ++++ = very good, and +++++ = excellent.

Table 3. Protein content (µg/gfw) of different organs in mulberry species.

Plant part	*Morus nigra*	*Morus alba*
Leaves	2.129	0.973
Reproductive	0.436	0.707
Shoot	1.249	0.222
Root	1.560	0.942

Table 4. Glutamine synthetase activity in the leaf and root extracts of mulberry species.

Cultivar	Plant organ	GS (µmol.gfw^{-1}.min-^{1})
Morus alba	Leaves	0.464
Morus alba	Roots	1.164
Morus nigra	Leaves	1.007
Morus nigra	Roots	1.37

tion reaction was most potent with blood groups A, B and O. Its reproductive organs reaction with blood groups A and B were particularly strong, and its relatively weaker reaction with blood group O was clearly visible without any ambiguity. The agglutination reactions of the shoot and root extracts of *M. nigra* were almost equally strong with all four blood groups (Table 1).

The potency of the agglutinating activities in *M. alba* can be seen in the data presented in Table 2, where following a 10 fold dilution of extracts, a very strong reaction with blood group O was detected in the shoot extract. In addition, strong reactions of leaf extract with blood group B and AB, reproductive organ extract with blood group O and A, and root extract with blood groups A, B, AB, and O were observed (Table 2).

In *M. nigra* all the agglutination activities were detectable in the original plant extracts, and following a 10 fold dilution with 150 mM NaCl the activities were higher in leaves extract with blood group A and B. The reaction was found strong with Blood group A in reproductive organs and blood group B and O in shoots extract. Root extract react with blood group A and O in sufficient amount (Table 2).

Protein content

Table 3 shows the protein content profile of different organs of the two mulberry species expressed as µg/gfw. These variations in the bulk proteins of various tissues are related to the differences in water content, the fibrous nature of the tissue as well as the distribution of storage proteins and soluble protein fractions.

The total protein contents were found highest as 2.129 and 0.973 µg/gfw in the leaves of *M. nigra* and *M. alba*, respectively. While reproductive organs of *M. nigra* and shoots of *M. alba* shows the lowest figure.

Glutamine synthetase assay

Table 4 shows the levels of glutamine synthetase activity in the leaf and root extracts of *M. alba* and *M. nigra*. Both species exhibited the presence of a highly active glutamine synthetase enzyme in their roots and shoots, and the activity of this ammonium assimilating enzyme was higher in roots (Table 4).

DISCUSSION

Lectins were detected in two indigenous cultivars of mulberry through haemagglutination test with different blood groups. The amount of lectins could be predicted by the visual grading of agglutination. The haemagglutination test is an established protocol for the detection of lectins (Lin et al., 1981; Yeasmin et al., 2001). Lectins have been reported in mulberry species before and isolated as well (Yeasmin et al., 2001). High level potent lectins were also detected through present study in local cultivars of mulberry species. As per haemagglutination, *M. nigra* is highly enriched in diverse form of lectins in different tissues. In *M. alba*, some tissues posses highly active lectins with considerable amount while the extract of some tissue reveal that it contain somewhat less amount by showing weaker reaction.

Both white mulberry and black mulberry species possess highly active glutamine synthetase enzyme in their root and leaf tissues. Glutamine synthetase is the key enzyme of nitrogen assimilation, playing an exclusive

role in ammonium incorporation for amino acid biosynthesis, and thus its activity is directly implicated in amino acid pool for protein synthesis, which in turn would be associated with the synthesis of plant lectins.

Conclusion

M. nigra and *M. alba* extracts contained potent phytoagglutinins in various tissues with highest contents in *M. nigra*. These highly potent lectins could be isolated and characterized according to their molecular weight, carbohydrates moieties, toxicity and antimicrobial activity. The ammonium assimilating enzyme activity was higher in roots (1.37 µmol.gfw-1.min-1), directly involved in amino acids synthesis through nitrogen fixation and correlating with lectins content. The total protein contents correlated with the degree of haemaglutination activity in the case of both the species which in turn indicates the presence of lectins as per protein contents in indigenous mulberry species.

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