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Technical Note

Biosynthesis of Lectins in Developing Seeds of Common Bean

ABSTRACT

Two cultivars of Phaseolus vulgaris *L., flor de mayo FM-RMC, showing resistance to common mosaic virus and to bean rust (*Uromyces phaseoli*), and FM-C, without such resistance, were used to study the synthesis of lectins during seed development as measured by affinity chromatography of protein fractions. Seeds at different stages of development were sorted out by size from 4–12 mm length, in addition to mature samples. The specific haemagglutinating activity (SHA) of isolated protein fractions from mature seeds, expressed as haemagglutinating units/mg protein, and the theoretical agglutination capacity (TAC), calculated from the SHA and the total protein fraction, were also assessed. The peak lectin level as measured by affinity chromatography was reached at a seed size of 10 mm and decreased thereafter. The lectin content and TAC of all samples of the disease-resistant cultivar were superior to that of the non-resistant variety.*

INTRODUCTION

The problems associated with common bean consumption are well known, particularly those related to the presence of some antinutritional factors and the production of gastrointestinal distress. Raw beans may be toxic and the principal toxicants are lectins or phytohaemagglutinin proteins (Jaffé, 1979; Pusztai *et al.*, 1979). This antinutritional aspect of lectins may be eliminated by moist heat treatment applied during cooking (Grant *et al.*, 1982; Kadam *et al.*, 1987). The seed lectins represent up to 11% of the storage proteins (Pusztai & Watt, 1974; Liener, 1976; Etzler, 1985; Osborn *et al.*, 1985). Lectins are carbohydrate-binding proteins with haemagglutinating and

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mitogenic properties that occur in many plant species, especially *Leguminosae* (Liener, 1976; Felsted *et al.*, 1981). It is worth noting that the physiological role of lectins in plants is still unknown. It has been postulated that they might be involved in sugar transport and storage, in the binding of the nitrogen-fixing bacteria to the root nodules of leguminous plants, in facilitating seed germination and in the resistance mechanisms to plant pathogens (Goldstein, 1981).

There is a lack of information on the biosynthetic patterns and deposition of lectins during seed development. Also, to our knowledge, no investigations have been performed concerning the postulated protective role of lectins against plant pathogens. This preliminary study was undertaken to investigate the biosynthesis of lectins during bean seed formation and to compare the seed lectin levels present in a normal bean variety with another genetically improved cultivar resistant to some plant pathogens. Biosynthesis is referred to here as the net result of synthesis and breakdown.

MATERIALS AND METHODS

Bean samples

The cultivars used for this study (*Phaseolus vulgaris* L., flor de mayo) were sown in plots at the experimental farm of CIAB–INIFAP (Celaya, Guanajuato, Mexico) during the spring of 1987. The normal variety flor de mayo (FM-C) without resistance to the common mosaic virus and to the bean rust *Uromyces phaseoli* was genetically improved to the newly derived cultivar flor de mayo (FM-RMC) with resistance to the above pathogens (Paredes-López *et al.*, 1989). Green pods were sampled during seed formation starting 15 days after flowering. Mature seeds were collected 45 days after flowering. The seeds were extracted from the pods with the aid of a scalpel, sorted out by size and freeze-dried. The predominant seed size at each sampling time after flowering was (in mm/day) 4/15, 6/16, 8/23, 10/28, 12/34 and mature seed/45. From now on immature samples will be designated by their average seed size. The green beans and mature samples were ground with a Udy cyclone mill (Udy Corp., Fort Collins, CO, USA) into a flour capable of passing a 100-US mesh sieve. The flours were stored in tightly covered containers at $-40°C$.

Protein content and extraction

The total crude protein content in the flour samples ($n \times 6{\cdot}35$, this indicates a factor of 6·25 is used to convert % nitrogen into total crude protein) was determined according to a microKjeldahl procedure (AACC, 1984).

The phosphate-buffered saline (PBS; 0·136M NaCl–2·7mM KCl–8·1mM Na_2HPO_4–1·5mM KH_2PO_4) -soluble protein was extracted from the seed flour as described previously (Paredes-López *et al.*, 1988*a*). The homogenate was magnetically stirred for 16 h at 4°C and centrifuged at 40 000*g* for 15 min at 4°C. An aliquot of the supernatant containing 100–200 μg of protein, by microKjeldahl, was made up to 1 ml with the addition of de-ionised water and 0·2 ml of 72% trichloroacetic acid (TCA) (Peterson, 1977; Naczk *et al.*, 1985). The mixture was thoroughly shaken, centrifuged at 3000*g* for 15 min and the supernatant discarded. The TCA treatment was repeated and the protein precipitate was resuspended with 1 ml of 0·1M NaOH, and used to determine protein content by the Lowry *et al.* (1951) procedure with crystalline bovine serum albumin as standard (Sigma Chemical Co., St Louis, MO, USA). The PBS-soluble protein was also extracted for affinity chromatography studies. In order to protect the column, the tannins were removed from the bean flour by adding 1·5 g polyvinylpyrolidone/g protein (Sievwright & Shipe, 1986). The rest of the extraction was performed as described above.

Haemagglutinating activity

The specific haemagglutinating activity (SHA), expressed as haemagglutinin units/mg protein, was determined according to Felsted *et al.* (1981). Aliquots of samples containing from 20 to 1600 μg of protein were increased to 200 μl with PBS and then the agglutination procedure was followed as described elsewhere (Paredes-López *et al.*, 1988a). The theoretical agglutination capacity (TAC) was calculated from the SHA and the total amount of protein fraction present in the seed flour and was expressed as haemagglutinin units $\times 10^3$/100 g dry flour.

Affinity chromatography

The cyanogen bromide activation of Sepharose 4B (Pharmacia, Uppsala, Sweden) was carried out according to March *et al.* (1974). The activated Sepharose was coupled to porcine thyroglobulin (Sigma Chemical Co.), as described by Felsted *et al.* (1975). The Sepharose–thyroglobulin was stored at 4°C in PBS with 0·02% sodium azide. Aliquots containing about 20 mg of PBS-extracted protein were added to 10 ml Sepharose–thyroglobulin, the suspension gently mixed for 2 h and then poured into a 2·5 × 30 cm column (Felsted *et al.*, 1975). The column was washed with PBS at a flow rate of 2 ml/min until the absorbance (280 nm) of the effluent was below 0·02. Elution of lectins was accomplished with 0·05M glycine containing 0·5M NaCl (pH 3). The appropriate fractions were TCA-precipitated and the protein content determined by Lowry *et al.* (1951).

RESULTS AND DISCUSSION

It is interesting to note the progressive decrease observed in microKjeldahl nitrogen content during seed formation of the bean varieties FM-C and FM-RMC as is shown in Fig. 1. There was also a gradual increase in PBS-soluble/TCA-insoluble protein as determined by the Lowry *et al.* (1951) method in seeds longer than 6 mm. At harvest time this protein represented 64% and 77% of the total crude protein from FM-C and FM-RMC, respectively.

The lectins isolated by affinity chromatography from the PBS-extracted proteins appear in Table 1. At the initial stages, 4–6 mm, no lectin could be isolated by this technique. For both varieties the maximum bound lectin (in g/100 g of PBS-soluble proteins) was reached at 10 mm length and declined thereafter. In other words, at the last stages of seed development more nonlectin proteins were synthesised. The lectin content, expressed as g/100 g of dry flour, increased throughout seed formation for the two cultivars. Osborn *et al.* (1985), using the same chromatographic technique, recovered similar amounts of lectin from various *Phaseolus vulgaris* L. cultivars in a mature stage. The FM-RMC had a significantly ($P < 0{\cdot}05$) higher content of lectins and TAC in mature seeds compared to the FM-C variety. In other words, the disease-resistant cultivar exhibited, in a mature stage, a higher lectin content and a higher theoretical agglutination capacity than that without resistance.

In summary, it was observed that the high nonprotein nitrogen evolved into 'true' protein content at progressive stages of seed maturation. The peak lectin level, isolated by affinity chromatography, was reached at the seed

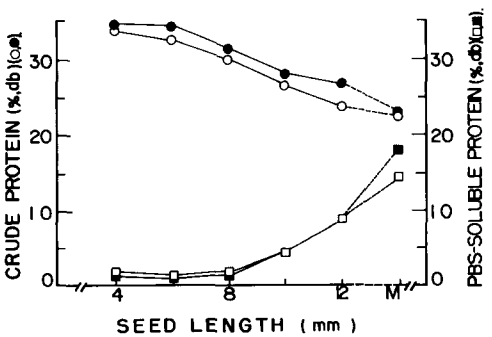

Fig. 1. Changes in the crude protein content ($n \times 6{\cdot}25$) and PBS-soluble protein during seed maturation. The PBS-extracted protein was determined by the Lowry procedure. Least significant difference (0·05): crude protein = 0·9; PBS-soluble protein = 0·8. M = Mature bean samples. ○, □, Cultivar without resistance to the common mosaic virus and to the bean rust (FM-N). ●, ■, Cultivar with resistance to the common mosaic virus and to the bean rust (FM-RMC).

TABLE 1
Lectins Isolated by Affinity Chromatography during Seed Maturation[a]

Seed length (mm)	*FM-C*				*FM-RMC*			
	Lectin content[b]	*Lectin content*[c]	*SHA*[d]	*TAC*[e]	*Lectin content*[b]	*Lectin content*[c]	*SHA*[d]	*TAC*[e]
8	$3{\cdot}9 \pm 0{\cdot}2^{b}$	0·07	—	—	$3{\cdot}3 \pm 0{\cdot}5^{c}$	0·05	—	—
10	$4{\cdot}6 \pm 0{\cdot}3^{a}$	0·20	—	—	$4{\cdot}1 \pm 0{\cdot}1^{b}$	0·18	—	—
12	$2{\cdot}6 \pm 0{\cdot}2^{d}$	0·23	—	—	$3{\cdot}1 \pm 0{\cdot}3^{c}$	0·28	—	—
Mature sample	$2{\cdot}7 \pm 0{\cdot}1^{d}$	0·39	695 ± 53	271	$3{\cdot}2 \pm 0{\cdot}1^{c}$	0·57	543 ± 40	309

[a] Lectins were not detected in earlier stage (4 and 6 mm).
[b] Lectin content expressed in g protein/100 g of protein extracted by PBS. Means of three determinations ± standard deviation by Lowry procedure. Results with the same letter are not significantly different by Duncan's multiple range test ($P < 0{\cdot}05$).
[c] Lectin content expressed in g protein/100 g dry flour. It was calculated from lectin content of 100 g of PBS-soluble protein.
[d] Specific haemagglutinin activity (SHA) values, expressed in haemagglutinin units/mg protein, are means of two determinations ± mean difference.
[e] Theoretical agglutination capacity (TAC) is expressed in haemagglutinin units × 10^3/100 g dry flour.

length of 10 mm. The TAC of the mature seeds of the FM-RMC cultivar was higher than that of the corresponding samples of the FM-C variety. Further studies are required to determine if there is a relationship between agglutination capacity of bean cultivars and resistance to pathogens.

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