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The changes of protein patterns during one week of germination of some legume seeds and roots

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This study was carried out in order to evaluate some Egyptian legume seeds (*Vicia faba, Cicer arietinum* and *Lupinus termes*) as raw and germinated foods, as sources of plant proteins. The work was extended to study the changes of the seed and root proteins during 7 days of germination. The results are summarised as follows: (1) The proteins of ungerminated seeds were resolved on SDS–PAGE into a number of bands and their molecular weights were determined: chick-peas, 19 (8–78 kDa); faba beans, 20 (11–96 kDa) and lupins 19 (14–86 kDa). (2) The changes in the seed and root proteins during 7 days of germination of the *V. faba, C. arietinum and L. termes* showed the following:

- The number of seed bands of *C. arietinum, V. faba* and *L. termes* at the first day were 19 (12–89 kDa), 25 (12–98 kDa) and 16 (12–98 kDa); they decreased up to the seventh day of germination to 12, 22 and 9 bands, respectively. The number of root bands were 13 (13·5–89 kDa), 23 (12–98 kDa) and 19 (13–96 kDa), respectively.
- The number of seed stable (constant) proteins during the 7 days of germination were 8, 4 and 0 proteins for *C. arietinum, V. faba* and *L. termes*, respectively. The roots contained 4, 3 and 1 proteins, respectively.
- *V. faba* and *L. termes* were the predominant seeds, containing high-molecular-weight reserve proteins which degraded and disappeared within the first 3 days of germination to give smaller peptides or amino acids. New synthesised proteins were found and this was especially marked in *L. termes* seeds.
- It was noticed that all three seeds contained newly synthesised proteins, mainly up to the third day of germination and the numbers of these proteins were found to be 4, 4 and 3 proteins for *C. arietinum, V. faba* and *L. termes*, respectively. In roots there were 5, 4 and 0 proteins, respectively.

INTRODUCTION

In Egypt, it is common to germinate some legume seeds which are rich in protein (20–50%) such as termes (*Lupinus termes*), broad bean (*Vicia faba*) and chick-pea (*Cicer arietinum*) before direct eating, cooking or use in a salad dressing. Germination improves the nutritional value of the proteins which are hydrolysed into easily assimilable polypeptides and essential amino acids, and decreases trypsin inhibitors. Sprouted chick-pea, lupin and broad beans, therefore, are better sources of easily assimilable protein and vitamin C than ungerminated seeds (Waris *et al.*, 1977) .

Protein content of the legume seeds can be categorised in order of increasing protein content. First, *C. arietinum* is close to 22%; second, *V. faba* is 25%, and third, *L. albus*, is 30% (Arora, 1982). The albumin proteins (AP) are minor and the globulin proteins (GP) are major proteins of legume seeds. Unlike the GP, which are storage proteins, the AP are mostly enzymic or non-storage proteins (Bhatty, 1982). AP of cotyledons were degraded during germination and thus behaved like the GP or storage proteins (Murray, 1979).

The protein breakdown was observed at 72 h of germination in all the legumes (Kumar & Venkataraman, 1975).

On SDS–PAGE, degradation was visible at earlier germination stages of faba beans, indicating hidden endoproteolytic cleavages (Boulter, 1970; Lichtenfeld *et al.*, 1979). After germination (for 3 days) of faba bean seeds, some bands disappeared and new ones appeared (Youssef *et al.*, 1987). In chick-peas the number of fast-moving components increased (Kumar & Venkataraman, 1975). At day 6, maximum degradation of protein occurred and the newly formed bands may represent

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the degradation product of the high-molecular-weight proteins (Kumar & Venkataraman, 1978). The protein reserves of cotyledons were depleted by 50% in 7 days of germination (Azhar *et al.*, 1972). Evidently, protein components of lower molecular weight were degraded later than those of higher molecular weight during seed germination (Konopska-Waliszkiewicz, 1988). The band intensities varied among the species; all species had a heavily stained protein band mostly in the middle of the gels (Bhatty, 1982).

This investigation was conducted to study the effect of germination throughout 7 days for *L. termes, V. faba* and *C. arietinum* on the changes of protein content and pattern distribution.

MATERIALS AND METHODS

Source of samples

Three different types of legume seeds (*Vicia faba*, Giza 402; *Lupinus termes*, Giza 2 and *Cicer arietinum*, Giza 2) were obtained from the Field Crops Research Institute, Agriculture Research Centre, Giza, Egypt.

Germination

The three types of legume seeds were first washed with distilled water, then surface-sterilised by washing for 60 s in 0·01% (w/v) sodium azide solution. Seeds were thinly spread on moistened cotton towels (0·5 cm thick) and daily sprayed with distilled water in plastic dishes (30 × 40 cm) for all samples. Germination was carried out at 25°C in an aired and dark incubator. Germinated samples, cut seeds and primary roots, were frozen and kept at –20°C.

Extraction of proteins

Seeds were first defatted and protein was extracted according to the method of Stegemann *et al.* (1987).

Fractionation of protein

Protein was fractionated by its solubility in $(NH_4)_2SO_4$ according to the method of Stegemann *et al.* (1987), and the 50% fraction was used. The concentration of the protein was measured by UV spectrophotometry at 280 nm.

SDS–PAGE

Proteins of sample extracts were identified by plotting on 12% SDS–PAGE according to the method of Laemmli (1970).

Preparation of protein marker

Low-molecular-weight (LMW) protein marker mixture (SERVA) was dissolved in the sample buffer and heated in the same manner as the extract samples according to the method of Laemmli (1970).

Molecular weight estimation

The protein migration (in cm) was plotted against the protein markers (LMW) to obtain standard curves of molecular weight, and the molecular weights of sample proteins were estimated.

RESULTS AND DISCUSSION

This experiment was set up to examine the effect of germination on proteins using SDS–PAGE to follow the changes in protein patterns during 7 days of germination in seeds and roots of the investigated legume seeds.

The seed and root proteins of *V. faba, L. termes* and *C. arietinum* were extracted through 7 days of germination, and fractionated by ammonium sulphate, the 50% ammonium sulphate fraction was subjected to 12% SDS–PAGE and stained with Coomassie blue. The results showed that ungerminated chick-pea contained 19 bands (12–89 kDa), faba bean 20 bands (14–98kDa) and *L. termes* 16 bands (95–12 kDa); as shown in Fig. 4. lane 0 (see below), these results are in line with those reported by Bhatty (1982).

During germination the results showed that *C. arietinum* seeds contained 19 protein bands ranging from 12 to 89 kDa (Fig. 1). *V. faba* seeds contained 25 bands ranging from 12 to 98 kDa (Fig. 2) and *L. termes* seeds contained about 16 bands ranging from 12 to 98 kDa as shown in Fig. 3 at the first day of germination. The roots contained 15 bands from 13·5 to 89 kDa, 23 bands from 12 to 98 kDa and 19 bands from 13 to 96 kDa for *C. arietinum, V. faba* and *L. termes*, respectively.

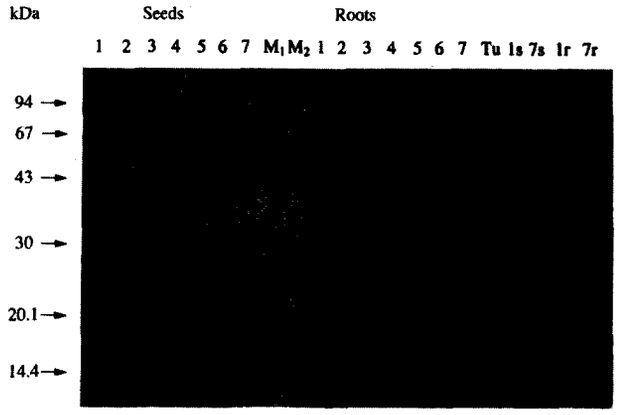

Fig. 1. SDS–PAGE — changes in the protein patterns during 7 days of germination in seeds and primary roots of *Cicer arietinum*. 1–7 seeds — seeds from 1 to 7 days of germination; 1–7 roots — roots from 1 to 7 days of germination; M_1 — LMW protein marker in kDa: phosphorylase b, 94·0; albumin, 67·0; ovalbumin, 43·0; carbonic anhydrase, 30·0; trypsin inhibitor (soya bean), 20·1; α-lactalbumin, 14·4. M_2 — P_5 protein marker in kDa: trypsin inhibitor (lung), 6·5; cytochrome c, 12·5; trypsin inhibitor (soya bean), 21·0; carbonic anhydrase 29·0 Tu — elongation factor has a molecular weight of 43·0 kDa and isolated from frog oocyte (used as a marker). 1s and 7s — first and seventh day of germination of seeds; 1r and 7r — first and seventh day of germination of roots.

At the seventh day of germination the number of bands was drastically decreased to 12, 22 and 9 bands for the seeds; and 7, 17 and 7 bands for the roots of *C. arietinum*, *V. faba* and *L. termes*, respectively, (Figs 1–3) as a result of proteolytic degradation during germination.

The number of constant proteins did not change in amount (concentration) or mobility on the gel during the germination period. The seeds contained eight proteins (64, 55, 27, 24, 20·1, 16, 13·5 and 12 kDa), four proteins (54, 34, 21 and 19 Kda) and nil, respectively. Also, the roots contained four constant proteins (80, 72, 42 and 16 kDa), three proteins (36, 21 and 19 kDa) and only one protein (19 kDa) for *C. arietinum*, *V. faba* and *L. termes*, respectively.

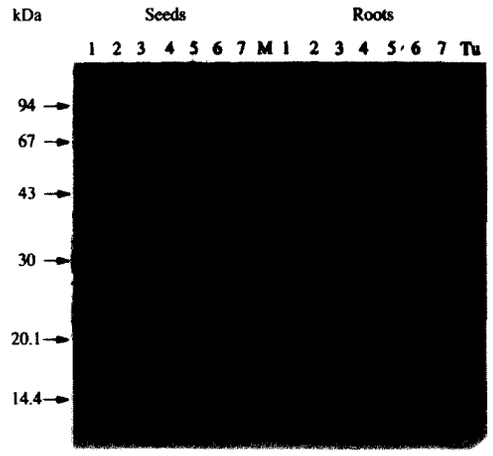

Fig. 2. SDS–PAGE — changes in the protein patterns during 7 days of germination in seeds and primary roots of *Vicia faba* 1–7 seeds — seeds from 1 to 7 days of germination; 1–7 roots — roots from 1 to 7 days of germination; M — LMW protein marker in kDa: phosphorylase b, 94·0; albumin, 67·0; ovalbumin, 43·0; carbonic anhydrase, 30·0; trypsin inhibitor (soya bean), 20·1; α-lactalbumin, 14·4. Tu — elongation factor has a molecular weight of 43·0 kDa and extracted from frog oocyte (used as a marker).

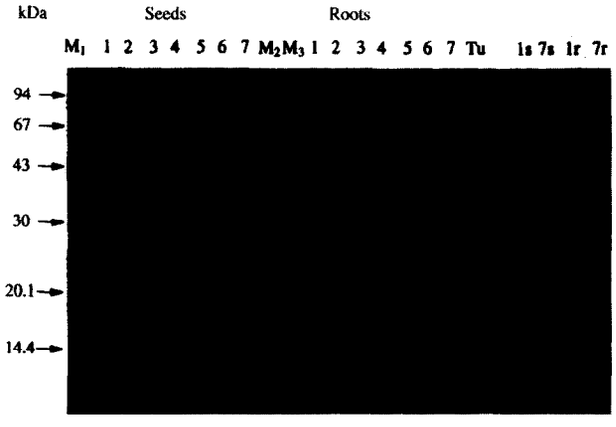

Fig. 3. SDS–PAGE — changes in the protein patterns during 7 days of germination in seeds and primary roots of *Lupinus termes*. 1–7 seeds — seeds from 1 to 7 days of germination; 1–7 roots — roots from 1 to 7 days of germination; M_2 — LMW protein marker in kDa: M_1, P_4 marker; phosphorylase b, 94·0; albumin, 67·0; ovalbumin, 43·0; carbonic anhydrase, 30·0; trypsin inhibitor (soya bean), 20·1; α-lactalbumin, 14·4. M_3 — P_5 protein marker in kDa; trypsin inhibitor (lung), 6·5; cytochrome c, 12·5; trypsin inhibitor (soya bean), 21·0; carboanhydrase, 29·0. Tu — elongation factor has a molecular weight of 43·0 kDa and isolated from frog oocyte (used as a marker). 1s and 7s — first and seventh day of germination of seeds; 1r and 7r — first and seventh day of germination of roots.

Our results are supported by other investigators who have found a change in reserve protein or its subunits of seeds caused by germination (Azhar *et al.*, 1972; Kumar & Venkataraman, 1975, 1978).

The protein synthesis elongation factor Tu (43 kDa) was also noticed, only in *V. faba* seeds (as constant protein) and roots (it decreased gradually up to the sixth day then disappeared at the seventh day). It was found in the root of *L. termes* only at the first day of germination in high concentration and in very low concentration at the second day, indicating that probably most of the protein synthesis occurred the first day of germination. Our results are in accordance with those found by Lichtenfeld *et al.* (1979), Kumar and Venkataraman (1978) and Youssef *et al.* (1987).

It was also noticed that seeds and roots contain some gradually decreasing protein bands during the germination period, where *C. arietinum* had only one band for seeds and one for roots with molecular weights of 86 and 27 kDa, respectively. *Vicia faba* seeds contained six proteins (98, 92, 72, 64, 60 and 12 kDa), while roots were found to contain five proteins (98, 92, 72, 17 and 14·4 kDa). On the other hand, no detectable proteins in the seeds and only two proteins in the roots (32, 15 kDa) of *L. termes*.

CONCLUSION

A comparison between the changes in the proteins during the 7 days germination of *Vicia faba, Cicer arietinum* and *Lupinus termes* seeds is presented in Figs 4, 5(a) and 5(b) and is summarised as follows.

(1) The number of bands of *C. arietinum, V. faba* and *L. termes* at the first day were 19, 25 and 16, and decreased up to the seventh day of germination to be 12, 22 and 9 bands, respectively.

(2) The number of stable (constant) proteins during the 7 days of germination were 8, 4 and 0 proteins for *C. arietinum, V. faba* and *L. termes*, respectively.

(3) The number of gradually decreasing proteins from the first day to the seventh day of germination were 1, 6 and 0 for *C. arietinum, V. faba* and *L. termes*, respectively.

(4) The gradually increasing proteins from the first day to the seventh day were found only in *L. termes* (four proteins), and this change was not observed in the other two seeds.

(5) *Vicia faba* and *L. termes* were the seeds containing most high-molecular-weight reserve proteins which degraded and disappeared within the first 3 days of germination to give smaller peptides or amino acids as well as newly synthesised proteins; this was especially marked in *L. termes* seeds.

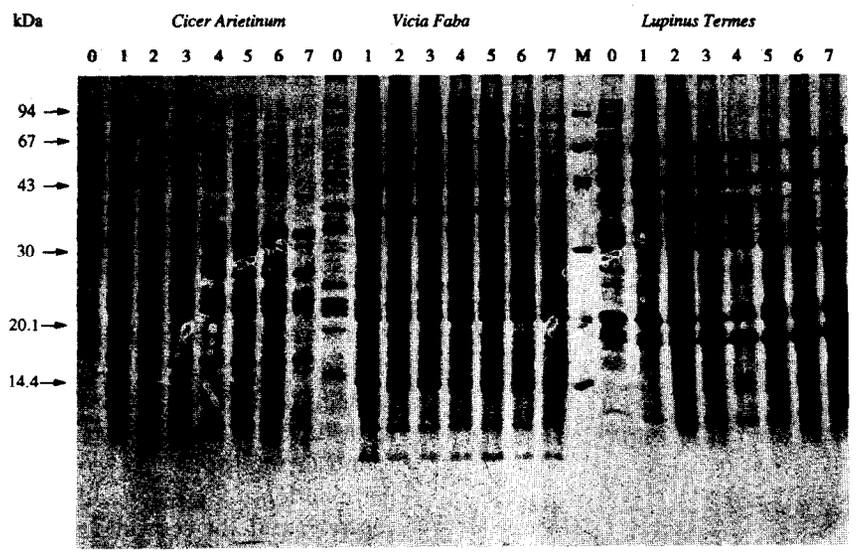

Fig. 4. Comparison between the total extracted protein from 0 to 7 days of germination for the three investigated seeds on SDS–PAGE 12%.

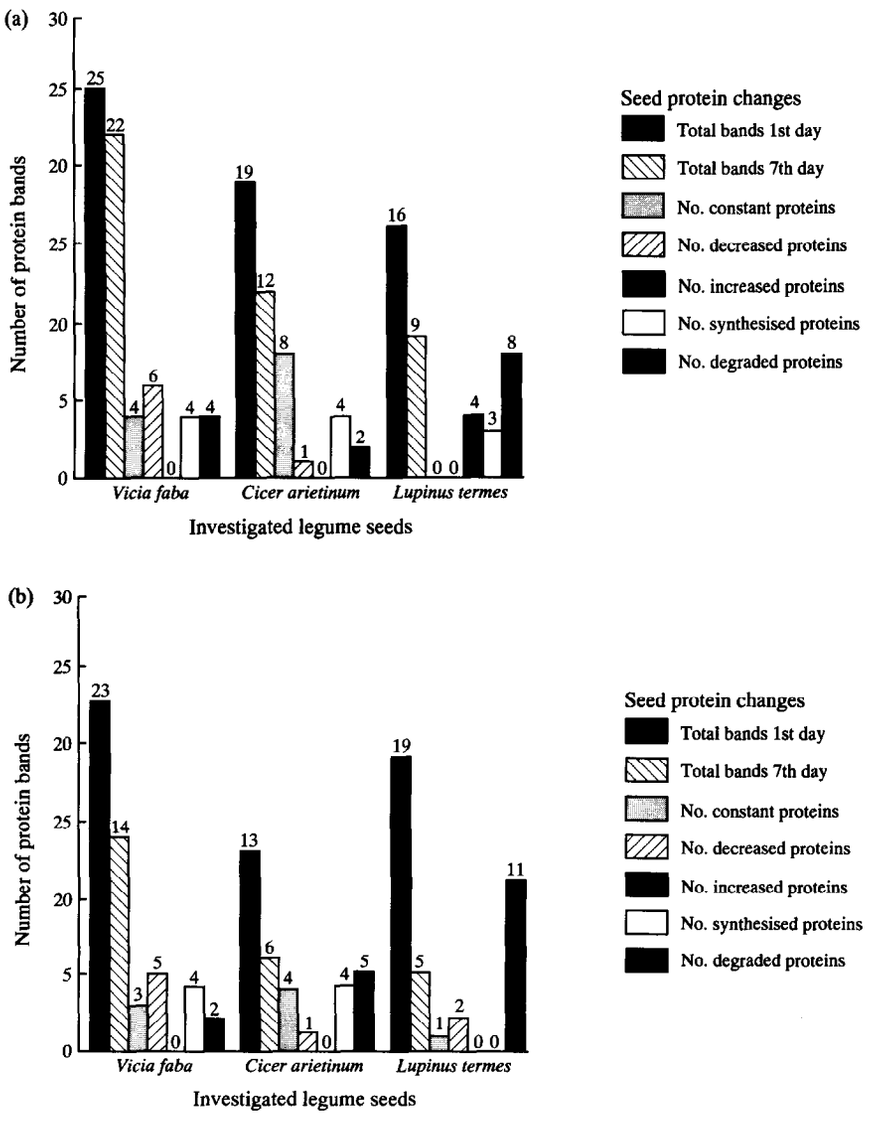

Fig. 5. Comparison between the changes in the protein patterns during 7 days of germination of (a) the investigated seeds; and (b) the investigated roots.

(6) It was noticed that all three seeds contained newly synthesised proteins mainly up to the third day of germination; the numbers of these proteins were found to be 4, 4 and 3 proteins for *C. arietinum, V. faba* and *L. termes* , respectively.

(7) Degradation of some proteins indicates that these proteins are reserve proteins, and appearance of some other proteins on specific days during germination indicates that these proteins might be enzymes or subunits of enzymes needed at this stage of germination. On the other hand, the constant proteins might also be enzymes or proteins which build the structure of the cells, while the gradually decreasing proteins indicate that these proteins are sources for amino acids required to build up other proteins or enzymes during the germination time.

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