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ELECTROPHORETIC PATTERNS OF PROTEINS, ISOLATED FROM SOYBEAN SEEDS GROWN UNDER CONDITIONS OF SOME MINERAL DEFICIENCY AND AFTER DIFFERENT PERIODS OF STORAGE

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Summary. In an earlier paper we showed that depending on seed yield, the variants of mineral deficiency studied could be set in order as follows: control (100%), Fe-deficiency (100%), deficiency of N, S and K (30–40%), B and complete mineral deficiency (0–1%). This study demonstrates that N, K, B and S deprivation causes alterations in all cases of mineral deficiencies where the yield is reduced – the quantitative changes in protein and polypeptide spectra are established. The degree of these changes does not correlate with the degree of yield reduction. The most drastic are the changes in electrophoretic spectra of S-deficient seeds. On the other hand, both N and K deficiencies provoke similar yield reduction whereas the changes in the protein spectra are not so significant. On the contrary, no differences between the protein spectra of the B-deficient and the control seeds are observed. Electrophoretic patterns of complete mineral deficiency seeds are similar to those of S-deficient seeds. Based on these data the following conclusions are made: 1) the production of small number of seeds not in all cases overcomes the adverse effect of mineral deficiency on seed quality, 2) the degree of changes in protein spectra of seeds depends mainly on the type of mineral element under deficiency, 3) in sulfur-deficient seeds the effect of natural aging on polypeptide spectrum is additive to the effect of S-deficiency itself.

Key words: electrophoretic spectra, mineral deficiency, seed storage, soybean seeds

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Introduction

Studies on plant responses to mineral nutrition are important because appropriate nutrition is essential for proper growth of plants. Changes in nutritional conditions cause a wide variety of morphological, physiological and biochemical responses. On the molecular level these responses are manifested as changes in the pattern of gene expression (Fujiwara et al., 1992; Wei et al., 1998; Leustek and Saito, 1999; Kim et al., 1999; Fabre and Planchon, 2000). The mineral deficiency during seed development usually influences seed yield and only under conditions of severe stress the seed composition is affected (Harrington, 1960; Austin, 1972; Randall et al., 1979). The manner by which the mineral deficiency affects the seed characteristics depends on the type of mineral element and its concentration in the medium (Marschner, 1986). Under limited sulfur availability, plants maintain overall levels of seed storage protein, accumulating more storage proteins with low content of sulfur-containing amino acids and less of those with high content (Rahman et al., 1983; Higgins, 1984; Fujiwara et al., 1992; Kim et al., 1999; Fabre and Planchon, 2000). On the other hand, the effect of a given mineral element deficiency depends on plant species. In wheat seeds sulfur deficiency affects the rate of protein biosynthesis but in pea seeds the protein breakdown rate changes (Castle and Randall, 1987). The degree of disturbances in protein biosynthesis rate and the type of protein fractions influenced depend also on plant species (Randall et al., 1979; Castle and Randall, 1987). It has been established that the lack of sulfur in the nutrient medium causes a significant decrease in legumine levels in pea seeds whereas in wheat seeds the gluten level decreases (Randall et al., 1979; Castle and Randall, 1987). A deficient supply of one element usually produces correlative changes in the concentration of other elements, in spite of their presence in the nutrient medium at normal concentration (Harrington, 1960; Benedycka et al., 1996; Sunarpi and Anderson, 1997; Blake-Kalff et al., 1998; Kim et al., 1999). This fact impedes the interpretation of the data concerning the effect of deficient supply of a single mineral element on the composition of seeds and their viability. Many studies on seed viability loss have been reported (Roberts, 1972; Bewley and Black, 1978) but less information is available about the relationship between adverse conditions during seed development, seed protein quality and susceptibility to alterations in the course of seed storage (Welch, 1986).

The purpose of this investigation was to study the electrophoretic patterns of proteins, isolated from soybean seeds, formed under mineral deficiency conditions and stored for different periods.

Materials and Methods

Investigations were carried out with dry mature soybean (*Glycine maximum* L., cv. Beason) seeds, produced by plants, grown as a hydroponic culture in the glasshouse

on the modified nutrient solution of Hogland and Arnon with addition of microelements from A to Z (Tyankova et al., 1978). The variants of mineral deficiency were as follows: N, Fe, B, S, K and complete mineral deficiency (deionized water). Seeds, produced by plants grown on a complete nutrient medium were used as a control. All seeds were inoculated with *Bradyrhizobium japonicum* strain 273 before sowing. The plants showed the typical symptoms characteristic of the respective deficits until the 20th day from the beginning of vegetation. During this period, plants grown under Fe-deficiency conditions showed the symptoms of severe chlorosis and for this reason they were supplied with 1/4 dose of Fe.

Seeds were analysed after storage from 1 to 5 years. Freshly harvested seeds were used as a control in this case.

Soluble proteins were extracted with 0.01 M phosphate buffer, pH 7.4 (seed material:buffer 1:12 w/v). Protein content of the extracts was determined by the method of Lowry et al. (1951). Native proteins were separated electrophoretically in 7.5% PAAG according to the method of Davis (1964). Polypeptides were resolved by electrophoresis of proteins under denaturing (SDS) and reducing (β-mercaptoethanol) conditions in 12.5% PAAG by the method of Laemmli (1970). Quantitative differences between protein spectra were evaluated by the intensity of staining of bands and qualitative differences were estimated by the number and Rm (relative mobility) values of protein bands. Electrophoretic patterns were scanned densitometrically.

Results and Discussion

The comparison of protein electrophoretic patterns of the investigated seeds allowed us to identify the individual effects of applied mineral deficiencies, the longevity of seed storage and the complex effect of both factors on the composition of seed proteins. Significant changes in seed protein patterns were observed only after 3 years of seed storage. Data presented in the figures refer to 5–3 years of seed storage. For control seeds, the whole investigation period is presented.

Twenty four protein bands with Rm values from 0.03 to 0.97 appeared in the electrophoretic patterns of proteins, isolated from control soybean seeds (Fig. 1). Storage longevity of control seeds from 1 to 5 years did not influence significantly the type of protein spectra. The ratio between intensities of staining of different bands of control seeds including all investigated years of storage remained constant. The amount of proteins was highest in bands No 2, 5, 7, 9 and 19 with Rm values 0.04, 0.14, 0.22, 0.30 and 0.75, respectively. The quantity of protein in bands No 16 and 24 (Rm values – 0.60 and 0.97) was lower.

The electrophoretic patterns of proteins, isolated from seeds, produced under conditions of N- (Fig. 2), B- (Fig. 3), Fe- (Fig. 4) and K-deficiency (Fig. 5) were similar in number and Rm values to those of control seeds. Moreover, the protein pat-

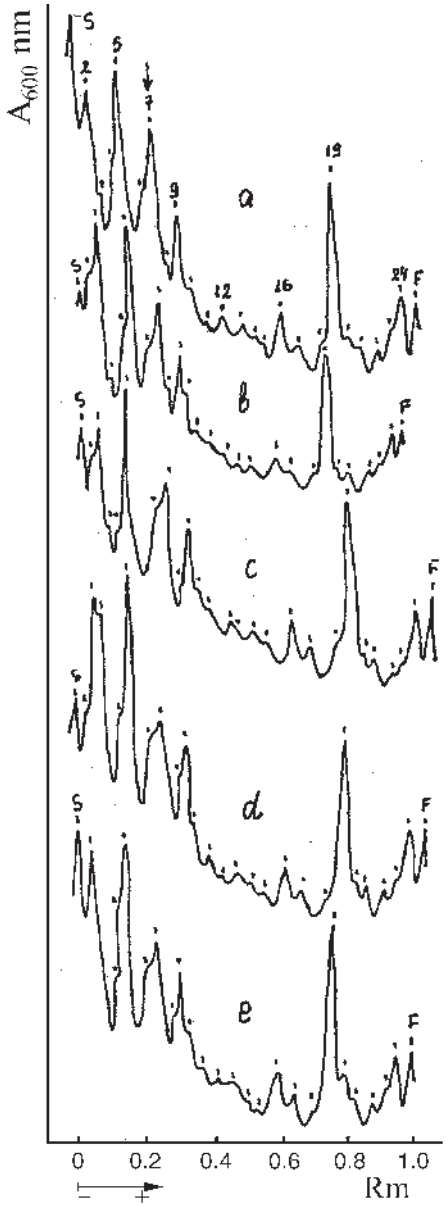

Fig. 1. Densitometric scans of protein spectra (native PAGE) of control soybean seeds, produced by plants, grown on complete mineral nutrient medium. a – 5 years of storage; b – 4 years of storage; c – 3 years of storage; d – 2 years of storage; e – 1 year of storage. Band number 1–24. The arrows indicate the position of bands, underwent quantitative changes.

terns of seeds, produced by N- and Fe-deficient plants were similar in intensity of staining of protein bands to those of control seeds. These results were in contrast with the results of Suzuki et al. (1998). They reported that Fe-deficiency stress specifically induced an appearance of several new proteins in barley roots. The lack of effect observed in our study might be due to the fact that the deficiency applied was only partial. In the case of Fe-deficiency the supply of plants with 1/4 dose of Fe added to the nutrient solution probably allowed plants to recover by the end

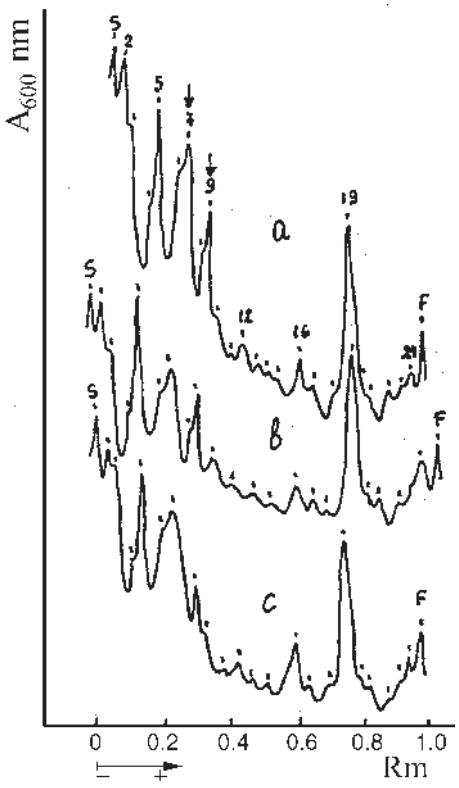

Fig. 2. Densitometric scans of protein spectra (native PAGE) of soybean seeds, produced by plants, grown under N-deficiency conditions. Symbols as in Fig. 1.

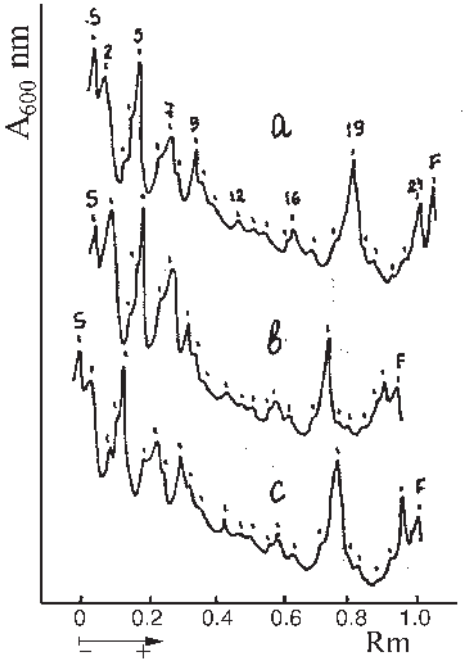

Fig. 3. Densitometric scans of protein spectra (native PAGE) of soybean seeds, produced by plants, grown under B-deficiency conditions. Symbols as in Fig. 1.

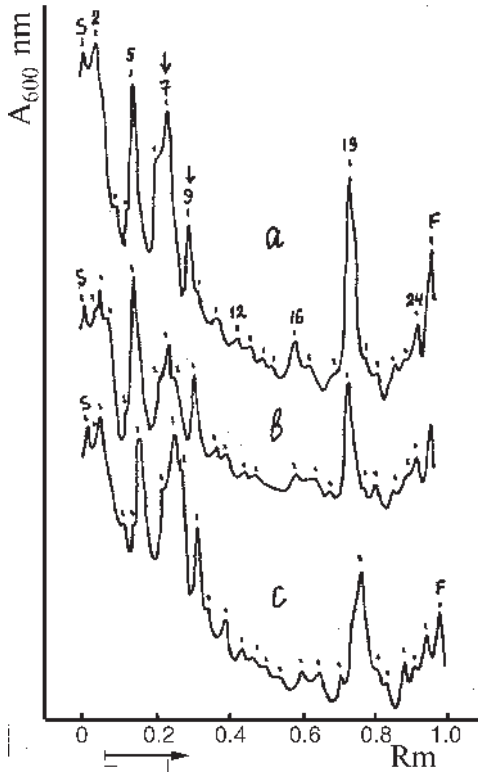

Fig. 4. Densitometric scans of protein spectra (native PAGE) of soybean seeds, produced by plants, grown under Fe-deficiency conditions. Symbols as in Fig. 1.

of the vegetation period. In the case of N-deficiency, the seeds were inoculated with *Bradyrhizobium japonicum* strain 273 prior to sowing, so that a part of the required N might be supplied by nitrogen fixation. The intensities of staining of protein bands from the electrophoretic patterns of B-, K-, S-deficiency (Fig. 6) as well as complete mineral deficiency (Fig. 7) variants were lower. These results indicate that probably the synthesis of the major soybean seed proteins is somehow affected during seed formation. Leigh and Wyn Jones (1984) and Walker et al. (1998) have proposed that a decline in cytosolic K^+ below the optimum level leads to protein synthesis reduction and this is the initial cause of growth reduction under K deprivation.

In our previous study we have established that the viability of all investigated seed lots, assayed immediately after their harvest is normal – above 90% (Tyankova et al., 1994). The viability of seeds produced by plants grown under deficiency declined more rapidly during storage periods as compared to control plants. A more significant decline was observed in K-, S- and complete deficiency variants. In spite of

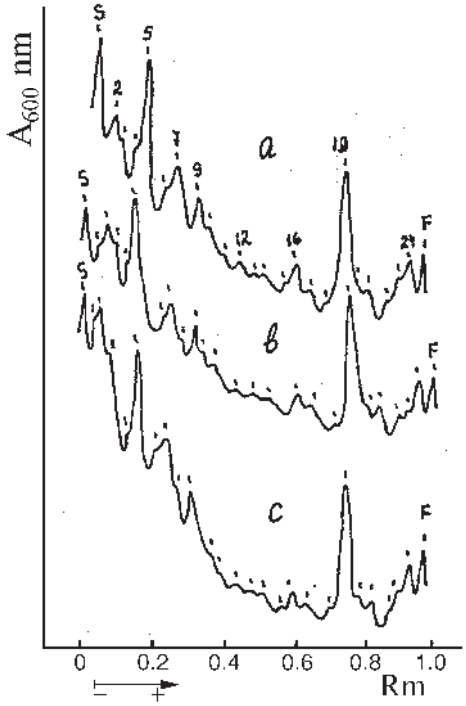

Fig. 5. Densitometric scans of protein spectra (native PAGE) of soybean seeds, produced by plants, grown under K-deficiency conditions. Symbols as in Fig. 1.

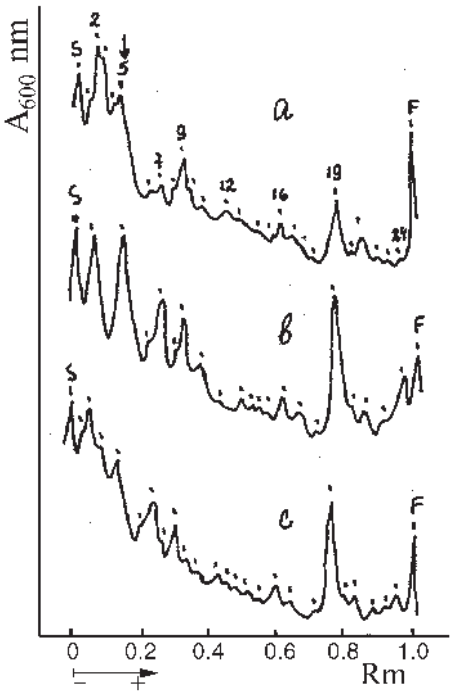

Fig. 6. Densitometric scans of protein spectra (native PAGE) of soybean seeds, produced by plants, grown under S-deficiency conditions. Symbols as in Fig. 1.

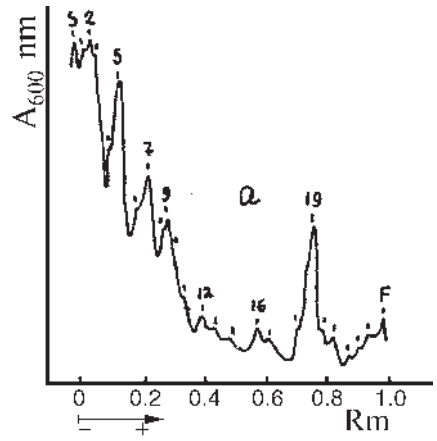

Fig. 7. Densitometric scans of protein spectra (native PAGE) of soybean seeds, produced by plants, grown under complete mineral deficiency conditions. Symbols as in Fig. 1.

viability deviations, no changes in protein patterns due to the natural aging of K-deficient seeds, stored for 5 years (Fig. 5), were observed. In general, the electrophoretic profile of the proteins from S-deficient seeds (Fig. 6) was similar to the profile of proteins, isolated from seeds of the other variants in number (24) and Rm values of protein bands, but it differed in protein amount assessed by the intensity of staining. This difference could be explained by the changes of protein set synthesized during seed development or by partial protein degradation. It is known that in a number of plant species sulfur nutrition affects the composition of seed

storage proteins. Generally, the levels of sulfur-poor proteins are elevated under conditions of limited sulfur supply, whereas the accumulation of sulfur-rich proteins is reduced (Kim et al., 1999). Moreover, remobilization of S from leaf proteins does not take place under conditions of S starvation unless N is also deficient (Sunarpi and Anderson, 1997). Soybean seed storage protein glycinin, which consists of approximately 50% of total seed proteins, is relatively rich in sulfur-containing amino acids and β-conglycinin is relatively poor in these amino acids. Under sulfur deficiency, more β-conglycinin accumulates than glycinins. Subunit composition of β-conglycinin is also affected, and there is a threefold increase in the accumulation of the β-subunit protein (which has an especially low sulfur content), whereas the other two subunits are little affected (Fujiwara et al., 1992). Our results showed that the intensity of staining of the fast moving band No 19 lowered specifically only in S-deficient seeds. The quantity of this band decreased most significantly in seeds, stored for 5 years, and characterized also by the lowest degree of viability. The S-deficiency conditions did not influence the qualitative composition of the spectrum. A significant decrease of viability to 64% was also established in seeds, produced under conditions of Fe-deficiency and stored for 5 years, however, no alterations in protein bands were observed (Fig. 4). These results showed that amongst all deficiency variants investigated, only in S-deficient seeds an individual protein band changed quantitatively. This fact could be explained by the complex effect of both S-deficiency and natural aging of seeds.

In order to characterize more precisely soybean seed proteins SDS-PAGE was used. In the polypeptide spectrum of control seeds 28 bands were registered (Fig. 8). This number of polypeptides coincides with the number, reported by Savoy (1977) for soybean seeds. The bulk of polypeptides were localized in the areas of slow (No 5, 6, 7), medium (No 13) and fast (No 20 and 21) moving groups. Most probably, these dominating polypeptides are similar to the fractions with MM of 80, 36 and 18.5 kDa, estab-

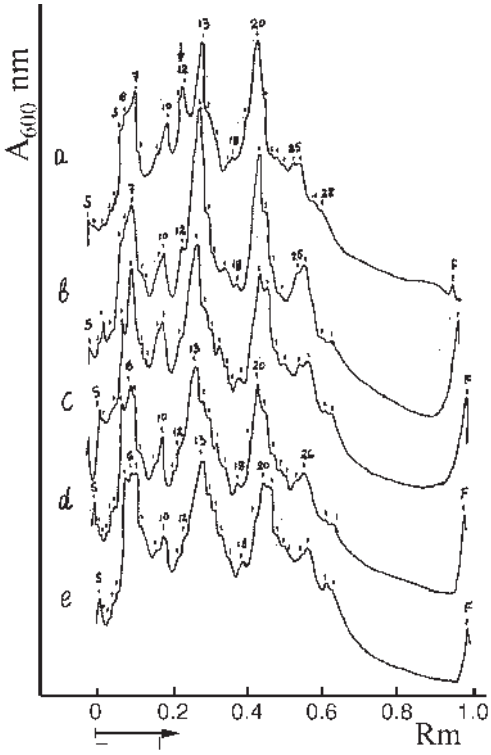

Fig. 8. Densitometric scans of polypeptide spectra (SDS-PAGE) of control soybean seeds, produced by plants, grown on complete mineral nutrient medium. Band number 1–28. The arrows indicate the position of bands, underwent quantitative changes.

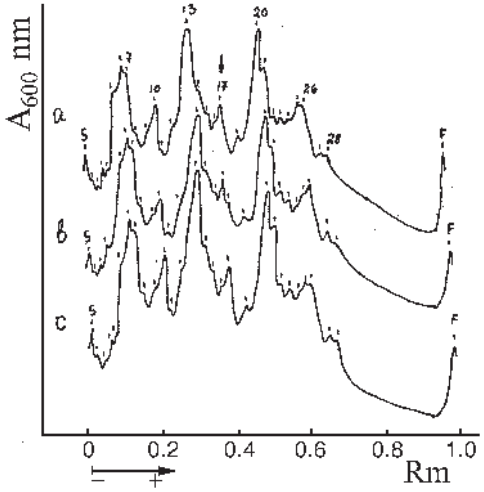

Fig. 9. Densitometric scans of polypeptide spectra (SDS-PAGE) of soybean seeds, produced by plants, grown under N-deficiency conditions. Symbols as in Fig. 8.

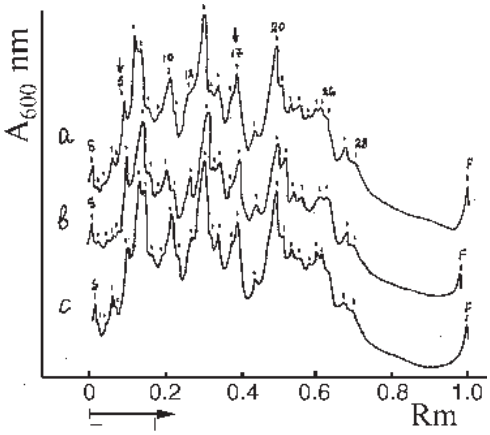

Fig. 10. Densitometric scans of polypeptide spectra (SDS-PAGE) of soybean seeds, produced by plants, grown under B-deficiency conditions. Symbols as in Fig. 8.

lished by Lassocinsky and Knypl (1978) using SDS-PAGE. No changes in the polypeptide spectra of control seeds after 2 and 3 years of storage were observed. A slight enhancement in the quantity of band No 12 was established after 4 years of storage. This trend was more pronounced after 5 years of storage but in these seeds the quantity of band No 13 decreased. Similar electrophoretic picture was obtained for Fe-deficient seeds (Fig. 14). In the rest variants specific changes in the polypeptide spectra were

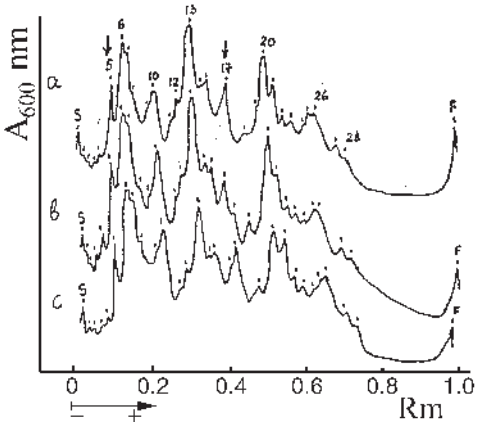

Fig. 11. Densitometric scans of polypeptide spectra (SDS-PAGE) of soybean seeds, produced by plants, grown under K-deficiency conditions. Symbols as in Fig. 8.

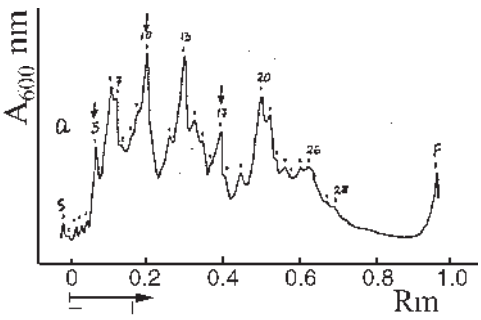

Fig. 12. Densitometric scans of polypeptide spectra (SDS-PAGE) of soybean seeds, produced by plants, grown under S-deficiency condition. Symbols as in Fig. 8.

observed. Depending on the type of mineral deficiency, the amount of some polypeptides was changed. As a general rule, the minor bands in a spectrum are better differentiated on the gels, which is especially emphasized among fast moving bands.

In seeds, produced under conditions of nitrogen (Fig. 9), boron (Fig. 10), potassium (Fig. 11), sulfur (Fig. 12) and complete mineral deficiencies (Fig. 13), the quantity of polypeptide No 17 increased in spite of seed storage longevity. The polypeptide band No 5 was better distinguished on the gels also in seeds, produced under B-, S-, K- and complete mineral deficiencies. In control seeds as well as in B-, N-, K-deficient seeds the medium migrating band No 13 was more intensive, whereas in Fe-deficient seeds all the three dominating polypeptides were comparable with respect to the intensity of staining. Significant changes in the polypeptide spectrum as a result of mineral deficiency were found in seeds from plants grown on complete mineral deficiency and on a S-deficient medium. A significant enhancement of the amount of polypeptide No 10 in comparison with control seeds was observed. According to Randall et al. (1979), the deficiencies of S, P and K lead to significant and specific changes in the ratio of protein levels in pea seeds. The S-deficiency decreases the quantity of proteins with unknown function, involving 22 kDa polypeptides. The results presented are in agreement with the data of Wrigley et al. (1984) showing a relative increase of the quantity of 55 kDa polypeptides with low content of sulfur. Masaki and Soejima (1972) prove that these polypeptides are products of dissociation of sulfur-poor 7S globulins. Based on these considerations, a suggestion could be

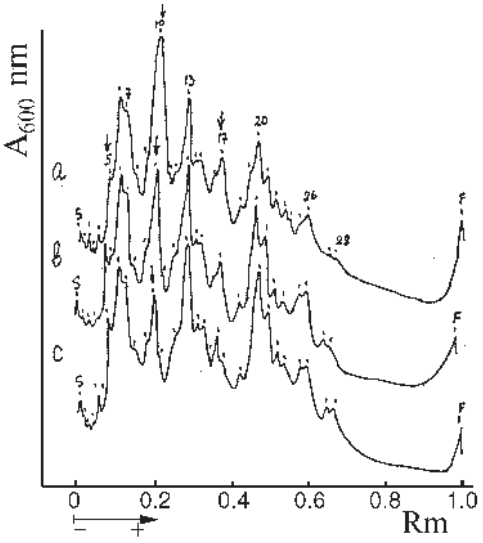

Fig. 13. Densitometric scans of polypeptide spectra (SDS-PAGE) of soybean seeds, produced by plants, grown under complete mineral deficiency conditions. Symbols as in Fig. 8.

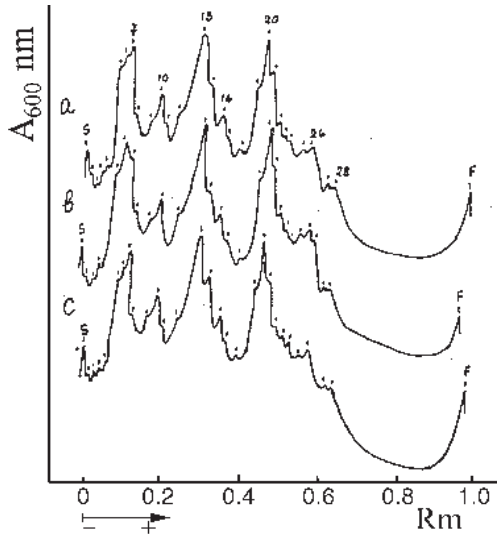

Fig. 14. Densitometric scans of polypeptide spectra (SDS-PAGE) of soybean seeds, produced by plants, grown under Fe-deficiency condition. Symbols as in Fig. 8.

made that the polypeptides in S-deficient seeds, which have underwent alterations, most probably belong to this group of proteins. In contrast to Randall and Wrigley (1986) as well as Castle and Randall (1987) we established no significant changes among the low molecular polypeptides. In seeds from S-deficient plants, stored for 5 years, the effect of natural aging was added to that of S-deficiency. As a result the intensity of band No 10 significantly exceeded that of all other bands. Thus, K-deficiency influenced the amount of band No 5, while N-, B- and S-deficiencies affected the quantity of band No 17. Only S-deficiency specifically induced changes in the quantity of band No 10. In this case a very close negative correlation between seed viability and quantity of this band was observed. The smallest changes in the polypeptide band No 10 were registered in the most viable seeds (91%). The most significant was the enhancement observed in the quantity of the same band in seeds with the lowest degree of viability (21%).

One could assume that in seeds, formed under full mineral deficiency conditions, absolute disorder in biosynthesis and accumulation of proteins will occur. The results obtained showed that electrophoretic spectra of these seeds were really affected in respect to the quantity of bands. These spectra reflected the effects of the different mineral deficiencies applied, however, in qualitative respect they were similar to those of control seeds.

The data presented in this investigation are contradictory to the statement that under severe mineral deficiency conditions plants form and grow up small number of seeds, but these seeds show the qualitative and quantitative composition similar to that in seeds from control plants (Harrington, 1960; Austin, 1972). In our previous investigation (Tyankova et al., 1994) we have established that depending on yield (seed number and weight), the variants studied could be set in order as follows: control (100%), Fe-deficiency (100%), deficiency of N, S and K (30–40%), B- and complete mineral deficiency (0–1%). The electrophoretic analysis performed in the present investigation showed that in all cases of mineral deficiencies where the seed yield was reduced, quantitative changes in protein and polypeptide spectra were established. The degree of these changes did not correlate with the degree of yield reduction. The most drastic were the changes in electrophoretic spectra of S-deficient seeds. Other two variants of deficiency – N and K, provoked similar yield reduction but the changes in the protein spectra were not so significant. On the contrary, there was no difference between the protein spectra of B-deficient (0–1% of seed yield) and control seeds (100% seed yield). Electrophoretic patterns of complete mineral deficient seeds were identical with those of S-deficient seeds. Based on these data the following conclusions could be made: 1) the production of small number of seeds not in all cases could overcome the adverse effects of mineral deficiency on seed quality, 2) the degree of changes in the protein and polypeptide spectra depends mainly on the type of mineral element under deficiency, 3) in the case of sulfur-deficient seeds the effect of natural aging of seeds on polypeptide spectrum is additive to the effect of S-deficiency itself.

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