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FTIR SPECTROSCOPY OF PROTEIN ISOLATES OF SALT-TOLERANT SOYBEAN MUTANTS

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The effect of salinity on the conformation of proteins of four salt-tolerant M_2 generation mutants of soybean plants (S04-05/150-2, S04-05/150-8, S04-05/150-106, and S04-05/150-114) was investigated using Fourier transform infrared (FTIR) spectroscopy. Salinity is one of the important abiotic stress factors that limits growth and productivity of plants. The mutants belonging to the M_2 generation were determined as tolerant to 90 mM NaCl. The relative contents of α-helix, β-sheet, turn, and irregular conformations for the soybean protein isolates were determined depending on the analysis of the amide I region. The comparison of the secondary structures of soybean proteins of the mutants with those of the control group indicated that the α-helix structure percentage was diminished while β-turn and disordered structures were increased as a result of the salt stress.

Keywords: *IR spectroscopy, soybean protein isolates, salt-tolerant mutants.*

Introduction. Soybean protein isolate possesses a wide range of functional properties that play an important role in food processing in addition to its high nutritional value [1]. The increasing use of poor-quality water for irrigation causes soil salinization. Thus, soil salinity is the major environmental problem that reduces crop yield in the cultivated lands. For this reason the development of salinity-tolerant plants is getting more important in recent years. Proteins are biologically active macromolecules that have functions in several biological processes. The functions depend on the molecule three-dimensional structure, which can be affected via alterations in folding processes at different physiological conditions. It is known that plants enable folding and prevent misfolding of the protein by developing various mechanisms under stress [2]. Previously, in our Molecular Biology and Genetics Department, radiation sensitivity [3] and salt sensitivity experiments were performed on soybean varieties [4, 5], and proline metabolism-related genes in salt-tolerant mutant soybean plants were investigated [5]. In our previous study, the molecular structure and elemental composition of soybean seeds of the S04-05 variety together with its salt-tolerant mutants were investigated by FTIR and EDXRF spectrometry. It was shown that the salt-tolerant mutants (M_2) have less protein but more lipid content [6]. As continuation of our work, in this study protein isolates of four M_2 salt-tolerant mutant soybean plants of the S04-05 variety (150-2, 150-8, 150-106, and 150-114) were investigated with respect to S04-05 control in order to determine the effect of protein conformations on salt tolerance of the mutants. The leaves are the most appropriate plant tissues to evaluate the level of salinity tolerance. Kongngern et al. [7] reported the presence of some salt stress-induced proteins in stressed plant leaves. For this reason in this study the conformational alterations of the proteins of the leaves of the salt-tolerant soybean mutants are investigated.

Experimental. In this study, protein isolates of four salt-tolerant M_2 generation mutants of soybean plants of the S04-05 variety (150-2, 150-8, 150-106 and 150-114), which were determined as tolerant to 90 mM NaCl, and the protein isolates of the control group (S04-05 variety) plants were investigated. The investigated mutants demonstrated genetic diversity [4, 5]. Details of the production and selection of the salt-tolerant M_2 generation mutants of soybean plants of S04-05 variety were from our unpublished data [8].

For total protein isolation, 0.5 g of soybean leaves were ground by liquid nitrogen and homogenized in 1.25 mL of 50 mM Tris-HCl (pH 7.8) containing 0.1 mM EDTA, 0.2% Triton X-100, 1 mM PMSF, and 2 mM DTT. After extraction, the samples were centrifuged at 14.000 rpm for 30 min, and supernatants were stored at 4^{o}C until measurement. Total protein contents were calculated using bovine serum albumin as protein standard according to the method of Bradford [9]. The protein isolates were lyophilized before IR spectral analyses.

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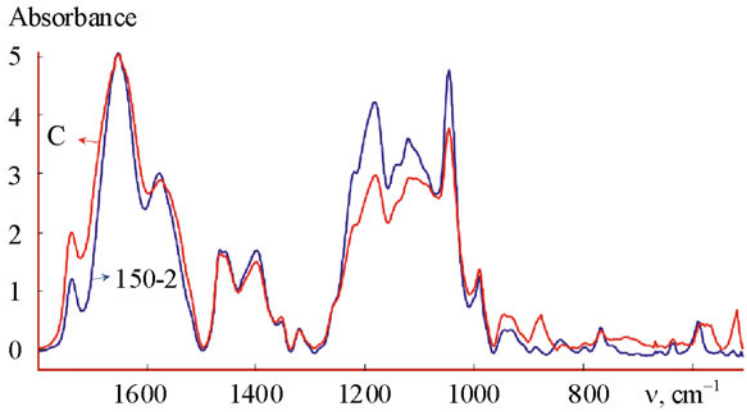

Fig. 1. The 1800–500 cm^{-1} region of FTIR spectra of the protein isolates of the 150-2 mutant and the control group.

The IR spectra of the samples were recorded on a Bruker Tensor FTIR spectrometer in the 4000–500 cm^{-1} range. A drop of protein isolate (*ca.* 50 μL) was placed between two ZnSe plates, and 200 scans were collected with a resolution of 1 cm^{-1}. For each species three independent measurements were performed. Vector-normalized spectra were used for analysis and comparison.

Spectral manipulations such as baseline adjustment, smoothing, obtaining the second derivative, and band fitting procedures were performed using GRAMS/AI 7.02 (Thermo Electron Corporation) software package. Band fitting was done using a Gaussian function, the fitting was undertaken until reproducible, and converged results were obtained with squared correlations greater than $r^2 \sim 0.99999$. The second derivative profile gives valuable information about the position of the bands and band widths. Thus, for the band fitting procedure (to locate the position of the peaks), the second derivative of the absorption spectrum was used as a guide. The position of each band component was chosen from the second derivative spectrum, then a shift of the position was allowed for fitting, leading to a lower error. The second derivatives of the spectra were obtained using a Savitzky–Golay function (two polynomial degrees, 13 points).

Results and Discussion. The 1800–500 cm^{-1} region of the FTIR spectra of the protein isolates of the 150-2 mutant and the control group are shown in Fig. 1. A comparison between the FTIR spectra of the mutant and control group shows some frequency and intensity changes, which implies some alterations in the protein conformation and amino acid contributions. In particular, the alterations in the 1500–500 cm^{-1} region indicate the differences in protein side chains and in free amino acid composition, probably occurring due to salt stress.

FTIR spectroscopy provides information about the secondary structure content and conformational changes of the proteins. The polypeptide and protein repeat units give rise to nine characteristic IR absorption bands, namely, amide A, B, and amide I–VII. The most sensitive spectral region to the protein secondary structural components is the amide I band (1700–1600 cm^{-1}), which is primarily C=O stretching motion [10, 11]. It may have some contributions from CN stretching and CCN deformation. The vibrational wavenumbers of amide I band components are widely used as probes for conformational preferences of peptides and proteins. Amide II occurs in the 1580–1510 cm^{-1} region and derives mainly from in-plane NH bending and from the CN stretching vibration. It is more complex than amide I, and, for this reason, it is less used to quantify the secondary structure of proteins even though it is conformationally sensitive [10].

Although the protein isolates were lyophilized before the IR spectral analyses, the IR spectra of the samples show the presence of water, which can affect particularly the amide I band. In order to eliminate the contribution of the water bands to the whole spectrum, computer subtractions of water bands were performed based on the 2150 cm^{-1} water association band. The FTIR spectra of the protein isolate of the control group before and after subtraction of water bands are shown in Fig. 2. The amide A band at 3275 cm^{-1} is clearly seen in the difference spectrum (Fig. 2b). The enlarged 1780–1492 cm^{-1} region of the water subtracted FTIR spectra of the investigated protein isolates are shown in Fig. 3. Some alterations in intensity and wavenumbers of the amide I and amide II bands in comparison to that of the control group are observed. The result indicated the presence of alterations in the protein conformations.

The secondary structure of the investigated soybean protein isolates was determined based on the amide I band found between 1700 and 1600 cm^{-1} of the water subtracted FTIR spectrum. Prior to the band component analysis, a straight

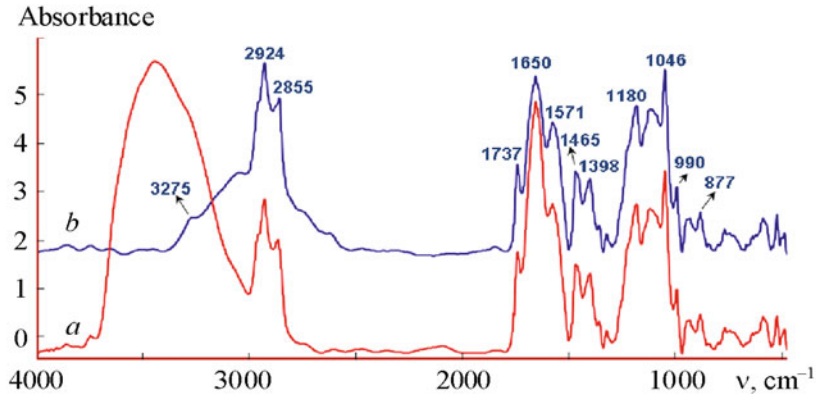

Fig. 2. The mean FTIR spectra of the control group, obtained by averaging three individual spectra, before (a) and after (b) subtraction of water bands.

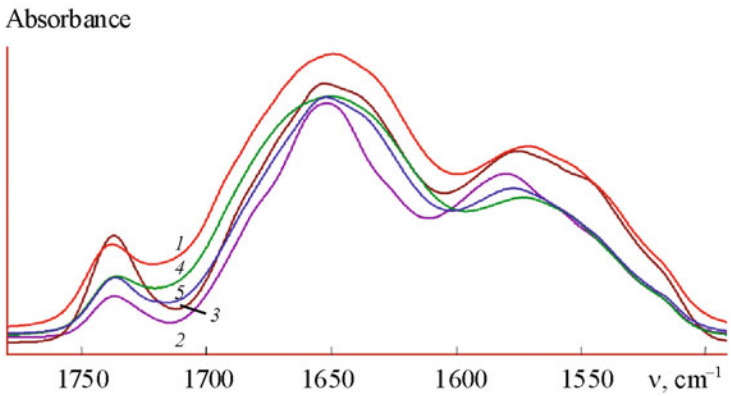

Fig. 3. The enlarged 1780–1492 cm^{-1} region of the water subtracted FTIR spectra of the investigated protein isolates. The control (1) and 150-2 (2), 150-8 (3), 150-106 (4), and 150-114 (5) salt-tolerant mutants.

baseline correction was performed in this region. The band fitting was performed according to the second derivative of the original spectrum, since the second derivative profile gives valuable information about the position of the bands and band widths. Therefore the position of each secondary structural component was chosen from the second derivative, and only a shift of the position was allowed for fitting, leading to a lower error. The areas of all bands assigned to a given secondary structure were then summed up and divided by the total area in order to obtain the contribution of each element. According to previous studies [10, 12–14], we assumed that the absorption coefficients were equal for all the secondary structures. Although there may be some differences between the absorptions coefficients of the secondary structural elements [15], several works suggest that the absorptivities are essentially independent of the secondary structural elements [14, 16–18]. On the other hand, since we investigated the relative contents, this assumption may be justified. The band component analysis of the 1709–1600 cm^{-1} region of the water subtracted FTIR spectra of the protein isolates of control and 150-2 mutant are shown in Fig. 4. The analysis of the amide I region of the control group protein isolate revealed six bands (Fig. 4a); the 1636 and 1619 cm^{-1} bands together with a high wavenumber component at 1696 cm^{-1} are characteristic of the β-sheet conformation, whereas the 1681 and 1665 cm^{-1} components can be attributed to the β-turn structure [10, 11]. The 1653 cm^{-1} component is characteristic for an α-helical conformation [10, 11]. On the other hand, the band fitting procedure of the amide I region of the water subtracted FTIR spectrum of the 150-2 mutant reveals seven components which can be attributed to the amide I structures (Fig. 4b). The 1629 and 1621 cm^{-1} bands together with the 1693 cm^{-1} component are characteristic of the β-sheet conformation, whereas the 1660, 1668, and 1679 cm^{-1} components can be attributed to the β-turn structure [10, 11, 19]. The strong band at 1646 cm^{-1} is attributed to an amide-I band of the disordered structure [10, 11]. The secondary

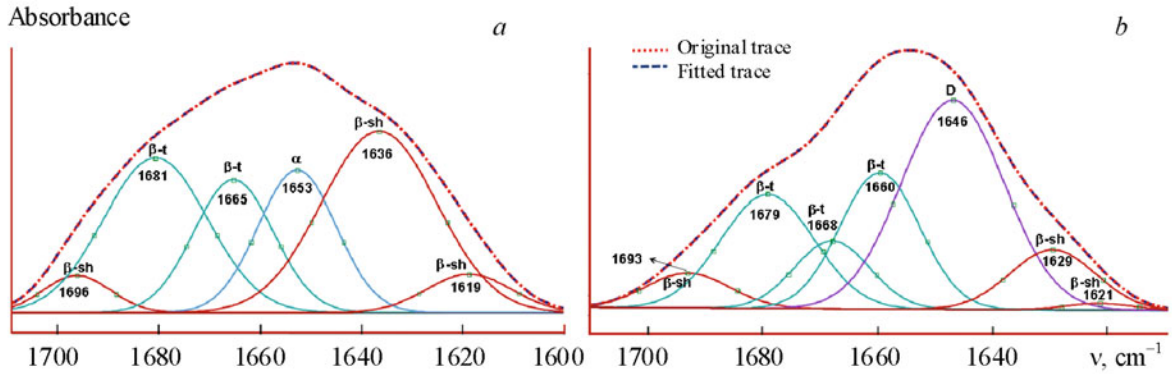

Fig. 4. The band component analysis of the 1709–1600 cm^{-1} region of the water subtracted FTIR spectra of the protein isolates of the control (a) and 150-2 mutant (b).

TABLE 1. The Results (%) of the Secondary Structure Composition of Protein Concentrates

Structure	C	150-2	150-8	150-106	150-114
α-helix	17.4	–	–	–	15.1
β-sheet	42.5	27.3	33.6	26.0	35.5
β-turn	40.1	42.5	43.1	43.4	40.2
Disordered	–	30.2	23.3	30.6	9.2

structure composition of protein concentrates, obtained as a result of the calculations of the individual component peak area divided by the total area, is presented in Table 1. A comparison of the band component analysis results of the control group protein isolate with those of mutants (see Fig. 4) indicated that the α-helical contribution of proteins decreased in favor of the disorder structure in the salt tolerant mutants. The secondary structure composition of the soybean protein isolate of the 150-2 mutant was found to be the most different from that of the control, whereas the 150-114 mutant was the most similar one. The results are consistent with the obtained results on expression analysis of proline metabolism-related genes in the salt-tolerant mutants of soybean plants [5]. The maximum proline accumulation level was obtained for the mutant 150-2 as the 2.43 fold, and the minimum proline accumulation level was obtained as the 1.14 fold for the 150-114 mutant plants after 7 days of salt treatment in accordance with the control groups by Celik and Unsal [5]. Since proline is an important osmolyte that is accumulated as a final product in the leaf tissues of the salt-tolerant soybean mutants under 90 mM NaCl stress, the result shows that the tolerance mechanisms of the mutant plants (150-2 and 150-114) are different under the salt stress factor [5]. In this study the protein conformations of the 150-2 and 150-114 mutants were found to be the most altered and the least altered conformations, respectively, in comparison to those of the control group. Combining our results with the previous findings [5], we can conclude that protein conformation alterations increase with increasing free proline accumulation, and thus with increasing salt stress. The results pointed out that alteration of the protein conformation was a result of the salt stress.

Conclusions. The effect of salinity on the conformation of protein isolates of four salt-tolerant M_2 generation mutants of soybean plants (150-2, 150-8, 150-106, and 150-114) was investigated via FTIR analysis. The results show that the protein secondary structure of the mutants was different from each other and there were also differences between the individual four mutants and the control group of the same variety (S04-05). According to the results of FTIR spectroscopy, the mutants 150-2 and 150-114, which show the maximum differences between secondary structure contents of the protein isolates, have been selected to investigate their proteomic profiles to discriminate their protein patterns under salinity conditions in further studies.

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