Changes of Kunitz trypsin inhibitor in soybean during germination at different temperature

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Abstract
Changes in KSTI-Ti⁰ during germination of seeds of three soybean varieties contain the genetic Ti⁰ variant of KSTI, at optimal condition (25ºC) and stress condition of germination at temperatures 5ºC and 10ºC were studied. The modified form of KSTI occurs in soybean seeds germinated at optimal temperature of 25ºC but not occur in seeds germinated for 10 days at 5ºC and 10ºC. Low temperatures resulted in significant differences of the process of KSTI modification in relation to the process in seeds exposed immediately to 25ºC. Modified forms occur in seeds germinated 10 days at low temperature and then transferred to 25ºC.

Key words: germination, KSTI, modification, soybean, temperature

Introduction
Several different trypsin inhibitors have been reported to be present in soybeans, but much of the soybean trypsin inhibitor activity is thought to be due to the protein SBTI-A₂ (Ricks et al., 1962), which was crystallised by Kunitz (1945) and is commonly known as the Kunitz trypsin inhibitor. In the soybean seeds three genetically different variants of Kunitz trypsin inhibitor (KTI), Ti⁰, Ti⁰ and Ti⁰ are present. These types are inherited as codominant alleles in a multiple allelic system at a single locus (Hymowitz and Hardy, 1972; Orf and Hymowitz, 1976). During seed germination a number of different changes take place in the seed proteins. Some band decrease, others increase and some show little change. Several reports of the appearance or disappearance of electrophoretically distinct proteinase inhibitors have appeared (Orf and Hymowitz, 1977, Fred and Rayan, 1978). Orf et al. (1977) showed the appearance of a new band with proteinase inhibitory activity during germination of soybean seeds and suggested that it may be a modified form of the Kunitz inhibitor. The appearance of new forms of KSTI during germination can be a result of different gene expression during germination and maturation. According to studies performed by Papastoitsis and Wilson (1991), a cysteine protease, designated as K1, initiates the appearance of the new form of KSTI. Protease K1 is essentially absent from dry soybean seeds, but appears at the beginning of germination and peaks at the third to fourth day of growth, when the new form of KSTI occurs. It is assumed that the initial proteolysis of KSTI is catalysed by protease K1 during germination and that it removes 5-carboxyl-terminal residues (Asp-Lys-Glu-Ser-Leu) of KSTI-Ti⁰ to produce KSTI-Ti⁰ₘ. According to Kumar et al (2006) trypsin inhibitor was continuously degraded during germination and the degradation was faster at higher germination temperature. Our objective was to determine, by the use of PAGE, if changes occurred in KSTI-Ti⁰ during germination of soybean seeds at optimal condition (25ºC) and stress condition of germination at temperatures 5ºC and 10ºC.
**Material and methods**

Seeds of three varieties, ZPS Bosa, ZPS 015 and Hodgson were used to monitor modifications of KSTI. Seeds were germinated in sterile sand in a growth chamber at 5°C, 10°C and 25°C. Five seedlings of each variety were harvested on the 1st, 3rd, 5th, 7th and 9th day from the beginning of germination. The rest of sowed material after 10 days from the beginning of germination were moved from growth chamber at 5°C and 10°C to growth chamber at 25°C. Seedlings were harvested on 11th, 13th, 15th, 17th and 19th day. The tissue was extracted in 0.5 M Tris-HCl buffer containing PMSF. Polyacrylamide gel electrophoresis with 10% gel in Tris-glycine buffer was performed according to Davis, 1964.

**Results and discussion**

Changes in KSTI-Ti during germination of soybean seeds at optimal condition (25°C) and stress condition of germination at temperatures 5°C and 10°C were studied. During germination on 25°C the new form of KSTI, although of a lower intensity, occurred in the variety ZPS 015 as soon as the third day of germination. In other two varieties, the modified form of KSTI of a high intensity occurred by the fifth day of germination (fig 1.). The intensity decrease of the native form of KSTI starts in the variety Hodgson from the third day of germination, i.e. prior to the production of the new KSTI form. This native form in this variety disappears on the ninth day of germination. The native form in the varieties ZPS Bosa and ZPS 015 beginning disappear on the 5th day of germination, but disappears almost completely on the 7th day. Both, modification and degradation of KSTI significantly differ over varieties. The intensity decrease of the native form of KSTI starts very early in the variety Hodgson, but this intensity although lower, lasts longer than in the other two varieties, in which the disappearance starts later but the native form disappears quickly and completely. The obtained results indicate that two bands, occurring on polyacrylamide gels one beside another during germination are identical. Freed and Ryan (1978) performed immunoelectrophoresis and achieved similar results.

![Fig.1. Modification of KSTI in three soybean variety after 1, 3, 5, 7, 9 germination day at 25°C. KSTI is nature form of Kunitz trypsin inhibitor.](image)

At both temperatures, 5°C and 10°C, during first 10 days germination modified form have not appeared, but during germination at 5°C intensity of KSTI decrease starting from 5th day for ZPS Bosa and Hodgson, and 7th day for ZPS 015 (fig.2). During germination on 10°C intensity of KSTI has not be significantly changed (fig.3). From obtained results could be concluded that KSTI during germination at low temperature behaves similarly to
other storage proteins of soybean. According to Kumar et al. (2006) early appearance of the modified form of Kunitz inhibitor at 35°C as compared to 25°C, confirms that the faster quantitative reduction at higher temperature is due to faster degradation of the original Kunitz inhibitor form at higher temperature.

Fig.2 Degradation of KSTI in three soybean variety after 1, 3, 5, 7, 9 germination day at 5°C

Fig.3 Degradation of KSTI in three soybean variety after 1, 3, 5, 7, 9 germination day at 10°C

After expose seed to low temperature during 10 days, seedlings were transferred at 25°C to study effect of low temperature during germination on KSTI forms. When seedlings were transferred form 5°C to 25°C nature form of KSTI was only 1st day in variety ZPS Bosa and Hodgson and 1st and 3rd day in variety ZPS 015. After that period activity of nature form have not be observed. Modified form of KSTI in seed of all varieties transferring from chamber at 5°C to higher temperature (25°C) appeared after 3 days, 11th day from start of germination. The maximum intensity of that form was 3rd day in variety ZPS Bosa and Hodgson, and 5th day in variety ZPS 015. In all varieties modification form, although weak intensity, was observed on 19th day of germination (fig.4).

Fig.4 Modification of KSTI after transfer seed from 5°C at 25°C.

Fig.5 Modification of KSTI after transfer seed from 10°C at 25°C.

Both nature and modification form of KSTI in all varieties was 1st to 5th day from transfer from 10°C on 25°C but they differ in intensity. Modified form KSTI appeared 1st day after
transfer on optimal germination condition, and disappeared 5\textsuperscript{th} day. Maximum intensity of modified form was 3\textsuperscript{rd} and 5\textsuperscript{th} day in variety ZPS Bosa and ZPS 015 as well as 1\textsuperscript{st} and 3\textsuperscript{rd} day in variety Hodgson (fig. 5).

\textbf{Conclusion}

Low temperatures of 5\textdegree C and 10\textdegree C resulted in significant differences of the process of KSTI modification in relation to the process in seed exposed, immediately after sowing, to temperature of 25\textdegree C. According to obtained results we can conclude that KSTI does not affect increased resistance of seeds germinated at low temperature, but also we can not draw a conclusion on a role of the modified form of KSTI in soybean seeds.

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\textbf{References:}

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