Heat-induced accumulation of chloroplast protein synthesis elongation factor, EF-Tu, in winter wheat

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Received 12 February 2007; accepted 28 March 2007

Summary

Chloroplast protein synthesis elongation factor, EF-Tu, has been implicated in heat tolerance in maize (\textit{Zea mays}). Chloroplast EF-Tu is highly conserved, and it is possible that this protein may be of importance to heat tolerance in other species including wheat (\textit{Triticum aestivum}). In this study, we assessed heat tolerance and determined the relative levels of EF-Tu in mature plants (at flowering stage) of 12 cultivars of winter wheat experiencing a 16-d-long heat treatment (36/30 °C, day/night temperature). In addition, we also investigated the expression of EF-Tu in young plants experiencing a short-term heat shock (4 h at 43 °C). Heat tolerance was assessed by examining the stability of thylakoid membranes, measuring chlorophyll content, and assessing plant growth traits (shoot dry mass, plant height, tiller number, and ear number). In mature plants, relative levels of EF-Tu were determined after 7 d of heat stress. High temperature-induced accumulation of EF-Tu in mature plants of all cultivars, and a group of cultivars that showed greater accumulation of EF-Tu displayed better tolerance to heat stress. Young plants of all cultivars but one did not show significant increases in the relative levels of EF-Tu. The results of the study suggest that EF-Tu protein may play a role in heat tolerance in winter wheat.

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Introduction

Chloroplast protein synthesis elongation factor, EF-Tu is a protein (45–46 kD) that plays a key role in polypeptide elongation during protein synthesis. This protein promotes the GTP-dependent binding...
of aminoacyl-tRNA to the A site of the ribosome (Brot, 1977; Riis et al., 1990). Chloroplast EF-Tu is a member of a nuclear-encoded multi-gene family (Baldauf et al., 1990; Ursin et al., 1993; Sugita et al., 1994; Maurer et al., 1996; Lee et al., 1997), and in land plants this protein is synthesized in the cytosol (Baldauf and Palmer, 1990).

Chloroplast EF-Tu has been implicated in the development of heat tolerance in maize (Zea mays) (Bhadula et al., 2001; Momcilovic and Ristic, 2004; Rao et al., 2004; Ristic et al., 2004). This protein accumulates in maize chloroplasts after 2 h of heat stress (Bhadula et al., 2001; Momcilovic and Ristic, 2004), and the heat-induced accumulation of EF-Tu correlates with the heat tolerance phenotype (Bhadula et al., 2001; Ristic et al., 2004). The heat-induced buildup (upregulation) of EF-Tu occurs over a broad temperature range starting from 35 °C upwards (Bhadula et al., 1998) and has been reported in both young (7–21-d-old plants) and mature (plants at flowering stage) maize (Zea mays) plants (Momcilovic and Ristic, 2007). It has been suggested that chloroplast EF-Tu confers heat tolerance by acting as a molecular chaperone and protecting chloroplast proteins from thermal aggregation and inactivation (Momcilovic and Ristic, 2004; Rao et al., 2004).

The implication of chloroplast EF-Tu in heat tolerance has been, so far, reported only in maize (Bhadula et al., 2001; Rao et al., 2004; Momcilovic and Ristic, 2004; Ristic et al., 2004). Chloroplast EF-Tu is highly conserved (Baldauf et al., 1990; Ursin et al., 1993; Sugita et al., 1994; Maurer et al., 1996; Lee et al., 1997; Bhadula et al., 2001), and it is possible that this protein may be of importance to heat tolerance in other plant species including wheat (Triticum aestivum). In this study, we tested the hypothesis that EF-Tu is upregulated in wheat under elevated temperatures and that wheat cultivars with contrasting tolerance to heat stress differ in the expression/accumulation of this protein under heat stress conditions. We determined the relative abundance of EF-Tu and assessed heat tolerance in mature plants (at flowering stage) of 12 cultivars of winter wheat after exposure to a 16-d-long heat stress. We chose mature plants because under field conditions wheat is more likely to experience prolonged exposure to elevated temperatures at flowering stage. In addition, we also investigated the expression of EF-Tu in young plants experiencing a short-term (4 h) heat shock (43 °C). Here we report that the long-term heat stress induced accumulation of EF-Tu in mature plants of winter wheat, and that a group of cultivars that showed greater accumulation of this protein displayed better tolerance to heat stress.

Materials and methods

Plant material and experimental conditions

Experiment with mature plants

Seeds of 12 cultivars of winter wheat (Triticum aestivum L.) were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Seeds were germinated in 4 cm deep trays containing potting soil (Metro Mix 200; Hummitt Intnl., Topeka, KS) in a greenhouse. Ten-day-old seedlings were vernalized at 4 °C for 8 weeks. Following vernalization seedlings of each cultivar were transplanted into ten pots (3 seedlings per pot; pot diameter at the top and the bottom was 21 and 16 cm, respectively; pot height was 20 cm) containing Metro Mix 200 potting soil. Plants were grown in a green house and watered daily in the winter of 2006. “Miracle Gro” fertilizer (24:8:16; Stern’s Miracle-Gro Products, Inc., Port Washington, NY) was added (according to manufacturer instructions) once every week during the entire duration of the experiment. At the beginning of flowering stage [growth stage Feekes 10.5.1 (Miller, 1992)], plants of each cultivar were divided into control (five pots) and treatment/heat-stress (five pots) groups. The control group was maintained under growth conditions in a greenhouse and the treatment group was exposed to heat stress for 16 d [day/night temperature of 36/30 °C; RH, 90–100%; and photoperiod, 16/8 h; PPF, 280 µmol m⁻² s⁻¹ (Sylvania cool white fluorescent lamps)] in a growth chamber. The heat treatment started by gradual increase in temperature from the ambient temperature to 36 °C over 1 h. Air temperature, relative humidity and light level were continuously monitored in the growth chamber, and in the greenhouse air temperature was measured at hourly intervals [the average daily temperature in the greenhouse was 22.7 ± 2.8 °C; during the period of heat stress treatment, the PPF and RH in the greenhouse ranged from 270 to 320 µmol m⁻² s⁻¹ (photoperiod, 16/8 h; supplemental light was used to extend day-light period) and 55–70%, respectively]. To minimize/avoid possible dehydration of the leaf tissue during heat treatment, pots of the treatment and control group were kept in trays containing ~1 cm deep water. Following heat treatment, plants were transferred to a greenhouse and allowed to recover at ambient temperature until harvest maturity. Relative levels of EF-Tu were determined after 7 d of heat stress, and plant heat tolerance was assessed after 16 d of heat treatment and at harvest maturity. For EF-Tu analysis, samples of leaf tissue were obtained from the flag leaf blades from two randomly selected plants (each plant was taken from a different replicate pot) from both control and heat-stressed group. Collected leaves were immediately frozen in liquid nitrogen and stored at −80 °C until further use.

Experiment with young plants

The experimental design was similar to that for mature plants with some modifications. Seeds of each cultivar were sown in three pots (5 seeds per pot) containing
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Metro Mix 200 potting soil. Plants were grown in a growth chamber (day/night temperature of 22/17°C; RH, 90–100%; and photoperiod, 16/8 h; PPF, 280 μmol m⁻² s⁻¹ (Sylvania cool white fluorescent lamps)) and watered daily. “Miracle Gro” fertilizer (Stem’s Miracle-Gro Products, Inc., Port Washington, NY) was added (according to manufacturer instructions) once every week during the entire duration of the experiment. Thirty-six-day-old plants were divided into a control (1 pot) and a treatment group (2 pots). The control group was maintained under growth conditions, and the treatment group was exposed to 43°C (RH, 90–100%) for 4 h in a growth chamber (Gallie et al., 1997). The temperature was gradually increased from 22 to 43°C over 1 h. Exposure time for heat shock treatment started when the temperature reached 43°C. For EF-Tu analysis, leaf tissue was collected from both control and heat-shocked plants immediately after heat treatment. The youngest, fully expanded leaves were collected from two randomly selected plants from each group. Collected leaves were immediately frozen in liquid nitrogen and stored at −80°C until further use.

Assessment of heat tolerance

In mature plants, heat tolerance was assessed by examining the stability of thylakoid membranes (Ristic et al., 1996) and measuring chlorophyll content (Reynolds et al., 1998) on the 16th day of heat treatment, and by assessing plant growth traits (shoot dry mass, plant height, tiller number, and ear number) at harvest maturity. The stability of thylakoid membranes was determined by measuring chlorophyll a fluorescence in intact 1-h dark adapted flag leaves (Ristic and Cass, 1993). Leaf samples were obtained from one randomly selected plant from each of five replicate pots from both control and heat-stress group. Fluorescence was measured in the middle portion of the flag blade (half-way between the base and the tip of the blade). The ratio of constant fluorescence (O) and the peak of variable fluorescence (P) (O/P) was used to assess the stability of thylakoid membranes (Krause and Weis, 1984; Ristic and Cass, 1993). Fluorescence measurements were conducted at room temperature using a pulse modulated fluorometer (Model O55-FL, Opti-Sciences, Hudson, NH).

Chlorophyll content was measured on the same flag leaves, in the same blade area that was used for fluorescence measurements, using a self-calibrating SPAD chlorophyll meter (Model 502, Spectrum Technologies, Plainfield, Illinois) (Fanizza et al., 1991; Reynolds et al., 1998). Five flag leaves (from five different pots) per cultivar/treatment (control and heat stress) were used for measurements.

At maturity, plants from five different pots (replicates) of each cultivar/treatment (control and heat stress) were harvested and data on plant growth traits (plant height, tiller numbers, ear number and shoot dry mass) were measured. Dry mass was measured after oven drying the shoots (leaves and stems) at 60°C for 7 d.

EF-Tu analysis

Chloroplast EF-Tu was analyzed using 1-D SDS-PAGE and immunoblotting (Bhadula et al., 2001). Total soluble proteins were extracted from the leaf tissue, and protein content was determined using the RC DC Protein Assay (BioRad, CA). Extracted proteins were separated on 10% polyacrylamide gels. Equal amounts of protein (15 μg per well) were loaded on the gels. Following electrophoresis, proteins were transferred to a PVDF membrane (BioRad, CA), and blots were probed for EF-Tu using maize anti-EF-Tu polyclonal antibody (Bhadula et al., 2001) and the ECL immunoblot kit (Amersham Biosciences, NJ); a preliminary study had shown that maize anti-EF-Tu antibody cross-reacts with wheat EF-Tu, as determined by mass spectrometry of immuno-precipitated EF-Tu from wheat leaf protein extracts. The relative levels of EF-Tu were estimated by determining band volume using Quantity One software (BioRad, CA).

Statistical analysis

All data were analyzed using PROC TTEST procedures in Statistical Analyses System (SAS, 2003) to determine the influence of heat stress on different cultivars and/or groups. The data on chlorophyll a fluorescence, chlorophyll content and growth traits had five replicates for each cultivar (five different plants in different pots). The data on EF-Tu had two replicates for each cultivar (two different leaves from different pots). Initially, the data on chlorophyll a fluorescence were analyzed using t-test assuming unequal variance and treatment (control vs. heat stress) as class variables with five replications. The results of Welch’s t-test assuming unequal variance, which contrasted the heat stress treatment mean to twice that of the control for each cultivar, showed that the cultivars could be broadly divided into two distinct groups (group 1, cultivars that were significantly susceptible to heat stress; and group 2, cultivars that were not significantly susceptible to heat stress). Thereafter, all the other data were analyzed with group as a class variable to see if there were significant differences in relative chlorophyll content and growth traits between the groups using PROC TTEST procedures in SAS.

Results

Heat tolerance and EF-Tu expression in mature plants

Exposure to heat stress caused damage to the thylakoid membranes in all wheat cultivars as indicated by increases in the ratio of constant fluorescence (O) and the peak of variable fluorescence (P) (Krause and Weis, 1984; Ristic and Cass, 1993) (Fig. 1A). The cultivars, however, differed in the extent of the damage. The damage (greater
increase in O/P) to thylakoid membranes under heat stress conditions, when compared to control, was significantly greater in cultivars Zlatka, Stepa, NS2-4523, Rana Niska and Kompas than in cultivars Proteinka, Ljiljana, Partizanka, Stamena, Jefimija, NS2-4992 and Dragana (Fig. 1A). Based on this analysis we classified the cultivars into two groups: a heat susceptible group (wheat cultivar group 1) and a heat tolerant group (wheat cultivar group 2) (B). Bars on individual columns in graph A indicate standard errors [n = 5 for all cultivars except cultivar Kompas (n = 10)].

Fig. 1. Influence of heat stress on the ratio of constant fluorescence (O) and the peak of variable fluorescence (P) (O/P) (Krause and Weis, 1984) in 12 cultivars of winter wheat after exposure to heat stress (A). Mature plants (at flowering stage) were exposed to 16-d-long heat stress (day/night temperature, 36°C/30°C; RH, 90–100%) in a growth chamber. On the 16th day of heat stress chlorophyll a fluorescence was measured in the flag leaves. Increases in O/P ratio indicate damage to thylakoid membranes, the higher the damage the lower the tolerance to heat stress (Krause and Weis, 1984; Ristic and Cass, 1993). Based on the damage to thylakoid membranes, cultivars were grouped into two groups: a heat susceptible group (wheat cultivar group 1) and a heat tolerant group (wheat cultivar group 2) (B). Bars on individual columns in graph A indicate standard errors [n = 5 for all cultivars except cultivar Kompas (n = 10)].

and a heat tolerant group (wheat cultivar group 2) (Fig. 1B).

Comparison of these two groups revealed that under heat stress conditions the average increase in the O/P ratio was over 300% higher in the heat susceptible (wheat cultivar group 1) than in the heat tolerant group (wheat cultivar group 2) (Fig. 2A). The heat susceptible and the heat tolerant cultivar groups also differed in the content of chlorophyll after exposure to heat stress (Fig. 2B). On the 16th day of heat treatment, the average chlorophyll content in the stressed plants of the heat susceptible cultivar group was 35% of that in control. In contrast, the average chlorophyll content in the stressed plants of the heat tolerant group was 85% of that in control. When the heat-induced changes in chlorophyll content were plotted against the heat-induced changes in the O/P ratio of chlorophyll \( a \) fluorescence, a strong negative linear correlation was observed (\( R = -0.957; P < 0.0001 \)). Cultivars with higher increases in O/P ratio (lower tolerance to heat stress) showed greater reduction in chlorophyll content than cultivars with lower increases in O/P ratio (higher tolerance to heat stress).

The assessment of plant growth traits at harvest maturity was consistent with the results of damage to thylakoid membranes (O/P ratio) and chlorophyll content after exposure to heat stress. The heat susceptible cultivar group showed slower growth after heat treatment than the heat tolerant group, as indicated by smaller shoot dry mass, plant height, and tiller and ear numbers (Fig. 2C).

Immunoblot analysis of protein extracts from the flag leaf tissue of mature plants revealed that the wheat cultivars differed in the relative amount of EF-Tu. Under control conditions (22 °C), cultivars showed variation in the relative level of EF-Tu with cultivars Proteinka, NS2-4523, Stamena, and Dragana having relatively low and cultivars Rana Niska, Partizanka, and Ljiljana relatively high...
amount of this protein (Fig. 3). Exposure to heat stress induced accumulation of EF-Tu in all cultivars (Fig. 3) but the two cultivar groups differed in the extent of accumulation. Under heat stress conditions, the heat susceptible cultivar group (wheat cultivar group 1) increased the relative level of this protein by 81% (Fig. 4). In contrast, the heat tolerant group (wheat cultivar group 2) augmented the relative level of EF-Tu by 186% (Fig. 4).

Fig. 3. Relative amounts of chloroplast EF-Tu in flag leaf blades from 12 cultivars of winter wheat. Mature plants (at flowering stage) were exposed to high temperature (day/night temperature, 36 °C/30 °C; RH, 90–100%) in a growth chamber. For EF-Tu analysis leaf tissue was collected after 7 d of heat stress. Total proteins were extracted and analyzed using immunoblotting. The blot was probed with anti-EF-Tu antibody (Bhadula et al., 2001). An equal amount of protein (15 μg) was loaded in each lane. The relative amounts of EF-Tu were estimated by determining band volume using Quantity One software (BioRad, CA). The plotted data (band volumes/relative amounts of EF-Tu) represent averages of two replicate samples. Bars on individual columns indicate standard errors. Columns assigned different letter indicate significant difference (P<0.05) between control (22 °C) and heat-stressed (36 °C) plants.

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Fig. 4. Relative amount of chloroplast EF-Tu in wheat cultivar group 1 (heat susceptible) and wheat cultivar group 2 (heat tolerant) (for more details on cultivar groups see legend of Fig. 1) after exposure to heat stress (day/night temperature, 36°C/30°C; RH, 90–100%) for 7 d. Mature plants (at flowering stage) were exposed to heat stress in a growth chamber. Relative amount of EF-Tu was determined in flag leaf blades. Total proteins were extracted and analyzed using immunoblotting. The blot was probed with anti-EF-Tu antibody (Bhadula et al., 2001). An equal amount of protein (15 μg) was loaded in each lane. The relative amount of EF-Tu was estimated by determining band volume using Quantity One software (BioRad, CA). The relative amount (band volume) of EF-Tu in heat-stressed plants was expressed as a percentage of that in control plants. Bars on individual columns indicate standard errors; n = 10 (5 cultivars × 2 replications) for wheat cultivar group 1; n = 14 (7 cultivars × 2 replications) for wheat cultivar group 2. Columns assigned different letter indicate significant difference between two cultivar groups. Note that the two cultivar groups significantly differ in the relative amount of EF-Tu (P < 0.05).

**EF-Tu expression in young plants**

The expression of EF-Tu in young plants was different from that in mature plants. Under control conditions, young plants showed relatively less variation in relative amounts of EF-Tu than mature plants (Fig. 5). Exposure to heat shock did not cause significant increase in the relative levels of this protein in all cultivars except one, Dragana (Fig. 5). In this cultivar, heat-shocked plants had 30% higher relative amount of EF-Tu than control plants (Fig. 5).

**Discussion**

Thylakoid membranes and their associated photosystem II (PS II) are primary targets of many plant stresses including heat and it is generally accepted that the maintenance of their integrity and stability under high-temperature stress conditions is a major component of heat tolerance in plants. Thylakoid membranes are considered the most heat-labile cell structure and they are one of the first components to be damaged by heat (Santarius, 1974; Schreiber and Berry, 1977). In wheat and related species, for example, thylakoids are more affected than the chloroplast envelope, stromal enzymes or the integrity of cell compartments (Monson et al., 1982; Thebud and Santarius, 1982; Kobza and Edwards, 1987; Al-Khatib and Paulsen, 1989; Sayed et al., 1989).

Quantification of damage to thylakoid membranes is often used as an indicator of the plant’s ability to tolerate heat stress (Berry and Bjorkman, 1980; Krause and Weis, 1984; Al-Khatib and Paulsen, 1990; Ristic and Cass, 1993; Ristic et al., 1996, 1998, 2004). Damage to thylakoid membranes is indicated by increase in the O/P ratio of chlorophyll a fluorescence (Berry and Bjorkman, 1980; Krause and Weis, 1984; Ristic and Cass, 1993; Ristic et al., 1996, 1998, 2004). Measurements of chlorophyll a fluorescence and determination of the O/P ratio can, thus, provide a reliable means of determining the heat tolerance of the leaf tissue (Bilger et al., 1984; Ristic and Cass, 1993; Ristic et al., 1996, 1998, 2004).

In this study, we used chlorophyll fluorescence and O/P ratio to assess heat tolerance of the leaf tissue in mature plants of 12 cultivars of winter wheat. As indicated by damage to thylakoid membranes (increases in O/P ratio), heat stress affected all wheat cultivars but the cultivars differed in their ability to tolerate stress. Cultivars from group 2 showed better ability to tolerate 16-d-long heat stress than cultivars from group 1. Wheat cultivars from group 2 also showed better ability to retain chlorophyll under heat stress than cultivars from group 1. This is consistent with Wardlaw et al. (1980) and Blum (1986) who demonstrated the presence of genetic variability in chlorophyll content in wheat cultivars under heat stress. Furthermore, chlorophyll loss in the heat-stressed wheat correlated negatively with the O/P ratio of chlorophyll a fluorescence. This negative correlation may be a consequence of damage to thylakoids caused by heat. Chlorophyll is primarily located in thylakoid membranes where it forms complexes with proteins of PS II and PS I (Schreiber and Berry, 1977; Vacha et al., 2007) and damage to thylakoid membranes would eventually lead to chlorophyll loss. Alternatively, chlorophyll loss may be due to possible heat-induced premature senescence (Al-Khatib and Paulsen, 1984; Harding et al., 1990).

Exposure of mature wheat plants to a long-term (16 d) heat stress resulted in increased accumulation of chloroplast EF-Tu. The heat-induced accumulation of EF-Tu was evident in all wheat cultivars...
but the two cultivar groups, however, differed in the expression of this protein. The heat tolerant group (wheat cultivar group 2) showed greater accumulation of EF-Tu than the heat susceptible group (wheat cultivar group 1). Similar differential expression of EF-Tu was also seen in maize (Momcilovic and Ristic, 2004, 2007). A heat tolerant maize line, ZPBL 1304, synthesized and accumulated greater amounts of EF-Tu under heat stress than a heat sensitive maize line, ZPL 389 (Momcilovic and Ristic, 2004, 2007). Also, a group of maize hybrids with higher tolerance to heat stress

Fig. 5. Relative amount of chloroplast EF-Tu in heat shocked young plants of 12 cultivars of winter wheat. Thirty-six-day-old plants were exposed to 43 °C for 4 h (RH, 90–100%) in a growth chamber. Chloroplast EF-Tu was analyzed in the youngest, fully expanded leaf blades. Total proteins were extracted and analyzed using immunoblotting. The blot was probed with anti-EF-Tu antibody (Bhadula et al., 2001). An equal amount of protein (15 μg) was loaded in each lane. The relative amount of EF-Tu was estimated by determining band volume using Quantity One software (BioRad, CA). The plotted data (band volumes/relative amounts of EF-Tu) represent averages of two replicate samples. Bars on individual columns indicate standard errors. Columns assigned the same letter indicate no significant difference between control (22 °C) and heat-shocked (43 °C) plants. Columns assigned different letter indicate significant difference between control and heat-shocked plants (P<0.05).
displayed greater accumulation of EF-Tu under heat stress conditions than a group of maize hybrids with lower tolerance to heat stress (Ristic et al., 1996).

The results of this research on wheat cultivars are consistent with our previous research on maize (Ristic et al., 1998), where heat-induced accumulation of EF-Tu was associated with the heat tolerance phenotype. Ristic et al. (1998) analyzed F$_2$ progeny from a heat tolerant maize line that accumulates EF-Tu (ZPBL 1304) and a heat sensitive maize line that does not accumulate EF-Tu under heat stress (ZPL 389). They observed heat-induced accumulation of EF-Tu in F$_2$ plants that displayed increased tolerance to heat stress.

The young and mature wheat plants differed in the expression of EF-Tu under heat stress. In all cultivars but one, young plants did not show significant heat-induced increase in the relative amounts of EF-Tu. In contrast, mature plants of all cultivars showed a significant heat-induced increase in the levels of EF-Tu. Unequal expression of EF-Tu in plants of different age was also observed in Arabidopsis (Gallardo et al., 2001) and maize (Momcilovic and Ristic, 2007). It is possible that differential expression of EF-Tu in young and mature wheat plants may, in part, be the result of different stress treatments, as the young plants experienced a short-term heat shock (43°C) and mature plants experienced prolonged exposure to high temperature (36/30°C, day/night).

The heat-induced accumulation of EF-Tu in mature plants implies that this protein may be of importance to heat tolerance in wheat. The mechanism by which wheat EF-Tu may confer heat tolerance is not clear. There is evidence that EF-Tu acts as a molecular chaperone and protects heat-labile proteins from thermal aggregation in prokaryotes (Caldas et al., 1998) and maize (Momcilovic and Ristic, 2004; Rao et al., 2004; Ristic et al., 2004). Also, our preliminary in vitro experiments showed that purified native wheat EF-Tu displays chaperone activity, as it protected Rubisco activase from thermal aggregation (Momcilovic et al., unpublished). We do not know if EF-Tu displays chaperone activity in wheat chloroplasts, and additional studies are needed to investigate this possibility. Furthermore, wheat EF-Tu may be involved in heat tolerance through its conventional function in polypeptide chain elongation (Riis et al., 1990; Nissen et al., 1995; Willson and Noller, 1998). Increase in the level of EF-Tu under heat stress may enhance the overall efficiency of protein synthesis and this, in turn, may have an effect on heat tolerance. Further studies are needed to investigate the mechanism of action of wheat EF-Tu in relation to heat tolerance.

In conclusion, the results of this study showed that heat stress induced accumulation of chloroplast EF-Tu in a flag leaf of mature plants of winter wheat. The heat-induced accumulation of EF-Tu was greater in a group of wheat cultivars that showed better tolerance to heat stress. The young and mature plants differed in the expression of EF-Tu under stress. The results support the hypothesis that chloroplast EF-Tu may play a role in heat tolerance in winter wheat.

Acknowledgments

The authors are grateful to Dr. Novica Mladenov and Dr. Radivoje Jevtić, Institute of Field and Vegetable Crops, Novi Sad, Serbia for generously providing seeds of cultivars of winter wheat. The authors are also thankful to Dr. Melvin J. Oliver, USDA-ARS, Columbia, MO, Dr. Janet P. Slovin, USDA-ARS, Beltsville, MD, Dr. Tom Cheesbrough, South Dakota State University, Brookings, SD, and Dr. Thomas E. Elthon, The University of Nebraska, Lincoln, NE for critical reading of the manuscript, and Dr. Mark S. West, USDA-ARS, Fort Collins, CO for his help on statistical analysis. This publication is approved as Kansas Agriculture Experiment Station No: 07-129-J. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products which may also be suitable.

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