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TEMPERATURE EFFECTS ON PROTEIN AND CARBOHYDRATES METABOLISM AND THE VEGETATION INDEX IN WHEAT (*Triticum durum* L.)

Efectos de la temperatura en el metabolismo de proteínas y carbohidratos y el índice de vegetación en trigo (*Triticum durum* L.)

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ABSTRACT. An experiment, under field conditions, in the Valle del Yaqui, Sonora, Mexico, was established which consisted of raising the temperature of the wheat at 2 °C above the ambient canopy temperature during the crop phenology to determinate warming effect on total soluble proteins (PST) and carbohydrates (CST) in stems and grain and the total soluble carbohydrates mobilization rate within the stem (TMC), and the NDVI. CIRNO C2008, a crystalline wheat variety was used as an experimental model. The PST, due to warming effect, decreased significantly in the heading phenophase in more than half of the Control treatment, aspect that denotes an early senescence. However, the CST content in the stem was higher in the heat treatment when it was determined 10 days after flowering. In the maturity there were no significant differences. The imposed heat did not affect the TMC. In the grain, heat did not affect the PST content but the CST content increased. The NDVI, due to heat effect, decreased from the stem elongation to maturity. The maximum value occurred, in the treatments, in flowering, demonstrating warming tolerance of the used variety to the occurrence of this phenophase. Such results suggest the feasibility of using CIRNO C2008 as a possible progenitor for breading in front heat stress.

Key words: heat, phenology, climate change

RESUMEN. Se estableció un experimento en condiciones de campo en el Valle del Yaqui, Sonora, México, que consistió en elevar la temperatura del trigo en 2 °C por encima de la temperatura ambiente del dosel durante la fenología del cultivo y determinar su efecto en los contenidos de proteínas (PST) y carbohidratos solubles totales (CTS) en tallos y en grano, la tasa de movilización de los carbohidratos (TMC) del tallo, y el índice de vegetación de diferencia normalizada (NDVI). Se utilizó como modelo experimental la variedad de trigo cristalino CIRNO C2008. El contenido de PST, por efecto del calor disminuyó significativamente en la fenofase de espigamiento respecto al tratamiento de Control, lo que denota una senescencia temprana. El contenido CST en el tallo fue mayor en el tratamiento de Calor cuando se determinó 10 días después de la floración. En la maduración no existieron diferencias significativas. El calor impuesto no afectó la TMC. En el grano, el calor no afectó el contenido de PST, pero sí se incrementó el contenido de CST. El NDVI, debido al calor, disminuyó a partir de la fenofase de elongación del tallo hasta la maduración. El máximo valor de NDVI ocurrió, en los tratamientos en la floración, demostrando tolerancia de la variedad al calor para la ocurrencia de esta fenofase. Tales resultados sugieren la factibilidad del uso de CIRNO C2008, como posible progenitor para programas de mejora genética ante el estrés térmico.

Palabras clave: calor, fenología, cambio climático

INTRODUCTION

The predicted climate change scenarios for recent years, according to IPCC reports, constitute a potential threat to the development of agriculture in various regions of the world (1).

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Mexico, due to its geographical location, has a great diversity of climates and ecosystems (2); however, arid and semi-arid climates are the ones that predominate, covering almost 50 % of their total territorial extension (3). In such climatic conditions the development of the vegetation, the microbial activity of the soil and the ecosystem is hindered by the negative incidence of abiotic factors, where the increase of the temperature has a marked incidence (4).

The current vulnerability of agriculture in these semi-arid regions of Mexico could be exacerbated by the effects expected from climate change scenarios, which mainly include the reduction of the number of cold hours of many crops and the wide and significant variability of temperatures, which will probably affect the physiological performance of crops, whose relationship between canopy temperature and yield is very close (5).

The Yaqui Valley in Sonora State is one of the regions with the greatest contribution to the national production of wheat in Mexico (6). This region will be impacted by climate change scenarios, where temperature increases of up to 2 °C are forecast for the next 10 years (7). There are some reports, based on parameterized predictive models that conclude that wheat will be one of the most affected crops. However, at the plant level, taking into account the genotype-environment interaction, the variability of the physiological, biochemical and agronomic performance of this species, in this region, is not fully described. Their detailed study, under field conditions will contribute to the understanding of physiological and biochemical mechanisms during their adaptation to climate change (8).

The morphology of plants is the reflection of complex physiological and biochemical processes that take place at the level of organs and tissues, which vary depending on the edaphoclimatic conditions existing in crop ecosystems (9). Slight variations in climatic conditions can cause a significant reduction in the rate of development of their organs, mainly leaf area and yield in plants (10). Thermal stress could then affect the chlorophyll content and modify the carbohydrate and protein metabolism of plants, as well as grain quality (11). The leaf surface of the plant intensely reflects the energy in the near infrared range (12). The reflectance is determined by the properties of the foliar tissues; that is, by its cellular structure and the interfaces between the outer cell wall, the protoplast and the chloroplast, which is indicative of its physiological state (9).

These anatomical characteristics of the plants once affected by stressors, such as heat, could cause variations in the values of vegetation index of normalized difference (13), so that their *in situ* evaluation offers reliable information on the nutritional status of the plant (14), which can be corroborated with the variations in the contents of total soluble proteins, as evidence of the nitrogen mineral nutrition during the development and in the grain and the content of total soluble carbohydrates in the stem and in the grain, as evidence of the efficiency of photosynthetic activity before the possible adverse effect of heat. In this sense, the present investigation was carried out with the objective of evaluating the effect of heating based on 2 °C above the ambient temperature of the culture canopy, on the contents of proteins (TSP) and total soluble carbohydrates (TSC) in stems and grain, the rate of mobilization of carbohydrates (TMC) of the stem, and the standardized vegetation difference index (NDVI).

MATERIALS AND METHODS

Location of experimental area

The experiment was carried out during the cultivation campaign from December 2016 to April 2017, under field conditions, at the Technology Transfer Experimental Center (CETT-910) of the Sonora Technological Institute (ITSON), located in the Valle del Yaqui. 27 ° 22’0.4 ‘’ N and 109 ° 54’50.6 ‘’ W (UTM: 607393.24 m E; 3027508.34 m N).

Treatments and temperature control

Two experimental variants were established, T1: increase of 2 °C at the ambient temperature of the crop canopy in the plots and T2: ambient temperature of the canopy in the control plots (control treatment). Such treatments were distributed following a completely randomized experimental design with 12 repetitions.

To raise the temperature of the canopy of the crop, from the 15 days after germination, six thermal radiators were used per plot (model FTE-1000, 1000 W, 240 V, 245 mm long x 60 mm wide, constructed by Mor Electric Company Heating Association Inc. Comstock Park, MI, USA), which were located on triangular equilateral structures of 5.2 m each (Figure 1).

Two radiators were installed on each side of the triangular structures forming a regular hexagon, which emitted heat until the temperature rose 2 °C above the ambient temperature of the canopy.

Figure 1. Top view of 12 repetitions of the triangular structure that was used to install the heat radiators, separated at a distance of 1.20 m from the canopy of the crop

The calculation area of each plot was a 3 m circle circumscribed in the regular hexagon formed by the six radiators (1). For thermal control, infrared temperature sensors (ITSR Apogee Instruments Inc., Logan, UT, USA) were installed directed at the center of each calculation surface, with an inclination angle of 45° from the horizontal surface of the soil . The ITSR sensors were coupled to a data logger (CR1000 Campbell Sci, Inc. Logan, UT, USA) that sends a voltage signal to an interface (MAI-05V, Avatar Instruments) which, in turn, translates the voltage signal to milliamps and sends it to a regulator (Attenuator A1P-24-30-S05, Avatar Instruments). This regulator controls the current sent to the heaters, so that the amount of heat emitted increases or decreases as a function of the temperature difference between the Heat and Control parcels, through the proportional, integrative and derivative routine described (15).).

AGROCLIMATIC VARIABLES

During the biological cycle of the crop, the average monthly temperature remained between 17 and 24 °C (average 18.6 °C). The monthly precipitation was less than 15.0 mm and the relative humidity varied from 50 to 63 % (Figure 2).

VARIETY USED

The wheat variety CIRNO C2008, classified as crystalline or hard wheat (*Triticum durum* L.). It originated from the selection in segregating populations of crossbreeding SOOTY-9 / RASCON-37 // CAMAYO, carried out in the International Center for the Improvement of Maize and Wheat (CIMMYT).

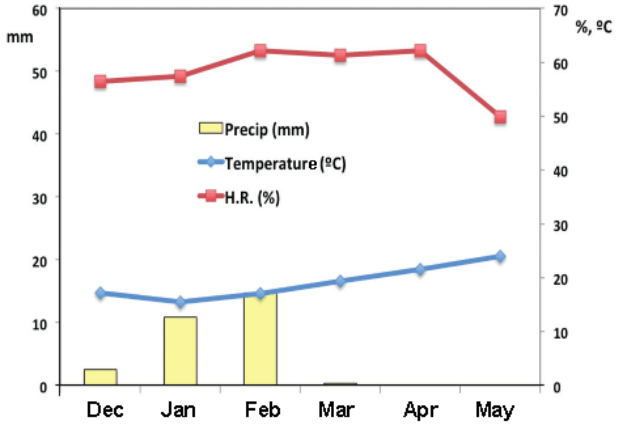

Figure 2. Climatic variables (temperature, precipitation and relative humidity) during the crop cycle December 2016-May 2017 in the experimental site

This variety was released for cultivation since 2008, being widely used in Mexico and particularly in Sonora. The grain yield when the variety was released in Sonora reached 5.6 t ha^{-1} and 6.3 t ha^{-1} with two and three irrigations, respectively (16).

SOWING, FERTILIZATION AND IRRIGATION

The sowing was carried out in a mechanized way with a seeder (SUB-24) on December 8, 2016, in a vertisol soil (17), three rows in the furrows and a planting density of 170 kg ha^{-1}. Background fertilization was carried out on the basis of 250 kg ha^{-1} of urea +100 kg ha^{-1} of monoammonium phosphate (MAP), 11-52-00, during the first irrigation (development phenophase).

Nitrogen fertilizer was applied at a dose of 50 kg ha^{-1} of urea in each applied one. In the three irrigation treatments were applied with an average lamina of water of 14 cm for each one and with a watering interval of 25 days.

CONTROL OF PESTS, DISEASES AND WEEDS

During the development of the crop, slight presence of foliar aphid (Schizaphis graminum) was found and the pesticide Muralla Max (ia Imidacloprid + Betaciflutrin) was applied at a rate of 0.20 L ha^{-1} in the periphery of the plots at a distance of 3 m from the calculation surface. Also, a slight presence of broadleaf weeds was observed and manually controlled before the irrigation.

VARIABLES EVALUATED

Content of total soluble proteins in the phenophases of tillering and flowering

A sample of 1.0 g of fresh foliar material was deposited in a mortar and macerated. The extraction was performed with 40.0 mL of a phosphate buffer solution at pH 6.86 prepared from potassium phosphate, a solution that was added slowly while the extraction was carried out (18). The content of total soluble proteins was quantified by absorption photometry in a Hewlett Packard 8452 spectrophotometer, at a wavelength of 750 nm.

The carbohydrate content was determined based on the dry mass of the last two upper internodes of the main stem (Ent2 and Ent3), plants of each treatment harvested 10 days after the beginning of the flowering phenophase (10 DAF) and in the phenophase of maturation (before harvest). Each of the internodes was divided into segments of 1-2 mm with a scalpel. Three repetitions per internode were processed, which were averaged to obtain a final sample for each treatment. For this trial three plants were taken per repetition of each treatment.

For the extraction of carbohydrates (19), three extractions were performed with 5 mL of ethanol (80 %) at 0.05 g of dry mass of the previously described stem segments (10 DAF and at maturity). The content of total soluble carbohydrates (mg g MS^{-1}) (MS: dry matter), was determined by the anthrone method (20). The absorbance was measured at a wavelength of 625 nm in a spectrophotometer (JENWAY 6405 UV / Vis) of the Technological Institute of Sonora.

After harvesting the samples and evaluating their biomass and grain yield, a random sample consisting of 100 grains of each replication was taken in both treatments, which was ground until a flour was obtained and the contents of proteins and soluble carbohydrates were determined again total in the grain following the same methodologies previously described.

The rate of mobilization of the total carbohydrates in the stem (TMC) (%), to the 10 DAF and the maturation, was calculated following the following formula (21):

$$TMC= [(A-B)\ (A)^{-1}] * 100$$

where: A and B represents the concentration of total carbohydrates (mg g MS^{-1}) at 10 DAF and at maturity, respectively.

NORMALIZED DIFFERENCE VEGETATION INDEX (NDVI)

The standardized difference vegetation index was measured with a portable sensor (Green Seeker, Trimble brand, from 15 days after germination, in each phenophase to harvest for a total of 11 measurements.) For this measurement an experimental variant was introduced without apply nitrogen fertilizer according to the sensor specifications, 10 measurements were taken in each repetition of treatment, at a height of 0.60 m from the crop canopy, according to the sensor reference manufacturer: -1 <NDVI> 1, higher NDVI values represent a better nutritional status (22).

STATISTICAL ANALYSIS

The fulfillment of the theoretical assumptions of homogeneity of variance (23) was checked and the means, their respective deviations and the standard error were determined. The contents of proteins and total soluble carbohydrates and the remobilization of carbohydrates in a total of six repetitions per treatment, as well as, the contents determined in the grain were compared by means of a theoretical distribution of probabilities for continuous quantitative variables of t-Student for a level of significance of 1 %.

For the processing of the NDVI data obtained in each phenophase, they were compared by means of a simple classification variance analysis based on a linear model of fixed effects (24) and when there were differences between the means these were compared by the multiple comparison test of Tukey for significance levels of 1% (25). The STATISTICA professional statistical package, version 8.4 for WINDOWS (26) was used for these analyzes.

RESULTS AND DISCUSSION

TOTAL SOLUBLE PROTEINS PHENOPHASES TILLERING AND TASSELING

The content of total soluble proteins was significantly increased in the control treatment in the spike phenophase; however, in the Heat treatment an opposite response was observed and its decrease in the spike phenophase, with respect to that of tillering, was 11.4% of concentration in the tillering phenophase (Figure 3).

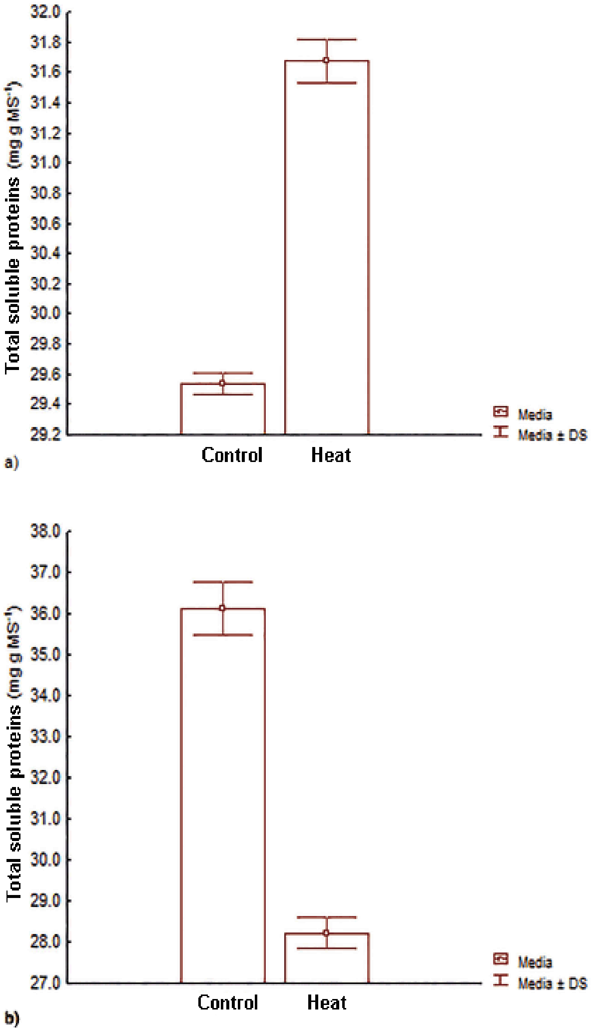

Figure 3. Foliar content of total soluble proteins phenophases of a) and b tillering) tasseling

The increase in total soluble proteins found in the Control treatment, in the spike phenophase, indicated the ability of the variety to accumulate metabolic reserves for the reproductive stage, where energy consumption and cellular dynamism are high (26). On the other hand, it was observed in the Heat treatment showed its adverse effect on the accumulation of total soluble proteins, perhaps favoring protein catabolism. In the spike phenophase, the metabolic integration between carbohydrates and proteins is high to ensure pollen quality fundamentally (27). Therefore, the decreases in the indicator values in the heat treatment were due to the accelerated hydrolysis of the proteins to their precursors to raise the concentration of free osmolytes in the cytosol and thus decrease their osmotic and water potential (28).

Under conditions of abiotic stress, mainly due to salinity and drought, in many plant species, first, there is an increase in the content of total soluble proteins (29), but these values are decreasing, depending on the intensity of the stress and the degree of stress and tolerance of the variety (30).

TOTAL SOLUBLE CARBOHYDRATES IN THE FLOWERING AND RIPENING PHENOFASES

The total carbohydrate content in the stem of the plants was higher in the Heat treatment when 10 DAF was determined; However, in the maturation phenofase there were no significant differences, but a reduction of its content was found between the phenofases in which it was evaluated (Table). The result found suggests a positive effect of the heat imposed on the accumulation of carbohydrates in the variety used as an experimental model. On the other hand, there were no differences in the mobilization rate demonstrating that the heat did not offer any biochemical impediment for this process.

There are reports on the adverse effect of heat on the mobilization of carbohydrates in other cereals (31) and in wheat (32). The present study constitutes the first report in the Yaqui Valley on the biochemical stability of carbohydrate metabolism in view of the increase in canopy temperature in wheat.

Table 1. Content of soluble carbohydrates 10 days after flowering and during the maturation of wheat. Carbohydrate mobilization rate

Treatments	Total soluble carbohydrate content (mg g MS^{-1})		Mobilization rate (%)
	10 DDF	Maduration	
Control	64,2 ± 0,86	1,85 ± 0,03	97,14
Heat	69,5 ± 0,26	1,8 ± 0,02	97,39
t-value	8,70	-2,40	
p	0,0001	0,07	

[arithmetic mean ± standard deviation]

CONTENT OF TOTAL SOLUBLE PROTEINS IN THE GRAIN

The content of total soluble proteins in the grain did not show significant variation due to the effect of the imposed heat, although, there was a tendency to decrease its values in the Heat treatment (Figure 4). This result demonstrated the possibility that the variety CIRNO C2008, transport protein sources and protein precursors to the fruit under stress conditions.

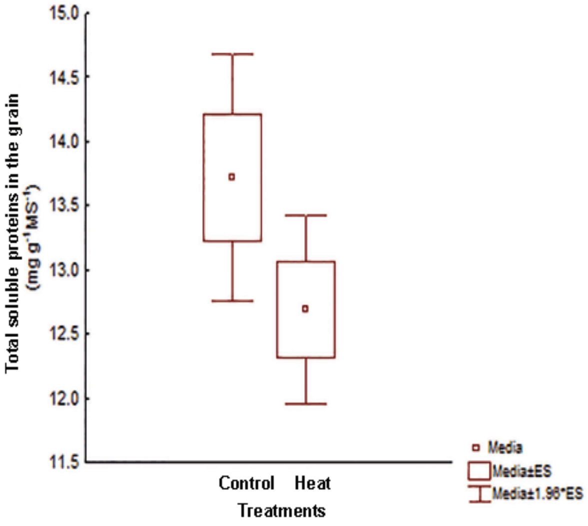

Figure 4. Content of total soluble proteins in the grain in the established treatments

This result was due to an increase in the activity of the enzymes nitrate reductase and glutamine synthase, favoring the accumulation and metabolism of nitrogen in the heat treatment (33). The protein concentration in the wheat grain (*Triticum estivum* L.) is one of the most important variables for the agricultural and industrial yield, because it is a determinant element of the grain price due to its nutritional value (34).

The protein in the wheat grain is the product of the accumulation of nitrogen absorbed by the plant during its vegetative stage until the grain is filled (35), and the final percentage is a direct relation of the nitrogen available from the plant and the yield of the plant culture (protein / carbohydrate ratio) (36). During the first phases of the filling, the moment of greater accumulation of proteins in the grain occurs and the speed of this will depend on the genotype, the simplistic availability of nitrogen and the ambient temperature (37)

CONTENT OF TOTAL SOLUBLE CARBOHYDRATES IN THE GRAIN

The highest content of total soluble carbohydrates was found in the Heat treatment and was significantly superior to the Control treatment (Figure 5). This result was due to an increase in photosynthetic activity of the crop. The capacity of some species and plant varieties to accumulate in significant adverse conditions amounts of soluble carbohydrates in the grain is a mechanism for the protection of gluten for subsequent germination (38).

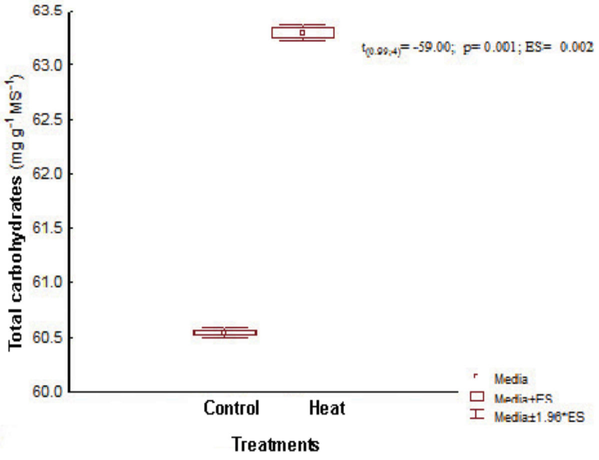

Figure 5. Concentration of total soluble carbohydrates in the grain in the established treatments

An important aspect in the evaluation of the quality of the grain is the concentration of soluble carbohydrates, given its effect during the processing for the preparation of pasta and flour (35). Crops that have been affected by abiotic stress, mainly water stress, reduce their yield and decrease grain quality (39,40). In this reduction of yield, the decrease in carbohydrate content in the grain has been more significant than the mobilization of total soluble proteins (41,42). However, in the present study, although it is about thermal stress, quality was not affected and the carbohydrate content increased.

NDVI

The NDVI in the Heat treatment was significantly superior to the Control and the non-fertilizer variant, from germination to tillering. However, from this phenophase and until the development of the nodes, the indicator did not show significant differences between treatments. From this phenophase the NDVI in treatment Control statistically exceeded the Heat treatment until the end of the crop phenology (Figure 6).

The maximum value of the polynomial curve described by the NDVI values (convex parable of negative quadratic coefficient) occurred in both treatments in the flowering phenophase. This result demonstrates the genetic stability of the variety evaluated for the occurrence of this phenophase (43), even in conditions of deficiency of nitrogen fertilizer and in the face of possible heat stress. The fact of having obtained in this phenophase the highest value of NDVI in the Control treatment, evidenced the good physiological and nutritive state of the plant and its favorable condition for the beginning of the reproductive stage.

The NDVI study, which has been correlated with nitrogen content and foliar chlorophyll in the cultivation of maize (*Zea mays*) (44) and wheat (45), showed that, although there was a decrease in spectral reflectance of the heat, the variety used as experimental model reached its maximum values in the phenophases of tillering, flowering and filling of the grain in the treatments, where high values were found content of proteins and total soluble carbohydrates.

The NDVI has been proposed as a predictive indicator of the photosynthetic activity of the plant, since it is determined mainly by the content and activity of the chlorophylls, which contributes to the carbonated nutrition and leads to the obtaining of a high content of carbohydrates in the grain (46).

The biochemical analyzes developed in the CIRNO C2008 wheat variety subjected to the increase of the temperature of the canopy of the crop in 2 °C from the 15 days after the germination, under field conditions, demonstrated that the heat does not exert significant effects on the accumulation of carbohydrates.

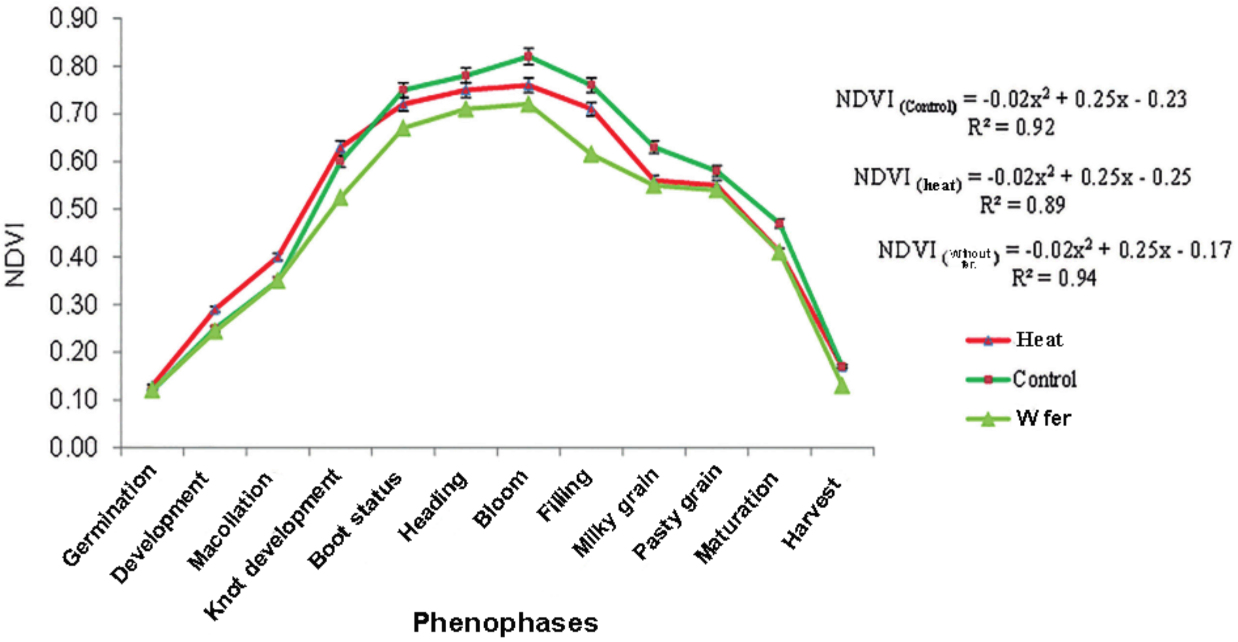

Figure 6. Normalized difference vegetation index during crop phenology in plants exposed to 2 ºC above room temperature, control plants and unfertilized plants

This result indicated minor affectations in the photosynthetic apparatus and in the nitrogen metabolism, although in the grain a tendency to decrease the content of total soluble proteins was observed. Such a result in the grain; however, it suggests a possible decrease in the quality of the seed and the industrial quality for the preparation of flour and pasta due to the increase in temperature during the phenology of the crop.

CONCLUSIONS

- The content of PST, due to the effect of heat, decreases in the spike phenophase in the stem and is not affected in the grains.
- The imposed heat increases the CST content in the stem 10 days after flowering and during maturation decreases as evidence of the occurrence of its mobilization towards the grains, while increasing the CST content in the grains.
- The NDVI, due to the effect of heat, decreases from the development phase of the knots to maturity. The maximum value occurs in this variety of wheat, in the established treatments, during flowering and the values describe a quadratic convex function that only differs in the vertex.

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