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Original scientific paper

CHARACTERIZATION OF VEGETATIVE AND GRAIN FILLING PERIODS OF WINTER WHEAT BY STEPWISE REGRESSION PROCEDURE.
I. VEGETATIVE PERIOD

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Modeling plant growth by mathematical functions is important for understanding plant development and growth. Most of the models of dry matter accumulation in small cereals simulated the period of grain filling while small attention has been devoted to mathematical simulation of vegetative period till anthesis. The aim of this research was to determine the most appropriate polynomial non-linear regression for dry matter accumulation till anthesis in winter wheat. Pobeda, a medium early variety, was used as model genotype for this research. A 5-year field data were analyzed by the forward procedure of stepwise regression. Although the procedure requires the maximum power of the polynomial regression to be used, we suggest using a lower power since it is easier for understanding and explanation and it is taking into account literature sources and

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biological laws. It can be accepted that quadratic regression model appropriately fits the process of dry matter accumulation till anthesis in winter wheat.

Key words: dry matter accumulation, model, stepwise regression, vegetative period

INTRODUCTION

Anthesis is the central phenological stage with regard to yield and yield components formation. The spike number per unit area is established during tillering and the kernel number per spike during stem elongation, i.e., one of the principal yield component, the number of kernels per unit area, is determined before anthesis. Changing the duration of the phases before anthesis by means of breeding, but without modifying the total time to anthesis, could be suggested as a prospective breeding tool (BORRÀS *et al*., 2009). The second yield component, kernel weight, is determined after anthesis, during grain filling.

It is accepted that grain number and grain weight develop under independent environments in the case of both wheat (MCMASTER, 2005) and barley (ARAUS, 2002). However, the amount of dry matter accumulated during vegetative period is an important prerequisite for yield, especially when stress conditions occur during grain filling (PRŽULJ and MOMČILOVIĆ, 2001a; 2001b; KUMAR *et al*., 2006; DORDAS, 2009).

Modeling of plant growth is crucial in understanding plant development and growth and prognosticating the length of certain phenological phases (WHITE, 2006). Description of plant development by mathematical functions can help producers to predict time of specific phenological phases. Field crop practices, e.g., fertilization and pest control, could be efficiently scheduled on the basis of the growth model (STRECK *et al.,* 2003).

Most of the models that have been developed simulated grain filling in small cereals, linear, quadratic, cubic, and logistic and its different forms (PRŽULJ, 2001; YIN *et al*., 2003; ZAHEDI and JENNER, 2003). To our best knowledge, little attention has been devoted to mathematical simulation of the vegetative period.

The objective of this study was to suggest the most appropriate polynomial regression for describing dry matter accumulation in the period before anthesis. The medium early cultivar Pobeda has been used as model cultivar in this research.

MATERIALS AND METHODS

Experimental design: A 5-years field trial was conducted under rainfed conditions at Institute of Field and Vegetable Crops in Novi Sad (45^{o}20'N, 15^{o}51'E, altitude 86 m), on the calcareous chernozem soil, during 2002/03-2006/07 growing seasons. Three winter wheat cultivars, Prima (early-maturing), Pobeda (medium-maturing), and Diplomat (late-maturing) were used in the investigation. The cultural practices applied were those regularly used in large-scale winter wheat production. Experimental plots were 10 m long and consisted of 6 rows 20 cm apart. To avoid confounding effects of diseases and wrong results and conclusions, the trial were sprayed with the fungicide Tilt 250 EC at Zadoks phase 34, with the fungicide Artea plus at Zadoks phase 64 and with the insecticide Karate zeon against *Lema melanopus.*

Measurements: Plant samples were taken at 2-4 day intervals from the emergence till anthesis. The samples that consisted of 3 plants per plot, taken from second and third rows, were collected in plastic bags, weighed in the lab and oven-dried at 80°C for 48 hours till constant weight. In the 2002/03 growing season, samples were collected 14 times, in 2003/04 22 times, in 2004/05 11 times, in 2005/06 9 times, and in 2006/07 17 times. Accumulated growing-degree days (GDD) were used as the time scale. The GDD was calculated as GDD=$\Sigma(T_n-T_b)$, where T_n was daily mean air temperature and it was calculated as $T_n = (T_7 + T_{14} + 2T_{21})/4$. T_7, T_{14}, and T_{21} were temperatures measured at 7am, 14pm, and 21pm, respectively. The base temperature (T_b) used in this study was 0°C (PRŽULJ, 2001; SANTIVERI *et al.*, 2002). When T_n was less than T_b, T_n was supposed to be 0°C.

Statistical analysis. For statistical analysis a special type of regression, that concerns a polynomial expression, was used:

$$Y_i=\alpha+\beta_1X_i+\beta_2 X_i^2+\beta_3 X_i^3+\ldots+\beta_mX_i^m+\varepsilon_i$$

and a model with parameters estimated in the expression:

$$\hat{Y}_i=a+b_1X_i+b_2 X_i^2+b_3 X_i^3+\ldots+b_mX_i^m$$ (ZAR, 1999).

If $m=1$, then the polynomial regression is reduced to a simple linear regression. The maximum power (m) of the polynomial that has statistical significance must be determined that fits a polynomial regression. A stepwise regression procedure was used for determination of maximum power, m, where maximum power should not be greater than $n-1$ if a polynomial is to fit the data, and, more practically, not greater than $n-2$ if statistical analysis is to be performed on the resulting polynomial fit (Zar, 1999). In this work the “forward selection” procedure for fitting the polynomial model was used. This model starts by fitting a linear regression ($\hat{Y}_i=a+bX_i$) to the data, and after that a second degree polynomial is fitted to the data. Quadratic equation ($\hat{Y}_i=a+b_1X_i+b_2X_i^2$) is made by adding quadratic term ($b_2X_i^2$) to the simple regresion. To test whether the added quadratic term improves the precision of *Y* values prediction, the *t* test is applied. If the *t* test does not reject the null hypothesis, $H_0 : \beta = 0$, that means that the simple regression model characterizes the relationship between Y and X, i.e., the quadratic term contributes insignificantly to this characterization. If the null hypothesis is rejected, a fit is considered with a third-degree polynomial, which is made by adding a cubic term ($b_3X_i^3$). This procedure of adding higher terms ($b_4X_i^4$, $b_mX_i^m$) and testing the null hypothesis fit continues untill it is accepted, which means that the polynomial with m-1 term is the best model.

RESULTS AND DISCUSSION

Our aim was to determine the maximum power of the polynomial that has statistical significance. When linear regression is fitted to the data and the null hypothesis is rejected, it means that linear regression cannot properly describe the amount of dry matter accumulated till anthesis (Table 1, Figure 1a).

Table 1. ANOVA of linear regression for dry matter accumulation till anthesis in the winter wheat variety Pobeda in the 2002/03 growing season

Source of variation	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	F-test	p
Linear regression	78.030	1	78.030	102.64*	0.000
Error	9.123	12	9.123		
Total	87.153	13			
a=-3,23327	b=0,00781	s_b=0,00077	t_b=10,131	$t_{(0,05;\ 12)}$=2,179 $t_{(0,01;\ 12)}$=4,318	

Biological concepts and literature references suggest that a non-linear relationship exists between GDD and accumulated dry matter (PRŽULJ, 2001; ZAHEDI and JENNER, 2003). Based on previous experience and biological concepts, it seems that quadratic and cubic functions are preferred because the regression parameters can be easily associated with some known biological phenomena and their biological implication is easily identified (Figure 1b,c).

Table 2. ANOVA of quadratic regression for dry matter accumulation till anthesis in the winter wheat variety Pobeda in 2002/03 growing season

Source of variation	SS	DF	MS	F-test	p
Linear regression	1.168	1	1.168	12.029*	0.005
Quadratic regression	8.055	1	8.055	82.969*	0.000
Error	1.068	11	0.097		
Total	10.291	13			
a=0.77094	b_1=-0.00495 b_2=0.000009	s_{b1}=0.00143 s_{b2}=0.000001	t_{b1}=-3.46827 t_{b2}=9.10874	$t_{(0.05;\ 11)}$=2.201 $t_{(0.01;\ 11)}$=4.637	

However, since the *p* value for both quadratic and cubic regression is lower than 0.05 for α=0.05, it means that the null hypothesis is rejected, i.e., in accordance with the stepwise regression analysis, the fit is tested with the next, quatric regression.

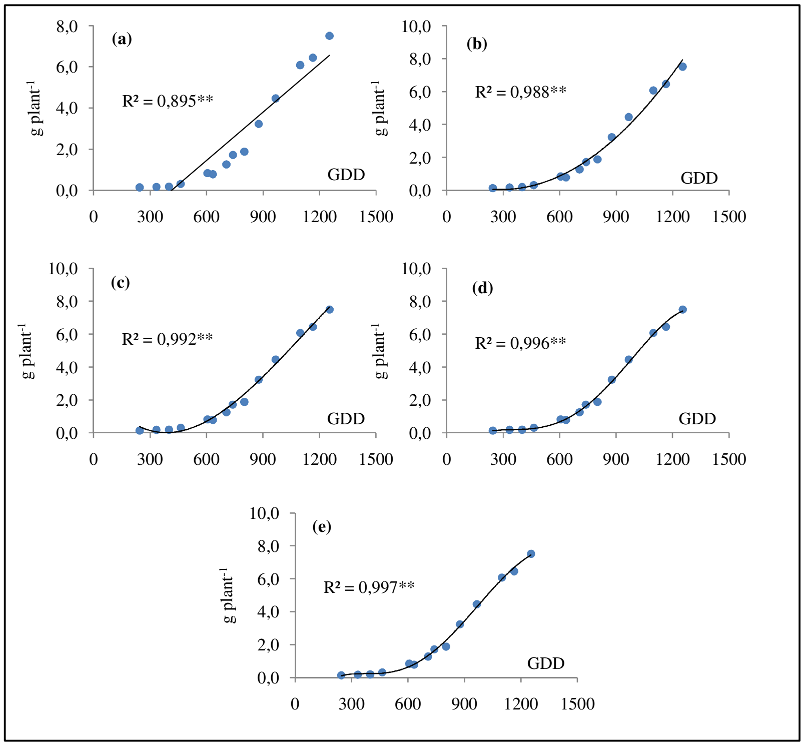

Figure 1. Fitting polynomial regression models for vegetative dry matter accumulation till anthesis in winter wheat. (a) Linear: $\hat{Y}$=-3.23327+0.00781x. (b) Quadratic: $\hat{Y}$=0.77094-0.00495x+ 9E-6x^2. (c) Cubic: $\hat{Y}$=2.98876-0.016589x+ 26E-5x^2+ 1E-8x^3. (d) Quartic: $\hat{Y}$=-1.63738+0.01632x- 5E-5x^2+ 7E-8x^3- 25E-11x^4. (e) Quintic: $\hat{Y}$=- 3.66627 +0.03484x- 11E-4x^2+ 16E-7x^3- 92E-9x^4+02E-14x^5.

Table 3. ANOVA of cubic regression for dry matter accumulation till anthesis in the winter wheat variety Pobeda in 2002/03 growing season

Source of variation	SS	DF	MS	F-test	p
Linear regression	0.777	1	0.777	11.745*	0.006
Quadratic regression	0.889	1	0.889	13.445*	0.004
Cubic regression	0.406	1	0.406	6.142*	0.033
Error	0.662	10	0.066		
Total	2.734	13			
a=2.98876	b_1=-0.016589	s_{b1}=0.004841	t_{b1}=-3.42708		$t_{(0.05; 10)}$=2.228
	b_2=0.000026	s_{b2}=0.000007	t_{b2}=3.66669		$t_{(0.01; 10)}$=4.587
	b_3=0.1x10^{-7}	s_{b3}=0.031x10^{-7}	t_{b3}=-2.47839		

In the four-power regression, the linear and quadratic terms fit the data but the cubic and quartic do not, so the null hypothesis for those terms is rejected (Table 4). Then, the quintic regression fits the data that shows no difference between experimental and predicted values, suggesting an acceptance of the null hypothesis (Table 5). Also, all experimental *t* values (t_{b1}-t_{b5}) were lower than the theoretical ones (Table 5).

Therefore, according to the stepwise regression analysis theory, the quartic polynomial is the optimum regression function for vegetative dry matter accumulation till anthesis in winter wheat Pobeda (Figure 1d). ZAR (1999) suggested adding one more term to the last one, i.e., to test the fit of the sextic polynomial regression to the data. In this paper, this was not done.

Table 4. ANOVA of quartic regression for dry matter accumulation till anthesis in the winter wheat variety Pobeda in 2002/03 growing season

Source of variation	SS	DF	MS	F-test	p
Linear regression	0.078	1	0.078	2.260	1.187
Quadratic regression	0.150	1	0.150	4.376	0.066
Cubic regression	0.280	1	0.280	8.157*	0.019
Quartic regression	0.352	1	0.352	10.246*	0.011
Error	0.309	9	0.034		
Total	1.169	13			

a=-1.63738	b_1=0.01632	s_{b1}=0.010858	t_{b1}=1.50334	$t_{(0.05;\,9)}$=2.262
	b_2=-0.00005	s_{b2}=0.000025	t_{b2}=-2.09191	$t_{(0.01;\,9)}$=4.781
	b_3=0.7x10^{-7}	s_{b3}=0.2x10^{-7}	t_{b3}=2.85609	
	b_4=-0.25x10^{-10}	s_{b4}=0.08x10^{-10}	t_{b4}=-3.29400	

A simple equation is preferred to characterize and model the duration and final weight as determinants of growth processes (YIN *et al*., 2003). Total duration of the life cycle of an organ, a plant or a crop can be divided into three sub-phases: first - an early accelerating phase, second - a linear phase and third- a saturation phase for ripening (GOUDRIAAN and VAN LAAR, 1994). That means that the growth pattern of total life cycle follows a sigmoid curve and the rate of growth a bell-shaped curve (YIN *et al.,* 2003). The sigmoid curve consists of three parts that can be presented by an exponential, a linear and a convex equation sequentially. Since we investigated a part of an unfinished biological process, i.e., dry matter accumulation from emergence till anthesis, where dry matter accumulation continues beyond anthesis, it is clear that the period before anthesis can be determined as a combination of the exponential and linear sub-phases.

The coefficient of determination (R^2) indicates that <90% of total variance was explained by the linear model and >90% by non-linear models in the 2002/03 growing season (Figure 1). In the other growing seasons, the applied non-linear models explained >94% of the total variance (Figure 2).

Table 5. ANOVA of quintic regression for dry matter accumulation till anthesis in the winter wheat variety Pobeda in 2002/03 growing season

Source of variation	SS	DF	MS	F-test	p
Linear regression	0.036	1	0.036	0.955	0.357
Quadratic regression	0.036	1	0.036	0.972	0.353
Cubic regression	0.032	1	0.032	0.866	0.379
Quartic regression	0.021	1	0.021	0.566	0.473
Quintic regression	0.011	1	0.011	0.300	0.599
Error	0.298	8	0.037		
Total	0,434	13			

a=-3.66627	b_1=0.03484	s_{b1}=0.035647	t_{b1}=0.977412	$t_{(0.05;\,8)}$=2.306
	b_2=-0.00011	s_{b2}=0.000115	t_{b2}=-0.986187	$t_{(0.01;\,8)}$=5.041
	b_3=1.6x10^{-7}	s_{b3}=1.73x10^{-7}	t_{b3}=0.930968	
	b_4=-0.92x10^{-10}	s_{b4}=1.22x10^{-10}	t_{b4}=-0.752352	
	b_5=0.2 x10^{-13}	$s_{b4=}$0.3 x10^{-13}	t_{b5}=0.547789	

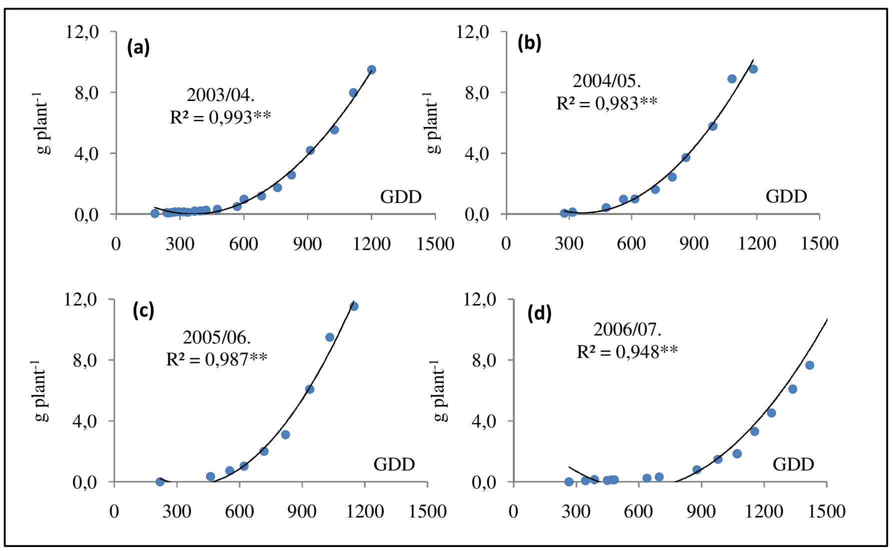

Figure 2. Optimum regression functions for vegetative dry matter accumulation till anthesis in winter wheat in the four growing seasons. (a) 2003/04: Ŷ=0.38125-0.00139x-1E-6x^2 + 7E-9x^3. (b) 2004/05: Ŷ= 2.058549- 0.010904x + 15E-5x^2. (c) 2005/06: Ŷ= 2.058549-0.010904x+0.000015x^2. (e) 2006/07: Ŷ=4.25139-0.01587x+13E-5x^2.

Although stepwise analysis select quadratic function, which fits best the data of dry matter accumulation, the plotted line did not simulate well the early dates, especially in the 2005/06 and 2006/07 growing seasons (Figure 2c, d). It is not possible to have a negative accumulation of dry matter in the period of the beginning of tillering in November. According to literature data and biological laws, the value of *a* coefficient should be just above zero, so the value of the *a* coefficients in the quadratic model for the 2002/03 season (Figure 1b) and in the cubic model for the 2003/04 season could be accepted, while in the other three seasons they are not acceptable (Figure 1a, c, d, e; Figure 2b, c, d). Regardless of the *a* value, some plotted curves matched the observed data quite well (Figure 1d, e; Figure 2a, b). WANG end ENGEL (1998) obtained scattered values for the early stages and poor simulation by the curve.

It was previously pointed out that our task was to use the maximum power of the polynomial that has statistical significance in order to present dry matter accumulation in winter wheat. It is obvious that the strict application of the stepwise regression procedure, without taking into account the previous experience and biological concepts of dry matter accumulation, generates results that should be critically scrutinized. In the 2002/03 growing season, the stepwise analysis suggested the use of the quartic regression, although the quadric curve fitted the data quite well, with a high value of the coefficient of determination, so the quadric curve can be used as model instead of the quartic one (Figure 1b). MONTGOMERY and PECK (1992) suggested using polynomial regression with lower power since X_i correlated with powers of X_i (i.e., X_i^2, X_i^3) in polynomial regressions with higher powers.

There are unequivocal reports that grain number in wheat and barley is diminished by high temperature during the pre-anthesis period. SINCLAIR and JAMIESON (2006) mentioned that grain number in wheat is determined 20 days before anthesis, and that grain number per spike is determined from 20 days before anthesis till 10 days after anthesis. UGARTE *et al.* (2007) found that high temperature during stem elongation decreased the yield by 46%, during booting-anthesis by 27% and during heading-anthesis by 15%.

Although it is not a direct topic of this investigation, the effect of dry matter accumulation before anthesis on grain weight should be mentioned in this paper. Many authors demonstrated the importance of dry matter accumulated before anthesis for yield level (PRŽULJ and MOMČILOVIĆ, 2001a; KUMAR *et al.*, 2006; DORDAS, 2009). The environment before anthesis also influences grain weight, although it is actually determined after anthesis, during grain filling. UGATE *et al.* (2007) showed that high temperature before heading, *i.e.*, before pollination, decreased the grain weight of bread wheat. CALDERINI *et al.* (1999) and CALDERINI and REYNOLDS (2000) found that the effect of high temperatures on the spikes for a few days before anthesis and during booting and anthesis decreased grain weight in wheat. Pre-anthesis temperature directly affected the growth of florets and indirectly the grain weight (CALDERINI *et al.*, 2001). High temperature before anthesis reduced the size of floret cavity in wheat and indirectly grain weight, since a positive relationship exists between floret cavity and grain weight (UGATE *et al.,* 2007).

Carpel weight and grain weight are in positive relation and carpel weight reduction by high temperature before anthesis decreases the grain weight in barley and wheat (CALDERINI *et al.*, 1999: CALDERINI and REYNOLDS, 2000).

CONCLUSION

The mathematical model for dry matter accumulation till anthesis depended to some extent on the year, so that different regressions can fit the data for the same genotype grown in different environments. Nevertheless, the quadratic model can be suggested as appropriate for describing dry mater accumulation till anthesis. The value of the *a* coefficient should be accepted with reserve.

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ANALIZA VEGETATIVNOG PERIODA I PERIODA NALIVANJA ZRNA KOD OZIME PŠENICE HIJERARHIJSKIM MODELOM VIŠESTRUKE REGRESIJE.
I. VEGETATIVNI PERIOD

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I z v o d

Modelovanje rasta biljke matematičkim funkcijama važno je za razumevanje razvoja i rasta biljaka. Većina modela akumulacije suve materije kod strnih žita simuliraju period nalivanja zrna, dok je malo pažnje posvećeno matematičkoj simulaciji perioda do cvetanja. Cilj ovog istraživanja bio je da se odredi najprikladnija funkcija za simuliranje akumulacije suve materije do cvetanja ozime pšenice hijerarhijskim modelom višestruke regresije (stepwise polynomial regression). U istraživanju je korišćena srednje rana sorta Pobeda. Hijerarhijski model korišćen je za analizu petogodišnjih rezultata. Iako procedura zahteva primenu polinoma sa najvišim odgovarajućim stepenom, predlažemo da se koristi niži stepen, koji ipak adekvatno opisuje proces nakupljanja suve materije do cvetanja. Može se zaključiti da kvadratna regresija opisuje na prihvalji način proces nakupljanja suve materije do cvetanja ozime pšenice.

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