

Assessing genetic variation for canopy temperature in wheat by linear mixed models

By

Mulatya Caroline Munindi

Internal Supervisors:

Mevrouw Annouschka Laenen

Mevrouw Liesbeth Bruckers

External Supervisors:

Prof. dr. Fred van Eeuwijk

Mr. Marcos Malosetti

Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Biostatistics

(2007 - 2008)

Certification

This is to certify that this report was written by Caroline Munindi Mulatya under our supervision.

Date.....

Mevrouw Annouschka Laenen

Internal Supervisor

Date.....

Mevrouw Liesbeth Bruckers

Co-Internal Supervisor

Date.....

Prof. dr. Fred van Eeuwijk

External Supervisor

Date.....

Mr. Marcos Malosetti

Co-External Supervisor

Date.....

Caroline Munindi Mulatya

Student

September 2008

Acknowledgement

I thank almighty God for granting me VLIR (Vlaamse Interuniversitaire Raad) scholarship to fulfil my childhood dream of becoming a professional biostatistician.

Sincere appreciation goes to my supervisors from University of Hasselt, Belgium and Wageningen University, Netherlands for their guidance and support during this study. Special thanks to Annouschka Laenen for her advice during preparation of this report as well as for the time spent going through my initial drafts and providing comments and points for improvement.

I would like to thank my fellow students and the whole team of Center for Statistics at the University Hasselt for their support and encouragement during the entire time of my studies in Belgium. Lastly, but not the least, I thank my family especially my mother, Grace Kalunda Mulatya for her spiritual and moral support during my studies.

Caroline M. Mulatya
September 2008

TABLE OF CONTENTS

Acknowledgement.....	iii
Abstract	vii
1 Introduction	1
1.1 Background	1
1.2 Organization of the report	2
1.3 Research Questions	3
1.4 Data and variables' Description	3
2 Methodology	5
2.1 Exploratory Data Analysis	5
2.2 Linear Mixed – Effects Model	5
2.2.1 Fitted Models for the five trials.....	6
2.2.2 Estimation and Inference on Random Effects	8
2.3 Marginal Testing for the Need of Random Effects	8
2.4 Software	9
3 Results.....	10
3.1 Descriptive Statistics	10
3.2 Individual Profiles, Mean, Variance and Correlation Structures.....	12
3.2.1 Individual profiles using standardized residuals	12
3.2.2 The Mean Structure	13
3.2.3 The Variance Structure using raw residuals.....	14
3.2.4 The Correlation Structure using standardized residuals.....	14
3.3 Implications of Exploratory Results to Statistical Modelling	15
3.4 Model Building.....	16
3.4.1 Selection of preliminary mean structure.....	16
3.4.2 Selection of preliminary random-effects structure	16
3.4.3 Selection of a Residual Covariance Structure	16
3.4.3 Test for need of heterogeneous residual variance per stages of growth.....	17
3.5 Model Reduction	18

3.5.1 Reduction of preliminary random-effects structure.....	18
3.5.2 Reduction of preliminary mean structure.....	20
3.5.3 Parameter estimates of fixed effects.....	20
3.6 Bayes Estimates of Random Intercepts.....	25
3.6.1 Histograms of random intercepts.....	25
3.6.2 Scatter plots of random intercepts across the trials.....	26
4 Discussion and Conclusion.....	28
5 Recommendations.....	30
6 References.....	31
7 Appendices.....	I

LIST OF TABLES

Table 1: Description of the five environments.....	4
Table 2: Summary Statistics of Canopy Temperature per Environment.....	10
Table 3: Summary Statistics of Canopy temperature against Stage of growth.....	10
Table 4: Summary Statistics of Canopy Temperature against the day time.....	11
Table 5: Test for the need of heterogeneous residual covariance per stage.....	18
Table 6: Results from models to test significance of random intercepts.....	19
Table 7: Hypothesis testing for the need of random effects.....	19
Table 8: Parameter Estimates of fixed effects with associated standard errors trial D02.....	21
Table 9: Parameter Estimates of fixed effects with associated standard errors trial D05.....	23
Table 10: Parameter Estimates of fixed effects with associated standard errors trial H05.....	23
Table 11: Parameter Estimates of fixed effects with associated standard errors trial H06.....	24
Table 12: Parameter Estimates of fixed effects with associated standard errors trial I06.....	24

LIST OF FIGURES

Figure 1: Individual Profiles using standardized residuals for trial D05 and I06.....	12
Figure 2: Mean Profile of Canopy Temperature for trial D05 and I06.....	13
Figure 3: Variance plot by raw residuals for trial D05 and I06.....	14

Figure 4: Scatter plots of standardized residuals for trial D05 and I06.....	15
Figure 5: Histograms of Bayes Estimates trial D02 and D05.....	25
Figure 6: Histograms of Bayes Estimates trial H05 and H06.....	26

Abstract

One way to enhance wheat farming in stressed environments is to develop genotypes that can tolerate unfavourable conditions like drought and heat. To achieve this goal, advanced plant breeding programs have been set up. In plant breeding and selection for drought resistance the interest is in finding genotypes that maintain lower canopy temperature as compared with other genotypes under the same field conditions. The main objective of this study was to assess if there exists significant genetic variation among genotypes exposed to similar environmental conditions. Specific objective was to study the evolution of canopy temperature of the genotypes over time. The dataset contained measurements of canopy temperature taken over time from genotypes exposed to five different environments/ trials. The dataset was explored by observing the individual profiles, mean, variance and correlation structures. The individual profiles indicated presence of large within variability as compared to between variability. The profiles showed large differences in measurements taken in the morning and in the evening from the same genotype. This could be due to difference in air temperature which needs to be corrected for in analysis. The mean structures suggested need to include linear, quadratic and cubic time effects in the models. The variances were not constant over time which suggested use of unstructured covariance structures. The correlation between measurements within a genotype was low in most of trials. The statistical analysis was done by use of linear mixed effects models. The need for genotype specific intercepts was tested and found significant except in trial I06. In this trial, the estimated variance of random intercepts was negative. Since the covariance matrix was not positive definite the model interpretation for this trial was done at marginal level only. At both genotype – specific level (omitting trial I06) and trial (population) level, the following covariates were found significantly associated with the evolution of canopy temperature: Anthesis in all trials, stage of growth in all trials except in trial D05, time of the day when the measurements were taken in all trials except in I06 and finally, Rye in trial H05, H06 and I06. In conclusion, there was significant genetic variation among genotypes exposed to similar environmental conditions. Genotype specific intercepts implied presence of genetic variation among genotypes. This means the genotypes performed differently within the trials. In addition, the evolution of canopy temperature changed over time and it depended on the anthesis, stage of growth and the time of the day when the measurements were taken in most of trials.

Keywords: Wheat genotypes, Canopy temperature, Multi-environmental trials, Longitudinal data analysis, Linear Mixed Model

1 Introduction

1.1 Background

Wheat is one of the most important food crops in the world. It is believed to have originated from Middle East, in the area called Mesopotamia now part of Iraq. Nowadays, it is grown all over the world, with United States, Canada, European Union and Australia being the major four exporters in the world trade [7]. Wheat is found in different varieties though, all these fall in two major categories based on growing season; that is, *winter wheat* and *spring wheat*. Winter wheat is planted in the fall and is harvested the next spring while spring wheat is planted in cold areas in the spring and harvested in summer [7].

Generally, wheat grows best in dry, mild climates. Climates that are too hot or too cold ruin the crop which leads to poor yields posing a challenge in wheat farming. One way to enhance wheat farming in stressed environments is to develop genotypes that can tolerate unfavourable conditions like drought and heat. A physiological approach would be the most attractive way to develop new varieties rapidly (Turner and Nicolas, 1987), but breeding for specific, suboptimal environments involves a deeper understanding of the yield-determining process [7]. This is where knowledge of crop responses to water deficits may be best put to use.

One way to quantify wheat water stress response is by use of canopy temperature (CT). Canopy temperature is the temperature of plants and/or the vegetative cover. It is often used to indicate vegetative water status and is used in models for estimating transpiration rates and sensible heat transport from vegetation. Ehrler *et al.* (1978) reported that the canopy temperature provided a good indication of the plant water potential of wheat when comparing environment with varying degrees of water stress. Canopy is often considered to be the outer surfaces of the vegetation. Plant height and the distribution, orientation, and shape of plant leaves within a canopy influence the atmospheric environment and many plant processes within the canopy.

Advanced plant breeding programs have been set up to develop wheat genotypes that can thrive under stressed environments. In plant breeding and selection for drought resistance the interest is in finding genotypes that maintain lower canopy temperature as compared with other genotypes under the same field conditions. Relatively lower canopy temperature in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture

and for maintaining a relatively better plant water status. This capacity, as expressed in relatively lower canopy temperatures, is believed to be correlated with final yield under stress or other parameters drought resistance in terms of yield such as various plant yield indices under stress. Canopy temperature is also believed to be negatively correlated with relative water content (RWC) across diverse wheat genotypes. Canopy temperature is also affected by the relative amount of desiccated and dead leaf in the canopy and thus is positively correlated with ‘leaf death score’ which is a visual rating of drought stress [11].

In this study, we apply the concept of longitudinal data analysis to assess if there exists significant genetic variation among genotypes exposed to similar environmental conditions. The focus is on the bread wheat (*Triticum aestivum*) whose genotypes are being experimented in the International Maize and Wheat Improvement Center (CIMMYT) [12]. The general objective of the CIMMYT wheat breeding program is to develop improved wheat varieties for farmers in developing countries, with special attention on the development of wheat varieties for production areas where environmental factors are limiting due to, for example, drought stress, high temperatures, etc.

1. 2 Organization of the report

The report is organized into seven sections. Section one introduces the background of wheat plants and advanced plant breeding programs, research questions and data description. Section two presents the methodology and the software applied in the analysis. In section three, we present the results of the analysis. Discussion and conclusion from the results are presented in section four. Recommendations, references and appendices follow thereafter.

1.3 Research Questions

The main objective of this study was to assess if there exists significant genetic variation among genotypes exposed to similar environmental conditions. Specific objective was to study the evolution of canopy temperature of the genotypes over time. The genotypes were exposed to five different environments and their canopy temperature measured over time. The two objectives were studied correcting for some factors (covariates) which will be discussed under the data description section. The objectives were explored per environment since the main interest was to compare genotypes exposed to similar environmental conditions over time.

1.4 Data and variables' Description

The dataset consists of 165 genetically distinct wheat plants from CIMMYT wheat program located in Mexico. In this population of genotypes, for a particular wheat genotype, identical copies could be created that were used across the different field trials. Thus, there existed a factorial structure of genotypes by environments for the data, with for each genotype by environment combination a longitudinal series of canopy temperature measurements.

The genotypes (wheat plants) were evaluated in five different environments (field trials). The trials were conducted in different years and under different water and temperature regimes. In each basic unit of observation, that is field plot, canopy temperature of genotypes was measured. This was measured on a number of days throughout the crop cycle. CT was measured by walking through the plots in a serpentine fashion, making sure that the angle of the measurement was the same across plots. On some days, the CT readings were taken twice, in the morning (am) and in the afternoon (pm). Table 1 presents a description of the trials and the number of measurements taken per genotype.

Table 1: Description of the five environments

Field trial	Regime	Year	Measurements taken
DRIP02	Drought	2002	6
DRIP05	Drought	2005	9
HEAT05	Heat	2005	9
HEAT06	Heat	2006	10
IRRI06	Irrigation	2006	5

Canopy temperature was the variable of interest (response) variable. Other variables recorded were: Time of the day when the measurements were taken (morning or evening), the stage of growth (vegetative or grain filling), anthesis (flowering period of plants) and rye which is a grass closely related to wheat. Oftenly, rye cross breeds with wheat plants during pollination which results to some parts of wheat chromosome having rye DNA. Presence of rye DNA in wheat chromosome is contrasted with absence (pure wheat).

2 Methodology

2.1 Exploratory Data Analysis

The statistical tools employed in this section are mainly descriptive statistics and graphical illustrations like box plots and scatter plots. In addition, average evolution (mean structures) and individual profiles were plotted to gain more insight into the dataset. The individual profiles were plotted using standardized residuals to see how genotype varies within itself (within variability) and between genotypes (between variability) in the same trial. The mean structures were plotted to investigate how in average the genotypes changed overtime in each trial. The variance structures were investigated by plotting raw residuals as a function of time. The residuals were obtained from a regression model after correcting for time effect and any other relevant covariates. Pairwise correlation coefficients were examined to get insight regarding the correlation structure. The structure was further examined by the use of scatter plots obtained from standardized residuals [5]. Further exploration was done by use of scatter plots of raw residuals. The residuals were from linear mixed model with a specified preliminary mean structure and random-effects.

2.2 Linear Mixed – Effects Model

The canopy temperature was measured over a period of time across the two stages of crop growth. This implies that, the data in hand is longitudinal hence appropriate techniques should be used for analysis. To account for these features, linear mixed –effects models were fitted on the dataset.

The linear mixed (effects) model is a generalization of the standard linear model where data are allowed to exhibit correlation and nonconstant variability. It is the routine framework of analysis for longitudinal data (subject responses are repeatedly measured over time) but can very easily be adopted for other data structures like clustered data (correlated within cluster), multivariate data (several responses measured for each experimental unit or subject), etc.

The linear mixed model, linear since the mean of the response is linear in terms of certain parameters, is called mixed because it incorporates both fixed and random effects. The fixed effects are the population-averaged parameters associated with known explanatory variables while the random effects are subject-specific parameters associated with randomly drawn subjects from a population. One common phenomenon with longitudinal data is that different subjects exhibit different patterns of evolution. Some subjects may start evolving below or above the average starting point (population-averaged intercept) with a rate of evolution faster or slower than the average rate (population-averaged slope). This phenomenon is accounted for in the linear mixed model by the random effects. They reflect the between-subject variation or the deviation of the subject-specific evolution from the average evolution [5 and 8].

2.2.1 Fitted Models for the five trials

General linear mixed-effects model for longitudinal data analysis was fitted for the canopy temperature. In general the model as given by Laird and Ware (1982) is formulated as:

$$\mathbf{Y}_i = \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b}_i + \boldsymbol{\varepsilon}_i \quad \text{model (2.1)}$$

Where i is an index denoting individual genotypes, \mathbf{Y}_i is the n_i dimensional response vector for genotype i , $1 \leq i \leq N$ (in this case \mathbf{Y}_i is the canopy temperature) and $N=165$ i.e. the number of subjects (genotypes) in the study. \mathbf{X}_i and \mathbf{Z}_i are respectively $(n_i \times p)$ and $(n_i \times q)$ dimensional matrices of known covariates (or design matrices), $\boldsymbol{\beta}$ is a p -dimensional vector containing the fixed effects (i.e., the parameter estimates of the covariates used in the models), $\mathbf{b}_i \sim \mathcal{N}(\mathbf{0}, \mathbf{D})$ is the q -dimensional vector that contains the random effects (i.e., random intercepts and slopes representing variability between the genotypes), $\boldsymbol{\varepsilon}_i \sim \mathcal{N}(\mathbf{0}, \boldsymbol{\Sigma}_i)$ is an n_i -dimensional vector of residual components and $\mathbf{b}_1, \mathbf{b}_2, \dots, \mathbf{b}_N, \boldsymbol{\varepsilon}_1, \boldsymbol{\varepsilon}_2, \dots, \boldsymbol{\varepsilon}_N$ are assumed to be independent. Finally, \mathbf{D} is a general $(q \times q)$ covariance matrix with (i, j) elements where $d_{ij} = d_{ji}$. This covariance matrix contains the covariance parameter estimates of the random effects. $\boldsymbol{\Sigma}_i$ is $(n_i \times n_i)$ covariance matrix that represents variability due to measurement errors.

From the description above, it is usually assumed that ε_i are independent and normally distributed with mean vector zero and covariance matrix Σ_i . It is also assumed that b_i follow a q -dimensional normal distribution with mean vector zero and covariance matrix D . ε_i and b_i are further assumed to be independent [5].

Model (2.1) implies that Y_i is normally distributed with mean vector $X_i\beta + Z_i b_i$ and covariance matrix Σ_i thus $Y_i|b_i \sim N(X_i\beta + Z_i b_i, \Sigma_i)$. The linear mixed model (2.1) implies the marginal model

$$Y_i \sim N(X_i\beta, V_i), V_i = Z_i D Z_i^T + \Sigma_i \quad \text{model (2.2)}$$

and inference, unless analysis is done in a Bayesian framework, is based on this marginal distribution of Y_i .

The residual variability ε_i in model (2.1) is assumed to have a constant variance and can be decomposed as; *Measurement error*: which reflects the variation added by the measurement process. *Serial Correlation*: This represents the belief that part of genotypes's observed profile is a response to time-varying stochastic process operating within the genotype where by times closer together are more correlated than times further apart. It results in a correlation between serial measurements, which is usually a decreasing function of the time separation between these measurements. The distinction above then leads to the decomposition [5].

$$\varepsilon_i = \varepsilon_{(1)i} + \varepsilon_{(2)i} \quad \text{where} \quad \varepsilon_{(1)i} \sim N(0, \sigma^2 I_{n_i}) \quad \text{and} \quad \varepsilon_{(2)i} \sim N(0, \tau^2 H_i).$$

Where, $\varepsilon_{(1)i}$ is the measurement error as explained above and $\varepsilon_{(2)i}$ captures serial correlation.

The serial covariance matrix H_i only depends on i through the n_i observations and through the time points t_{ij} at which the measurements are taken and i denotes the i^{th} genotype.

We refer to Verbeke and Molenberghs (2000, Chapter 5 and 6) for details on estimation and inference of parameter estimates of θ where θ contains all the parameters $(\beta^T, \alpha^T)^T$, α being a vector of all variance and covariance parameters in V_i .

2.2.2 Estimation and Inference on Random Effects

Prediction of random effects was done in order to achieve the main objective of this study which was to assess if there exists significant genetic variation among genotypes exposed to similar environmental conditions. The random effects will reflect deviations of genotype-specific profiles from the population-averaged profile. The obtained estimates of the random effects will be used to detect genotypes with outlying profiles over time and in prediction of genotype-specific profiles [5]. The random effects are estimated from model (2.1) since model (2.2) does not imply that the variability in the data can be explained by random effects. The random effects are estimated by bayesian techniques considering the marginal distribution of $b_i \sim N(0, D)$ as a prior distribution for b_i . Given the observed values y_i for Y_i , the distribution of b_i conditional on the observed values thus $f(b_i | y_i)$ is then defined as

$$f(b_i | Y_i = y_i) = \frac{f(Y_i | b_i) f(b_i)}{\int f(Y_i | b_i) f(b_i) db_i}$$

We refer to Verbeke and Molenberghs (2000, Chapter 7) for more details on inference for the random effects.

2.3 Marginal Testing for the Need of Random Effects

Random effects in a linear mixed model represent the variability in subject- specific intercepts and slopes, not explained by the covariates included in the model. Under the hierarchical interpretation of the model, it may therefore be of scientific interest to test for the need of (some of the) random effects in the model. In this study, we investigated whether the random effects, included in the preliminary random-effects structure, are really needed in the model. Using results of Self and Liang (1987) on nonstandard testing situations, Stram and Lee (1994, 1995) have been able to show that the asymptotic null distribution for the likelihood ratio test statistic for testing hypotheses of this kind is often a mixture of chi-squared distributions rather than the classical single chi- squared distribution.

This test statistic was used to test for the need of random intercepts in the fitted models. The hypothesis of interest was formulated as:

$$H_0 : D = 0 \text{ versus } H_A : D = d_{11}$$

where d_{11} is a non-negative scalar representing the variability in random intercepts. The test statistic is given by -2 (REML log-likelihood value for model without random effects-REML log-likelihood value for model with random intercepts). The null distribution of the test statistic is a mixture of χ_0^2 and χ_1^2 distributions, with equal weights of 0.5. The p-value is thus given by:

$$\begin{aligned} p\text{-value} &= P(\chi_{0,1}^2 > -2(L_0 - L_1)) \\ &= \frac{1}{2}P(\chi_0^2 > -2(L_0 - L_1)) + \frac{1}{2}P(\chi_1^2 > -2(L_0 - L_1)) \end{aligned}$$

Where L_0 and L_1 are restricted maximum likelihood under null and alternative hypothesis respectively [5]. Inclusion of random slopes in the models did not yield fruits due to convergence problem.

2.4 Software

The software used in this study are Microsoft Excel (for data manipulation) and SAS version 9.1 for the analysis. The tests were carried out based on 5% level of significance.

3 Results

3.1 Descriptive Statistics

As seen in Table 2, the highest canopy temperature was from genotypes exposed to heat in 2005 (H05). Similarly, the measurements taken from genotypes in this trial have the highest variability. The lowest canopy temperatures were reported from genotypes exposed to irrigation in 2006. Similar observation can be made from the box plots in Figure A, Appendix A. These findings imply presence of genotype environment (trial) interaction though this needs to be proved statistically.

Table 2: Summary Statistics of Canopy Temperature per Environment

Environment	Mean	Standard Deviation	Minimum	Maximum
D02	25.3295	2.0631	21.1250	32.8318
D05	26.8134	2.8152	22.0301	32.4363
H05	28.7952	4.8244	21.2121	38.8699
H06	28.7517	2.5368	22.9192	33.4868
I06	23.6150	2.8365	18.8005	28.7792

Table 3 shows the summary of canopy temperatures measured on genotypes at the two stages of growth. There is notable difference in the measurements taken during the two stages especially in trial H05 and I06. In general we observe lower canopy temperatures during the vegetative stage. This could imply that, the stage of growth is a potential factor associated with canopy temperature.

Table 3: Summary Statistics of Canopy Temperature against Stage of growth

Trial	Stage	Mean	Standard Deviation	Minimum	Maximum
D02	Vegetative	24.6165	1.3533	22.0412	27.5853
	Grain filling	25.6861	2.2564	21.1250	32.8318
D05	Grain filling	26.8134	2.8152	22.0301	32.4363
H05	Vegetative	26.7881	3.3559	21.2121	33.8973
	Grain filling	35.8199	1.3575	32.5217	38.8699
H06	Vegetative	28.3939	2.5683	22.9192	33.4567
	Grain filling	29.5865	2.2528	25.8255	33.4868
I06	Grain filling	26.5210	1.4644	23.9106	28.7792
	Vegetative	21.6776	1.6093	18.8005	24.5728

From Table 4, we observe differences in measurements of canopy temperature within trials with regard to day time. Much discrepancy is seen from Trial H05 like in the case of stage

variable (Table 3). In general, we observe lower canopy temperature in the morning. The effect of this variable to canopy temperature will be determined in formal analysis.

Table 4: Summary Statistics of Canopy Temperature against the time of day

Trial	Day time	Mean	Std Dev	Minimum	Maximum
D02	Morning	24.8590	2.0683	21.1250	29.0346
	Evening	25.5648	2.0213	22.0412	32.8318
D05	Morning	25.5035	2.2865	22.9903	30.4598
	Evening	27.8614	2.7601	22.0301	32.4363
H05	Morning	26.7008	4.0879	21.2121	37.1686
	Evening	32.9840	3.1763	27.8491	38.8699
H06	Morning	28.2572	2.5974	22.9192	33.4868
	Evening	29.9056	1.9534	25.9496	33.4567
I06	Evening	23.6150	2.8365	18.8005	28.7792

3.2 Individual Profiles, Mean, Variance and Correlation Structures

In this section, we present the plots from exploratory data analysis. Plots from trial D05 and I06 will be presented in this section and the rest are as shown in Appendix A. It should be noted that, the five datasets used in this study had different number of measurements taken per genotype as shown on Table 1 hence using equal axes was not plausible.

3.2.1 Individual profiles using standardized residuals

Individual profiles of 10 randomly selected genotypes from each trial were plotted to explore the variability between and within genotypes. The profiles from most of trials seem to be having different canopy temperatures at the beginning of the experiment. In each trial, the genotypes seem to follow similar trend over time. There is a notable difference in measurements within genotypes. The variability within genotypes seems to be higher as compared with the variability between the genotypes. It appears that, genotypes with high canopy temperature at the beginning tend to retain high temperatures throughout the study period in most of trials. The correlation within genotypes suggests need to consider random effects in the model. Figure 1 presents individual profiles obtained using standardized residuals for trial D05 and I06.

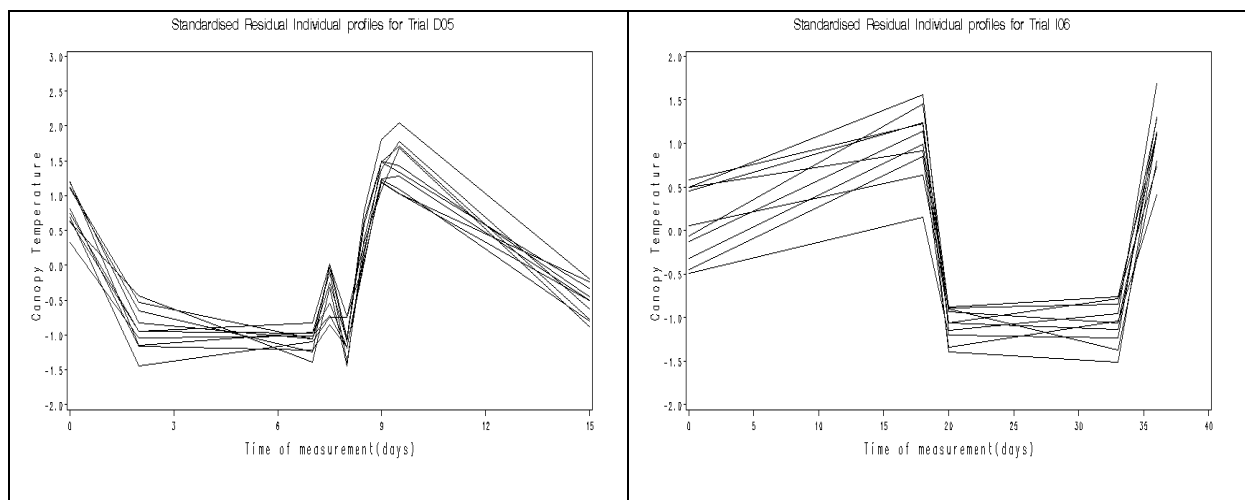


Figure 1: Individual Profiles using standardized residuals for trial D05 and I06

All the plots of individual profiles show patterns that are not clear and of no specific direction. This can be attributed to large differences between measurements taken in the morning and in the evening within a genotype. The differences might be caused by different air temperatures between the two periods that needs to be corrected for in analysis. In general, the profiles suggest that, an important covariate like ‘air temperature’ could be missing in the datasets.

3.2.2 The Mean Structure

The mean change of canopy temperature over time for each trial was examined. Figure 2 presents the average evolution plots with standard errors of means from trial D05 and I06. In trial D02 (appendix A), the mean structure seems to stabilize from day one to day 24 .There is high variability in mean canopy temperature at day 25 as indicated by larger standard errors. The mean structure for trial I06 seems to have an increasing trend over time. Plots of trial D05, H05 and H06 do not have obvious trend of increase or decrease over time. Therefore, it would be plausible to include quadratic and cubic time effects in the models.

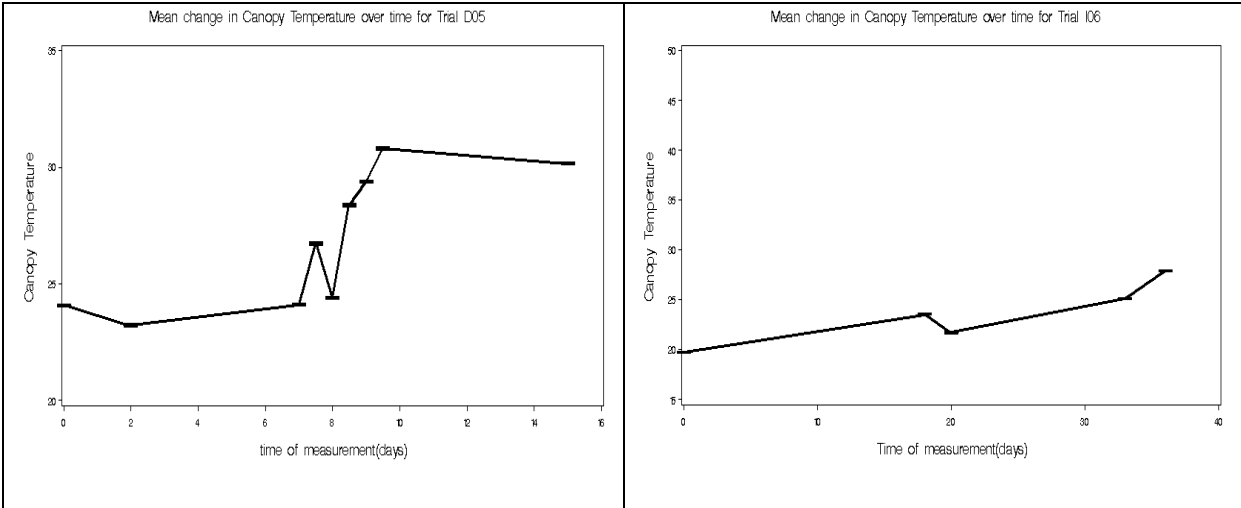


Figure 2: Mean Profile of Canopy Temperature for trial D05 and I06

3.2.3 The Variance Structure using raw residuals

In addition to the average evolution, a plot of the variance is also important in building an appropriate longitudinal model. In the individual profiles of Figure 1, we observed presence of notable between and within–subject variability. In addition to that, the variance structures were obtained by plotting raw residuals as a function of time. These residuals as explained in section 2.1 were obtained from regression model after correcting for time and relevant covariates. The variance structures for trial D05 and I06 are as presented in Figure 3.

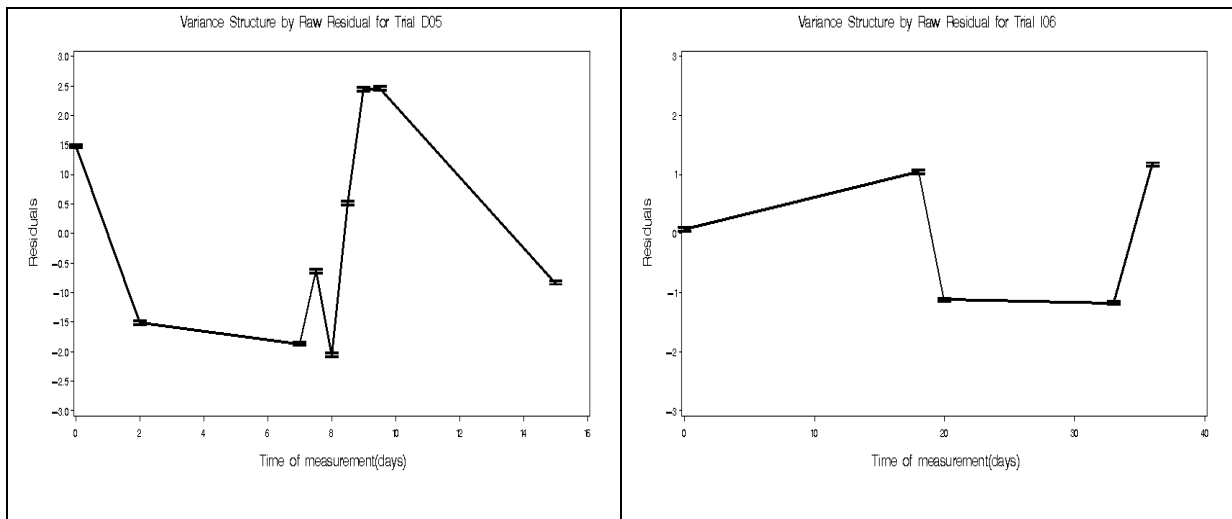


Figure 3: Variance plot by raw residuals for trial D05 and I06

In general the five variance structures show non-stable variance over time. This implies that structures that allow for non-constant variance be considered in the modelling process.

3.2.4 The Correlation Structure using standardized residuals

The correlation structures were used to study the correlation of measurements within a genotype. This was done using scatter plots of standardized residuals as explained in section 2.1. From Figure 4, the off diagonal elements picture the standardized residuals obtained from pairs of measurements within a genotype. The correlation seems to decay as we move away from the main diagonal. Therefore, going by that, we can conclude that a pair of measurement taken in two time points that are very close is having a high relationship than that taken in two time points that are far apart. However this observation is not clear in some trials. The correlation was further explored numerically and by use of scatter plots of residuals from

linear mixed model with a specified preliminary mean structure and random effects, (see Table A to E and Figure F to G in the Appendix A). Generally, the correlation between measurements was weak except in trial D02.

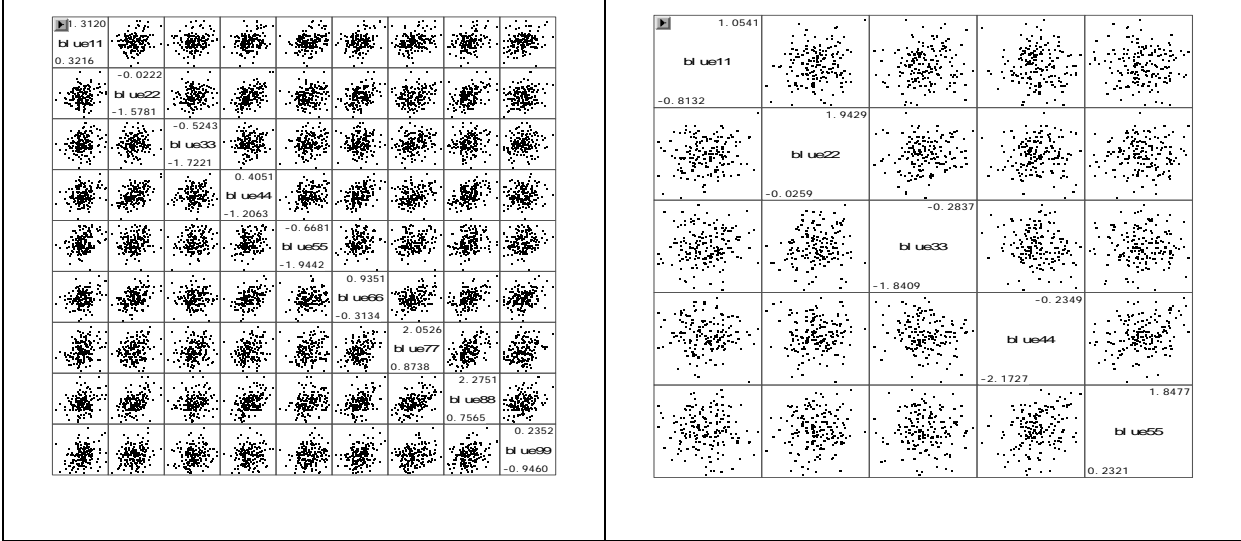


Figure 4: Scatter plots of standardized residuals for trial D05 and I06

3.3 Implications of Exploratory Results to Statistical Modelling

From the mean average exploration, it seems plausible to start with a model with linear trend for trial (DRIP02). However, it was not clear if linear time effect was adequate since at the beginning the trend appeared to rise and decrease at the end of the study. Therefore, a model with quadratic time effect was fitted at the initial stage of model building. Similarly, the exploration of mean structures of the other trials supported need of quadratic and cubic time effects because the trends did not seem linear over time. The individual profile plots indicated presence of both within and between variability which suggested need to include random effects in the model. In trial D02, the variance seems to decrease sharply at day 25 and in trial D05 is decreasing gradually as from day 10 onwards. However, in trial H05, the variance is stabilising from day 28 while in both H06 and I06 trials is fluctuating over time. In general, the variances were not constant over time. This implies starting with unstructured covariance structure might be a plausible beginning and look for further simplification in later steps.

3.4 Model Building

3.4.1 Selection of preliminary mean structure

In the initial stage of model building, all covariates and their interactions with linear, quadratic and cubic time effects were included in the model. Thus the models included linear, quadratic and cubic time effects plus their interactions with the covariates of interest. The covariates considered were: the stage of growth (vegetative or grain filling), the time of the day when the measurements were taken (morning or evening), rye and anthesis periods. The preliminary mean structures were specified in line with implications from exploratory data analysis.

3.4.2 Selection of preliminary random-effects structure

From the individual profiles, it was observed that genotypes have different starting points but relatively similar trends over time per trial. In addition, the variance plots were not stable over time. This implied a constant variance model could not be a plausible starting point and we included random effects in the preliminary models. Initially models were fitted including both random intercepts and slopes. Random slopes were omitted from the models later due to convergence problems.

3.4.3 Selection of Residual Covariance Structure

Having identified the random effects structure, the next step is to partition the remaining variability ε_i which is assumed to have constant variance into measurement error and serial correlation. From our datasets, the time points are not equally spaced. This suggests use of covariance structures which allow for unequally spaced observations. Possible structures would be spatial Gaussian, spatial Exponential and spatial Power which allow for unequally spaced observations. From the exploratory data analysis we observed that, the correlation coefficients do not suggest a decreasing function over time. This implies serial correlation

may not be present or is of less impact. Nevertheless, we explored the mentioned serial correlation structures by fitting models with the same mean and random effects structure but with different serial correlation and measurement error. Also fitted were models with unstructured, compound symmetry and simple error covariance structures. However, due to convergence problems in all models for the five trials, we assumed a simple residual covariance structure (diagonal structure). This structure assumes that residuals associated with observations on the same genotype are uncorrelated and have equal variance [8].

3.4.3 Test for need of heterogeneous residual variance per stage of growth

After specifying the residual covariance structure, the next step was to investigate if the genotypes had different variability in the two stages of growth (vegetative and grain filling). A model with homogeneous (equal variability in both stages) residual covariance was compared with a model with heterogeneous residual covariance (different variability per stage). The covariance structures were assumed to be simple (diagonal) as explained in section 3.4.3. The hypothesis of interest was formulated as:

$$H_0 : \sigma^2_{vegetative} = \sigma^2_{grain\ filling} \text{ versus } H_1 : \sigma^2_{vegetative} \neq \sigma^2_{grain\ filling}$$

Restricted Maximum Likelihood-based ratio test was used to test the hypothesis. The test statistic was obtained by computing: -2 (REML log-likelihood value for model with homogeneous residual variances minus REML log-likelihood value for model with heterogeneous residual variances). The asymptotic distribution of the test statistic under the null hypothesis is a chi-square with one degree of freedom (χ_1^2). The results are reported in Table 5.

Table 5: Test for the need of heterogeneous residual covariance per stage

Trial	Homogeneous	Heterogeneous	Difference	p-value
	-2 REML log-likelihood value			
DRIP 02	1575.5	1498.9	76.6	<.0001
DRIP 05
HEAT 05	2858.7	2854.4	4.3	0.0381
HEAT 06	3301.8	3032.7	269.1	<.0001
IRRI 06	6565.9	6228.8	337.1	<.0001

Based on 5% level of significance we reject the null hypothesis and specify heterogeneous residual variances for the two stages of wheat growth in all the trials apart from DRIP 05. All the genotypes from this trial were taken measurements during grain filling stage.

3.5 Model Reduction

3.5.1 Reduction of preliminary random-effects structure

The need of random effects included in the preliminary random-effects was investigated using a mixture of chi-square test as explained in section 2.3. Models with random intercepts were compared with models without random effects except for trial I06. From the fitted model of observations in this trial, it was observed that the value of the estimated variance of random intercepts equals zero. This could be due to small variance components or model misspecification. To overcome this problem, the option ‘*nobound*’ was specified in SAS and the model fitted again to see the true estimated value of the variance components. After giving flexibility to the variance components, it was found out that, the variance of random intercepts was negative. Since the covariance matrix was not positive definite the model interpretation was done at marginal level hence no hierarchical interpretation given to this model. The results of the tests for the other four trials are shown in Table 6.

Table 6: Results from models to test significance of random intercepts

Trial	Random Effects	
	Model1: Random intercept	Model2: Without random effects
	$-2\ln [L_{REML}(\hat{\theta})]$	
DRIP02	1498.9	1959.8
DRIP05	1620.3	1794.0
HEAT05	2854.4	3008.6
HEAT06	3032.7	3171.9

The p-values obtained under REML estimation for the comparison of these models are calculated from the mixture of two chi-square distributions with equal weights and degrees of freedom as shown in Table 7.

Table 7: Hypothesis testing for the need of random effects

Trial	Hypothesis	Asymptotic null distribution		p-value
		$-2\ln[\lambda_N]$		
DRIP02	Model 2 vs. Model 1	460.9	$\chi^2_{0.1}$	<.0001
DRIP05	Model 2 vs. Model 1	173.7	$\chi^2_{0.1}$	<.0001
HEAT05	Model 2 vs. Model 1	154.2	$\chi^2_{0.1}$	<.0001
HEAT06	Model 2 vs. Model 1	139.2	$\chi^2_{0.1}$	<.0001

All model comparisons had p-values less than 0.05. Therefore, the covariance structure for the four trials should not be simplified by deleting random intercepts from the model.

3.5.2 Reduction of preliminary mean structure

After selecting the final covariance structure of the models, the need of the fixed effects specified in the preliminary mean structure was examined. Approximate F-statistics with denominator degrees of freedom estimated by the Satterthwaite approximation were used to test the significance of the fixed effects. Inferences on average longitudinal evolutions are not robust under misspecification of marginal covariance structure. Hence the need of inferential procedures based on the so-called empirical variance estimators. These estimates can then be shown to be consistent, as long as the mean is correctly specified in the model [5]. In our case, the mean structure might not be correctly specified as explained in section (3.2.1). There could be an important missing covariate like ‘air temperature’ which needs to be corrected for, in the analysis. We present the parameter estimates with the associated model based and empirical standard errors. The comparison of model based and empirical standard errors can be used as an informal way of checking the plausibility of the specified variance covariance structure. In general, there were no much discrepancies between standard errors from model based and robust standard errors. This implies the specified covariance structures could be appropriate for the data at hand. However, this should be interpreted with caution since the mean structures might be incorrectly specified resulting in biased covariance parameter estimates.

3.5.3 Parameter estimates of fixed effects

We present the results of the final fitted models under this section. The parameter estimates are interpreted both at population (trial) level and subject-specific (genotype) level. We present the expected canopy temperature and the interpretation of associated parameter estimates in trial D02. The expected canopy temperature for genotypes exposed to other trials can be stated in a similar way with corresponding parameter estimates. The interpretation of the parameter estimates and their corresponding effect on the response follows as stated for trial D02. The covariance parameter estimates are reported on Table F in the Appendix B. Table 9 to 12 presents the parameter estimates from the other trials. The results from trial D02 are as shown in Table 8.

Table 8: Parameter Estimates of fixed effects with associated standard errors trial D02

Effect	Parameter	Parameter Estimate	Empirical (s.e)	Model based(s.e)	p-value
Intercept	β_0	15.3573	1.1668	1.0922	<.0001
Time	β_1	2.4454	0.0466	0.0425	<.0001
Time_squared	β_2	-0.1293	0.0011	0.0013	<.0001
Anthesis	β_3	0.0935	0.0135	0.0126	<.0001
Stage	β_4	20.1618	0.7886	0.7834	<.0001
Daytime	β_5	-190.61	1.8918	1.9420	<.0001
Time*Anthesis	β_6	0.0012	0.0003	0.0003	0.0013

The results show that, the effect of anthesis, stage of crop growth, the time of the day when the measurements were taken are significantly associated with the canopy temperature. Similarly, the effect of the interaction between anthesis and time was significant.

3.5.3. 1 Parameter Interpretation at Marginal level

The expected canopy temperature at population level is given as:

$$E[\text{canopy temperature}_{ij}] = 15.3573 + 2.4454t_{ij} - 0.1293t_{ij}^2 + 0.0935\text{anthesis}_i + 20.1618\text{stage}_i - 190.61\text{daytime}_i + 0.0012\text{anthesis} * t_{ij}$$

The parameter estimates at marginal level are as follows: $\hat{\beta}_0$ is the estimated average canopy temperature at the start of experiment and is common to all genotypes in this trial (D02). $\hat{\beta}_1$ and $\hat{\beta}_2$ are the estimated common average linear and quadratic effect of time respectively. $\hat{\beta}_3$ and $\hat{\beta}_4$ are the estimated common average effect of anthesis and stage of growth. $\hat{\beta}_5$ is the estimated common average effect of day time when the measurements were taken. $\hat{\beta}_6$ is the estimated common average effect of interaction between anthesis and linear time.

3.5.3. 2 Parameter Interpretation at Genotype-specific level

At subject-specific level, the fitted model is as stated below: given the random intercept b_{0i} , the expected canopy temperature is given by:

$$E[\text{canopy temperature}_{ij} | b_{0i}] = (15.3573 + b_{0i}) + 2.4454t_{ij} - 0.1293t_{ij}^2 + 0.0935\text{anthesis}_i + 20.1618\text{stage}_i - 190.61\text{daytime}_i + 0.0012\text{anthesis} * t_{ij}$$

Where, b_{0i} represents the deviation of genotype i from the average response at the start of experiment.

The parameter estimates can be interpreted as: $\hat{\beta}_0 + b_{0i}$ is the estimated response of genotype i at the start of the experiment. The estimated effect of the other parameters on the canopy temperature is as defined for population (marginal) model but conditioned on the random intercepts.

3.5.3. 3 Parameter estimates of fixed effects for trial D05, H05, H06 and I06

In this section we give general interpretation of the estimated parameters of the fixed effects from the other trials. As mentioned in section 3.5.3 the interpretation of the parameter estimates and their corresponding effect on the response at marginal and genotype-specific level follows as stated for trial D02.

Table 9 presents the results from genotypes exposed to drought in 2005 (trial D05). The results indicated a significant effect of the time of the day when the measurements were taken, anthesis and its interaction with linear, quadratic and cubic time effects.

Table 9: Parameter Estimates of fixed effects with associated standard errors trial D05

Effect	Parameter	Parameter Estimate	Empirical (s.e)	Model based(s.e)	p-value
Intercept	β_0	32.8476	0.6899	0.8958	<.0001
Time	β_1	-6.3604	0.5641	0.6670	<.0001
Time_squared	β_2	1.1964	0.0986	0.1131	<.0001
Time_cubic	β_3	-0.0518	0.0041	0.0048	<.0001
Anthesis	β_4	-0.0376	0.0089	0.0116	<.0001
Daytime	β_5	-5.9053	0.1328	0.1432	<.0001
Time*Anthesis	β_6	0.0180	0.0074	0.0087	0.0154
Time ² *Anthesis	β_7	-0.0042	0.0013	0.0014	0.0013
Time ³ *Anthesis	β_8	0.0002	0.0001	0.0001	0.0002

Table 10 presents the results from genotypes exposed to heat in 2005 (trial H05). From the results it can be observed that, the effects of anthesis, rye, stage were significantly associated with the canopy temperature. The interaction between anthesis and time was significantly associated with the canopy temperature.

Table 10: Parameter Estimates of fixed effects with associated standard errors trial H05

Effect	Parameter	Parameter Estimate	Empirical (s.e)	Model based(s.e)	p-value
Intercept	β_0	26.2655	0.5813	0.6725	<.0001
Time	β_1	4.2331	0.0509	0.0652	<.0001
Time_squared	β_2	-0.1943	0.0025	0.0035	<.0001
Time_cubic	β_3	0.0027	0.0001	0.0001	<.0001
Anthesis	β_4	0.0520	0.0098	0.0114	<.0001
Rye	β_5	-0.2005	0.0568	0.0631	0.0004
Stage	β_6	-122.71	5.5505	7.6613	<.0001
Daytime	β_7	-27.3145	0.2672	0.3414	<.0001
Time*Anthesis	β_8	-0.0038	0.0003	0.0002	<.0001

Table 11 presents the results from genotypes exposed to heat in 2006 (trial H06). From the results it can be observed that, the effect of anthesis, rye, stage and time of the day when the measurements were taken were significantly associated with the canopy temperature. Similarly, the interaction between time and anthesis, was significantly associated with the canopy temperature.

Table 11: Parameter Estimates of fixed effects with associated standard errors trial H06

Effect	Parameter	Parameter Estimate	Empirical (s.e)	Model based(s.e)	p-value
Intercept	β_0	24.3979	0.6947	0.6419	<.0001
Time	β_1	1.9023	0.0163	0.0237	<.0001
Time_squared	β_2	-0.1157	0.0006	0.0010	<.0001
Time_cubic	β_3	0.0019	0.0001	0.0001	<.0001
Anthesis	β_4	0.0334	0.0112	0.0103	0.0028
Rye	β_5	-0.1185	0.0445	0.0483	0.0078
Stage	β_6	79.5886	1.0276	1.1281	<.0001
Daytime	β_7	-1.9041	0.0439	0.0743	<.0001
Time*Anthesis	β_8	-0.0017	0.0002	0.0002	<.0001

Table 12 presents the results from genotypes exposed to irrigation in 2006 (trial I06). From the results it can be observed that, the effect of anthesis, rye and stage were significantly associated with the canopy temperature. The interaction between time and anthesis was also found to be significantly associated with the canopy temperature.

Table 12: Parameter Estimates of fixed effects with associated standard errors trial I06

Effect	Parameter	Parameter Estimate	Standard errors	p-value
Intercept	β_0	21.0519	1.7423	<.0001
Time	β_1	3.0284	0.0553	<.0001
Time_squared	β_2	-0.1771	0.0020	<.0001
Time_cubic	β_3	0.0022	0.0001	<.0001
Anthesis	β_4	0.0456	0.0281	0.1043
Rye	β_5	-0.1003	0.0431	0.0203
Stage	β_6	24.2608	0.3534	<.0001
Time*Anthesis	β_7	-0.0020	0.0006	0.0034

3.6 Bayes Estimates of Random Intercepts

Empirical bayes estimates of the random intercepts were obtained to check potential outlying genotypes by use of histograms. Scatter plots of the estimates were used as an informal way to check the performance of genotypes across the trials. The scatter plots would reveal if the genotypes perform similarly across the trials. A section of the estimates is presented in table G in Appendix B.

3.6.1 Histograms of random intercepts

Histograms of random intercepts were plotted in order to see if there existed outlying genotypes in the trials. There seems to be no outlying genotypes as shown by the histograms except in trial D05. In this trial, three genotypes (9, 71 and 117) seem to be outlying and have extreme high canopy temperature than in the population. The effect of these genotypes on the results was examined by removing them from the dataset and fitting the model again. However, there was no notable difference in parameter estimates obtained compared to those from initial model. Therefore, the final inferences were based on the full dataset. Table H in appendix B presents results from both models (reduced and full dataset).

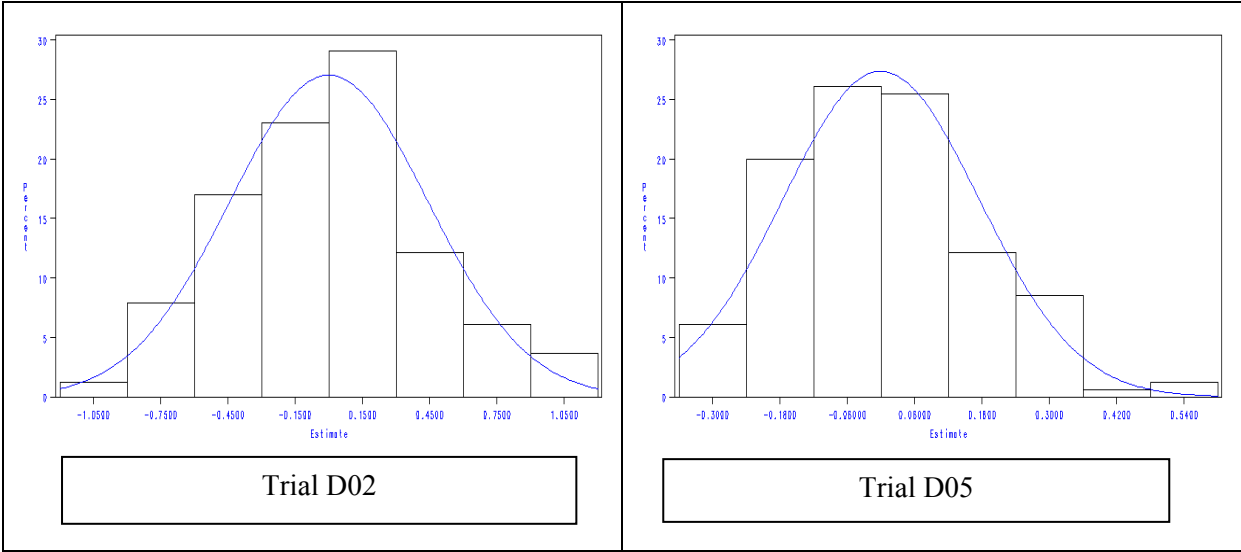


Figure 5: Histograms of Bayes Estimates trial D02 and D05

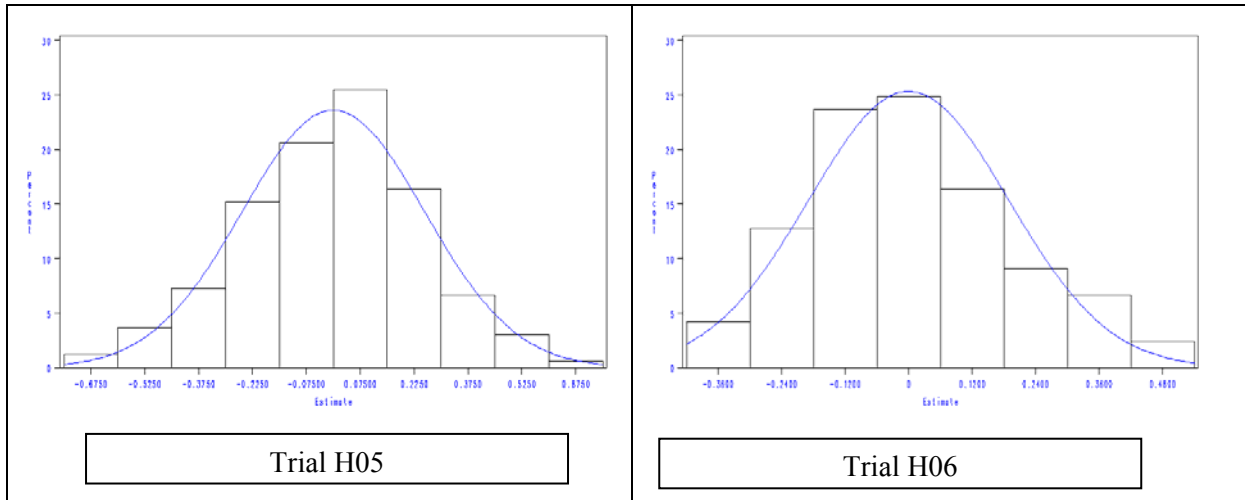


Figure 6: Histograms of Bayes Estimates trial H05 and H06

3.6.2 Scatter plots of random intercepts across the trials

Figure 7 shows the scatter plot of bayes estimates of genotypes from the four trials.



Figure 7: Scatter plots of bayes estimates of random intercepts across the trials

Figure 7 in the previous page, shows a linear correlation of the estimates between trial D05, H05 and H06. This could imply that, the genotypes behave the same across the three trials. That is, those with high canopy temperature tend to have similar traits across the trials and vice versa. This finding could have been due to similarities in environmental factors in H05 and H06 (heat- stressed conditions) and the fact that, D05 (drought-stressed conditions) and H05 were conducted in the same year (2005). The correlation could also symbolise the capability of genotypes to perform well/ poor across the trials. There was no correlation observed between the estimates from trial D02 with the other trials. These findings as explained, are based on exploratory data analysis and need to be verified by use of statistical analysis.

4 Discussion and Conclusion

This study applied the concept of longitudinal data analysis to analyse data on canopy temperature of bread wheat whose genotypes are being experimented in CIMMYT to assess their response to various environmental factors.

The datasets used in this study came from five different environments. The data was first explored at environmental level and at the level of covariates of interest within the environments. The exploratory analysis gave some insight into the datasets in regard to some variables like the stage of growth and time of the day when the measurements were taken.

The data exploration like individual and average profiles presented useful features that were used to narrow down and guide on the selection of the analysis techniques to be used. The individual profiles indicated presence of both within and between variability of the genotypes. The profiles also indicated large differences in the measurements taken in the morning and in the evening within a genotype. This could be due to differences in the air temperature which might be an important covariate missing from the dataset and should be corrected for during the analysis. The mean structures suggested inclusion of linear, quadratic and cubic effects of time in the models. The variance structures were not constant over time hence, unstructured covariance structures were explored in the initial stages of model building. Due to convergence problems, the residual covariance structures were assumed to be simple and heterogeneous variability of measurements in the two stages of growth was specified. This meant that, the variability in canopy temperature was different in the two stages. The correlation between measurements within a genotype was low except in trial D02.

Linear mixed models were fitted for the five datasets separately. The models included fixed effects and random intercepts. The need for genotype specific intercepts was tested and found significant except in trial I06. In this trial, the estimated variance of random intercept was negative. Since the covariance matrix is not positive definite the model interpretation was done at marginal level only. From the other trials, it was observed that, the largest percentage of total variability was due to the measurement error. The random intercept explains (39%) in D02, (23%) in D05, (11%) in H05 and (7.9%) in H06.

The statistical results from the five trials showed a significant effect of anthesis period on canopy temperature. There was positive effect of anthesis on canopy temperature when genotypes were exposed to drought-stressed conditions in 2002, heat period in 2005 and 2006 and irrigation in 2006. This implied that, late onset of flowering leads to high canopy temperatures. The effect was negative under drought-stressed conditions in 2005. The effect of stage of growth (grain filling) was positively associated with canopy temperature under drought-stressed conditions in 2002, heat and irrigation in 2006. Positive correlation was expected due to the fact that, at grain filling period, the vegetative content of the plant is low and the plant is not able to regulate its temperature well. This results in high canopy temperatures. The effect was negative for heat-stressed conditions in 2005. The absence of rye DNA in chromosomes of the genotypes was negatively associated with canopy temperature when genotypes were exposed to heat conditions in 2005, 2006 and irrigation in 2006. This implied that, pure genotypes have low canopy temperature as compared to those which crossbred with rye species. There was no significant difference in rye species crossbred with genotypes in drought-stressed conditions. The time of the day when the temperatures were recorded (morning) was negatively associated with canopy temperature under all environmental conditions. This is expected because in the morning the temperatures are low.

In conclusion, there was significant genetic variation among genotypes exposed to similar environmental conditions. Genotype specific intercepts implied presence of genetic variation among genotypes. This meant the genotypes performed differently within the trials. The genotypes with negative deviations from the population mean canopy temperature were considered better and able to survive in harsh environmental conditions like drought and heat.

The canopy temperature of genotypes was highly influenced by the anthesis period, the stage of growth and the time of the day when the measurements were taken. The genotypes had low canopy temperatures when there was no rye DNA in their chromosome. Low canopy temperatures meant the genotypes were able to regulate their temperatures well under harsh conditions.

5 Recommendations

From the individual profiles we observed large differences in measurements taken in the morning and in the evening within a genotype. This could be due to differences in the air temperatures. If so, then future analysis should be done correcting for air temperature or by using centered canopy temperature at each time point as the response variable. Alternatively, leaf water potential or leaf relative water content can be used as the response variable.

The ultimate goal of many advanced plant breeding programs is to evaluate the performance of individual genotypes across multi-environmental trials and to detect the quantitative trait locus (QTL) associated with the genetic variation. We have found out that, there exists genetic variation for canopy temperature in wheat. This means there are good prospects for quantitative trait locus analysis. Therefore future studies should investigate the performance of genotypes across trials using formal statistical techniques and do QTL analysis to localize the QTL/genes that are responsible for the observed genetic variation.

6 References

1. AlKhatib, K. and Paulsen, G.M. (1990). Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. *Crop Sci.* **30**: 1127-1132.
2. Ehrler, W.L., Idso, S.B., Jackson, R.D. and Reginato, R.J. (1978) Wheat canopy temperature: Relation to plant water potential. *Agron.* **70**: 251-256.
3. Fischer, R.A. and Sanchez, M. (1979). Drought resistance in spring wheat cultivars. II. Effects on plant water relations. *Aust. J. Agric. Res.* **30**: 801-814.
4. Molenberghs, G. and Verbeke, G. (2007). *Longitudinal Data Analysis*, Diepenbeek, Universiteit Hasselt, Belgium, unpublished course notes.
5. Verbeke, G. and Molenberghs, G. (2000). *Linear Mixed models for longitudinal data*. Springer, New York.
6. Verbeke, G. and Molenberghs, G. (2006). *Correlated and Multivariate Data*, Diepenbeek, Universiteit Hasselt, Belgium, unpublished course notes.
7. Siddique, M.R., Hamid, A. and Islam, M.S. (2000). Drought stress effects on water relations of wheat. *Bot. Bull. acad. Sin.* **41**: 35-39.
8. West, B. T., Welch, B. K. and Galecki, A. T. (2007). *Linear Mixed Models: A Practical Guide Using Statistical Software*. Chapman & Hall/CRC.

Websites

9. <http://www.rochedalss.qld.edu.au/wheat.htm> accessed on 2/7/2008
10. <http://library.thinkquest.org/TQ0312380/wheat.htm> accessed on 20/7/2008
11. http://www.plantstress.com/methods/IRT_protocol.htm accessed on 12/7/2008
12. <http://www.cimmyt.org> (International Maize and Wheat Improvement Center) accessed on 4/7/2008

7 Appendices

APPENDIX A

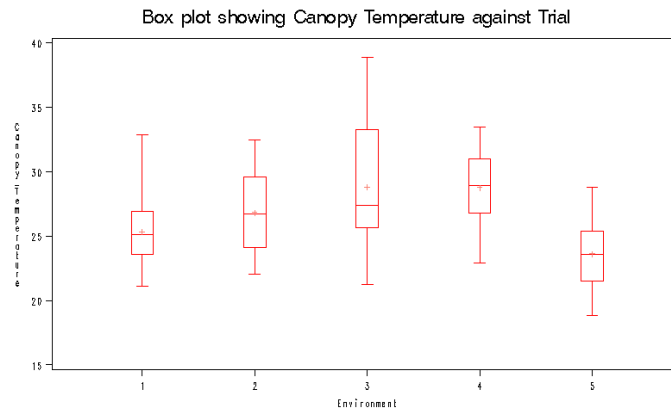
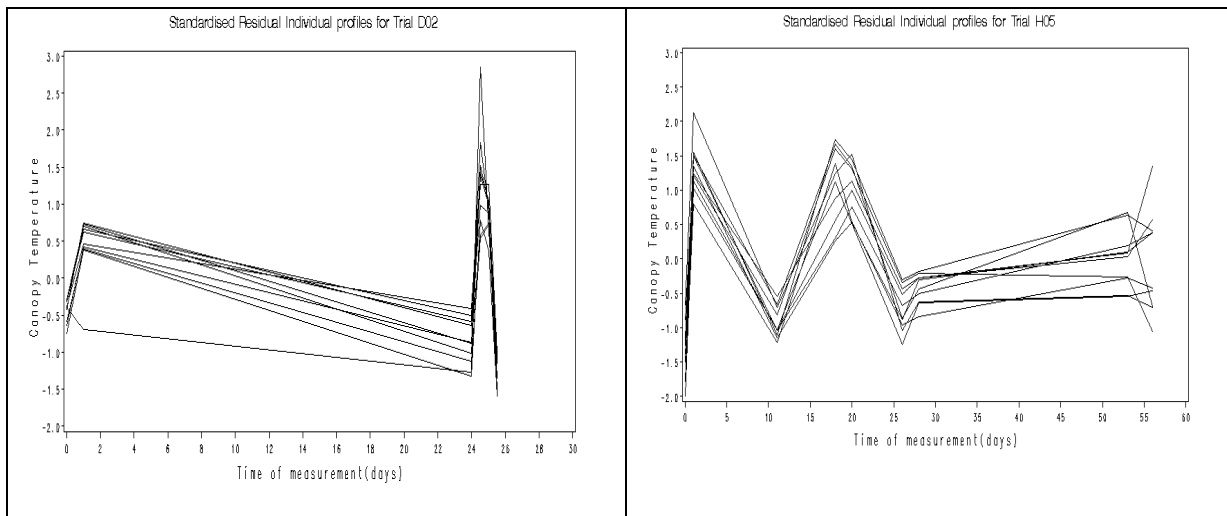


Figure A: Box plots of Canopy Temperature per Trial



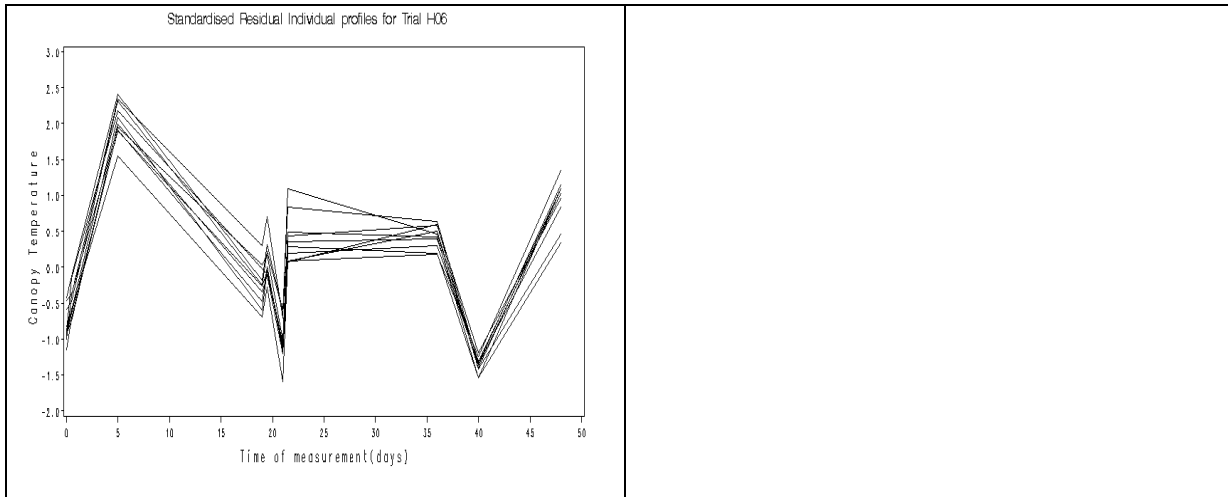


Figure B: Standardized Residuals individual Profiles for trial D02, H05 and H06

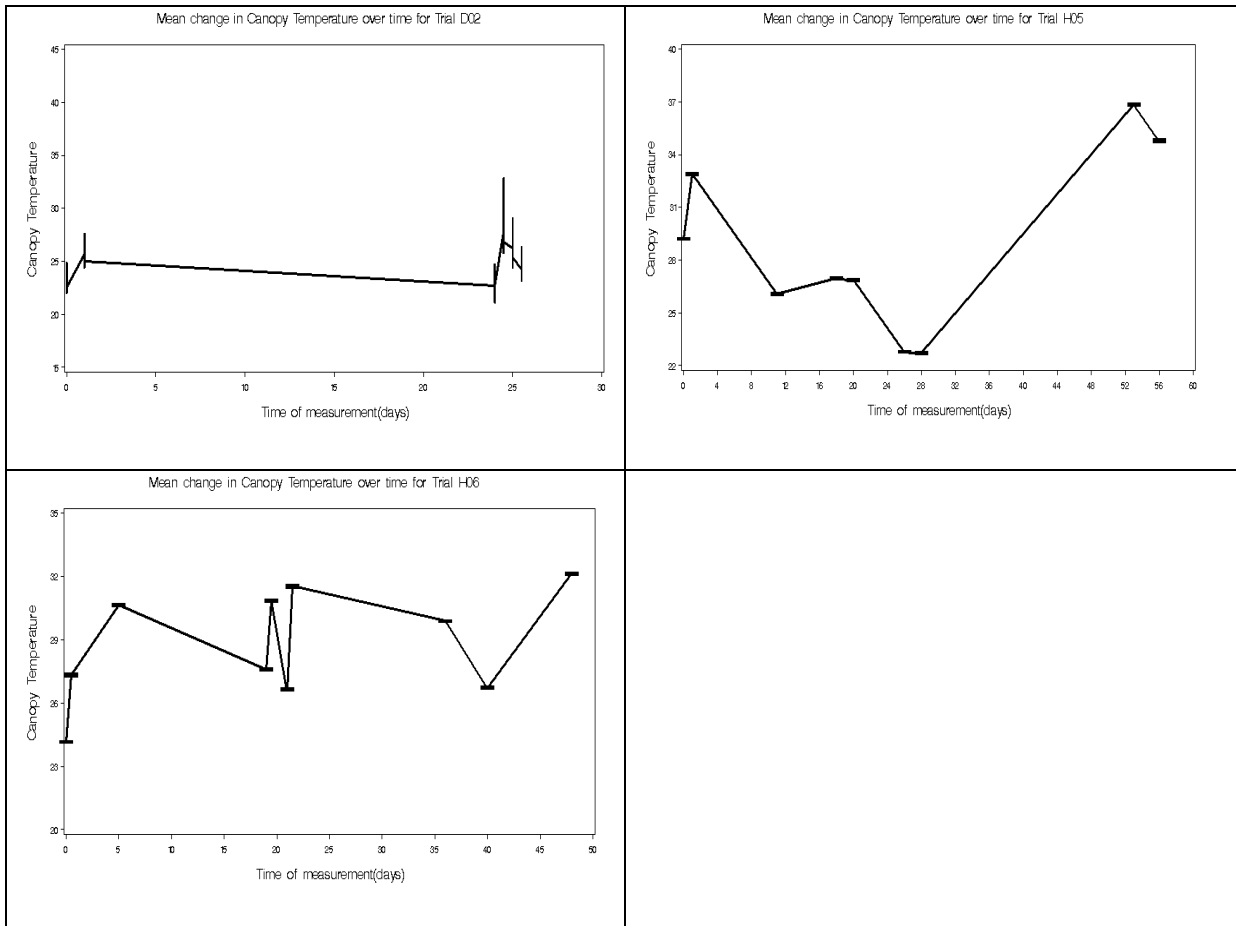


Figure C: Mean Profile of Canopy Temperature for trial D02, H05 and H06

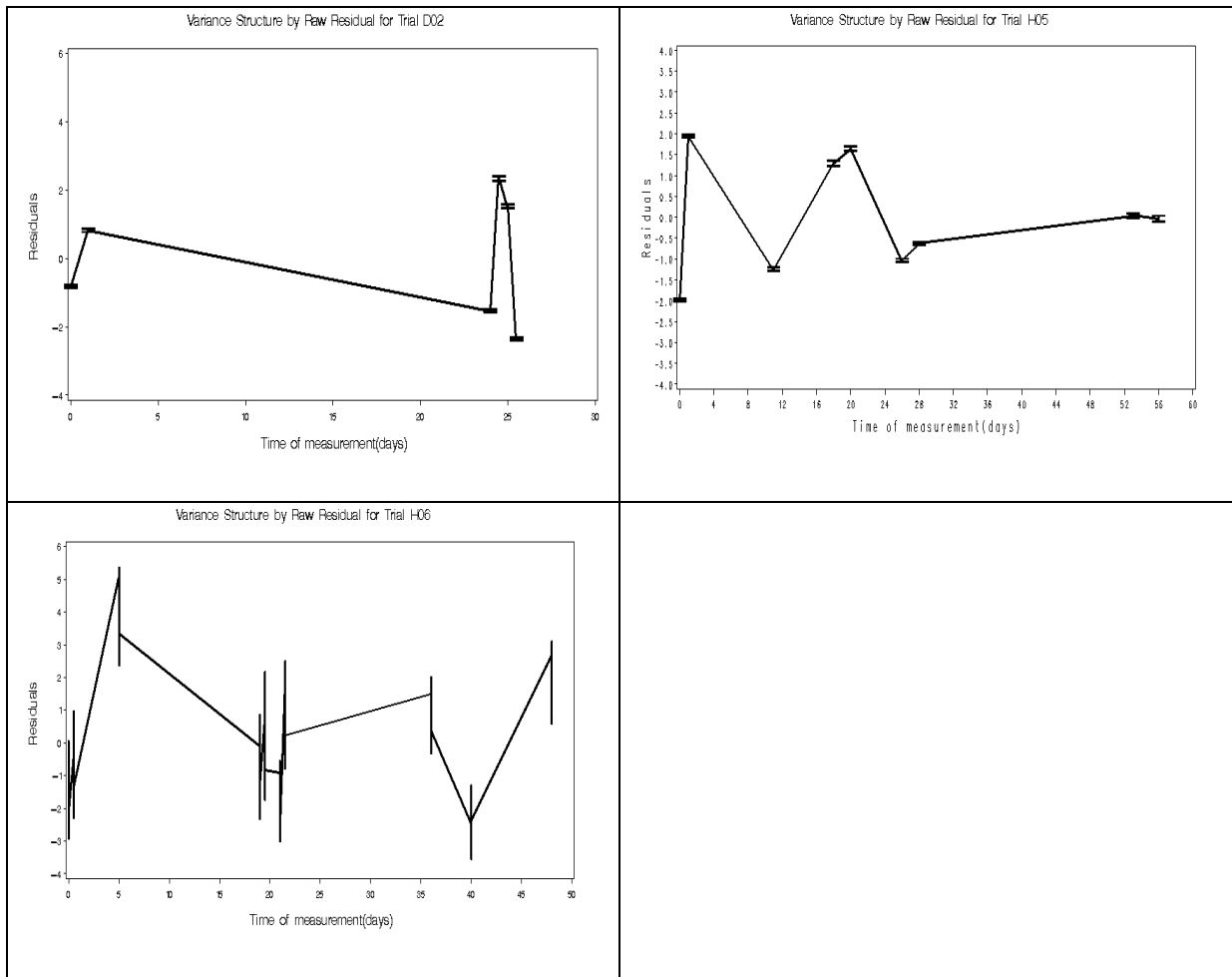
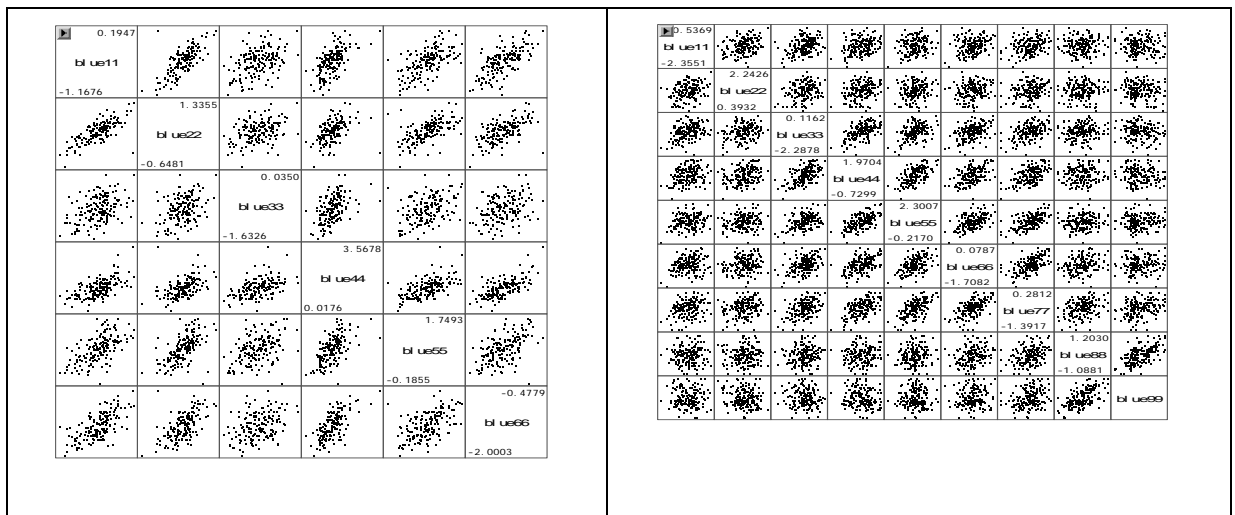


Figure D: Variance plots by raw residual for trial D02, H05 and H06



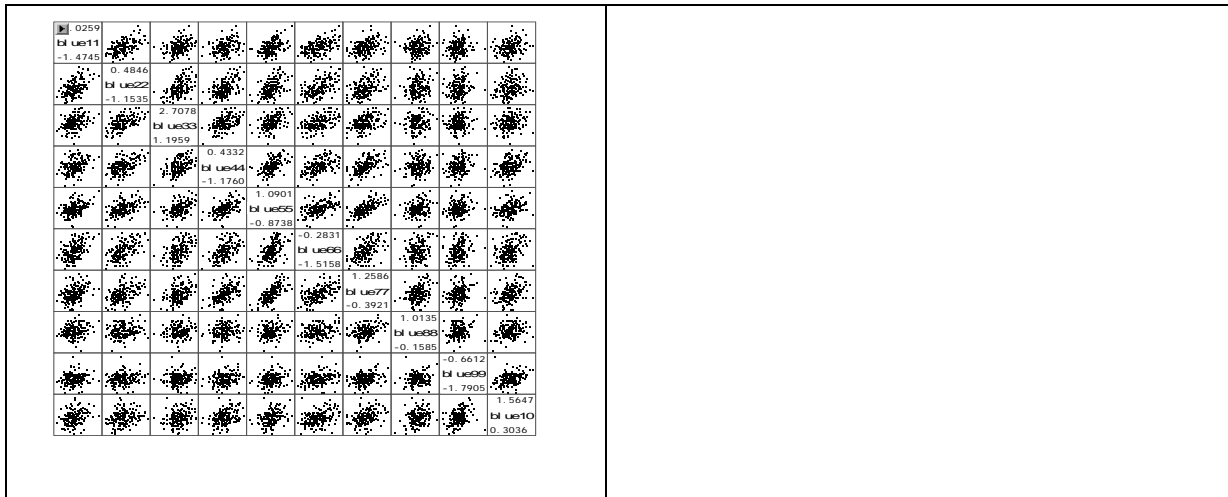


Figure E: Scatter plots of standardized residuals for trial D02, H05 and H06

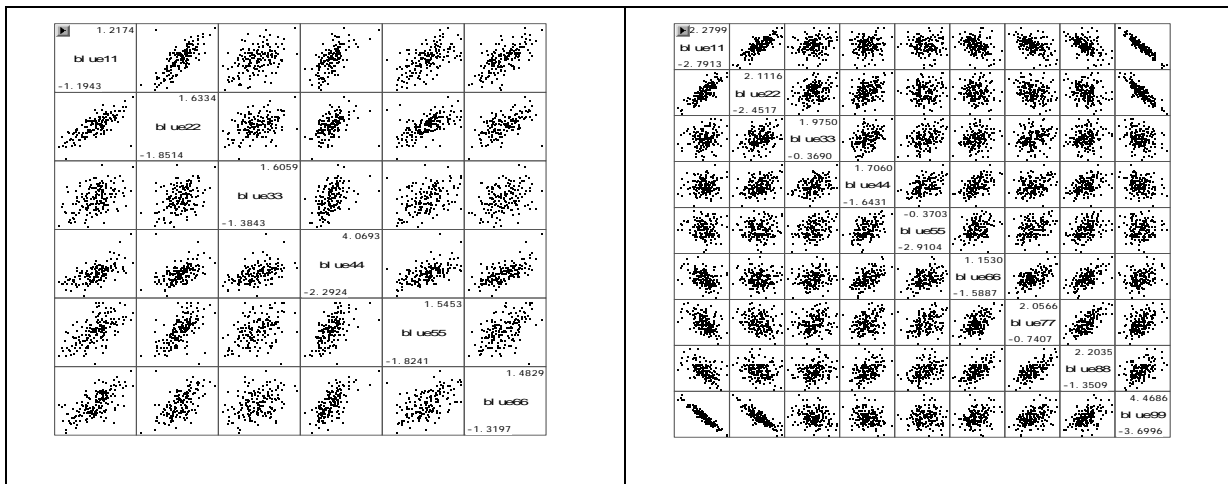


Figure F: Scatter plots of raw residuals from the linear mixed model trial D02 and D05

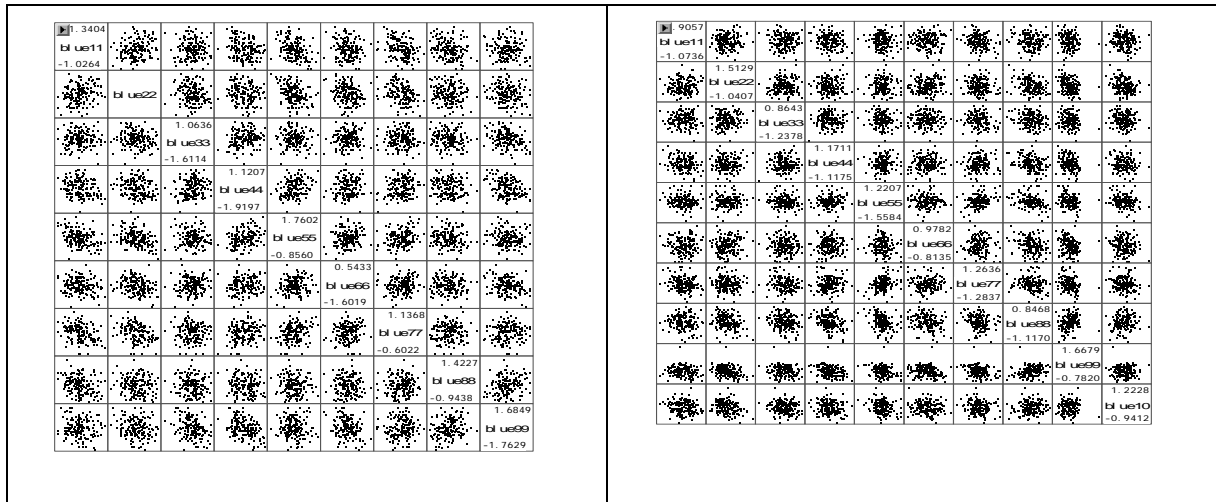


Figure G: Scatter plots of raw residuals from the linear mixed model trial H05 and H06

Table A: Correlation coefficients trial D02

	Day 0	Day 1	Day 24	Day 24.5	Day 25	Day 25.5
Day 0	1	0.8082	0.6100	0.7538	0.7190	0.7716
Day 1	0.8082	1	0.5467	0.6215	0.6910	0.6959
Day 24	0.6100	0.5467	1	0.6188	0.5838	0.5453
Day 24.5	0.7538	0.6215	0.6188	1	0.7003	0.7734
Day 25	0.7190	0.6910	0.5838	0.7003	1	0.6582
Day 25.5	0.7716	0.6959	0.5453	0.7734	0.6582	1

Table B: Correlation coefficients trial D05

	Day 0	Day 2	Day 7	Day 7.5	Day 8	Day 8.5	Day 9	Day 9.5	Day 15
Day 0	1	0.2173	0.2321	0.3103	0.3246	0.3327	0.3982	0.3871	0.2735
Day 2	0.2173	1	0.1876	0.5403	0.2104	0.4194	0.3017	0.4888	0.1944
Day 7	0.2321	0.1876	1	0.1764	0.2629	0.1428	0.3036	0.2416	0.2034
Day 7.5	0.3103	0.5403	0.1764	1	0.3514	0.5047	0.3546	0.4886	0.1429
Day 8	0.3246	0.2104	0.2629	0.3514	1	0.2250	0.2748	0.3411	0.1891
Day 8.5	0.3327	0.4194	0.1428	0.5047	0.2250	1	0.4705	0.5222	0.3127
Day 9	0.3982	0.3017	0.3036	0.3546	0.2748	0.4705	1	0.5635	0.3657
Day 9.5	0.3871	0.4888	0.2416	0.4886	0.3411	0.5222	0.5635	1	0.3695
Day 15	0.2735	0.1944	0.2034	0.1429	0.1891	0.3127	0.3657	0.3695	1

Table C: Correlation coefficients trial H05

	Day0	Day 1	Day 11	Day 18	Day 20	Day 26	Day 28	Day 53	Day 56
Day 0	1	0.2558	0.3575	0.2578	0.1635	0.2649	0.2911	0.1369	0.0839
Day 1	0.2558	1	0.2508	0.2805	0.1044	0.1536	0.2044	0.1195	-0.0005
Day 11	0.3575	0.2508	1	0.5363	0.4117	0.4510	0.4616	0.1834	-0.0076
Day 18	0.2578	0.2805	0.5363	1	0.5952	0.4732	0.5549	0.1427	-0.0522
Day 20	0.1635	0.1044	0.4117	0.5952	1	0.4816	0.5762	0.1881	0.1442
Day 26	0.2649	0.1536	0.4510	0.4732	0.4816	1	0.6007	0.3943	0.2430
Day 28	0.2911	0.2044	0.4616	0.5549	0.5762	0.6007	1	0.4628	0.4212
Day 53	0.1369	0.1195	0.1834	0.1427	0.1881	0.3943	0.4628	1	0.6648
Day 56	0.0839	-0.0005	-0.0076	-0.0522	0.1442	0.2430	0.4212	0.6648	1

Table D: Correlation coefficients trial H06

	Day0	Day0.5	Day 5	Day19	Day19.5	Day21	Day21.5	Day36	Day 40	Day 48
Day0	1	0.5294	0.4184	0.3708	0.4561	0.5025	0.3882	0.1814	0.1185	0.2540
Day 0.5	0.5294	1	0.5278	0.5477	0.5344	0.5690	0.4685	0.2142	0.2350	0.2776
Day 5	0.4184	0.5278	1	0.4904	0.4452	0.4922	0.4205	0.1127	0.1233	0.2263
Day 19	0.3708	0.5477	0.4904	1	0.5599	0.5612	0.5746	0.1922	0.2368	0.3662
Day 19.5	0.4561	0.5344	0.4452	0.5599	1	0.5186	0.6828	0.2401	0.2711	0.3559
Day 21	0.5025	0.5690	0.4922	0.5612	0.5186	1	0.5353	0.2361	0.2564	0.3649
Day 21.5	0.3882	0.4685	0.4205	0.5746	0.6828	0.5353	1	0.3535	0.2910	0.4050
Day 36	0.1814	0.2142	0.1127	0.1922	0.2401	0.2361	0.3535	1	0.1947	0.2810
Day 40	0.1185	0.2350	0.1233	0.2368	0.2711	0.2564	0.2910	0.1947	1	0.3614
Day 48	0.2540	0.2776	0.2263	0.3662	0.3559	0.3649	0.4050	0.2810	0.3614	1

Table E: Correlation coefficients trial I06

	Day0	Day 18	Day 20	Day 33	Day 36
Day0	1	0.1058	0.0624	-0.0425	-0.0397
Day 18	0.1058	1	0.0789	0.0539	0.0625
Day 20	0.0624	0.0789	1	-0.1250	0.0134
Day 33	-0.0425	0.0539	-0.1250	1	0.3223
Day 36	-0.0397	0.0625	0.0134	0.3223	1

APPENDIX B

Table F: Covariance Parameter estimates

Trial	Covariance parameters	Stage	Estimates
D02	UN(1,1)		0.2115
	timeclas	stage 1	0.2591
	timeclas	stage 2	0.0777
D05	UN(1,1)		0.0421
	timeclas	Stage 1	0.1391
H05	UN(1,1)		0.0913
	timeclas	stage 1	0.3898
	timeclas	stage 2	0.3157
H06	UN(1,1)		0.0519
	timeclas	stage 1	0.0989
	timeclas	stage 2	0.5016
I06	UN(1,1)		-0.0890
	timeclas	stage 1	0.6031
	timeclas	stage 2	5.0447

Table G: Empirical Bayes estimates for random intercepts

Genotype	Effect	D02	D05	H05	H06
		Estimate	Estimate	Estimate	Estimate
1	Intercept	-0.00616	0.1710	0.4260	0.4005
2	Intercept	0.05208	0.2131	0.06188	-0.00495
3	Intercept	-0.5869	-0.1555	-0.2644	-0.09040
4	Intercept	0.2279	0.2620	0.3681	0.1941
5	Intercept	-0.5820	-0.1873	0.06310	-0.1871
6	Intercept	0.03809	-0.07689	0.07593	0.06115
7	Intercept	11.308	0.09114	0.2974	0.3413
8	Intercept	-0.1394	0.2963	0.5069	0.2746
9	Intercept	0.2015	0.4970	0.2789	0.3285
10	Intercept	-0.7610	0.007123	-0.1010	0.08068
156	Intercept	-0.8169	-0.1358	-0.3306	-0.2417
157	Intercept	-0.2329	0.01576	-0.2130	-0.2611
158	Intercept	0.6866	-0.1924	-0.08085	0.07780

159	Intercept	-0.1203	-0.00715	-0.2591	-0.01366
160	Intercept	0.9435	0.1615	0.1954	-0.02281
161	Intercept	0.1337	0.07684	-0.1903	-0.09567
162	Intercept	0.07863	0.2450	0.2730	0.1960
163	Intercept	-0.6063	0.1443	0.1863	0.007678
164	Intercept	0.1673	0.3294	0.2964	0.3022
165	Intercept	-0.6935	-0.1277	-0.4888	-0.2018

Table H: checking for influence for the outlying genotypes trial D05

Effect	Estimate	Reduced dataset		Estimate	Full dataset	
		Standard Error	P-value		Standard Error	P-value
Intercept	32.9406	0.8754	<.0001	32.8476	0.8958	<.0001
Time	-6.3751	0.6661	<.0001	-6.3604	0.6670	<.0001
Time ²	1.2002	0.1130	<.0001	1.1964	0.1131	<.0001
Time ³	-0.0520	0.0047	<.0001	-0.0518	0.0048	<.0001
Anthesis	-0.0394	0.0114	0.0006	-0.0376	0.0116	0.0013
Daytime	-5.8713	0.1439	<.0001	-5.9053	0.1432	<.0001
Time*anthesis	0.0184	0.0087	0.0348	0.0180	0.0087	0.0394
Time ² *anthesis	-0.0042	0.0014	0.0039	-0.0042	0.0014	0.0046
Time ³ *anthesis	0.0002	0.0001	0.0009	0.0002	0.0001	0.0011