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Uncovering a Regulatory Switch Controlling Tomato Fruit Ripening

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Abstract

AP2 is a major regulator of tomato fruit ripening and in the fruit pericarp, AP2a regulates the expression of CNR in a negative attitude [7]. Levels of CNR and AP2 gene expression in Wild-type tomato and Mutant type tomato were compared in order to investigate the mechanism of ethylene action [1]. This study is explained the relationship with types (Rin, Nor, Mutant and Wild) and time (breakpoints) for AP2 and CNR levels. The linear model and weighted least square model are created with the type and time variables for these levels. It was found that, AP2 level is not affected by time point. However, CNR level can be changed with type and time points.

Keywords: Linear Model; Tomato fruit ripening; Weighted Least Squares.

1. Introduction

In the late 1980s, Agrobacterium mediated genetic engineering techniques were developed and it could be successfully moved genetic material into the nuclear genome of tomatoes [8]. Genetic material can also be added into a tomato cell's chloroplast and chromoplast plastomes utilizing biolistic [6].

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Fruit ripening is a developmental and biochemical process that includes several metabolic changes and has improved as a mechanism of seed dispersal [2]. In the case of fleshy fruits the changes in colour, texture, flavour, aroma and nutritional characteristics not only make fruit attractive for seed spread organisms, but also supplies essential vitamins, minerals, phytonutrients and fiber important for human and animal diets [2]. The plant hormone ethylene influences numerous parts of plant advancement [4,10] and is supposed to control the ripening of many climacteric fruits, such as tomato [1,10,11,13]. For example, the beginning of tomato fruit ripening is related to an increase in ethylene bio-synthesis, the beginning is hastened when unripe fruit is uncovered to exogenous ethylene, and the deduction of ethylene from the fruit or exposure of fruit to specific inhibitors of ethylene bio-synthesis greatly retard ripening [10]. Subsequently, ethylene impacts the formative methodologies connected with tomato fruit ripening [10]. One hypothesis for the system of ethylene activity is that it controls the articulation of particular genes [10].1 In the work of Oserial and his colleagues it has pointed that systems biology of tomato fruit developed and research the combined transcription factor and metabolite analysis of tomato transcription factor and show the ethylene receptor Mutants reveals novel regulatory interactions [12]. The result of this work, Nor and Rin behaviour together in progressive to control ripening and also Nor has a more important effect on ripening gene expression than Rin [12]. Lincoln J.E. and Fischer R.L. describe the regulation of gene with ethylene in Wild type and rin tomato fruit [10]. They found that the rin mutation causes ethylene levels throughout fruit development [10]. According to another researcher show to AP2 is a major regulator of tomato fruit ripening and in the fruit pericarp, AP2a regulates the expression of CNR in a negative attitude [9]. Tomato is the essential model for clinical fruit ripening for a synthesis of scientific and agricultural causes [9]. Its products of the fruits a critical part of the human diet and gives wellbeing profits as the wellspring of vitamins, minerals and antioxidants. Fruit ripening has a complex design [9]. Genetically programmed process end of dramatic alterations in colour, texture, flavour, and aroma of the fruit freshly [9]. There are various colour mutants in tomato. The Cnr tomato mutant has a colourless, non-ripening phenotype. AP2 plays a critical role in fruit ripening [9]. This gene was described as a negative regulator of ripening and of ethylene production [9, 5]. In this work, carotene formation in the Cnr and AP2 mutant has been studied at the biochemical level. The data are taken from Hodgman and his colleagues ' work which is uncovering a regulatory switch controlling tomato fruit ripening. Levels of CNR and AP2 gene expression in Wild-type tomato (Lycopersiconesculentum), non-ripening (Nor) type tomato, ripening (Rin) type tomato and Mutant type tomato were compared in order to investigate the mechanism of ethylene action [2]. Every type is divided eight breakpoints (Br, Br+1, Br+2, Br+3, Br+4, Br+5, Br+6, Br+7). For Wild type is observed two variables in the each first (Br) and third (Br+2) breakpoints and in other each breakpoints have three variables. Totally, Wild type has twenty two variables. Other each type has three observations for each breakpoint, totally they have twenty four variables. The aim of the study is investigating weather significant to breakpoints and Wild and Mutant types for CNR and AP2 level. Linear model is used for data and looking significance of the time and types. (Wild, Mutant)

2. Methods

2.1. Linear Model

The first of regression type problems was to aid navigation with the use of astronomy in the 18th century [3].
The methods of least squares were developed by Legendre in 1805 [3]. Gauss was developed the method and he

achieved the least squares are the optimal solution when the errors are normally distributed [3]. Generally, the method was used in the physical sciences until later in the nineteenth century [3]. The linear model supposes to, response variable Y, $X_1, X_2, X_3, ..., X_n$ are forms of predictors [3]. The model generally would be,

$$Y = f(X_1, X_2, X_3, ..., X_n) + \varepsilon_i \quad (1)$$

where f is unknown function and is the error in this representation [3]. F is a smooth, continuous function so linear model is generally,

$$Y = \beta_0 + \beta_1 * x_{i1} + ... + \beta_p * x_{i(p-1)} + \varepsilon . \quad (2)$$

The regression equation is marked as:

$$y = X\beta + \varepsilon \quad (3)$$

where

$$y = \begin{bmatrix} y_1 \\ y_2 \\ . \\ . \\ . \\ y_n \end{bmatrix}, X = \begin{bmatrix} 1 & x_{11} & . & . & . & x_{1(p-1)} \\ 1 & x_{21} & . & . & . & x_{2(p-1)} \\ . & . & . & & & . \\ . & . & & . & & . \\ . & . & & & . & . \\ 1 & x_{n1} & . & . & . & x_{n(p-1)} \end{bmatrix},$$

$$\beta = \begin{bmatrix} \beta_1 \\ \beta_2 \\ . \\ . \\ . \\ \beta_{p-1} \end{bmatrix}, \varepsilon = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ . \\ . \\ . \\ \varepsilon_n \end{bmatrix}$$

Figure 1

The best estimate of β defined as the one which minimizes the sum of the squared errors [3]. $\hat{\beta}$ minimizes is called the least squares estimate of β

$$\sum \varepsilon_i^2 = (y - X\beta)^T (y - X\beta) \quad (4)$$

β find that satisfies:

$$\hat{\beta} = (X^T X)^{-1} X^T y$$

These are named Normal equations.

The null hypothesis are written

$$H_0 : \beta_j = 0$$

For test on a single predictor [5]. If

$$|T| = \left| \frac{\hat{\beta}_j}{SE(\hat{\beta}_j)} \right| > t_{n-p,\alpha/2} ,$$

Null hypothesis reject and coefficient is significant.

2.2 Weighted Least Squares

Linear model is assumed that the error term ε is that it is independent and identically distributed (i.i.d.) from case to case [5]. That is, $var\varepsilon = \sigma^2 I$. Weighted least squares are used when the errors are independent, but not identically distributed. Sometimes the errors are uncorrelated but have unequal variance where the form of the inequality is known [3]. When $\sum = diag(1/w_1, ..., 1/w_n)$ where w_i are the weights so. So $S = diag(\sqrt{1/w_1}, ..., \sqrt{1/w_n})$ we can regress $\sqrt{w_i y_i}$ $\sqrt{w_i x_i}$ on [5]. Cases with low variability should get a *high weight, high variability a low weight [5]*.

2.3 Compare model

There are a lot of ways for comparing models. In this project, Akaike Information Critertion is used for choosing better model. It is defined as -2maxloglikelihood+2p. Akaike Information Critertion prefer small result for the best fit model [3].

3. Results and Discussion

In this project, type and time columns are made. Type column is included the “0” and “1”. “0” represents the Wild type and “1” representing the Mutant type. Time column is included the “0,1,...,7” which represents breakpoints. Firstly, we will look model for CNR. General model is; Table 1 shows to R–squared is approximately 85 % of variation in CNR can be explained by our model (Time and type). First fitted model is If we look p value all significant over the model. Wild and Mutant types of CNR are oscillations look like sine and a period of oscillations is 2 days for Wild type, 3 days for Mutant type (Figure 1). R–squared is approximately 85 % of variation in CNR can be explained by our model in table 1. Model ’s p value is small and model is significant. The reason of using weighted least squares is Wild type has twenty two variables, however Mutant type has twenty four variables so the model can be non-constant variance of error. R –squared is approximately 84%. All p values are smaller than 0.05 so all parameters are significant. Akaike Information criterion values

and the first model (727.2861) is smaller than the second model (728.9092) and third model (731.5996) so the first model is the best model. In summary, oscillations are not significant for CNR level.

Table 1: Level of CNR linear models

Level of CNR linear model				Model with sine function for CNR			Weighted least square for CNR		
Parameter	Estimate	Standard error	p value	Estimate	Standard error	p value	Estimate	Standard error	p value
Intercept	4412.32	198.73	≈0	4200	152.2	≈0	4606.34	187.54	≈0
Time	-96.97	40.04	0.02	$5103*10^{11}$	$2.498*10^{11}$	0.047	-149.03	40.48	≈0
Type	-2893.47	181.91	≈0	-3041	202.7	≈0	-2905.30	194.98	≈0
Residual standard error: 615.5 on 43 degrees of freedom				Residual standard error: 626.5 on 43 degrees of freedom			Residual standard error: 1013 on 43 degrees of freedom		
Multiple R-squared: 0.856, Adjusted R-squared: 0.8493				Multiple R-squared: 0.8508 , Adjusted R-squared: 0.8439			Multiple R-squared: 0.843, Adjusted R-squared: 0.8357		
F-statistic: 127.8 on 2 and 43 DF, p-value: ≈0				F-statistic: 122.6 on 2 and 43 DF, p-value: ≈0			F-statistic: 115.5 on 2 and 43 DF,p-value: ≈0		

Table 2: Level of AP2 linear models

Level of AP2 linear model				Model with sine function for AP2			Weighted least square for AP2		
Parameter	Estimate	Standard error	p value	Estimate	Standard error	p value	Estimate	Standard error	p value
Intercept	1234.24	76.24	≈0	1240.11	77.01	≈0	1168.10	50.41	≈0
Time	-17.75	15.36	0.254	-19.32	15.69	0.225			
Type	-943.36	69.79	≈0	-943.72	71.16	≈0	939.33	71.49	≈0
Residual standard error: 236.1 on 43 degrees of freedom				Residual standard error: 360.4 on 43 degrees of freedom			Residual standard error: 362.54 on 44 degrees of freedom		
Multiple R-squared: 0.8097, Adjusted R-squared: 0.8009				Multiple R-squared: 0.8038, Adjusted R-squared: 0.7947			Multiple R-squared: 0.7969, Adjusted R-squared: 0.7923		
F-statistic: 91.48 on 2 and 43 DF, p-value: ≈0				F-statistic: 88.09 on 2 and 43 DF, p-value: ≈0			F-statistic: 172.6 on 1 and 44 DF, p-value: ≈0		

This paper has given information about the CNR and AP2 levels relationship with type (Wild, Mutant) and time (breakpoints). To sum of the analysis which is choosing the best model for Wild and Mutant types of CNR and AP2 levels. Type is a matrix which is occurred with "0" (Wild type) and "1" (Mutant type) and time is occurred

"0, 1, ... 7" (Br, Br+1, ..., Br+7). In this project, linear model and weighted least square model are used. The reason of use weighted least square is Wild type has twenty two variables; however Mutant type has twenty four variables so model can be non-constant variance of error. The results of linear model time and type variables are significant for CNR level. In addition, we reached constant variance and oscillations are not significant for CNR level of Mutant and Wild types. Our final fitted model is,

$$Y_{i1} = 4412.32 - 96.97 * x_{i1} - 2893.47 * x_{i2} + \varepsilon_i$$

for CNR level of Wild and Mutant types.

AP2 level of Wild and Mutant types have non-constant variance of errors so in this project applied the linear model and weighted least model. Two of models are time is not significant so time (breakpoints) is not important when the model explanations for AP2 level. Our final model is with type variable and weighted least square model and fitted model is shown;

$$Y_{i2} = 1168.10 - 939.33.97 * x_{i1} + \varepsilon_i$$

for AP2 level.

4. Recommendations

In this study, our data include time and type variables for AP2 and CNR levels. In future studies, more data will be collected like weather, another condition which is effective to AP2 and CNR levels. With this information, the project will be extended and give a clearer idea about CNR and AP2 levels.

Acknowledgment

The data are taken from Hodgman and his colleagues ' work which is uncovering a regulatory switch controlling tomato fruit ripening. Levels of CNR and AP2 gene expression in Wild-type tomato (Lycopersiconesculentum), non-ripening (Nor) type tomato, ripening (Rin) type tomato and Mutant type tomato were compared in order to investigate the mechanism of ethylene action. This work part of master thesis [14].

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