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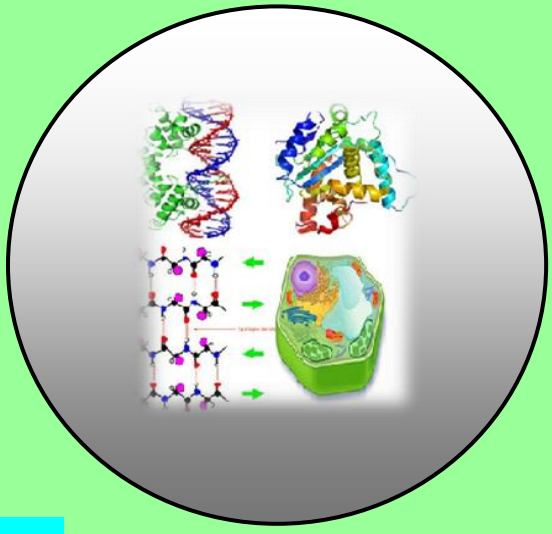
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66
Index Copernicus International Value
IC Value of Journal 47.86 Poland, Europe

J. Biol. Chem. Research
Volume 33 (1) 2016 Pages No. 454-465



Journal of Biological and Chemical Research

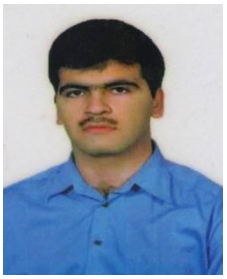
An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 33, No. 1: 454-565, 2016
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 33/2/21/2016

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)



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RESEARCH PAPER

Received: 24/04/2016 **Revised: 06/05/2016** **Accepted: 10/05/2016**

Evaluation of Effects of the 64 combined Hormone and Vitamin on shoot Induction in Agria Potatoes

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ABSTRACT

Due to the sensitivity of potato to virus, production of virus-free plants, reduction in production costs and increased performance, in vitro cultivation of this plant is very important. To evaluate the effect of six combined hormones and vitamins as treatments on main and secondary shoot length, the minimum and maximum of secondary shoot length and number of secondary shoot Agria potatoes, an experiment was conducted in 2014-2015 in a randomized complete block design with three replications in laboratory condition. Multivariate analysis of variance (multivariate) showed that in all traits between treatments (hormonal compounds) there is a significant difference at 1% probability level. Based on the obtained results, the main shoot length of treatment (Pyridoxine5ppm + thiamine 20ppm + biotin 10 ppm+ cytokinin 5ppm) and the lowest amount related to combined hormone treatment (Pyridoxine5ppm + thiamine 5ppm + biotin 5ppm + cytokinin 2ppm), respectively. Based on these results, treatment of thiamine 5ppm is the best combination to increase the minimum of secondary shoot length and the mean of secondary shoot length. The best treatment combination to increase the length of the side branch was 10ppm biotin. It is recommended to produce the greatest number of secondary shoot cytokinin 2ppm. It also became clear that in order to increase the main shoot length Pyridoxine5ppm + thiamine treatment combination 20ppm + 10 biotin ppm+ cytokinin 5ppm is appropriate.
Keywords: Hormonal Compounds Shoot Induction and Agria Potatoes.

INTRODUCTION

Potato is one of the most valuable herbs in the world. This plant in 140 countries and at a level equivalent to 20 million hectares cultivated. The most allocated cultivation areas of potatoes in the world are located in Asia and Europe that China is the largest area under potato cultivation and about 25 percent of the world's potatoes are produced in China (Mikeny, 2006). Potato production in Iran has special value. Iran ranks twelfth in the world in terms of potato production and in Asia, after China and India is in third place (FAO, 200). Potato has the supply of seeds, fertilizers, agricultural operations, pest control and labor in different areas, and is an expensive product. Thus the average cost of this product is high (Hooker, 1990; Seabrook, 2005). On the other hand, the variety of potato cultivars face viruses and viroid seriously. Potato virus tubular leaves, potato virus Y will decrease yield up to 95% and potato virus X cause reduction in tuber by 75-50 percent (Fatima et al., 2005).Since the potato multiplication by non-sexual organs (glands and micro-glands) and propagation by seed breeding true to occur, the availability of disease-free plants and tubers are important.

So in addition to classical and traditional methods, using modern techniques of genetic engineering and tissue culture, is the good news in the quickest way to potato breeding purposes. Reservation, germ plasm conservation, healthy and virus-free plants, saving time and costs, including the use of tissue culture of haploid production (Bajaj, 1987).

Due to the sensitivity of potato viruses, production of virus-free plants through in vitro cultivation and their proliferation, leads to reduced costs and increased performance (Fatima et al., 2005). Meristem culture for production of virus-free plants for the first time, 60 years ago by Morel and Martin were used. (Quote from Fatima et al., 2005; Espinoza et al., 1984).

In vitro propagation of potato(Production of virus-free plants, micro-glands) are reliable methods for seed production, is free from any contamination (Al-Safadiet al., 2000).Due to this, in vitro propagation of virus-free plants is important (Hussain et al., 2006; Taboriet al., 1999).Micro-glant production in vitro experiment for the first time as a tool for solving problems in potato pathology (Coleman et al., 2001), by culturing individual nodes with lateral buds to produce virus-free were in potato seed tubers (Gopal et al., 2004; 1998).Since the potato plantlets in vitro at a rapid rate during transportation, re-cultivation (compatibility) will be destroyed, therefore, to provide an alternate source to decrease damage during cultivation seems to be necessary.

The micro-glant production as a source of basic seed production through tissue culture is beneficial (Hussain et al., 2006; Taborietal., 1999). According to the sources, previous studies on the production of virus-free plants to study and Martin Morrell goes back to 1952 and considering the importance of potato production and propagation of virus-free plants, since then, a number of factors involved in proliferation of potato (In vitro production of virus-free plants and micro-glant) is studied.

In most reports, Good seedling production in vitro, have a lower success, because during transportation, and re-cultivation vanishes and on the other hand micro-glant produced using growth regulators and certain substances such as the CCC, however, the number, size and speed of tubers and not a good start.

This paper is optimized for shoot induction medium and plants in vitro on MS medium by varying the concentration of the three vitamins MS medium and conducted using BAP, till the best combination of BAP and vitamin obtain for shoot induction.

MATERIAL AND METHODS

In this survey first, the medium for MS was prepared and single-node cuttings were planted in Agria potatoes. To prepare the medium, ingredients for 6 solution's (stock) includes salts macro elements, salts, micronutrients, KI, CaCl2, iron salts and vitamins were prepared and was kept in the fridge.To prepare the medium, the required storage solutions are removed and with combinations of growth regulators required (which was prepared in reserve) were added to the medium.Volume of the solution become up to 1000 ml depending on the medium may be30 g of sucrose is added to the environment.After the addition of Miyoinozitol1.0 grams per liter, pH solution by using a normal NaOH or HCl was adjusted to 8.5.for preparing solid environment 8 g of agar was added to the medium.Hormonal treatment used in this study for the shoot induction, cytokinins, Pyridoxine, thiamine, biotin, respectively. BAP to dissolve completely in water a few drops to a normal NaOH or KOH was used. Single-node scion taken from the middle of the shoot, in order to have haemogenic explants, medium salts containing MS, hormonal treatments and vitamins were transferred.
In this experiment to study shoot induction and roots, under conditions of darkness and light in a medium consisting of salts of MS, vitamins treatments and BAP were studied. Cultivation in 16 h light and 8 hours in the dark at 2 ± 25 ° C for one month were maintained. In this experiment, in any cultivation, hormonal treatments effect, types and amounts of vitamins on the main shoot length, number of secondary shoot, the maximum number of secondary shoot, the maximum number of secondary shoot and the mean of secondary shoot length of Agria as a randomized complete block design with 3 replications was conducted.

Treatments include

NO. Treatment	Treatments(Hormone Levels)	Description of the treatments
0	Control	(0 ppm) CompoundsTreatmental
1	PBA2	2Cytokininppm
2	PBA3	3Cytokininppm
3	PBA4	4Cytokininppm
4	PBA5	5Cytokininppm
5	P5	5 Pyridoxineppm
6	P20	20 Pyridoxineppm
7	T5	5 Thiaminppm
8	T20	20 Thiaminppm

9	**B5**	**5 Biotinppm**
10	**B10**	**10 Biotinppm**
11	**P5 PBA2**	**2cytokininppm + 5 Pyridoxineppm**
12	**P20 PBA2**	**2cytokininppm + 20 Pyridoxineppm**
13	**T5 PBA2**	**2cytokininppm + 5 Thiaminppm**
14	**T20 PBA2**	**2cytokininppm + 20 Thiaminppm**
15	**B5 PBA2**	**2cytokininppm + 5 Biotinppm**
16	**B10 PBA2**	**2cytokininppm + 10 Biotinppm**
17	**P5 PBA3**	**3cytokininppm + 5 Pyridoxineppm**
18	**P20 PBA3**	**3cytokininppm + 20 Pyridoxineppm**
19	**T5 PBA3**	**3cytokininppm + 5 Thiaminppm**
20	**T20 PBA3**	**3cytokininppm + 20thiaminppm**
21	**B5 PBA3**	**3cytokininppm + 5biotinppm**
22	**B10 PBA3**	**3cytokininppm + 10biotinppm**
23	**B5 PBA4**	**5cytokininppm + 5biotinppm**
24	**P20 PBA5**	**5cytokininppm + 20pyridoxineppm**
25	**T5 PBA5**	**5cytokininppm + 5thiaminppm**
26	**T20 PBA5**	**5cytokininppm + 20thiaminppm**
27	**B5 PBA5**	**5cytokininppm + 5biotinppm**
28	**B10 PBA5**	**5cytokininppm + 10biotinppm**
29	**P5 PBA4**	**4cytokininppm + 5pyridoxineppm**
30	**P20 PBA4**	**4cytokininppm + 20pyridoxineppm**
31	**T5 PBA4**	**4cytokininppm + 5thiaminppm**

32	**T20 PBA4**	**4cytokininppm+ 20thiaminppm**
33	**B5 PBA6**	**cytokininppm6 + 5biotinppm**
34	**B10 PBA4**	**4cytokininppm + 10biotinppm**
35	**P5 T5 B5 PBA2**	**+ 5thiaminppm + 5pyridoxineppm 2cytokininppm + 5biotinppm**
36	**P20 T20 B10 PBA2**	**+ 20thiaminppm + 20pyridoxineppm 2cytokininppm + 10biotinppm**
37	**P20 T5 B10 PBA2**	**+ 5thiaminppm + 20pyridoxineppm 2cytokininppm + 10biotinppm**
38	**P5 T5 B5 PBA3**	**+ 5thiaminppm + 5pyridoxineppm 3cytokininppm + 5biotinppm**
39	**P20 T20 B10 PBA3**	**+ 20thiaminppm + 20pyridoxineppm 3cytokininppm + 10biotinppm**
40	**P20 T5 B10 PBA3**	**+ 5thiaminppm + 20pyridoxineppm 3cytokininppm + 10biotinppm**
41	**P5 T5 B5 PBA4**	**+ 5thiaminppm + 5pyridoxineppm 4cytokininppm + 5biotinppm**
42	**P20 T20 B10 PBA4**	**+ 20thiaminppm + 20pyridoxineppm 4cytokininppm + 10biotinppm**
43	**P20 T5 B10 PBA4**	**+ 5thiaminppm + 20pyridoxineppm 4cytokininppm + 10biotinppm**
44	**P5 T5 B5 PBA5**	**+ 5thiaminppm + 5pyridoxineppm 5cytokininppm + 5biotinppm**
45	**P20 T20 B10 PBA5**	**+20thiaminppm + 20pyridoxineppm 5cytokininppm + 10biotinppm**
46	**P20 T5 B10 PBA5**	**+ 20thiaminppm + 20pyridoxineppm 5cytokininppm + 10biotinppm**

Signs used in the treatment of hormone compounds include:
T (thiamin) = B1 vitamin,
P (pyridoxine) = B6 vitamin
PBA (cytokinin) = **[benzyl) -9- (2-Ttrahydrvpyranyl) 9- H purine)**
B(Biotin)=5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid

After sufficient growth of shoots (4 weeks after planting) notes were done.Evaluatedtrait include.
Main shoot length, number of secondary shoot, the maximum number of secondary shoot, the minimum number of secondary shoot and the mean of secondary shoot length.Length was measured with a ruler.Data in randomized complete block design was analyzed with SPSS software.Many treatments for mean comparison were drawn in the diagram.SPSS software was used for plotting graphs.

RESULTS AND DISCUSSION

Multivariate analysis of variance (multivariate) traits are listed in Table 1. According to Table 1, for all studied traits among treatments (hormonal compounds) are significantly different at the 1% level. Also among iterations only the number of secondary shoot had significant difference at 5% level. According to Table 2, all indicators and statistical criteria among treatment and hormonal combination have significant differences at 1% of the studied characters.

Table 1.Analysis of variance multiple traits in different hormone combinations.

Source of variant	df	Mean of square				
		Main shoot length(mm)	Number of secondary shoot	The maximum number of secondary shoot	The minimum number of secondary shoot	The mean of secondary shoot length(mm)
Replication	2	**1.799**	**3.814***	**2.830**	**.641**	**2.627**
Hormone Levels	46	**30.048****	**9.636****	**18.533****	**12.288****	**18.077****
Error	92	4.356	0.522	13.189	18.767	12.601
C.V(%)		3.73	25.32	33.38	49.17	35.39
***And **,significant in 5% and 1%,respectively.**						

Table 2. Test results of multiple experiments of assessed traits in multiple variance analysis.

Effect		Amount	Amount of F	df of Theory	df of Error	Significant level
Replication	**Pillai's Trace**	**.190**	**1.867^{ns}**	**10.000**	**178.000**	**.053**
	Wilks' Lambda	**.813**	**1.921***	**10.000**	**176.000**	**.045**
	Hotelling's Trace	**.227**	**1.975***	**10.000**	**174.000**	**.039**
	Roy's Largest Root	**.211**	**3.760****	**5.000**	**89.000**	**.004**
Hormone Levels	**Pillai's Trace**	**3.440**	**4.408****	**230.000**	**460.000**	**.000**
	Wilks' Lambda	**.000**	**7.327****	**230.000**	**443.351**	**.000**
	Hotelling's Trace	**33.883**	**12.728****	**230.000**	**432.000**	**.000**
	Roy's Largest Root	**22.229**	**44.459****	**46.000**	**92.000**	**.000**

. ***And **,significant in 5% and 1%,respectively.**

According to Figure 1, most of the main shoot length relate to treatment 45, (Pyridoxine 5ppm + thiamine 20ppm + biotin 10ppm + cytokinin 5ppm) and the lowest amount of that relates to the treatment and combined hormone 35 (Pyridoxine5ppm + thiamine 5ppm + biotin 5ppm + cytokinin 2ppm) respectively. Based on trait of the number of secondary shoot, the maximum amount relates to treatment 2 (cytokinin ppm2) and lowest one relates to control treatment and treatment7 (thiamine 5ppm) (Figure 2).

According to Figure 3, the maximum amount of secondary shoot length was observed in treatment 10 (biotin 10ppm). According to Figure 4, the maximum value of the minimum of secondary shoot length was observed in treatment 7 (thiamine 5ppm) and 10 (biotin 10ppm). According to Figure 5, the maximum value of the mean of secondary shoot length was observed in treatment 7 (thiamine 5ppm).

According to the results, the treatment of thiamine 5ppm as the best combination to increase the minimum of secondary shoot length and the mean of secondary shoot length was found. On the other hand, the best treatment combination to increase the maximum of secondary shoot length was biotin 10ppm. Also, cytokinin 2ppm is recommended to produce the greatest number of secondary shoot. To increase the main shoot length Pyridoxine5ppm + thiamine 20ppm + biotin 10ppm + cytokinin 5ppm are recommended.

Description of the Figures

NO. Treatment	Treatments(Hormone Levels)	Description of the treatments
0	**Control**	**0compoundsTreatmentalppm**
1	**PBA2**	**2cytokininppm**
2	**PBA3**	**3cytokininppm**
3	**PBA4**	**4cytokininppm**
4	**PBA5**	**5cytokininppm**
5	**P5**	**5pyridoxineppm**
6	**P20**	**20pyridoxineppm**
7	**T5**	**5thiaminppm**
8	**T20**	**20thiaminppm**
9	**B5**	**5biotinppm**
10	**B10**	**10biotinppm**
11	**P5 PBA2**	**2cytokininppm + 5pyridoxineppm**
12	**P20 PBA2**	**2cytokininppm + 20pyridoxineppm**
13	**T5 PBA2**	**2cytokininppm + 5thiaminppm**
14	**T20 PBA2**	**2cytokininppm + 20thiaminppm**
15	**B5 PBA2**	**2cytokininppm + 5biotinppm**

16	**B10 PBA2**	**2cytokininppm + 10biotinppm**
17	**P5 PBA3**	**3cytokininppm + 5pyridoxineppm**
18	**P20 PBA3**	**3cytokininppm + 20pyridoxineppm**
19	**T5 PBA3**	**3cytokininppm + 5thiaminppm**
20	**T20 PBA3**	**3cytokininppm + 20thiaminppm**
21	**B5 PBA3**	**3cytokininppm + 5biotinppm**
22	**B10 PBA3**	**3cytokininppm + 10biotinppm**
23	**B5 PBA4**	**5cytokininppm + 5biotinppm**
24	**P20 PBA5**	**5cytokininppm + 20pyridoxineppm**
25	**T5 PBA5**	**5cytokininppm + 5thiaminppm**
26	**T20 PBA5**	**5cytokininppm + 20thiaminppm**
27	**B5 PBA5**	**5cytokininppm + 5biotinppm**
28	**B10 PBA5**	**5cytokininppm + 10biotinppm**
29	**P5 PBA4**	**4cytokininppm + 5pyridoxineppm**
30	**P20 PBA4**	**4cytokininppm + 20pyridoxineppm**
31	**T5 PBA4**	**4cytokininppm + 5thiaminppm**
32	**T20 PBA4**	**4cytokininppm+ 20thiaminppm**
33	**B5 PBA6**	**6 cytokininppm + 5biotinppm**
34	**B10 PBA4**	**4cytokininppm + 10biotinppm**
35	**P5 T5 B5 PBA2**	**+ 5thiaminppm + 5pyridoxineppm**
36	**P20 T20 B10 PBA2**	**2cytokininppm + 5biotinppm + 20thiaminppm + 20pyridoxineppm**
37	**P20 T5 B10 PBA2**	**2cytokininppm + 10biotinppm + 5thiaminppm + 20pyridoxineppm**
38	**P5 T5 B5 PBA3**	**2cytokininppm + 10biotinppm + 5thiaminppm + 5pyridoxineppm**
39	**P20 T20 B10 PBA3**	**3cytokininppm + 5biotinppm + 20thiaminppm + 20pyridoxineppm**

40	**P20 T5 B10 PBA3**	**3cytokininppm + 10biotinppm + 5thiaminppm + 20pyridoxineppm**
41	**P5 T5 B5 PBA4**	**3cytokininppm + 10biotinppm + 5thiaminppm + 5pyridoxineppm**
42	**P20 T20 B10 PBA4**	**4cytokininppm + 5biotinppm + 20thiaminppm + 20pyridoxineppm**
43	**P20 T5 B10 PBA4**	**4cytokininppm + 10biotinppm + 5thiaminppm + 20pyridoxineppm**
44	**P5 T5 B5 PBA5**	**4cytokininppm + 10biotinppm + 5thiaminppm + 5pyridoxineppm**
45	**P20 T20 B10 PBA5**	**5cytokininppm + 5biotinppm +20thiaminppm + 20pyridoxineppm**
46	**P20 T5 B10 PBA5**	**5cytokininppm + 10biotinppm + 20thiaminppm + 20pyridoxineppm**
		5cytokininppm + 10biotinppm

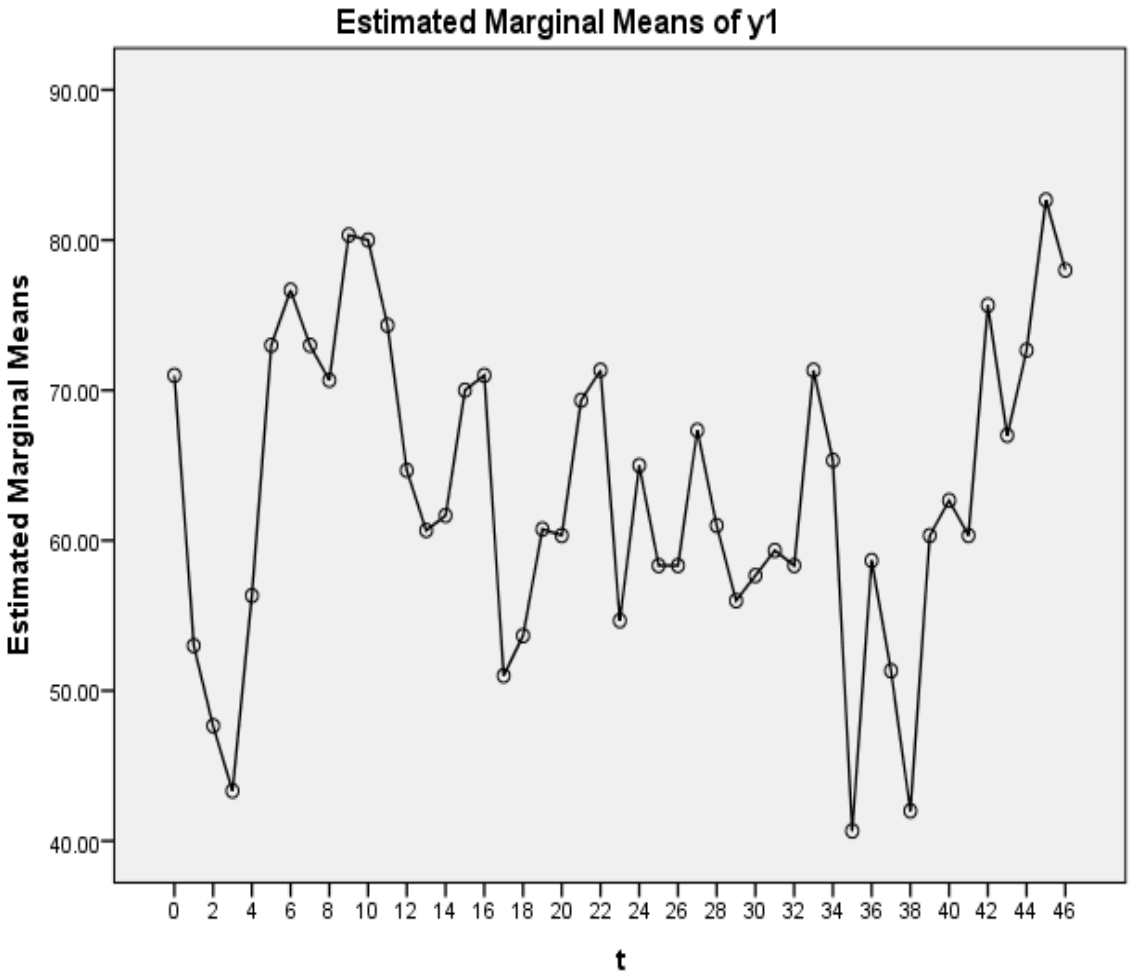

Figure 1. Diagram of hormonal treatments on main shoot length.

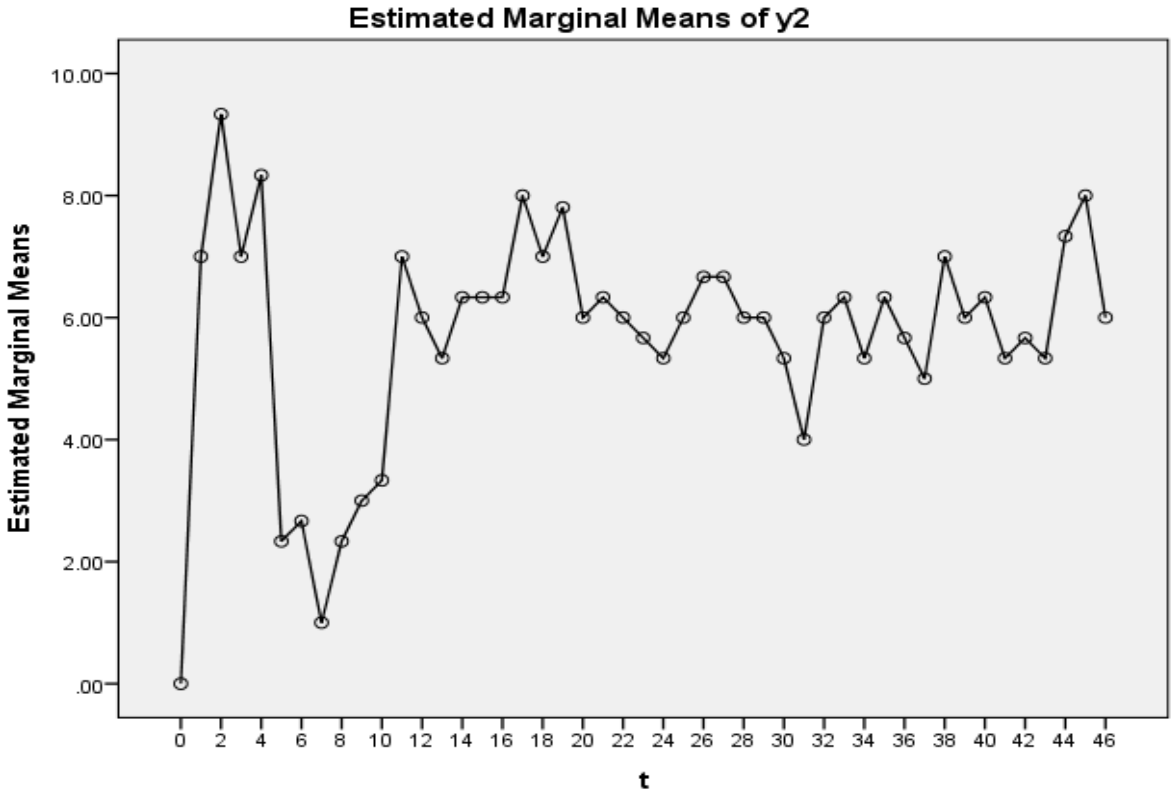

Figure 2. Diagram of hormonal treatments on the number of secondary shoot.

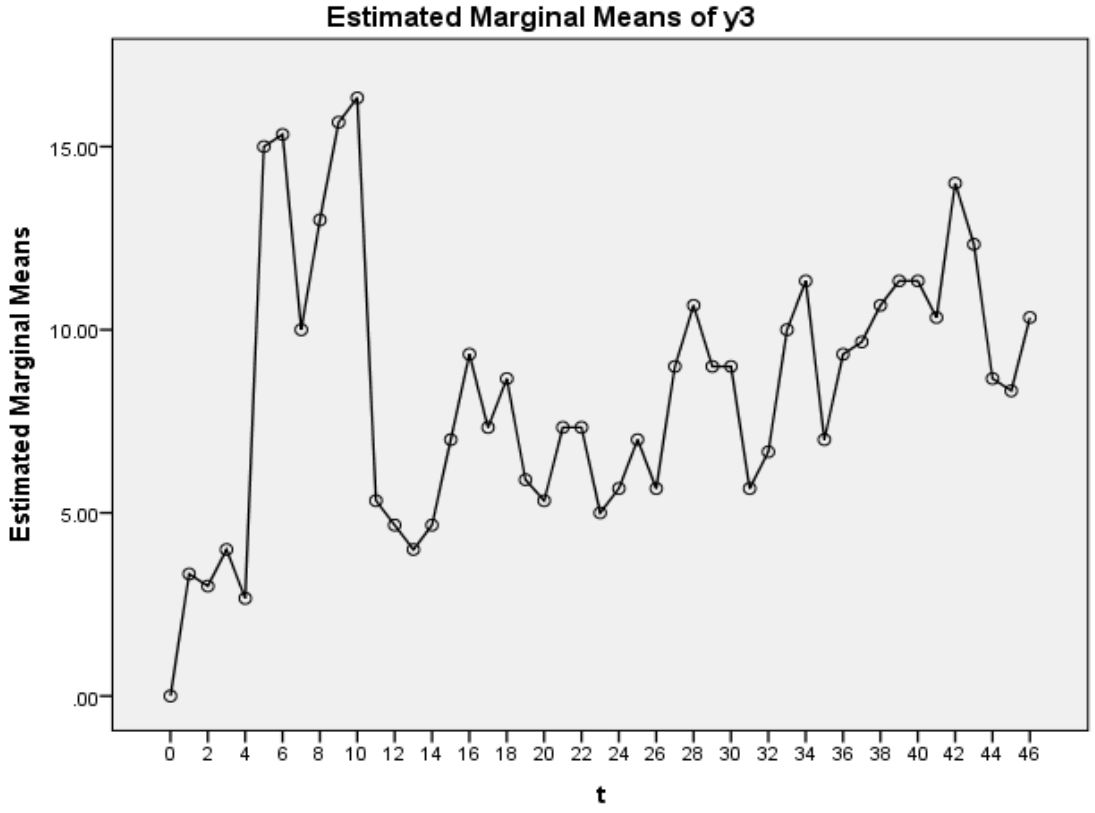

Figure 3. Diagram of hormonal treatments on the maximum of secondary shoot length.

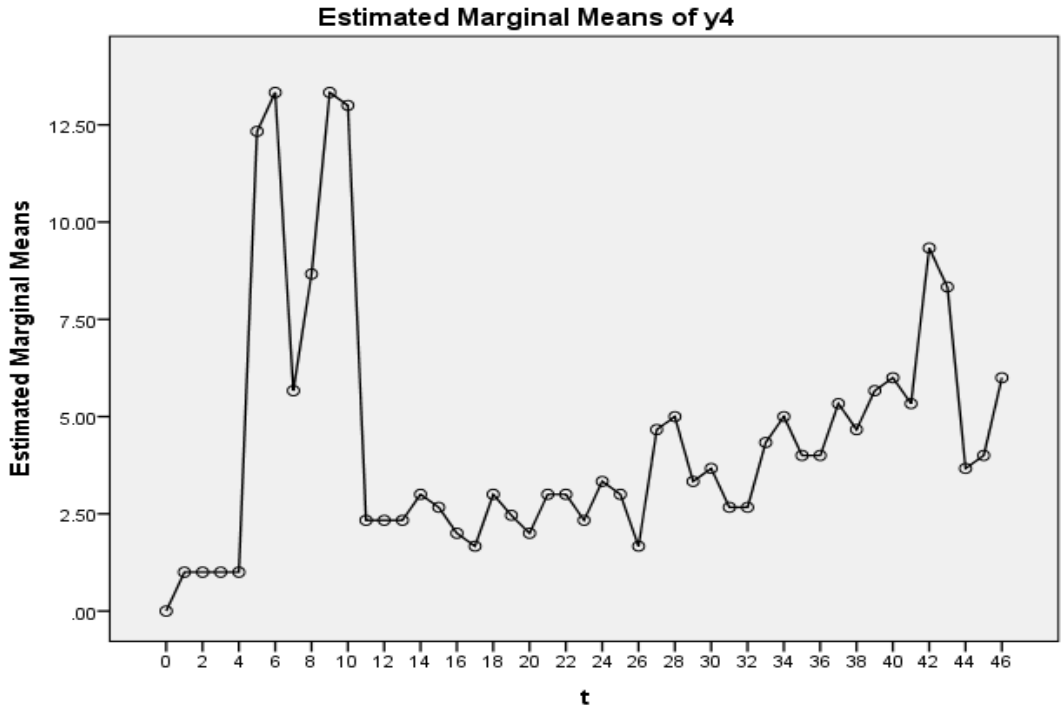

Figure 4. Diagram of hormonal treatments on the minimum of secondary shoot length.

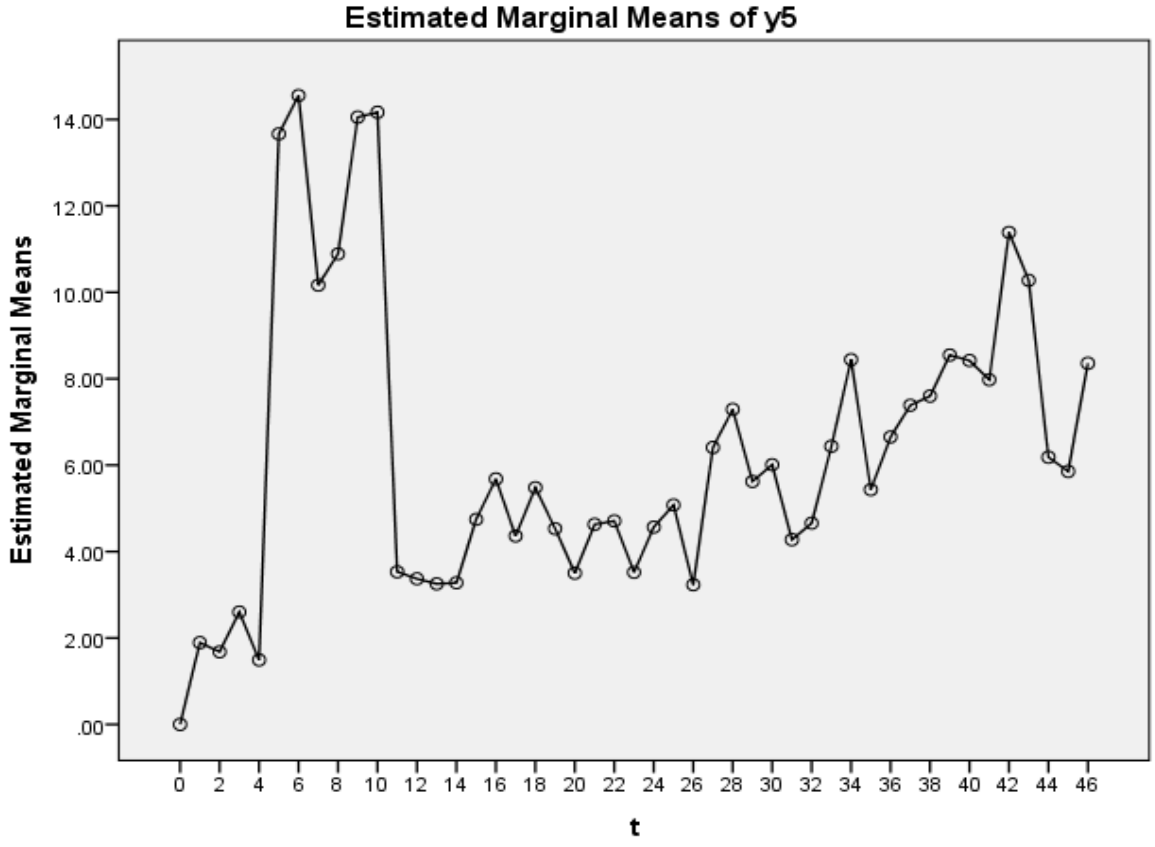

Figure 5. Diagram of hormonal treatments on the mean of secondary shoot length.

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