

# Use of microorganism as a biofertilizer on bean



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# Aim of study

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The objectives of this study are:

- 1-To estimate the effect of bacteria as a biofertilize on bean .
- 2-To understand the relationship between bacteria and bean .

# Introduction

- ▶ Legumes (Leguminosae) are economically important crop, Legumes are a high quality, protein-rich animal feed and whole some source of nutrition for people; moreover, they are a valuable crop in different crop rotation systems.
- ▶ Legumes are able to fix atmospheric nitrogen up to  $250 \text{ kg ha}^{-1}$ , thus reducing the load on the agro fertilizer.
  - ▶ For example plant associated micro-organisms nitrogen-fixing Rhizobium .
- ▶ one of the most important ways to take advantage of useful interaction of microorganisms and to maintain diversity in agricultural ecosystems is to use useful soil microorganisms which are living in rhizosphere environment and are capable of improving plant nutrition and soil fertility through biological fixation of nitrogen, phosphate solubilization, and generally enhancement of plant growth.

- ▶ Most cultivated soils contain large populations of such micro-organisms.
- ▶ Use of biofertilizers is one of the important components of integrated nutrient management.
- ▶ Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers.
- ▶ They can be grouped in different ways based on their nature and function, such as *Bacillus megaterium* var, *phosphaticum*, *Bacillus subtilis*, *Bacillus circulans*, *Pseudomonas striata* and *Em1*.
- ▶ In order to develop sustainable farming systems with reduced inputs of manufactured fertilizers, more attention should be given to the use of microorganisms that could have beneficial effects on crop production .


# Material and method

- ▶ Soil (sand : clay) 1:1
- ▶ 90 pots .
- ▶ Two type seed of bean (قرطاسي وبلدي) .
- ▶ Two type of bacteria 1)Em1 2) *Bacillus megaterium* .
- ▶ Chlorophyll meter (SPAD) .
- ▶ Meter .
- ▶ Balance .
- ▶ Water sources .



# Method

- ▶ Soil was a mixture sand : clay (1:1) .
- ▶ 90 pots were filled with soil mixture .
- ▶ Two type of bean seeds (قرطاسي و بلدي) was planting in pots at 22/12/2016 .
- ▶ Inoculation both type of bacteria Em1, *Bacillus megaterium* in plant bean twice with one month interval between the two inoculation.
- ▶ The first inoculation was do after one month from planting.
- ▶ Irrigation was carried out 3 times per week, each plant takes about 3 liters of water per week.
- ▶ Bean started flowering at 22/Feb. As a result ,we reduced the number of irrigation times per week.
- ▶ The pods stated formation at 13/March

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- ▶ The length of the plant and the length of the pod were measured and the number of branches, leaves and pod was calculated for each pot at 11/4/2017.
  - ▶ We use chlorophyll meter (SPAD) to determine the chlorophyll content in the leaves .
  - ▶ Harvested of plant at 16/4/2017, after that we take weight of pods and seed in pods then measure the number of seeds for each plant .
  - ▶ After we harvested, the plant root was washed to get rid of the soil mixture and get roots .
  - ▶ The weight of roots in each pot was measured .
  - ▶ After that, we determine the number of nodules in random samples of five plants from each variety and treatments.
  - ▶ Measure the weight of nodules .
  - ▶ The experimental design was CRBD
  - ▶ SAS V8 to conduct analysis of variance and mean separation.

# ANOVA test for chlorophyll content in plant

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	499.237311	249.618655	1.36	0.2616
bacteria	2	1634.979768	817.489884	4.47	0.0145
var	1	74.574074	74.574074	0.41	0.5251
bacteria*var	2	375.944228	187.972114	1.03	0.3628





# LSD for Chlorophyll Contents in Bean

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	8.4376

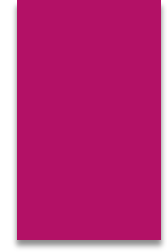
<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	5.7417

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	bacteria
	A	33.266	29	B.M
B	A	25.200	29	control
B		23.230	30	EM1

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	28.173	45	Qertas (1)
A	26.153	43	Local (2)

# ANOVA test for Number of leaves in Bean

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	372.558943	186.279472	3.74	0.0278
bacteria	2	2588.749419	1294.374710	26.02	<.0001
var	1	1081.891875	1081.891875	21.75	<.0001
bacteria*var	2	402.287515	201.143757	4.04	0.0212



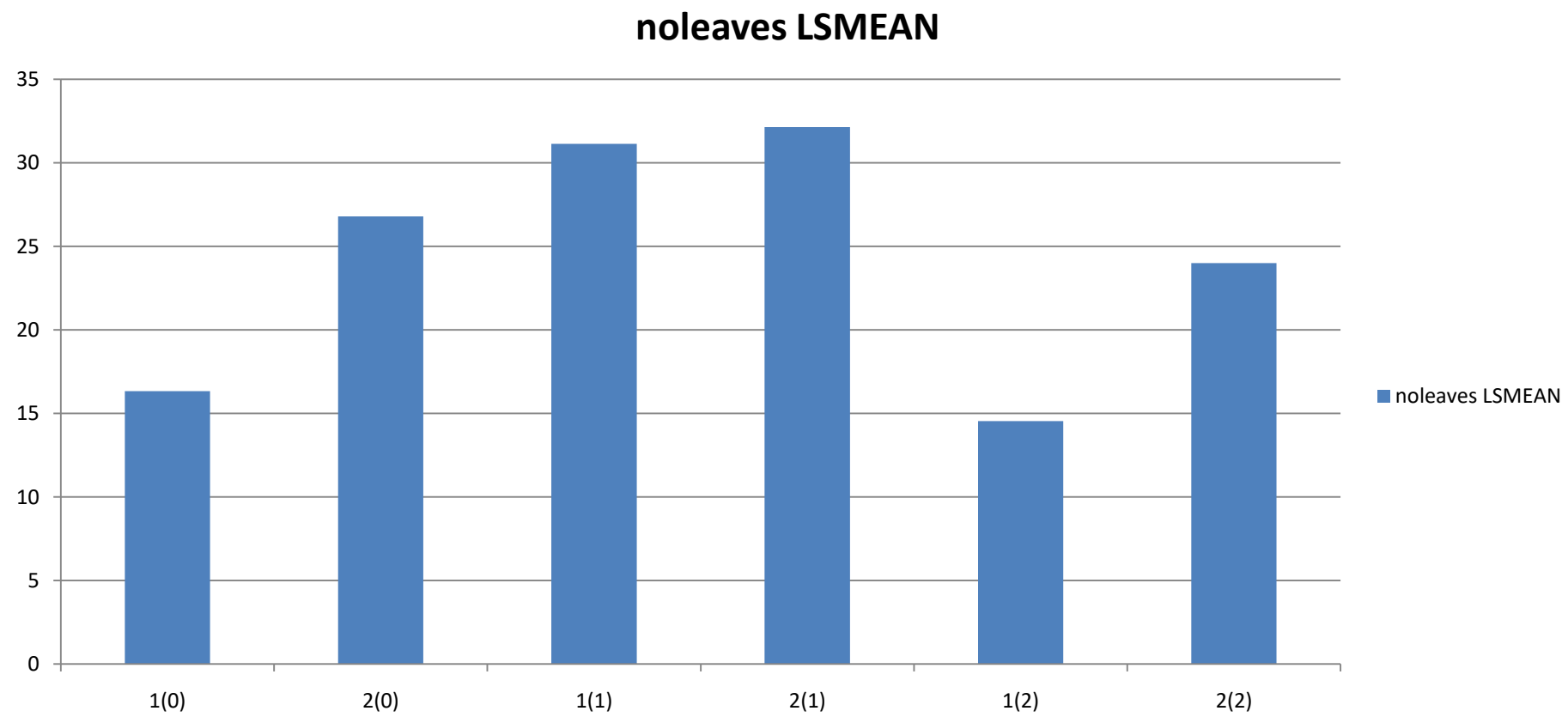
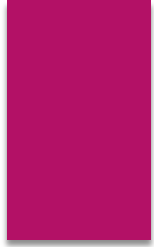
# LSD test for Number of leaves in Plant

Alpha	0.05
Minimum Significant Difference	4.3732

Alpha	0.05
Minimum Significant Difference	2.9755

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	31.633	30	1
B	21.483	29	0
B	19.267	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	27.727	44	2
B	20.667	45	1

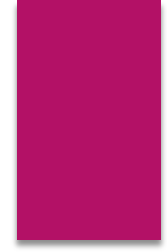


# ANOVA test for Pod length in Bean

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	0.355556	0.177778	0.05	0.9481
bacteria	2	8.022222	4.011111	1.20	0.3051
var	1	1681.344444	1681.344444	504.81	<.0001
bacteria*var	2	10.155556	5.077778	1.52	0.2238



# LSD test for Pod Length in Bean



Alpha	0.05
Minimum Significant Difference	1.1248

Alpha	0.05
Minimum Significant Difference	0.7654

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	10.6333	30	1
A	10.0000	30	0
A	10.0000	30	2

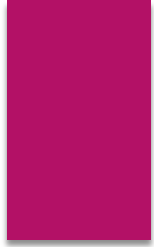
Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	14.5333	45	1
B	5.8889	45	2

# ANOVA test for Number of Pods in Bean

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	8.415447	4.207724	0.30	0.7393
bacteria	2	60.377352	30.188676	2.17	0.1202
var	1	1069.738473	1069.738473	77.07	<.0001
bacteria*var	2	23.935553	11.967776	0.86	0.4261



# LSD test for Number of Pods in Bean



Alpha	0.05
Minimum Significant Difference	2.3099

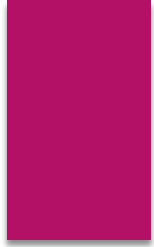
Alpha	0.05
Minimum Significant Difference	1.5716

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	9.1333	30	1
A	8.1379	29	0
A	7.1333	30	2

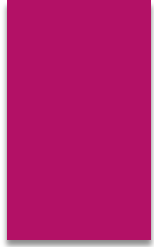
Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	11.6364	44	2
B	4.7111	45	1



# ANOVA test for Number of Branches in Bean



Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	2.37950058	1.18975029	1.24	0.2936
bacteria	2	0.82235772	0.41117886	0.43	0.6519
var	1	0.01124661	0.01124661	0.01	0.9139
bacteria*var	2	0.41389212	0.20694606	0.22	0.8058



# LSD test for Number of Branches in Bean

Alpha	0.05
Minimum Significant Difference	0.6062

Alpha	0.05
Minimum Significant Difference	0.4125

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	3.3667	30	1
A	3.2759	29	0
A	3.1333	30	2

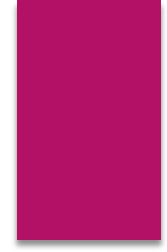
Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	3.2727	44	2
A	3.2444	45	1

# ANOVA test for Height

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	298.8666667	149.4333333	2.34	0.1029
bacteria	2	390.2000000	195.1000000	3.05	0.0526
var	1	348.1000000	348.1000000	5.45	0.0220
bacteria*var	2	163.4000000	81.7000000	1.28	0.2840



# LSD test for Height



Alpha	0.05
Minimum Significant Difference	4.927

Alpha	0.05
Minimum Significant Difference	3.3526

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	bacteri a
	A	63.533	30	0
B	A	60.933	30	1
B		58.433	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	62.933	45	2
B	59.000	45	1

# ANOVA test for *Days to flowering*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	27.2222222	13.6111111	3.34	0.0404
bacteria	2	767.4888889	383.7444444	94.11	<.0001
var	1	413.8777778	413.8777778	101.50	<.0001
bacteria*var	2	81.7555556	40.8777778	10.02	0.0001



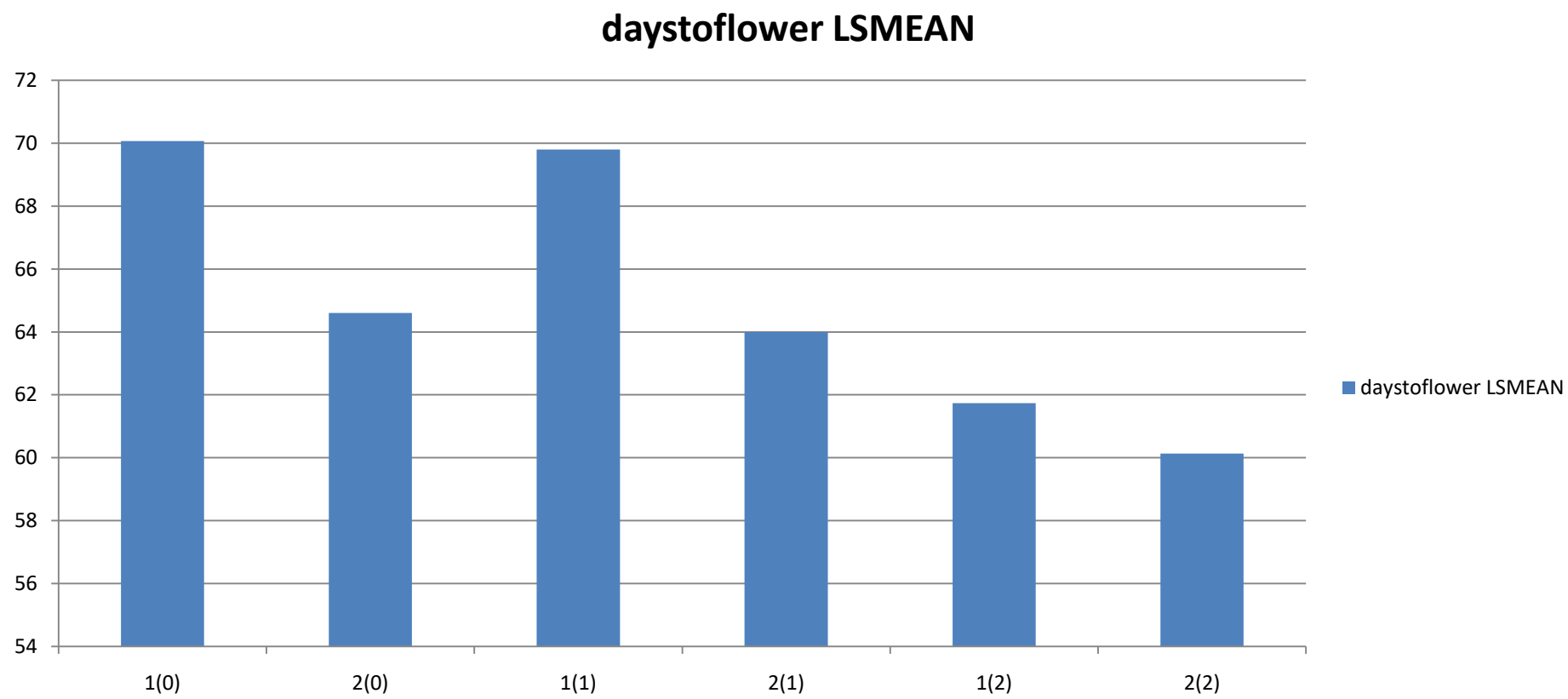
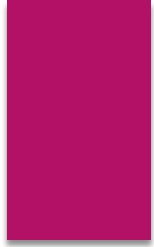
# LSD test for *Days to flower*

Alpha	0.05
Minimum Significant Difference	1.2446

Alpha	0.05
Minimum Significant Difference	0.8469

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	67.3333	30	0
A	66.9000	30	1
B	60.9333	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	67.2000	45	1
B	62.9111	45	2



# ANOVA test for period for *Pod* *initiation*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	2.066667	1.033333	0.21	0.8086
bacteria	2	1690.466667	845.233333	174.23	<.0001
var	1	11.377778	11.377778	2.35	0.1295
bacteria*var	2	96.288889	48.144444	9.92	0.0001





# LSD test for period for *Pod initiation*

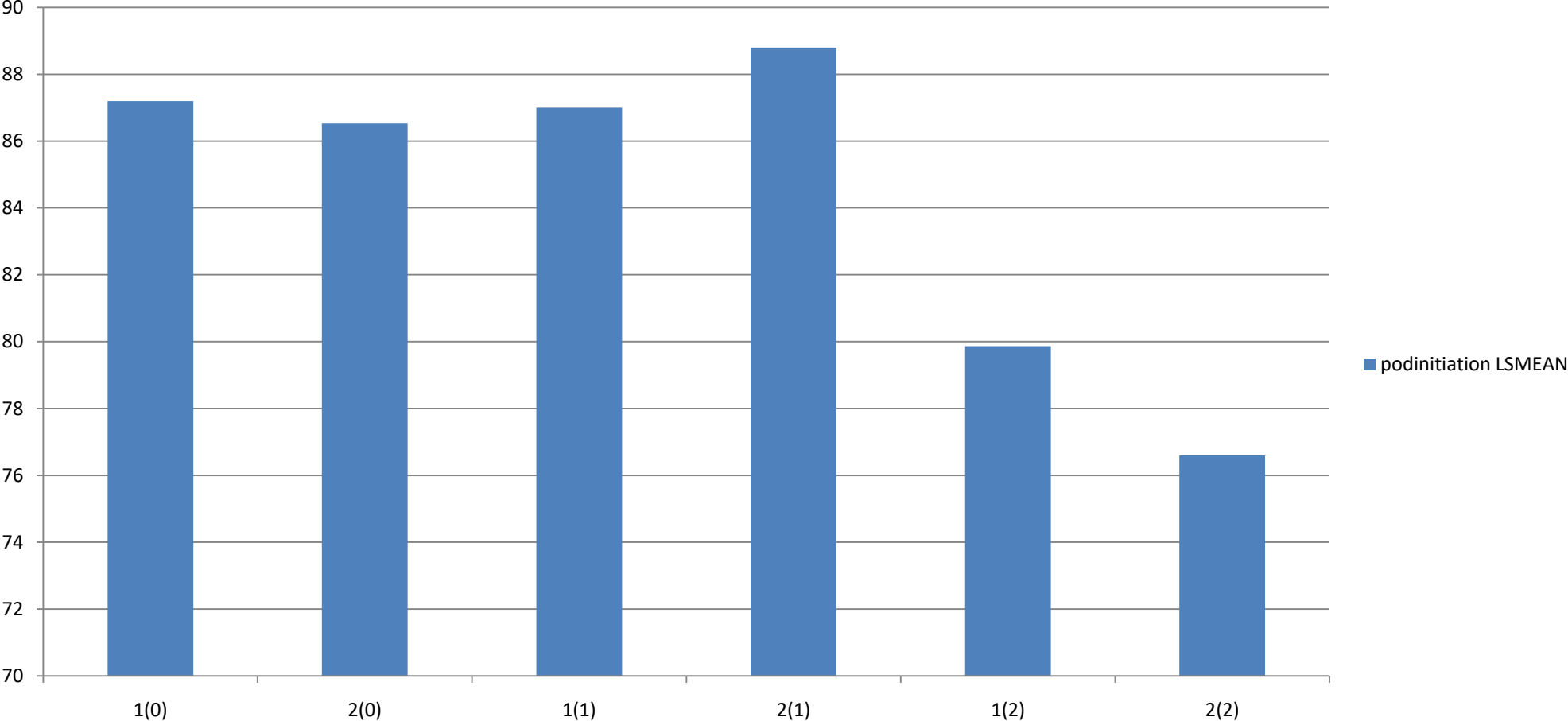
Alpha	0.05
Minimum Significant Difference	1.3575

Alpha	0.05
Minimum Significant Difference	0.9237

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	87.9000	30	1
A	86.8667	30	0
B	78.2333	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	84.6889	45	1
A	83.9778	45	2

podinitiation LSMEAN



# ANOVA test for *Interval between Flowering and Pod initiation*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	2.066667	1.033333	0.21	0.8086
bacteria	2	1690.466667	845.233333	174.23	<.0001
var	1	11.377778	11.377778	2.35	0.1295
bacteria*var	2	96.288889	48.144444	9.92	0.0001

# LSD test for *IntervalFloweringToPod*

Alpha	0.05
Minimum Significant Difference	1.5918

Alpha	0.05
Minimum Significant Difference	1.0832

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	21.0000	30	1
A	19.5333	30	0
B	17.3000	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	21.0667	45	2
B	17.4889	45	1

# ANOVA test for *Wt Plant*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	415.318772	207.659386	0.24	0.7895
bacteria	2	3511.241886	1755.620943	2.00	0.1416
var	1	2186.542416	2186.542416	2.50	0.1181
bacteria*var	2	1169.135406	584.567703	0.67	0.5159

# LSD test for *Wt Plant*

Alpha	0.05
Minimum Significant Difference	18.694

Alpha	0.05
Minimum Significant Difference	12.72

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	86.045	29	0
A	80.154	27	1
A	70.502	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	83.802	45	1
A	73.254	41	2

# ANOVA test for Wt Pods

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	748.256692	374.128346	1.17	0.3160
bacteria	2	1385.690050	692.845025	2.17	0.1216
var	1	6265.780618	6265.780618	19.58	<.0001
bacteria*var	2	112.459106	56.229553	0.18	0.8392

# LSD test for Wt Pods

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	11.3

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	7.6889

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	42.224	29	0
A	38.560	27	1
A	32.870	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	45.059	45	1
B	27.741	41	2



# ANOVA test for HI

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	355.824056	177.912028	0.98	0.3801
bacteria	2	540.297073	270.148537	1.49	0.2323
var	1	4034.412734	4034.412734	22.21	<.0001
bacteria*var	2	328.603591	164.301795	0.90	0.4090

# LSD test for HI

Alpha	0.05
Minimum Significant Difference	8.514

Alpha	0.05
Minimum Significant Difference	5.7931

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	47.800	29	0
A	46.742	30	2
A	42.398	27	1

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	52.308	45	1
B	38.520	41	2

# ANOVA test for Wt Seeds

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	50.815990	25.407995	0.15	0.8637
bacteria	2	6279.933766	3139.966883	18.15	<.0001
var	1	1020.810877	1020.810877	5.90	0.0174
bacteria*var	2	126.752491	63.376245	0.37	0.6945

# LSD test for wt Seeds

Alpha	0.05
Minimum Significant Difference	8.3089

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	35.350	27	1
B	22.776	29	0
B	17.700	30	2

Alpha	0.05
Minimum Significant Difference	5.6535

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	29.623	45	1
B	21.941	41	2

# ANOVA test for *Number of Seeds*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	71.6327187	35.8163593	0.98	0.3793
bacteria	2	452.6904283	226.3452142	6.20	0.0032
var	1	373.6736892	373.6736892	10.24	0.0020
bacteria*var	2	46.3857227	23.1928614	0.64	0.5323

# LSD test for *Number of Seeds*

Alpha	0.05
Minimum Significant Difference	3.8421

Alpha	0.05
Minimum Significant Difference	2.6106

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	bacteria
	A	17.186	29	0
B	A	14.615	26	1
B		11.767	30	2

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	16.583	41	2
B	12.534	44	1

# ANOVA test for Shoot to Root ratio

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	38.117102	19.058551	0.08	0.9247
bacteria	2	7873.628174	3936.814087	16.19	<.0001
var	1	1289.528656	1289.528656	5.30	0.0240
bacteria*var	2	60.202236	30.101118	0.12	0.8838

# LSD test for Shoot to Root ratio

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	9.9203

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	47.573	30	2
B	37.207	29	0
C	23.994	26	1

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	6.7405

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	40.340	44	1
B	33.050	41	2



# ANOVA test for Wt Root

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	70.532026	35.266013	0.26	0.7682
bacteria	2	2570.332239	1285.166119	9.64	0.0002
var	1	2308.852399	2308.852399	17.32	<.0001
bacteria*var	2	249.496168	124.748084	0.94	0.3966

# LSD test for Wt Root

Alpha	0.05
Minimum Significant Difference	7.3023

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	31.643	30	2
A	31.004	30	0
B	19.742	26	1

Alpha	0.05
Minimum Significant Difference	4.9581

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	32.771	44	1
B	22.638	42	2

# ANOVA test for Nodules Number

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	2	21.35690667	10.67845333	18.71	<.0001
bacteria	2	20.81768000	10.40884000	18.23	<.0001
var	1	1.61604000	1.61604000	2.83	0.0963
bacteria*var	2	1.74274667	0.87137333	1.53	0.2234

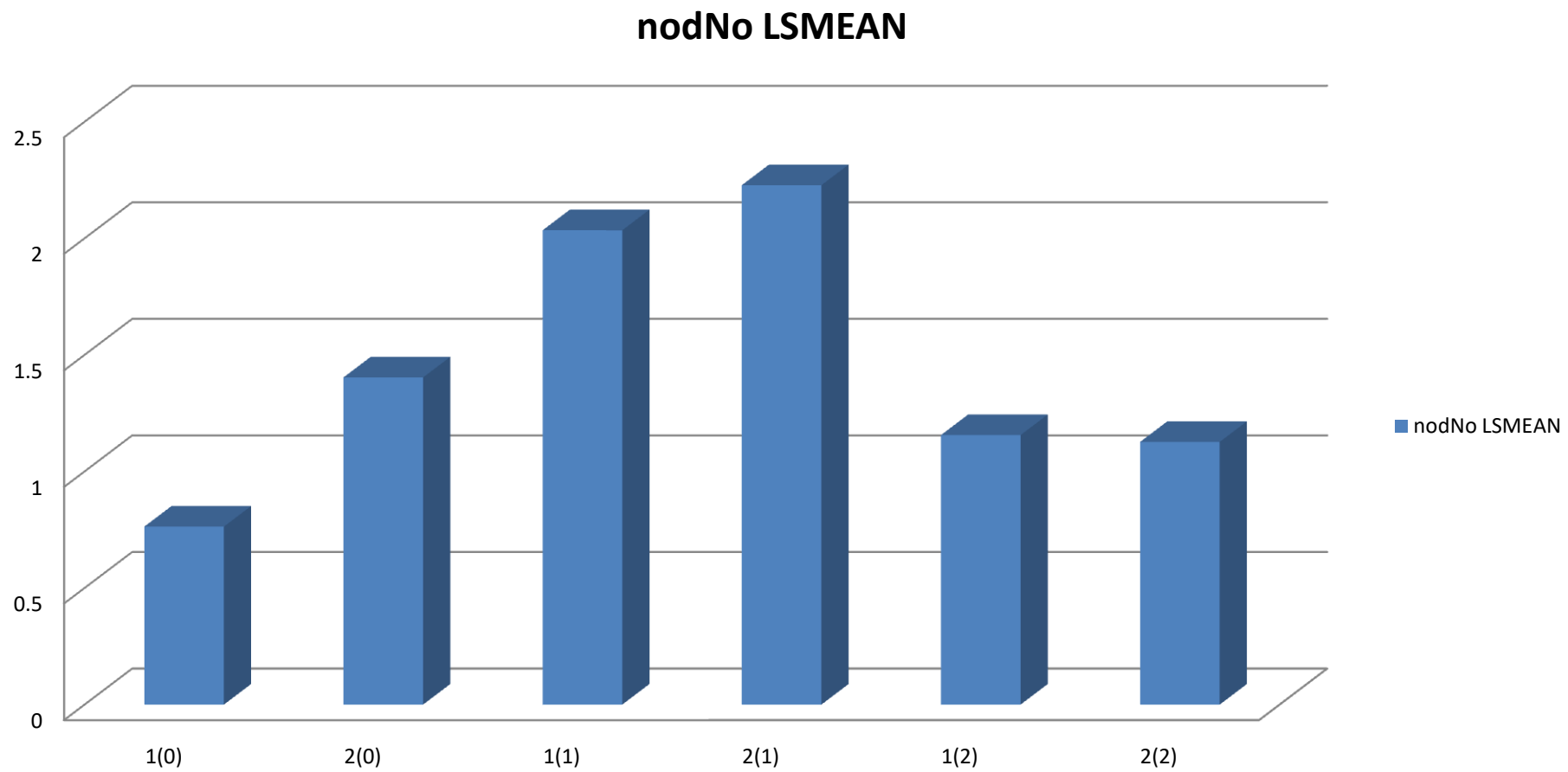
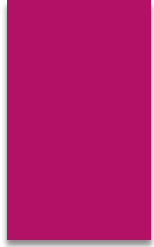
# LSD test for Nodules Number

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	0.4657

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	bacteria
A	2.1313	30	1
B	1.1413	30	2
B	1.0833	30	0

<b>Alpha</b>	0.05
<b>Minimum Significant Difference</b>	0.3169

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	var
A	1.5860	45	2
A	1.3180	45	1



	DMT	EMT	
V1 and V2	Chl		
V1	highest seed weight. Nodules Number	Short days to flowering Days to Pod initiation Shortest interval to initiation of pod Highest shoot to root ratio Weight of root	
Length pod. Lower Height. Weight of pods Highest harvest index		Height	
V2	No.leaves Number of seeds Nodules Number	Shortest days to flowering Days to Pod initiation	
No.pod Higher Height Number of seeds			

# *Thank You*

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100%  
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