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RESPONSE OF SUPERIOR GRAPEVINES TO SOIL INOCULATION WITH SOME PHOSPHORUS SOLUBILIZING BACTERIA TRANSFORMATS

Omar F. Dakhly[1*] and M.A.M. Abada[2]

1. Genet. Dept., Fac. Agric., Minia Univ., Egypt

2. Viticulture Res. Dept., Hort. Res. Inst., ARC, Giza, Egypt

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ABSTRACT: During 2015 and 2016 seasons, laboratory and field experiments were carried out. Laboratory experiment aimed to produce three transformats of solubilizing P bacterial from exposing wild *Actinomyces sp*. to irradiation with Ultraviolet rays (UV) for one to five minutes. All newly transformats were derived from *bacillus megatherium* var. phosphaticum. In the other filed experiment as a slow release P fertilizer *i.e.* rock phosphate (19.3% P_2O) besides any one of the previous three newly P transformats were used as a partial replacement of mineral P (MP) namely triple calcium superphosphate (37.5% P_2O_5) in Superior vineyards. Results showed that using P at 50 to 75% mineral P, 25 to 50% Rock phosphate and any one of the produced P bacterial transformats (1,2 or 3) was very effective in enhancing leaf chemical components (chlorophyll a, b, total chlorophylls, total carotenoids as well as percentages cane total carbohydrates, N, P, K, Mg, Ca and S), berry set (%), yield and quality of the berries over the application of P as 100% mineral P or when P was added as 25% mineral P with 75% rock phosphate and any one of P microbial transformats. The best P microbial transformats in terms of increasing growth characteristics, vine nutritional status, berry setting (%), yield and cluster aspects could be arranged, in descending order as follows transformats 3, 1 and 2 (*Bacillus megatherium* var. phosphaticum). Quality of the berries was greatly improved in response to using transformats 3 and 2 and 1, in ascending order. Using rock phosphate at 50% and P bacterial transformats 3 at 10 ml/vine as a partial replacement of 50 % mineral P fertilizer in Superior vineyards gave the best results with regard to the yield and fruit quality.

Key words: Mineral P, rock phosphate, solubilizing P bacterial transformats, *Bacillus megatherium* var. phosphaticum, yield, berries quality, Superior grapevines.

INTRODUCTION

Substitution of chemical P fertilizers with bio organic sources will meet the requirements of exportation and expand the marketing of grapevine cv. Superior. P in the soil can be assimilated fertilizers have received a great interest, recently as (rock phosphate and P-coated urea), since they are natural inexpensive and available fertilizers. However, P solubilization rarely occurs in alkaline soils. Rock phosphate as a slow release fertilizer realized its properties in the rhizosphere under large microbial communities that facilitate weathering of minerals by numerous mechanisms such as lowering pH by producing organic acids, phenolic compounds oilserophores, in chelatin and exchange reactions and P solubilizing microorganisms. Previous studies gave good evidence about the organisms that responsible for enhancing the solubility P. Solving soil P fixation can be easily achieved using bacteria solubilizing P or biofertilizer phosphorene and mycoorhiza. Microbial transformats combined with slow release P fertilizers was essential for enhancing P uptake (**Padwal and Indi, 2003**).

Soloubility of P in triple calcium superphosphate and slow release P fertilizers in

* **Corresponding author: Tel. :** +20100192127
E-mail address: faissalfadel@yahoo.com

the soil can be governed by soil type, pH, organic matter and biofertilization. Using different organic manures enriched with *Bacillus megatherium* var. phosphaticum as well as the application of mycorrhiza substantially was associated with enhancing the availability of P to plants **(Mba, 1994; Dahama, 1999; El-Karamany *et al*., 2000; Motosugi *et al*., 2002; Cabrera *et al*., 2003; Kannaiyan, 2003).**

Previous studies showed that using mineral P along with slow release P fertilizer (rock phosphate) plus P bacterial transformats namely *Bacillius megatherium* var. phosphaticum (phosphorene and mycoorhizal) was very effective in stimulating growth aspects and vine nutritional status as well as improving yield and quality of the berries in various grapevine cvs. and another related crops **(Abd El-Hameed, 2002; Nikolaou *et al*., 2002; Padwal and Indi, 2003; El-Naggar, 2004; El-Shenawy and Stino, 2005a,b; Ibrahim, 2009; Ahmed and Abada, 2012; Shaheen *et al*., 2013; Shaaban, 2014).**

The target of this study was clearing the effect of using rock phosphate as slow release P fertilizer and some newly bacterial transfomats of P as a partial alternative to mineral phosphorus (MP) on growth aspects, yield as well as physical and chemical characteristics of Superior grapevine cv.

MATERIALS AND METHODS

Laboratory Experiment

Laboratory experiment was conducted at the Department of Genetics, Fac. Agric. Minia Univ. Egypt.

Trnasformats of microorganisms

1. *Azotobacter vinelandii* transformat was isolated from the soil of Minia Governorate. The growing crop was grape. This isolate was used as a receipt.
2. *Bacillus megaterium* var. phosphaticum was isolated from the soil of the experimental farm, Fac. Agric. Minia Univ. (**Abdel-Raheem *et al*., 1995)**. This isolation was used as a donor parent.

Media

1. Complete medium (CM) was used for *Bacillus megaterium* var. phosphaticum culturing. (**Abdel-Hafeth, 1966)**.
2. Complete medium (CM) was used for *Azotobacter vinelandii* culturing (**Strandberg and Wilson, 1968**).

Transformation procedure

Total DNA of *Azotobacter vinelandii* was extracted from 1 g of late log-phase growth according to the procedure of when the *Bacillus megatherium* var. phosphaticum transformants were obtained using procedure of (**Page and Vontigerstron, 1979**).

Filed experiment

This experiment was carried out during the two consecutive seasons of 2015 and 2016 on sixty uniform in vigour 8-years old Superior grapevines grown in a private vineyard located at El-Hawarta Village, Minia District, Minia Governorate. The soil texture is clay and well water drained since water table depth is not less than two meters. Analyses of the experiment soil are shown in Table 1. The chosen vines were planted at 2 × 3 meters apart, (700 vines/fad.) Cane pruning system was followed at the first week of Jan. leaving 84 eyes per vine (six fruiting canes × 12 eyes plus six renewal spurs × two eyes) with the assistance of Gable shape supporting system. The vines were irrigated through surface flood irrigation system using Nile water.

Except those dealing with the present treatments (all sources of P) and biofertilization, the selected vines (60 vines) received the usual horticultural practices which are commonly used in the vineyard.

This study included the following ten treatments:

1. Applying P as 100% mineral phosphorus (MP) (200 g/vine triple calcium superphosphate, 37.5% P_2O_5).
2. Applying P as 75% MP (150 g/vine triple calcium superphosphate) + 25% rock phosphate (RP) (97.8 g/vine) (19.3% P_2O_5)+ $transformat_1$ (*Bacillus megatherium* var. phosphaticum) at 5 ml/vine.
3. Applying P as 50% MP (100 g/vine triple calcium superphosphate)+ 50% RP (194.4 g/vine) + $transformat_1$ (*Bacillus megatherium* var. phosphaticum) at 10 ml/ vine.

Table 1. Analyses of the tested soil

Constituents	Values
Particle size distribution	
Sand (%)	7.0
Silt (%)	21.5
Clay (%)	71.5
Texture	Clay
pH (1:2.5 extract)	7.95
EC (1 :2.5 extract) (dsm^{-1}) 1 cm / 25°C.	0.97
OM (%)	2.01
$CaCO_3$ (%)	2.41
Total N (%)	0.11
Available P (Olsen, ppm)	3.11
Available K (ammonium acetate, ppm)	405.9

4. Applying P as 25% MP (50 g/vine triple calcium superphosphate) + 75% RP (291.6 g/ vine) + $transformat_1$ (*Bacillus megatherium* var. phosphaticum) at 20 ml/ vine.
5. Applying P as 75% MP (150 g/vine triple calcium superphosphate) + 25% RP (97.8 g/ vine) + $transformat_2$ (*Bacillus megatherium* var. phosphaticum) at 5 ml/vine.
6. Applying P as 50% MP (100 g/vine triple calcium superphosphate) + 50% RP (194.4 g/ vine) + $transformat_2$ (*Bacillus megatherium* var. phosphaticum) at 10 ml/ vine.
7. Applying P as 25% MP (50 g/vine triple calcium superphosphate) + 75% RP (291.6 g/ vine) + $transformat_2$ (*Bacillus megatherium* var. phosphaticum) at 20 ml/vine.
8. Applying P as 75% MP (150 g/vine triple calcium superphosphate) + 25% RP (97.8 g/ vine) + $transformat_3$ (*Bacillus megatherium* var. phosphaticum) at 5 ml/vine.
9. Applying P as 50% MP (100 g/vine triple calcium superphosphate) + 50% RP (194.4 g/vine) + $transformat_3$ (*Bacillus megatherium* var. phosphaticum) at 10 ml/vine.
10. Applying P as 25% MP (50 g/vine triple calcium superphosphate) + 75% RP (291.6 g/vine) + $transformat_3$ (*Bacillus megatherium* var. phosphaticum) at 20 ml/vine.

Each treatment was replicated three times, two vines per each replicate. Triple calcium superphosphate (37.5% P_2O_5) as a source of mineral P was added once just after winter pruning (3^{rd} week of January). The slow release P fertilizer (rock phosphate 19.3% P_2O_5) was added once just after winter pruning) in shallow holes under vine canopy (20 cm apart from vine trunk). All P bacterial transformats (*Bacillus megatherium* var. phosphaticum) was added once at growth start (1^{st} week of March) in shallow holes, 20 cm apart from the trunk and covered with moist soil. All the selected vines (60 vines) were received botanical compost fixed the same 0.25 kg/vine. Once just after winter pruning (3^{rd} week of January) 50 cm far from the vine trunk in drenches (50 × 50 × 50 cm dimensions). Analyses of botanical compost are given in Table 2.

Randomized complete block design (RCBD) was followed (**Rangaswamy, 1995),** where the experiment consisted of ten treatments, each treatment was replicated three times, two vine per each.

Table 2. Analyses of botanical compost

Parameter	Value
Cubic meter weight (kg.)	600.0
Moisture (%)	29.0
Organic matter (%)	30.7
Organic carbon (%)	28.56
pH (1: 2.5 extract)	6.20
EC (dsm^{-1}) (1: 2.5 extract)	1.25
C/N ratio	14.28
Total N (%)	2.0
Total P (%)	1.02
Total K (%)	1.21
Total Ca (%)	1.25
Total Mg (%)	1.30
Total Fe (ppm)	18.5
Total Mn (ppm)	37.55
Total Zn (ppm)	43.22
Total Cu (ppm)	17.40

During both seasons the following parameters were recorded:

1-Vegetative growth aspects *i.e.* main shoot length (cm), number of leaves/shoot, leaf area (**Ahmed and Morsy, 1999**) and pruning wood weight/vine, wood ripening coefficient (**Bouard, 1966**) and cane thickness (cm).

2-Percentage of total carbohydrates in the canes.

3-Leaf chemical components *i.e.*: chlorophylls a and b , total chlorophylls and total carotenoids (mg/1 g FW) (**Von-Wettstein, 1957**) as well as percentages of N, P, K, Mg, Ca and S (**Wilde *et al.*, 1985 ; Balo *et al.*, 1988**).

4-Percentage of berry setting.

5-Yield/vine expressed in weight (kg) and number of clusters per vine as well as cluster weight (g) and dimensions (length and shoulder, cm).

6-Percentage of shot berries.

7-Physical and chemical characteristics of the berries *i.e.* berry weight (g) and dimensions (longitudinal and equatorial) (cm), TSS (%), total acidity (%), reducing sugars (%) (**Lane and Eynon, 1965 ; AOAC, 2000**).

8-Total counts of bacteria in the soil was counted (cfug)/1.0 g soil (**Cochra, 1950**).

Statistical analysis was done and the treatment means were compared using new LSD at 5% according to **Rangaswamy (1995) and Rao (2007**).

RESULTS AND DISCUSSION

Results in Table 3 indicate that the highest frequencies of transformants were obtained when DNA of *Azotobacter vinelandii* was mixed with *Bacillus megatherium* var phosphatticum recipient cells for two hours at 30°C incubation. This may be attributed to the competence of the recipient cells **(Ali *et al.*, 1980)**.

Table 3. Number and percentages of *Bacillus megatherium* var. phosphaticum grown on complete medium of *Azotobacter vinelandii*

Number and percentage	Control	Transformants
Number/ml	2000	600
(%)	100	30%

Vegetative Growth Characteristics

It is clear from the obtained results in Table 4 that supplying Superior vines with P as 25 to 50% mineral P (MP) + the slow release P fertilizer (RP) at 50 to 75% + any one of the three bacterial transformants (*Bacillus megatherma* var. phosphaticum) each at 5 to 10 ml/vine significantly enhanced main shoot length, number of leaves/ shoot, leaf area, wood ripening coefficient, pruning wood weight and cane thickness relative to employing P *via* 100% MP or when P was added as 25% MP even with the application rock phosphate and any one of the three P transformats.

The stimulations on these characteristics was significantly related to reducing percentage of MP from 75 to 50% and at the same time increasing the percentage of RP from 25 to 50 % and the levels of P transformats from 5 to 10 ml/vine. The best P transformats in this respect was arranged in descending order as follows 3, 1, 2 (*Bacillus megatherium* var. phosphaticum). The maximum values of vegetative aspects were recorded for the vines received P as 50% MP + 50% rock phosphate + 10 ml/vine transformat 3. Supplying the vines with P as 25% MP + 75% rock phosphate + P transformat *i.e.* transformat 2 (*Bacillus megatherium* var. phosphaticum) gave the lowest values. These results were true during both seasons.

Leaf and Cane Chemical Components

Amending the vines with P as 50 to 75% mineral P (MP) + the slow release P fertilizer (RP) at 25 to 50% + any one of the three bacterial transformats (*Bacillus megatherium* var. phosphaticum) each at 5 to 10 ml/vine significantly was increased enhancing the eleven chemical traits *i.e.* cane total carbohydrates (%), percentages of N, P, K, Mg, Ca and S as well as chlorophylls a and b, total chlorophylls and total carotenoids in the leaves relative to employing P *via* 100% MP or when P was added as 25% MP even with the application of rock phosphate and any one of the three P transformats (Tables 5 and 6).

The promotion of these chemical components was significantly in proptional to reducing percentage of MP from 75 to 50% and increasing the percentage of RP from 25 to 50% and the levels of P bacterial transformats from 5 to 10 ml/vine. The best P transformats in this respect could be arranged in descending order as 3, 1 and 2 (*Bacillus megatherma* var. phosphaticum. The maximum values of these leaf components were recorded on vines received P as 50% MP + 50% RP + 10 ml/vine transformat 3 (*Bacillus megatherma* var. phosphaticum. Supplying the vines with P as 25% MP + 75% rock phosphate + P transformat microbial 2 (*Bacillus megatherium* var. phosphaticum) gave the lowest values. These results were true during 2015 and 2016 seasons.

Percentage of Berry Set, Yield/Vine and Cluster Aspects

It is evident from the obtained results in Table 7 that fertilizing Superior vines with P as 50 to 75% mineral P + the slow release P fertilizer at 25 to 50% + any one of the three P bacterial transformats (*Bacillus megatherium* var. phosphaticum) each at 5 to 10 ml/vine significantly was followed by enhancing berry setting (%), yield expressed in weight (kg) and number of clusters/vine as well as weight, length and shoulder of cluster relative to supplying P *via* 100% MP or when P was added as 25 (%) MP even with the application of rock phosphate and any one of the three P transformats.

The promotion in the previous parameters chemical components was significantly in proptional to reducing percentage of MP from 75 to 50% and increasing the percentage of rock

Table 4. Effect of replacing mineral P fertilizers partially using slow release P fertilizers and different microbial transformats on some vegetative growth characteristics of Superior grapevines during 2015 and 2016 seasons

Treatment	Shoot length (cm.)		No. of leaves/ shoot		Leaf area $(cm)^2$		Wood ripening coefficient		Pruning wood weight (kg)		Cane thickness (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
P as 100% (MP)	96.3	97.0	18.1	19.0	99.0	99.5	0.53	0.51	1.91	2.00	0.84	0.81
P as 75% MP + 25% RP + transformat 1 at 5 ml/vine	99.9	100.6	19.3	20.2	101.9	102.4	0.64	0.65	1.99	2.08	0.95	0.95
P as 50% MP + 50% RP + transformat 1 at 10 ml/vine	105.9	106.6	20.8	21.7	103.0	103.6	0.68	0.69	2.06	2.15	1.01	1.02
P as 25% MP + 75% RP + transformat1 at 20 ml/vine	90.0	90.7	16.0	16.8	94.0	94.5	0.46	0.47	1.90	1.89	0.71	0.72
P as 75% MP + 25% RP + transformat 2at 5 ml/vine	108.0	108.6	21.9	22.8	105.6	106.1	0.71	0.72	2.15	2.24	1.12	1.14
P as 50% MP + 50% RP + transformat 2 at 10 ml/vine	111.0	111.7	23.0	23.8	108.6	109.1	0.75	0.77	2.25	2.34	1.20	1.22
P as 25% MP + 75% RP + transformat 2 at 20 ml/vine	91.5	92.2	17.1	18.0	95.5	96.0	0.48	0.49	1.84	1.93	0.75	0.76
P as 75% MP + 25% RP + transformat 3 at 5 ml/vine	112.9	113.5	25.0	25.8	110.0	110.4	0.78	0.80	2.31	2.40	1.31	1.33
P as 50% MP+ 50% RP + transformat 3 at 10 ml/vine	116.7	117.5	27.0	27.8	112.0	122.6	0.81	0.83	2.41	2.50	1.40	1.42
P as 25% MP + 75% RP + transformat 3 at 20 ml/vine	92.9	93.6	17.8	18.4	97.0	97.6	0.50	0.51	1.87	1.96	0.78	0.79
New LSD at 5%	1.1	1.0	1.0	1.0	1.4	1.3	0.03	0.02	0.06	0.05	0.05	0.06

MP= Mineral phosphorus. RP= Rock phosphate. Transformats= *Bacillus megatherm* var. phosphaticum

Table 5. Effect of replacing mineral P fertilizers partially using slow release P fertilizers and different microbial transforamts on percentages of cane total carbohydrates, leaf N, P, K, Mg and Ca of Superior grapevines during 2015 and 2016 seasons

Treatment	Cane total carbohydrates (%)		Leaf N (%)		Leaf P (%)		Leaf K (%)		Leaf Mg (%)		Leaf Ca (%)		Leaf S (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
P as 100% (MP)	13.90	14.10	1.60	1.62	0.116	0.110	1.41	1.32	0.64	0.66	2.01	2.10	0.70	0.69
P as 75% MP + 25% RP + transformat 1 at 5 ml/vine	15.50	15.80	1.69	1.71	0.119	0.113	1.49	1.40	0.69	0.71	2.09	2.18	0.74	0.73
P as 50% MP+ 50% RP + transformat 1 at 10 ml/vine	16.00	16.20	1.75	1.80	0.125	0.119	1.59	1.50	0.73	0.75	2.20	2.28	0.79	0.78
P as 25% MP + 75% RP + transformat1 at 20 ml/vine	13.20	13.50	1.50	1.52	0.101	0.095	1.20	1.11	0.51	0.53	1.91	2.00	0.60	0.59
P as 75% MP + 25% RP + transformat 2at 5 ml/vine	14.50	14.60	1.85	1.87	0.131	0.126	1.66	1.57	0.76	0.78	2.31	2.40	0.84	0.83
P as 50% MP + 50% RP + transformat 2 at 10 ml/vine	15.00	15.30	1.94	1.96	0.141	0.135	1.72	1.62	0.80	0.82	2.41	2.50	0.90	0.89
P as 25% MP+ 75% RP + transformat 2 at 20 ml/vine	12.90	13.10	1.53	1.55	0.105	1.100	1.25	1.16	0.55	0.57	1.95	2.04	0.66	0.65
P as 75% MP + 25 % RP + transformat 3 at 5 ml/vine	16.60	16.80	2.01	2.03	0.151	0.146	1.79	1.69	0.85	0.87	2.51	2.60	0.95	0.94
P as 50% MP+ 50% RP + transformat 3 at 10 ml/vine	17.10	17.30	2.11	2.13	0.161	0.156	1.84	1.75	0.89	0.93	2.61	2.70	0.99	0.98
P as 25% MP + 75% RP + transformat 3 at 20 ml/vine	13.60	13.80	1.56	1.59	0.110	0.105	1.30	1.21	0.58	0.60	1.96	2.06	0.69	0.70
New LS D at 5%	0.60	0.40	0.05	0.04	0.02	0.02	0.04	0.03	0.03	0.03	0.05	0.04	0.03	0.03

MP= Mineral phosphorus. RP= Rock phosphate. Transformates= *Bacillus megatherm* var. phosphaticum

Table 6. Effect of replacing mineral P fertilizers partially using slow release P fertilizers and different microbial transformats on percentages of leaf pigments of Superior grapevines during 2015 and 2016 seasons

Treatment	Chlorophyll a (mg/ 1g FW)		Chlorophyll b (mg/ 1g FW)		Total chlorophyll (a+b) (mg/ 1g FW)		Total carotenoids (mg/ 1g FW)	
	2015	2016	2015	2016	2015	2016	2015	2016
P as 100% (MP)	2.11	2.20	1.71	1.75	3.82	3.95	1.60	1.63
P as 75% MP + 25% RP + transformat 1 at 5 ml/vine	2.21	2.30	1.74	1.78	3.95	4.08	1.65	1.68
P as 50% MP + 50% RP + transformat 1 at 10 ml/vine	2.31	2.40	1.80	1.83	4.11	4.23	1.71	1.74
P as 25% MP + 75% RP + transformat1 at 20 ml/vine	2.00	2.08	1.59	1.64	3.59	3.72	1.47	1.50
P as 75% MP + 25% RP + transformat 2at 5 ml/vine	2.41	2.50	1.88	1.92	4.29	4.42	1.80	1.83
P as 50% MP + 50% RP + transformat 2 at 10 ml/vine	2.51	2.60	1.98	2.02	4.49	4.62	1.86	1.89
P as 25% MP + 75% RP + transformat 2 at 20 ml/vine	2.05	2.14	1.64	1.68	3.69	3.82	1.52	1.55
P as 75% MP + 25% RP + transformat 3 at 5 ml/vine	2.61	2.70	2.11	2.15	4.72	4.85	1.90	1.93
P as 50% MP+ 50% RP + transformat 3 at 10 ml/vine	2.71	2.80	2.21	2.25	4.92	5.05	1.95	1.98
P as 25% MP + 75% RP + transformat 3 at 20 ml/vine	2.10	2.19	1.69	1.74	3.79	3.93	1.56	1.60
New LSD at 5%	0.06	0.05	0.03	0.03	0.04	0.04	0.04	0.04

MP= Mineral phosphorus. RP= Rock phosphate. Transformats= *Bacillus megatherm* var. phosphaticum

Table 7. Effect of replacing mineral P fertilizers partially using slow release P fertilizers and different microbial transformats on berry setting and yield/ vine of Superior grapevines during 2015 and 2016 seasons

Treatment	Berry setting (%)		No. of clusters/ vine		Yield/ vine (kg)		Cluster weight (g.)		Cluster length (cm.)		Cluster shoulder (cm.)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
P as 100% (MP)	10.1	10.5	25	26	9.3	9.9	375.0	382.0	19.3	19.0	8.8	9.0
P as 75% MP + 25 % RP + transformat 1 at 5 ml/vine	11.0	11.3	25	28	10.3	11.6	412.0	415.0	21.0	20.7	9.1	9.3
P as 50% MP + 50 % RP + transformat 1 at 10 ml/vine	11.9	12.9	26	28	10.8	11.7	418.0	420.0	21.8	21.5	9.4	9.7
P as 25% MP + 75 % RP + transformat1 at 20 ml/vine	8.3	8.7	25	27	9.2	10.3	368.0	382.0	17.1	16.6	7.9	8.2
P as 75% MP + 25 % RP + transformat 2at 5 ml/vine	12.5	12.9	25	27	9.9	10.8	395.0	400.0	22.9	22.4	9.7	10.0
P as 50% MP + 50 % RP + transformat 2 at 10 ml/vine	13.0	13.4	25	27	10.1	11.1	402.0	410.0	24.0	23.7	10.1	10.4
P as 25% MP + 75 % RP + transformat 2 at 20 ml/vine	8.8	9.2	26	28	9.5	10.4	365.0	372.0	17.8	17.5	8.2	8.5
P as 75% MP + 25 % RP + transformat 3 at 5 ml/vine	13.9	14.4	26	30	11.1	12.7	425.0	432.0	25.0	24.7	10.5	10.8
P as 50% MP+ 50 % RP + transformat 3 at 10 ml/vine	14.5	14.9	25	29	10.8	12.6	430.0	435.0	25.9	25.6	10.8	11.1
P as 25% MP + 75 % RP + transformat 3 at 20 ml/vine	9.2	9.6	26	29	9.7	11.2	372.0	385.0	18.1	17.7	8.8	8.1
New LSD at 5%	0.6	0.6	NS	2.0	0.4	0.6	6.1	6.4	0.6	0.5	0.4	0.4

MP= Mineral phosphorus RP= Rock phosphate Transformats= *Bacillus megatherm* var. phosphaticum phosphate from 25 to 50% and the levels of P transformats from 5 to 10 ml/vine. The best P transformats in this respect could be arranged in descending order as follows 3, 1 and 2 (*bacillus megatherma* var. phosphaticum.) The maximum values of these parameters were recorded for the vines amended with P as 50% MP + 50 % rock phosphate + 10 ml/vine transformat 3 (*Bacillus megatherma* var. phosphaticum). Supplying the vines with P as 25% MP + 75% rock phosphate + P transformat *i.e.* transformat 2 (*Bacillus megatherium* var. phosphaticum) gave the lowest values. These results were true during both seasons.

Percentage of Shot Berries

The obtained results in Table 8 show that amending Superior vines with P as 50 to 75% mineral P (triple calcium superphosphate) + the slow release P fertilizer (Rock phosphate) at 50 to 75% + any one of the three P bacterial transformats (*Bacillus megatherium* var. phosphaticum) each at 5 to 10 ml/vine significantly was followed by controlling the percentage of shot berries relative to amending P *via* 100% MP or when P was added as 25 % MP even with the application of rock phosphate and any one of the three P transformats.

The reduction on such unfavourable phenomenon shot berries was significantly related to reducing percentage of MP from 75 to 50% and increasing the percentage of rock phosphate from 25 to 50% and the levels of P transformats from 5 to 10 ml/vine. The best P transformats in this respect was arranged in descending order as 3, 1 and 2 (*Bacillus megatherium* var. phosphaticum). The lowest values were recorded on the vines that received P as 50% MP + 50 % rock phosphate + 10 ml/ vine transformat 3 (*Bacillus megatherium* var. phosphaticum. Supplying the vines with P completely *via* mineral P gave the highest values. These results were true during 2015 and 2016 seasons.

Some Physical and Chemical Characteristics of the Berries

It can be stated from the obtained results in Tables 8 and 9 that, fertilizing Superior vines with P as 50 to 75% mineral P (triple calcium superphosphate) + the slow release P fertilizer (Rock phosphate) at 25 to 50% + any one of the three P bacterial transformats (*Bacillus megatherium* var. phosphaticum) each at 5 to 10 ml/vine significantly was very effective in improving quality of the berries in terms of increasing weight, longitudinal and equatorial diameter of berry, TSS (%), reducing sugars (%) and decreasing total acidity (%) compared with supplying the vines *via* 100% MP or when P was added as 25% MP even with the application of rock phosphate and any one of the three P transformats.

The promoting on berries quality was significantly in proptional to reducing percentage of MP from 75 to 50% and at the same time increasing the percentage of rock phosphate from 25 to 50% and the levels of P transformats from 5 to 10 ml/vine. The best P transformats in this respect could be arranged in descending order as 3, 2 and 1(*Bacillus megatherium* var. phosphaticum). The best results with regard to both physical and chemical of the berries were obtained due to supplying the vines with P as 50% MP + 50% rock phosphate + 10 ml/vine transformats 3 (*Bacillus megatherium* var. phosphaticum). Supplying the vines with P as 25% MP + 75% rock phosphate + transformat 1 (*Bacillus megatherium* var. phosphaticum) gave unsatisfactory promotion on all physical and chemical diameters of the berries. These results were true during 2015 and 2016 seasons.

Total Counts of Bacteria in the Soil

Results in Table 9 clearly show that total counts of bacteria in the soil was significantly varied among the ten P management treatments. It was significantly enhanced by reducing the percentage of mineral P or increasing percentage of rock phosphate from 25 to 50% and P bacterial transformats from 5 to 10 ml/vine. A significant reduction was observed with reducing P mineral from 50 to 25% with the application of rock phosphate of P bacterial transformats. The lowest values were recorded on the soil under vines received mineral P at 25 to 75% rock phosphate + 20 ml P microbial transformats 3. The best P microbial transformats were transformat 3, transformat 2 and transformat 1, in descending order.

Table 8. Effect of replacing mineral P fertilizers partially using slow release P fertilizers and different microbial transformats on physical and chemical of the berries, bunch quality and percentages of TSS, of Superior grapevines during 2015 and 2016 seasons

Treatment	Shot berries (%)		Berry weight (g.)		Berry longitudinal (cm.)		Berry equatorial (cm.)	
	2015	2016	2015	2016	2015	2016	2015	2016
P as 100% (MP)	10.6	11.0	3.5	3.5	2.00	2.01	1.91	1.94
P as 75% MP + 25 % RP + transformat 1 at 5 ml/vine	9.6	9.4	3.7	3.8	2.06	2.08	1.95	1.96
P as 50% MP + 50 % RP + transformat 1 at 10 ml/vine	9.2	9.0	4.0	4.0	2.11	2.12	2.00	2.01
P as 25% MP + 75 % RP + transformat1 at 20 ml/vine	10.5	10.3	2.9	3.0	1.90	1.91	1.81	1.82
P as 75% MP + 25 % RP + transformat 2at 5 ml/vine	8.0	7.8	4.4	4.5	2.16	2.17	2.05	2.06
P as 50% MP + 50 % RP + transformat 2 at 10 ml/vine	7.4	7.2	4.8	4.9	2.22	2.23	2.10	2.11
P as 25% MP + 75 % RP + transformat 2 at 20 ml/vine	10.0	9.8	3.1	3.2	1.92	1.93	1.84	1.85
P as 75% MP + 25 % RP + transformat 3 at 5 ml/vine	6.0	5.8	5.0	5.1	2.27	2.29	2.14	2.14
P as 50% MP+ 50 % RP + transformat 3 at 10 ml/vine	5.5	5.2	5.3	5.4	2.33	2.35	2.18	2.19
P as 25% MP + 75 % RP + transformat 3 at 20 ml/vine	9.6	9.4	3.3	3.3	1.94	1.95	1.86	1.87
New LSD at 5%	0.8	0.8	0.2	0.3	0.04	0.04	0.04	0.03

MP= Mineral phosphorus RP= Rock phosphate Transformates= *Bacillus megatherm* var. phosphaticum

Table 9. Effect of replacing mineral P fertilizers partially using slow release P fertilizers and different microbial transformats on TSS, total acidity, reducing sugars of superior grapevines fruits and total count of bacteria in soil during 2015 and 2016 seasons

Treatment	TSS (%)		Total acidity (%)		Reducing sugars (%)		Total counts of bacteria (fig)	
	2015	2016	2015	2016	2015	2016	2015	2016
P as 100 % (MP)	17.1	17.0	0.684	0.690	14.8	15.0	151.0	152.7
P as 75% MP + 25 % RP + transformat 1 at 5 ml/vine	17.4	17.5	0.660	0.659	15.3	15.5	156.0	157.1
P as 50% MP + 50 % RP + transformat 1 at 10 ml/vine	17.8	17.9	0.640	0.639	15.6	15.8	160.0	161.4
P as 25% MP + 75 % RP + transformat1 at 20 ml/vine	16.1	16.2	0.696	0.696	13.9	14.1	125.0	126.6
P as 75% MP + 25 % RP + transformat 2at 5 ml/vine	18.2	18.3	0.610	0.609	16.0	16.2	164.7	166.0
P as 50% MP + 50 % RP + transformat 2 at 10 ml/vine	18.6	18.7	0.580	0.585	16.6	16.8	169.3	171.0
P as 25% MP + 75 % RP + transformat 2 at 20 ml/vine	16.4	16.5	0.690	0.679	14.3	14.5	131.0	132.8
P as 75% MP + 25 % RP + transformat 3 at 5 ml/vine	19.0	19.1	0.550	0.549	17.1	17.3	174.4	176.0
P as 50% MP+ 50 % RP + transformat 3 at 10 ml/vine	19.4	19.5	0.519	0.520	17.4	17.6	177.5	179.0
P as 25% MP + 75 % RP + transformat 3 at 20 ml/vine	16.6	16.7	0.688	0.687	14.5	14.7	140.0	141.5
New LSD at 5%	0.4	0.4	0.3	0.012	0.4	0.3	1.1	1.4

MP= Mineral phosphorus RP= Rock phosphate Transformates= *Bacillus megatherm* var. phosphaticum

Economical Study for the Recommended Treatment

As shown in Table 10, net profit gained by the application of the recommended treatment (50% Mineral P, 50% rock phosphate + 10 ml bacterial transformats 3) applied in one faddan contains 700 vines reached 12200 and 16850 (LE) while in the control vines (100% M.P) reached 9000 and 9600 (LE) during both seasons, respectively. The increase on net profit due to application of the recommended treatment over the control reached 3200 and 7250 (LE) during both seasons, respectively.

DISCUSSION

Intraspecific transformation and transformation between different genera of microorganisms and evalutation of the transformation frequency have been reported by many authors **(Altman and Philipposn, 1996; Mavingui *et al.*, 1997).** Homologous integration events of the transforming DNA into homologous positions of the recipient genome occur at hight frequency can be readily selected using selectable markers **(Mavingui *et al*, 1997)**. Studied transformation of an antibiotic resistant fungus (as donor) to Rhizobial cells (as recipient) and transformation between different genera of microorganisms of **Marekova *et al.* (1996).**

The promotion of solubility and release of P in mineral P fertilizers (Triple or double calcium superphosphate) due to using various solubilizing, bacterial transformats might be attributed to their effect in activating hydrolytic enzymes, weathering of minerals and lowering of soil pH by different mechanisms through production of organic acids, production of B vitamins, natural hormones like IAA, Cytokinianes, GA3 as well as antibiotics. These merits were reflected in enhancing organic matter availability of nutrients, water retention and root development (**Nijjar, 1985**). The previous merits of P bacterial transformats as plant physiology were supported by the results of **Mba (1994), Motosugi *et al.* (2002) and Kannaiyan (2003**).

These results are in concordance with those obtained by **Abd El-Hameed (2002), Nikolaou *et al.* (2002), Padwal and Indi (2003), El-Naggar (2004), El-Shenawy and Stino (2005a, b), Ibrahim (2009), Ahmed and Abada (2012), Shaheen *et al.* (2013) and Shaaban (2014)**.

Table 10. Economical study for the recommended treatment if it applied in one fadddan

Recommended treatment	2015	2016
Total of costs Hort. Practices (LE)	17000	18000
Costs of triple Ca. superphosphate (LE)	150	150
Costs of rock phosphate(LE)	35	40
Costs of B. megathanian phosphate (LE)	154	154
Total costs (LE)	17339	18344
Yield/fad. (tons)	7.4	8.8
Price of yield/fad.	29600	35200
New profit (LE)	12200	16850
Control		
Costs Hort. Practices (LE)	17000	18000
Yield/fad. (tons)	6.5	6.9
Price of yield/fad. (LE)	26000	27600
Net profit (LE)	9000	9600
Increase over control	3200	7250

*Price of ton grapes in the first (2015) and second season (2016) were 4000 (LE).

Conclusion

Using rock phosphate at 50% and P bacterial transformats 3 at 10 ml/vine as a partial replacement of mineral P fertilizer (50%) gave the best results with regard to the yield and berries quality of Superior grapevines.

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استجابة كروم العنب السوبيريور لتلقيح التربة ببعض متحولات البكتريا المذيبة للفوسفور

عمر فتحى داخلى[1] - محمد علي مجاور عباده[2]

١- قسم الوراثة - كلية الزراعة - جامعة المنيا - مصر

٢- قسم بحوث العنب - معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة- مصر

تم إجراء تجربة معملية وأخرى حقلية خلال موسمى ٢٠١٥، ٢٠١٦، أجريت التجربة المعملية لإنتاج ثلاث متحولات جديدة من البكتيريا الميسرة للفسفور من خلال تعريض بكتريا الأسيتومايبس الثلاثة للأشعة البنفسيجية لمدة ١-٥ دقائق ولقد اشتقت جميع هذه المتحولات من بكتريا الباسليس ميجاثيرم فوسفاتكم. أما فى التجربة الحقلية فقد تم استخدام السماد الفوسفاتى بطئ التحلل (صخر الفوسفات) مع أى متحولة من متحولات البكتريا المذيبة للفوسفور السابقة كبديل جزئى للتسميد الفوسفاتى المعدنى (سوبر فوسفات الكالسيوم الثلاثي) لكروم العنب السوبيريور، اظهرت نتائج الدراسة أن استخدام الفوسفور المعدنى بنسبة مئوية تتراوح ما بين ٥٠ إلى ٧٥% (سوبر فوسفات الكالسيوم الثلاثية)، ٢٥ - ٥٠% صخر الفوسفات مع واحد من متحولات البكتريا المذيبة للفوسفور (متحولات ١، ٢، ٣) كان فعالا جدا فى تحسين جميع صفات النمو الخضرى ومكونات الأوراق الكيميائية والنسبة المئوية لعقد الحبات وجودة الثمار وذلك بالمقارنة باستخدام الفوسفور فى الصورة المعدنية فقط أو عندما يستخدم السماد الفوسفاتى المعدنى فى صورة ٢٥% مع صخر الفوسفات وأى متحولة بكترية مذيبة للفوسفور، وكانت أفضل متحولة بكترية فوسفاتية فى تحسين الخصائص الخضرية ومكونات الأوراق وكمية المحصول وخصائص العناقيد هى متحولة ٣، ١، ٢ علي التوالى تنازليا، أما خصائص الجودة للحبات فقد تحسنت بوضوح عند استخدام متحولات ٢،٣، ١ مرتبة ترتيبا تنازليا، ويمكن استنتاج إن استخدام صخر الفوسفات بنسبة ٥٠% مع متحولة البكتريا المذيبة للفوسفور هى متحولة رقم ٣ بمعدل ١٠ مل/الكرمة كبديل جزئى للتسميد الفوسفاتى المعدنى بنسبة ٥٠% يعطى أفضل النتائج بخصوص كمية المحصول، وجودة حبات عنب السوبيريور.

المحكمون :

١- أ.د. سعد عبدالخالق مهنا	أستاذ الفاكهة – كلية التكنولوجيا والتنمية – جامعة الزقازيق.
٢- أ.د. طلعت على محمد أبو سيد أحمد	أستاذ الفاكهة المتفرغ – كلية الزراعة – جامعة الزقازيق.