

Original Research Article

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Effect of Phosphorus Solubilizing Bacteria on Growth, Yield, Quality and Nutrient Uptake in Nanasaheb Purple Grapes Grown under Semi-Arid Condition

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ABSTRACT

Keywords

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The field experiment was conducted to study the effect of phosphorus solubilizing bacteria (PSB) on growth, yield, quality and nutrient uptake in Nanasaheb Purple grapes during 2018-19. The PSB product (Jaiv Shakti P) was drenched through soil at 30th, 45th and 60th days after fruit pruning at a different concentration of 0.5, 1, 1.5, 2 and 2.5 ml/vine. The observations on growth, yield, quality, nutrient uptake and microbial load at rhizosphere were studied at different stages of development was recorded. Growth and quality, berry biochemical, petiole nutrient content and microbial load showed significant difference to the various levels of PSB application. The positive correlation between PSB concentration and growth, yield, quality, nutrient and microbial characters was recorded in the present study.

Introduction

Grape (*Vitis vinifera* L.) is one of the major important fruit crops earning maximum foreign exchange. The area under grape cultivation during 2017-18 was 1.39 lakh hectares with production of 2920 million tonnes (Anonymous, 2018). Nanasaheb Purple is promising black seedless variety developed by clonal selection from Sharad Seedless grapes. It has high demand in domestic market due to its quality and attractive colour and it is being grown in four

states viz. Maharashtra, Andhra Pradesh, Madhya Pradesh and Karnataka covering more than 21,000 hectares area (Anonymous, 2019). Berry development requires appropriate dosage of nutrients. Phosphorus is an important nutrient in producing the quality grape as it is also directly related to fruitfulness of vine. It is required mostly for plant growth in tropical areas due to its less availability in soil (Santana *et al.*, 2016). The concentration ranges between 0.2 -0.8 % dry weight of the plant and it is mainly constituent of nucleic acids, enzymes,

coenzymes, nucleotides, and phospholipids. Phosphorus is essential in each part of grapevine and its development, from the micro level to many physiological and biochemical plant activities including photosynthesis (Sharma *et al.*, 2013). It is also actively responsible for root growth, cell enlargement, formation of flowers and seeds, crop maturity, quality of crop, energy production, storage, transfer reaction and showed resistance to many plant diseases (Sharma *et al.*, 2013; Kumar and Patel, 2018; Khan *et al.*, 2009; Satyaprakash *et al.*, 2017; Walpola and Yoon, 2012 (a)).

Phosphorus is the second most important macronutrient required by the plants, next to nitrogen. At present, availability of soluble form of P for plants in the soil is limited because of its fixation as insoluble phosphates of iron, aluminium and calcium in soil (Walpola and Yoon, 2012 (b); Mehrvarz *et al.*, 2008; Alam *et al.*, 2002). About 75–90% of the added chemical P fertilizer is precipitated by metalcation complexes and it becomes fixed in soils and has long term impact on the environment in terms of eutrophication, soil fertility depletion and carbon footprint (Sharma *et al.*, 2013).

Microorganism which helps to solubilize insoluble phosphate are called as phosphate solubilizing microorganisms (PSMs). These are eco-friendly and helps in converting insoluble phosphate to soluble forms by acidification, chelation and exchange reaction (Zahoor *et al.*, 2017). There are many strains of bacteria (*Pseudomonas*, *Bacillus*, *Rhizobium*, *Enterobacter* etc.) and fungi (*Aspergillus* and *Penicillium*) found to be as important phosphate solubilizers (Whitelaw, 2000; Peela *et al.*, 2013). PSB not only provide P for growth of plant it also helps in increasing efficiency of N fixation and accelerate the accessibility of other trace elements by synthesizing essential growth

promoting substances like siderophores, antibiotics etc. (Walpola and Yoon, 2012 (a); Hussain *et al.*, 2013). Zahoor *et al.*, (2017) found that PSB application in tomato significantly increase plant height, fruit yield and P (%) in fruits over control. Purlak and Kose (2009) also reported that PSB application results in better yield, nutrition and plant growth enhancement in strawberry. Rolli *et al.*, (2017) noted that application of PSB improved shoot length and shoot diameter in grapevines of Italy. Considering importance of PSB and its eco-friendly nature, the experiment was conducted to study the effect of phosphorous solubilizing bacteria on growth, yield, quality and nutrient uptake in Nanasaheb Purple grapes grown under semi-arid condition.

Materials and Methods

The trial was conducted at grower's field in Pune district during 2018-19. The experimental site is situated in Mid-West Maharashtra at an altitude of 527 m above mean sea level. The trial was conducted on three-year-old vineyard of Nanasaheb Purple grapes grafted on Dogridge rootstock and trained to bower system. The vines were spaced at 9 feet between two rows and 4 feet between vines. The prepared PSB product (Jaiv Shakti P) by Embio Ltd., Mahad, M.S.) was applied through soil drenching at 30th, 45th and 60th days after fruit pruning at a concentration of 0.5, 1, 1.5, 2 and 2.5 ml/vine. The soil samples were collected in sterile polythene bags and brought into laboratory and stored at 4°C for microbial analysis (Liu *et al.*, 2016). The observations were recorded as below.

Growth and yield parameters

The growth parameters were recorded at 75th days after fruit pruning. Shoot length was measured with the help of measuring tape and

was expressed in cm. Shoot diameter was measured with the help of digital Vernier Calliper and was expressed in mm. Total chlorophyll (mg/g) and Leaf area (cm²) were measured using leaf area meter (CIB, Inc).

The yield parameters such as bunches per vine were counted after berry setting and mean was recorded. Average bunch weight (g) was recorded by averaging five bunches. Number of berries per bunch was counted and mean was recorded. Fifty berry weight (g) was recorded with the help of weighing balance and yield per vine (kg) was recorded at the time of harvesting.

Quality parameters

The quality parameters were recorded after harvesting. Berry length (mm) and berry diameter (mm) was recorded with the help of digital vernier calliper. The juice was extracted from berries using muslin cloth and TSS and acidity was estimated. Total soluble solids (°B) were measured using a hand-held temperature-compensated digital refractometer (ERMA, Japan), while titratable acidity (g/lit) was measured titrating a known volume of juice with 0.1 N NaOH using phenolphthalein as indicator.

Biochemical parameters

After the harvest, the whole berry was crushed and the sample was stored at -20 °C for further analysis. Total phenolic content (mg/g) was estimated using Folin-Ciocalteu reagent and by measuring the absorbance of the reaction mixture at 650 nm (Singleton and Rossi, 1965). Same samples were used for estimation of tannin (mg/g) and colour intensity (%). The results obtained were expressed as catechol equivalent. Reducing sugars (mg/g) were estimated by using Dinitro salicylic Acid (DNSA) method (Miller, 1972). Estimation of carbohydrate (mg/g) was

done by the Anthrone method (Hedge and Hofreiter, 1962). The anthocyanin (mg/lit) contents of the berry samples were analysed according to the method of Ticconi *et al.*, (2001). Proline content (mg g⁻¹) was colorimetrically estimated as per the method of (Bates *et al.*, 1973). Protein (mg/g) estimation was carried out by colorimetric method described by Lowry *et al.*, (1951).

Petiole nutrient content

The petiole samples were drawn from field at 35th, 45th and 65th days after fruit pruning. The collected petioles were washed with tap water followed by distilled water and then dried in oven. After drying, a fine powder was prepared and used for further nutrient analysis. The samples were dried at 70°C, wet digested and analyzed for N by Kjeldahl method. Another part of the sample was digested with HNO₃:HClO₄ (9:4 v/v) and P was estimated by vanado-molybdate method. The sodium and potassium were analysed by ICP OES (Agilent Make, Model -5110 SVDV). Potassium and sodium were determined by flame photometer. An atomic absorption Spectrophotometer was used for determining Ca, Mg and Zn in the absorption mode (Sharma *et al.*, 2005).

Microbial count

The quantitative estimation of phosphate solubilisation by soil microflora was measured by vanadomolybdate phosphoric method (Jackson, 1985). One gm of soil sample collected from grapes rhizosphere was added in 9 ml of distilled water, for each sample separately, vortexed and made up to 10⁻⁹ folds by serial dilution method. From the serially diluted samples, 0.1ml suspension was spreaded on Pikovskaya agar medium (Nautiyal, 1999) amended with tri calcium phosphate (TCP 5.0 g/L) as a sole source of P and incubated at 30°C for 7 days. The

growing bacterial colonies formed clear hallow zone. These colonies were counted as a phosphate solubilising bacteria.

Statistical analysis

The experiment was conducted in randomizer block design. Data were analyzed using Minitab software, version 16.0. Comparison of means was made by Duncan's multiple range test (P-0.05). The data represented in figures are expressed as means of three biological replicated \pm standard deviation (SD).

Results and Discussion

Growth parameters

The data recorded on various growth parameters are presented in table. With the application of different concentrations of PSB, significant differences were recorded among the growth parameters. Highest shoot length (92.06 cm), shoot diameter (8.43 mm), total chlorophyll (1.31mg/g) and leaf area (178 cm²) were recorded in PSB application of 2.5ml/vine treatment while lowest shoot length (78.24 cm), shoot diameter (7.33 mm), total chlorophyll (0.90) and leaf area (136.67 cm²) were recorded in control. Highest growth was noted in maximum concentration of PSB i.e. 2.5 ml/vine and decline in growth was observed as concentration of PSB decreased. Positive correlation between PSB concentration and growth of vine was noted in the present study. From the above results it can be stated that application of PSB significantly changes the growth of vine and higher concentration of PSB results better growth of vine. This might be due to the production of auxin by PSB and increased supply of phosphorus by PSB (Fankem *et al.*, 2008). Also the increase in growth characters might be due to stimulative effect of PSB on P solubilisation leading to higher P

availability and uptake by plants as reported by Sharma *et al.*, (2007) and Turan *et al.*, (2007). Zahoor *et al.*, (2017) reported improved growth characteristics by application of PSB in Tomato and Rolli *et al.*, (2017) noted improving shoot length and shoot diameter in grapevines of Italy.

Yield parameters

The yield parameters such as bunches per vine and number of berries per bunch showed non-significant effect on PSB application in the vineyard as the bunches per vine and number of berries per bunch are maintained keeping in view of export. Parameters like average bunch weight, 50 berry weight and yield/vine showed significant effect to PSB application. Highest average bunch weight (663.67 g), 50 berry weight (458.0 g) and yield (17.38 kg) were recorded in PSB application of 2.5 ml/vine while lowest average bunch weight (513.63 g), 50 berry weight (355.0 g) and yield (13.53 kg) were noted in control (No PSB application). Increase in average bunch weight, 50 berry weight and yield were observed as concentration of PSB application/vine (0.5, 1.0, 1.5, 2.0 and 2.5 ml/vine) increased. The positive correlation was observed between the PSB concentration and yield parameters. Availability of P and its active participation in shoot and root growth led to better plant growth, which later translated into higher yield attributes and resultant yield of grapes. The increase in fruit yield with inoculation of P solubilizing microorganisms might be due to increase in P availability through solubilization of insoluble inorganic phosphate by organic acid, decomposition of phosphate-rich organic compounds and production of plant growth promoting substances (Gaur and Sunita, 1999). Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen

et al., 2006). Similar results were reported in tomato (El-Tantawy and Mohamed, 2009; Poonia and Dhaka, 2012).

Quality parameters

Application of PSB to the vine showed positive effect on grape quality parameters. Berry length, berry diameter, TSS and acidity varied significantly among different concentration of PSB. Berry length was increased from 19.33 to 22.67mm while the berry diameter was increased from 18.33 to 23.33 mm with the increase in concentration of PSB from 0.5 to 2.5 ml/vine respectively. The treatment T3 (1ml PSB/vine) recorded highest TSS (18.67 °B) and lowest acidity (5.50 g/lit.) while lowest TSS (18.20 °B) and highest acidity (5.55 g/lit.) were observed in T₁ (control).

Biochemical parameters

Biochemical parameters like phenol, total tannin, colour intensity, reducing sugar, carbohydrates, anthocyanin, proline and protein studied in the present experiment are presented in table. Application of PSB resulted in significant variation in

biochemical content. Phenol content was highest (3.67 mg/g) in T₅ (2.0 ml PSB/vine), colour intensity was maximum (2.60 %) in T₄ (1.5 ml PSB/vine). Reducing sugar (103.57 mg/g) and proline (8.81 µmoles) were higher in T₃ (1.0 ml PSB/vine). Total tannin (4.01 mg/g), anthocyanin (476.12 mg/lit.) and protein (9.69 mg/g) were observed in T₆ (2.5 ml PSB/vine) while lowest phenol (2.07 mg/g), total tannin (2.27 mg/g), colour intensity (1.51 %), reducing sugar (66.33 mg/g), anthocyanin (218.52 mg/lit.), proline (5.50µmoles) and protein (5.66 mg/g) were recorded lowest in T₁ (control). From the above results it can be stated that PSB application significantly affects the biochemical content of berries as compared to control. The variation in biochemical content might be due to the P uptake that has resulted in quick metabolic transformation of starch to pectin into soluble compounds and rapid translocation of sugars from leaves to the developing berries (Mishra and Tripathi, 2011). The findings of the present investigations are in agreement with the result of Singh *et al.*, (2009) in ber, Baksh *et al.*, (2008) in guava, Mishra and Tripathi (2011) in strawberry.

Table.1 Effect of PSB on growth parameters

Treatments	Shoot length (cm)	Shoot diameter (mm)	Total Chlorophyll (mg/g)	Leaf area (cm ²)
T1 (Control)	78.24	7.33	0.90	136.67
T2	80.35	7.70	1.07	140.00
T3	83.62	7.87	1.14	154.00
T4	86.31	8.30	1.23	165.33
T5	91.07	8.43	1.30	175.00
T6	92.06	8.43	1.31	178.00
SEm ±	0.38	0.03	0.02	0.67
CD at 5%	1.15	0.10	0.06	2.02
Sig.	**	**	**	**

Table.2 Effect of PSB on yield parameters

Treatment	Bunches/vine	Average bunch weight (g)	No. of berries per bunch	50 berries weight (g)	Yield/vine (kg)
T1 (Control)	26.33	513.67	73.00	355.00	13.53
T2	27.00	529.33	72.33	364.67	14.30
T3	26.00	556.33	73.66	375.00	14.47
T4	27.00	602.00	72.66	432.67	16.25
T5	26.00	660.00	71.66	453.67	17.26
T6	26.33	663.67	72.33	458.00	17.38
SEm ±	0.31	3.55	0.59	3.41	0.23
CD at 5%	0.94	10.70	1.79	10.26	0.69
Sig.	NS	**	NS	**	**

Table.3 Effect of PSB on quality parameters

Treatment	Berry diameter (mm)	Berry length (mm)	TSS (⁰ Brix)	Acidity (g/lit)
T1 (Control)	18.33	19.33	18.20	5.55
T2	19.67	19.67	18.23	5.52
T3	21.33	20.00	18.67	5.50
T4	22.33	20.67	18.43	5.51
T5	23.67	22.67	18.33	5.53
T6	23.33	22.67	18.23	5.55
SEm ±	0.23	0.30	0.02	0.01
CD at 5%	0.70	0.90	0.07	0.02
Sig.	**	**	**	**

Table.4 Effect of PSB on biochemical quality parameters

Treatments	Phenol (mg/g)	Total Tannin (mg/g)	Colour intensity (%)	Reducing sugar (mg/g)	Carbohy drates (mg/g)	Anthocy anin (mg/lit.)	Proline (µmoles)	Protein (mg/g)
T1 (Control)	2.07	2.270	1.513	66.33	375.822	218.52	5.500	5.66
T2	2.07	3.274	2.071	71.70	371.433	315.43	7.800	6.87
T3	3.09	3.402	2.450	103.57	373.797	467.57	8.180	6.73
T4	3.40	3.674	2.607	92.42	372.380	470.19	6.475	9.00
T5	3.67	3.975	1.821	67.75	373.100	475.58	6.127	9.59
T6	3.66	4.013	1.922	67.46	373.557	476.12	6.247	9.69
S E m ±	0.01	0.02	0.01	0.31	0.29	0.58	0.06	0.02
CD at 5%	0.02	0.05	0.03	0.92	0.88	1.74	0.18	0.07
Sig.	**	**	**	**	**	**	**	**

Table.5 Effect of PSB on petiole nutrient status 35 DAP

Treatments	N (%)	P (%)	K (%)	Na (%)	Ca (%)	Mg (%)	Zn (ppm)
T1 (Control)	1.46	0.66	2.17	0.92	0.44	0.43	167.4
T2	1.46	0.73	2.27	0.15	0.68	0.30	186.2
T3	0.90	0.73	1.84	0.22	0.49	0.25	168.1
T4	1.18	0.71	1.71	0.24	0.54	0.32	203.8
T5	1.18	0.73	1.81	0.32	0.54	0.31	182.5
T6	1.23	0.72	2.13	0.17	0.68	0.29	161.2

Table.6 Effect of PSB on petiole nutrient status 65 DAP

Treatments	N (%)	P (%)	K (%)	Na (%)	Ca (%)	Mg (%)	Zn (ppm)
T1 (Control)	0.90	0.44	4.02	0.10	1.17	0.75	51.35
T2	0.76	0.60	3.42	0.07	0.89	0.54	30.68
T3	0.87	0.57	3.82	0.11	1.32	0.72	49.25
T4	0.92	0.52	4.45	0.08	1.29	0.75	51.40
T5	0.98	0.53	4.12	0.11	1.33	0.80	56.38
T6	0.92	0.55	2.72	0.11	1.26	0.68	44.93

Table.7 Effect of PSB on microbial load:
million CFU/gm of soil

Description	T ₁ (Control)	T ₂	T ₃	T ₄	T ₅	T ₆
Before application	0.2	0.1	0.3	0.2	0.1	0.3
30 days	0.6	0.8	0.8	0.9	1	1.2
45 days	3	3.1	3.4	3.7	3.8	4.1
60 days	4.2	4.9	5.6	5.9	6.7	7.2
75 days	5.2	5.8	6.8	6.3	7.2	7.4
At harvest	2.9	3.2	3.3	3.4	3.4	3.5

Nutrient uptake

At 65 DAP N (0.98 %), Ca (1.33 %), Mg (0.80 %) and Zn (56.38 ppm) were observed in T₅ (2.0 ml PSB/vine). Maximum Na (0.11 %) was noted in T₃ (1.0 ml PSB/vine), T₅ (2.0 ml PSB/vine) and T₆ (2.5 ml PSB/vine) treatments whereas, P (0.60 %) and K (4.45 %) were observed higher in T₅ (2.0 ml PSB/vine) and T₄ (1.5 ml PSB/vine). While lowest N (0.76 %), Na (0.07 %), Ca (0.89 %), Mg (0.54 %) and Zn (30.68 ppm) were recorded in T₂ (0.5 ml PSB/vine). Whereas,

lowest P (0.44 %) and K (2.72 %) were recorded in T₁ and T₂ respectively. The above results clearly indicated that application of PSB not only increased P level but also helped in improving uptake of other nutrients. This might be due to the phosphorus solubilizing bacteria (PSB) helped in growth of plants by stimulating the efficiency of biological nitrogen fixation, synthesizing phytohormones and enhancing the availability of some trace elements such as zinc and iron (Wani *et al.*, 2007).

Microbial load

Microbial growth around the rhizosphere is important for healthy crop and also for better nutrient uptake. In this investigation microbial count of soil was measured at 30, 45, 60, 75 DAP and at harvest stage where PSB application is applied. From the results it is observed that PSB application treatment showed the microbial count 0.3 CFU/gm soil to 3.5 CFU/gm soil from the 30th, 45th, 60th after application to harvest stage respectively. Microbial count showed significant changes among shift PSB application. This was mainly due to the purity of PSB and CFU count is to be maintained at recommended level. Which results increasing microbial activity at rhizosphere.

From the study it was concluded that T₅ and T₆ treatments were at par with each other. Nanasahab Purple grapes showed better performance to PSB application (Jaiv Shakti P). All the parameters such as growth, yield, quality and nutrient uptake showed positive effect to PSB. Application of PSB increases growth, improves quality, nutrient uptake, microbial growth at rhizosphere as compared to control. From the above investigation it can be stated that PSB application at a concentration of 2.0 ml/vine was proved to be the best for Nanasahab Purple grapes.

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