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Pre-harvest application of methyl jasmonate for improving nutritional quality of Pusa Navrang grape (*Vitis vinifera*)

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ABSTRACT

The effect of pre-harvest application of Methyl Jasmonate (MeJA) on berry quality, flavonoid content and antioxidant capacity in grapes (*Vitis vinifera* L.) *cv*. Pusa Navrang were studied under subtropical conditions of New Delhi. Different doses of MeJA (0, 5, 10, 15 mM) were applied at three different dates. There were no significant differences were recorded for bunch and berry weight. Grapes treated with MeJA had slightly higher soluble solid content, lower titratable acids than untreated berries. Methyl jasmonate significantly enhanced the content of total monomeric anthocyanins, total phenolics, flavonoids and the antioxidant capacities in the berries. These were found significantly different for dose of methyl jasmonate and time of application. In general, when MeJA(10 mM) applied at veraison stage was found most effective for improving the berry bioactive compounds and had significantly higher total monomeric anthocyanin (1434.43 C_3GE mg/kg fresh berry weight), total phenolic content (504.58 mg/100 g), total flavonoid content and antioxidant activity measured in terms of DPPH.

Key words: Antioxidant, Anthocyanins, Flavonoids, Grape, Methyl jasmonate, Phenolics

Grape (*Vitis vinifera* L.) is widely adapted crop and growing globally in varied agro-climatic regions. In India, it is being produced of 2.74 million tonnes from an area of 0.136 million ha (Anon 2017). More than 80% of the grapes are used for wine making globally, whereas, in India, the majority of produce are utilized for table purpose and a small quantity is processed for making juice and wine (Vijaya *et al*. 2018). Berries are delicious and rich source of energy, bioactive compounds and a range of phytochemicals (Jindal *et al*. 2004). The phytochemical composition of plant foods varies according to genetical, physiological and agronomical factors. The number of phytochemicals are primarily genotypic and growing condition dependent (Gutiérrez-Gamboa *et al*. 2019). However, the amount of phytochemicals can also be improved through the use of elicitors (Ruiz-Garcia and Gomez-Plaza 2013, Reyes-Diaz *et al*. 2016). The elicitor treatments trigger the synthesis of phytochemical compounds in fruits. Specific elicitor application can be used to increase metabolite production in the plant (Gowthami 2018).

Methyl jasmonate is a plant growth regulator plays an important role as a signalling molecule and modulating plant defense responses, including antioxidant systems (Creelman and Mullet 1997). The pre-harvest application of MeJA to fruit crops significantly increases the health promoting compounds black berries (Wang *et al*. 2008), grapes (Ruiz-Garcia *et al*. 2012, Gil-Munoz *et al*. 2017, Portu *et al*. 2017). It also enhances the accumulation of anthocyanins and total phenols in grapes (Flores *et al*. 2015). In India, Pusa Navrang is a high yielding grape variety, which is ideal for making of juice and coloured wine (Karibasappa and Adsule 2008, Sharma *et al*. 2018). The berries are widely used for improving chromatic properties of juice and coloured wines in India (Khurdiya *et al*. 1995). It is one of the early maturing, grape variety rich in anthocyanins and polyphenols (Fitrat *et al*. 2016). Given the technological significance of methyl jasmonate their use at pre-harvest application has been studied for improving the berry quality, phenolic content and antioxidant capacities.

MATERIALS AND METHODS

Pusa Navrang grape vines used in this study were grown at the Main Garden, Division of Fruits and Horticultural Technology, ICAR- Indian Agricultural Research Institute, New Delhi during 2016-18. The climatic conditions are characterized as subtropical. The experiment was laid out in factorial RBD with 3 replications. Three vines were randomly selected and the treatments were designated with four levels of MeJA (0, 5, 10, 15 mM) and three time of application (at veraison, 3 days after veraison and 6 days after veraison). Berry quality, phenolic content and antioxidant

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activities were recorded after harvest maturity. Three lots of 100 g of undamaged berries were randomly selected from each treatment and were then used for physico-chemical analyses. Bunch and berry weight was determined using the electronic precision balance. Bunch length and width with pedicel was determined by using measuring scale. Berry diameter and length was determined by using of Digimaticcaliper (Mitutoyo, Japan). The juice recovery was measured and expressed in percentage. The total soluble solids (TSS) of grape juice were measured with the help of digital refractometer. The digital *p*H meter was used to measure the *p*H. The titratable acidity of the juice present in all the samples was determined by titration against 0.1 N NaOH solution (AOAC 2000). The content of the ascorbic acid was analyzed by titration method (AOAC 2000). The quantity of the total monomeric anthocyanin was measured by UV-VIS Spectrophotometer (UV 5704SS, ECIL, India) at 520 nm and 700 nm wavelength, using the *p*H-differential method (Wrolstad *et al*. 2005). The total phenolics content was determined, using Folin–Ciocalteu reagent (FCR) method (Singleton *et al*. 1999). The total flavonoid content was measured spectrophotometrically (Zhishen *et al*. 1999). The antiradical capacity of the sample extract was measured using DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) with spectrophotometer at 515 nm (Brand-Williams *et al*. 1995, Sanchez-Moreno *et al*. 1999). The significant differences among treatments for each variable were assessed by analysis of variance (ANOVA) using SAS version 9.3 software (SAS 2013). LSD test was used to separate the means (P=0.05) when ANOVA test was significant.

RESULTS AND DISCUSSION

The pre-harvest application of methyl jasmonate to grapes has several effects. Results from 2016-17 and 2017-18 seasons were grouped and the mean data of physico-chemical characteristics, anthocyanins, total phenolics, total flavonoids and antioxidant capacity of grapes was measured.

Physico-chemical composition of grapes: Table 1 and 2 shows the physico-chemical data of the grapes at the moment of harvest. No changes in bunch weight were observed compared to control grapes with different concentrations of MeJA and time of application independently. However,

Table 1 Effect of methyl jasmonate on bunch weight, berry weight, juice *p*H, juice recovery, TSS and TSS: Acid ratio in Pusa Navrang grapes

Methyl jasmonate levels	Bunch weight (g)	Berry weight (g)	Juice *p*H	Juice recovery (%)	TSS (°Brix)	TSS: Acid ratio
MeJA 5 mM	121.49^{a}	1.23^{a}	3.98^{b}	72.72^{c}	18.36^{a}	20.32^{a}
MeJA 10 mM	128.28^{a}	1.28^{a}	3.93^{c}	77.22^{a}	18.29^{a}	21.31^{a}
MeJA 15 mM	124.21^{a}	1.21^{ab}	3.99^{a}	74.89^{b}	18.39^{a}	21.94^{a}
Control	125.16^{a}	1.13^{b}	3.86^{d}	69.67^{d}	18.00^{a}	21.80^{a}
LSD (P=0.05)	8.36	0.45	0.01	1.34	0.45	2.06
Time of application						
At veraison	128.41^{a}	1.23^{a}	3.97^{a}	73.30^{ab}	18.37^{a}	21.46^{a}
3 days after veraison	126.53^{a}	1.19^{a}	3.91^{c}	74.83^{a}	18.27^{a}	21.49^{a}
6 days after veraison	119.41^{a}	1.21^{a}	3.93^{b}	72.75^{b}	18.15^{a}	21.08^{a}
LSD (P=0.05)	7.24	0.39	0.01	1.16	0.39	1.78
MeJA × Time of application						
MeJA 5 mM at veraison	129.77^{abc}	1.27^{abc}	3.94^{f}	72.78^{de}	18.25^{a}	20.17^{a}
MeJA 5 mM at 3 DAV	115.87^{bc}	1.22^{abcd}	3.94^{ef}	71.95^{e}	18.35^{a}	20.58^{a}
MeJA 5 mM at 6 DAV	118.83^{abc}	1.20^{abcd}	3.97^{bc}	73.43^{cde}	18.48^{a}	20.21^{a}
MeJA 10 mM at veraison	135.83^{a}	1.32^{a}	3.96^{de}	78.43^{ab}	18.68^{a}	20.97^{a}
MeJA 10 mM at 3 DAV	124.20^{abc}	1.25^{abc}	3.88^{g}	79.82^{a}	18.12^{a}	21.56^{a}
MeJA 10 mM at 6 DAV	124.82^{abc}	1.28^{ab}	3.94^{f}	73.42^{cde}	18.08^{a}	21.40^{a}
MeJA 15 mM at veraison	128.17^{abc}	1.25^{abc}	4.03^{a}	75.90^{bcd}	18.53^{a}	21.82^{a}
MeJA 15 mM at 3 DAV	132.33^{ab}	1.17^{bcd}	3.99^{b}	76.28^{bc}	18.37^{a}	21.67^{a}
MeJA 15 mM at 6 DAV	112.13^{c}	1.20^{abcd}	3.97^{cd}	72.50^{e}	18.28^{a}	22.34^{a}
Control at veraison	119.87^{abc}	1.10^{d}	3.88^{g}	66.10^{f}	18.00^{a}	22.87^{a}
Control at 3 DAV	133.73^{ab}	1.13^{cd}	3.84^{h}	71.25^{e}	18.25^{a}	22.14^{a}
Control at 6 DAV	121.87^{abc}	1.15^{bcd}	3.84^{h}	71.65^{e}	17.75^{a}	20.38^{a}
LSD (P=0.05)	14.48	0.78	0.01	2.32	0.78	3.56

MeJA, methyl jasmonate; DAV, Days after veraison; LSD, Least significant difference. Different letters in the same row indicate significant differences according to the LSD test (P<0.05).

significant differences were recorded with interaction between MeJA and time of application. The slight significant differences were also recorded in berry weight parameters. The maximum significant difference was recorded in MeJA (15 mM) applied 6 days after veraison. Ruiz-Garcia *et al.* (2012) and Gil-Munoz *et al*. (2017) also confirmed the above findings that the pre-harvest application of MeJA did not affect much on berry weight. In contrast, larger berries were recorded in Monastrell (Paladine-Quezada *et al*. 2018). The juice *p*H and juice recovery were also less influenced by MeJA when applied on all the three dates. However, there was significant differences recorded, when applied 15 mM at veraison for juice *p*H and 10 mM MeJA applied 3 days after veraison for juice recovery.

Different concentrations of MeJA and time of application also did not showed significant differences for total soluble solids, titratable acidity (TA), TSS: Acid ratio and ascorbic acid content (Table 1 and 2). There were also not any changes recorded in interactions for TSS, TA, and TSS:TA ratio. However, treatments were differed significantly for ascorbic acid content when different concentrations were applied on all the three dates. The maximum significant differences were recorded when MeJA (10 mM) applied at veraison as compared to control and two different dates (3 days and 6 days after veraison). Above findings were in agreement with Portu *et al*. (2017) and Ruiz-Garcia *et al*. (2012), whereas, no significant differences between control grapes and grapes from grapevines foliar treated with MeJA. However, the effect on total acidity, *p*H, tartaric and malic acid content varied from one year which suggested that, the influence of MeJA application on grape physico-chemical parameters could therefore dependent on the climatic conditions of the year as well (Ruiz-Garcia *et al*. 2012).

Total monomeric anthocyanins content: The application of MeJA improved the quantities of grape total monomeric anthocyanins (TMA) by more than 40% (Table 2). The results were significant with different concentration, time of application and the interaction between two factors.

Table 2 Effect of methyl jasmonate on total titratable acidity, ascorbic acid, total monomeric anthocyanin, total phenolics, total flavonoids and antioxidant capacity in terms of DPPH activity in Pusa Navrang grapes

Methyl jasmonate levels	Total titratable acidity (%)	Ascorbic acid (mg/100 ml of juice)	TMA (C_3GE mg/kg fresh berry wt.)	Total Phenolics (GAE, mg/100 g)	Total flavonoids (QE, mg / 100 g)	DPPH (TE, µmol/g)
MeJA 5 mM	0.91^{a}	4.18^{b}	1058.56^{c}	316.24^{c}	245.99^{c}	30.70^{a}
MeJA 10 mM	0.86^{a}	5.88^{a}	1286.83^{a}	460.58 ^{a}	355.29^{b}	36.40^{a}
MeJA 15 mM	0.84^{a}	6.34^{a}	1250.07^{b}	406.27^{b}	359.74^{a}	37.33^{a}
Control	0.83^{a}	5.61^{a}	826.78^{d}	283.14^{d}	197.52^{d}	21.82^{a}
LSD (P=0.05)	0.08	0.90	4.35	4.69	2.47	1.70
Time of application						
At veraison	0.86^{a}	5.70^{a}	1172.87^{a}	384.66^{a}	317.05^{a}	35.27^{a}
3 days after veraison	0.86^{a}	5.47^{a}	1109.81^{b}	380.61^{a}	299.04^{b}	32.12^{a}
6 days after veraison	0.87^{a}	5.33^{a}	1034.00^{c}	334.41^{b}	252.82^{c}	27.30^{a}
LSD (P=0.05)	0.07	0.78	3.77	4.07	2.14	1.47
MeJA x Time of application						
MeJA 5 mM at veraison	0.91^{a}	4.27^{cd}	1074.33^{g}	332.50^{e}	222.86^{h}	36.44^{bc}
MeJA 5 mM at 3 DAV	0.91^{a}	4.51^{bcd}	1067.14^{g}	316.11^{f}	266.02^{f}	32.41^{d}
MeJA 5 mM at 6 DAV	0.92^{a}	3.76^{d}	1034.22^{h}	300.12^{g}	249.09^{g}	23.25^{e}
MeJA 10 mM at veraison	0.90^{a}	6.79^{a}	1434.43^{a}	504.58^{a}	418.33^{b}	40.01^{b}
MeJA 10 mM at 3 DAV	0.84^{a}	5.24^{abcd}	1317.11^{c}	469.58^{b}	353.61^{d}	37.70^{ab}
MeJA 10 mM at 6 DAV	0.85^{a}	5.63^{abcd}	1108.95^{f}	407.56^{d}	293.93^{e}	31.01^{d}
MeJA 15 mM at veraison	0.85^{a}	6.32^{abc}	1344.28^{b}	444.05^{c}	439.31^{a}	40.60^{a}
MeJA 15 mM at 3 DAV	0.85^{a}	6.62^{ab}	1237.93^{d}	437.58^{c}	379.58^{c}	38.10^{ab}
MeJA 15 mM at 6 DAV	0.83^{a}	6.07^{abc}	1167.99^{e}	337.20^{e}	260.32^{f}	33.28^{cd}
Control at veraison	0.79^{a}	5.42^{abcd}	838.45^{i}	257.50^{h}	187.68^{k}	23.57^{e}
Control at 3 DAV	0.83^{a}	5.52^{abcd}	817.05^{j}	299.17^{g}	196.94^{j}	20.26^{e}
Control at 6 DAV	0.88^{a}	5.87^{abc}	824.85^{j}	292.76^{g}	207.93^{i}	21.64^{e}
LSD (P=0.05)	0.14	1.55	7.53	8.13	4.28	2.94

MeJA, Methyl jasmonate; DAV, Days after veraison; TMA, Total monomeric anthocyanin; C_3GE, Cynidin-3-glucoside equivalent; GAE, Gallic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; QE, Quercetin equivalent; TE, Trolox equivalent; LSD, Least significant difference. Different letters in the same row indicate significant differences according to the LSD test (P<0.05).

The maximum content was recorded with 10 mM MeJA (1286.83 C_3GE mg/kg fresh berry weight) as compared to control (826.78 C_3GE mg/kg fresh berry weight). The time of application also significantly affected the accumulation of TMA in berries and recorded maximum, when applied at veraison (1172.87 C_3GE mg/kg fresh berry weight). The effect was found more pronounced when, MeJA 10 mM was applied at veraison (1434.22 C_3GE mg/kg fresh berry weight) as compared control (817.05 C_3GE mg/kg fresh berry weight) applied at 3 days after veraison. The increase in berry total anthocyanin and individual anthocyanins content through use of MeJA has been reported by several workers (Ju *et al*. 2016). Portu *et al*. (2017) also reported that, methyl jasmonate foliar application increased anthocyanins in grape through synthesis of 3-O-glucosides of petunidin and peonidin and *trans*-p-coumaroyl derivatives of cyanidin and peonidin. This might be due to the enhanced expression of key genes (*PAL, CHI and MYB*) in the synthesis pathway of polyphenols and anthocyanins after treatment with ABA and MeJA, which thus promote the synthesis of polyphenols and anthocyanins (Ruiz-Garcia *et al*. 2012, Ruiz-Garcia and Gomes-Plaza 2013, Gil-Munoz *et al*. 2017).

Total phenolics content: The grape phenolics content was increased significantly in the samples from MeJA treatment in comparison with control samples Table 2. Both, MeJA levels and time of application affected the content of total phenolic content significantly. The maximum total phenolics content was recorded when the MeJA was applied at 10 mM. The most effective time of application was at veraison stage. However, the highest content of total phenolics was recorded MeJA 10 mM was applied at veraison (504.58 GAE, mg/100 g) as compared to veraison control (257.50 GAE, mg/100 g). Increase in phenolic contents through pre-harvest application was also reported in Monastrell, Merlot and Cabernet Sauvignon grapes by Paladines-Quezada *et al*. (2018) due to activation of phenylalanine ammonia-lyase (PAL) enzymes. Similar findings were also suggested by Gil-Munoz *et al*. (2017) in grape berries treated with MeJA.

Total flavonoids content: The content of the total flavonoids differed significantly among all the treatments at 5% level of significance (Table 2). Within the MeJA concentrations, 15 mM was found most effective for increasing the total flavonoids content (359.74 mg/100 g in terms of quercetin equivalent). Whereas, time of application also affected significantly to the total flavonoids content, whereas, maximum content was recorded, when MeJA was applied at veraison (317.05 GAE, mg /100 g). However, the maximum increase in total flavonoids content was recorded, when 15 mM MeJA was applied at veraison (439.31 GAE, mg/100 g) as compared to control (Table 2). This increment was more than double over and above to rest of the control treatments. This increase in flavonoids content mainly takes place by elicitation of enzymatic activities for synthesis of major monomeric flavanols and phenolic acids (Portu *et al*. 2017).

Antioxidant activity in terms of DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate): The antioxidant capacity of grapes treated with MeJA was assessed through DPPH assay (Table 2). It was clearly revealed from the data that, MeJAalone also did not affect the antioxidant activity in terms DPPH. Similarly, there was no difference observed in days of application. However, significant differences existed when the MeJA (15 mM) was applied at veraison stage (40.60 μmol/g in terms of Trolox equivalent) followed by 10 mM at veraison stage. However, the minimum content was recorded in control, when applied 3 days after veraison. The significant differences among the different treatment combinations were also recorded. Similar increase in the antioxidant activity through use of methyl jasmonate has been reported by Wang *et al*. (2008), Flores *et al*. (2015) and Portu *et al*. (2017).

In the present study, the elicitor methyl jasmonate was found effective as biostimulant that can trigger defense reactions in grapevines through induction of the synthesis of secondary metabolites, as mainly phenolics. These are usually applied with the aim to improve phenolic properties of grapes. Currently, the adaptation of elicitors as biostimulants to viticulture is of greater interest because these compounds represent alternative strategies to improve the nutritional value of grapes. Methyl jasmonate is the most studied elicitor, and it is generally the most effective for improving grape berry quality. However, its large-scale application in the vineyard is not yet economically viable, and future research must be carried out to lower the cost of its synthesis. Future research should therefore investigate the chemical responses of grapevines to exogenous elicitors across different grapevine varieties over more than one harvest. In particular, these should include the subsequent impacts on the expression of the organic enzymes and their relationships with other viticultural factors.The results obtained suggest that, methyl jasmonate foliar application could be a simple and accessible practice, that allows to enhance grape quality.

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