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## Effect of CPPU Brassinolide and Prohexadione calcium on photosynthesis, transpiration rate and related parameters in new castle apricot

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**Abstract**

Apricot, although being the third most important stone fruit crops of India, still requires a great improvisation from production point of view. Growth regulators are being used by the growers to increase the yield by improving the yield attribute parameters. Positive impact of growth regulators on net photosynthetic rate and related parameters leads to better yield and quality of fruits. Keeping this in forefront, a study entitled "Effect of CPPU Brassinolide and Prohexadione calcium on photosynthesis, transpiration rate and related parameters in New Castle apricot" was carried out at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni with the objective of improvising the physiological parameters and thereby, increasing the yield. The study was conducted on twenty three-year-old experimental trees which were subjected to 10 treatments viz. CPPU at 2.5, 5 and 10 ppm applied at petal fall, prohexadione-calcium at 50, 100 and 200 ppm at pit hardening stage and brassinolide at 0.5, 1 and 2 ppm at petal fall. The results of the study showed that the treatment T<sub>6</sub> (200 ppm Pro-Ca) had the higher stomatal conductance (18.2 mmol m<sup>-2</sup> s<sup>-1</sup>), transpiration rate (22 mmol m<sup>-2</sup> s<sup>-1</sup>), net photosynthetic rate (52.1 μmol m<sup>-2</sup> s<sup>-1</sup>) and total chlorophyll content (3.39 mg g<sup>-1</sup>). Thus, it can be concluded that with the application of 200 ppm Pro-Ca growth regulator, the physiological parameters having the most dominant effect on increasing the yield of apricot, can be enhanced to get better returns.

**Keywords:** CPPU, net photosynthetic rate, stomatal conductance, brassinolide and prohexadione

**Introduction**

Apricot, although being the third most important stone fruit crops of India, still requires a great improvisation from production point of view. Apricot (*Prunus armeniaca* L.) is a deciduous tree fruit species belonging to family Rosaceae. It is one of the important stone fruit crops and grown worldwide in mild temperate to extreme cold regions. In India, it is cultivated in hilly regions of Himachal Pradesh, Jammu and Kashmir, Uttarakhand (Parmar and Kaushal, 1982)<sup>[14]</sup> and to a limited extent in the hill region of north-eastern states of the country. The area under apricot cultivation in India is around 5358 ha with annual production of 15072 MT (Anonymous, 2016)<sup>[2]</sup>. Total area under apricot cultivation in Himachal Pradesh is 3650 ha with production of 11514 MT during 2016-2017 (Anonymous, 2017)<sup>[3]</sup>. Solan, Shimla, Kullu, Mandi and Kinnaur are the main districts in Himachal Pradesh where apricot is grown extensively. Some drying type apricots are being grown in the dry temperate areas of Kinnaur, Lahaul & Spiti in Himachal Pradesh and in Ladakh region of Jammu & Kashmir. The low-moderate chilling "New Castle" is the most commercial cultivar of apricot in the mid-hills of Himachal Pradesh.

The purpose of using plant growth substances as foliar applications is to improve quality vegetative growth for producing maximum yield. Sitofex (CPPU){(N-(2-chloro-1-pyridinyl)-N-phenylurea)} at different concentrations enhanced cell division, increased cell size and ultimately leads to increased tree growth (Asaad, 2014). Pro-Ca is an inhibitor of GA synthesis with low toxicity and persistence (Smit *et al.* 2005)<sup>[18]</sup>, which reduces the levels of GA1 (the highly active form) and increases GA20 (inactive form) causes a reduction in shoot elongation, reducing the excessive vegetative growth. Brassinolide was the first isolated brassinosteroid in 1979 when it was shown that pollen from *Brassica napus* could promote stem elongation and cell divisions, and the biologically active molecule was isolated. BRs have been shown to be involved in numerous plant processes like promotion of cell expansion

and cell elongation synergism with auxin, play role in cell division and cell wall regeneration, promotion of vascular differentiation etc. Growth regulators are being used by the growers to increase the yield by improving the yield attribute parameters. Positive impact of growth regulators on net photosynthetic rate and related parameters leads to better yield and quality of fruits. The target of this study was achieving the possibility of improvising physiological processes (net photosynthetic rate, transpiration rate etc.) and ultimately the final yield and quality of fruit through spraying above mentioned growth regulators on "New Castle" apricots.

### Materials and Methods

The experiment was carried out on 23-year-old trees of apricot cultivar New Castle raised on wild apricot seedlings. The trees had been planted at a spacing of 3×3 meters and trained as open centres. For the present study, thirty trees were selected on the basis of uniform vigour and were maintained under uniform cultural practices during the entire course of investigation.

The experimental trees were subjected to different treatments of forchlorfenuron, prohexadione-calcium and brassinolide as per details given in Table 1.

**Table 1:** Description of forchlorfenuron, prohexadione-calcium and brassinolide treatments

Treatments	Chemicals	Concentration (ppm)	Time of application
T <sub>1</sub>	CPPU	2.5	Petal fall
T <sub>2</sub>	CPPU	5	Petal fall
T <sub>3</sub>	CPPU	10	Petal fall
T <sub>4</sub>	Pro-Ca	50	Pit-hardening
T <sub>5</sub>	Pro-Ca	100	Pit-hardening
T <sub>6</sub>	Pro-Ca	200	Pit-hardening
T <sub>7</sub>	Brassinolide	0.5	Petal fall
T <sub>8</sub>	Brassinolide	1	Petal fall
T <sub>9</sub>	Brassinolide	2	Petal fall
T <sub>10</sub>	Control	-	-

The experiment was laid out in Completely Randomized Block Design (CRBD) with three replications.

### Rate of stomatal conductance

The rate of leaf stomatal conductance was observed by randomly selecting ten mature leaves from each experimental tree from all over the tree periphery. The observations were recorded during active growth period in mid-June between 10:00 to 11:00 AM with the help of LI-COR 6200 Portable Photosynthesis System. The results were expressed in  $\text{m mol m}^{-2}\text{s}^{-1}$ .

### Rate of transpiration

The rate of transpiration was observed by randomly selecting ten mature leaves from each experimental tree from all over the tree periphery. The observations were recorded during active growth period in mid-June between 10:00 to 11:00 AM with the help of LI-COR 6200 Portable Photosynthesis System. The results were expressed in  $\text{m mol m}^{-2}\text{s}^{-1}$ .

### Net Photosynthetic Rate

The observations on net photosynthetic rate of ten mature leaves randomly selected from all over the periphery of each experimental plant were taken with the help of LI-COR 6200 Portable Photosynthesis System in mid- June between 10:00

and 11:00 AM. The results were averaged and expressed in  $\mu\text{mol m}^{-2}\text{s}^{-1}$ .

### Chlorophyll content

A sample of ten representative fully grown leaves from the current season's growth of each tree was detached in the morning hours, in the first week of July (Halfacre *et al.*, 1968), immediately placed in ice box and brought to the laboratory. The samples were then kept in the refrigerator below 0°C to avoid degradation of chlorophyll pigments.

### Extraction

Leaves from each sample were washed and chopped into fine pieces under subdued light and 100 mg of chopped material was placed in vial containing 7 ml of dimethyl sulphoxide (DMSO). The contents of the vials were incubated at 65 °C temperature for 30 minutes and then extract was transferred to graduated test tube and final volume was made to 10 ml with dimethyl sulphoxide (Hiscox and Israelstam, 1979).

### Estimation

Optical density (OD) of the above extract was recorded on Spectronic-20 D at 645 and 663 nm wave lengths against DMSO blank and total chlorophyll content was calculated by using the following formula:

$$\text{Total Chlorophyll (mg/g)} = \frac{20.2 A_{645} + 8.02 A_{663} \times V}{A \times 1000 \times W}$$

Where,

- V = Volume of extract used
- A = Length of light path in cell (1cm)
- W = Weight of the sample (g)
- A<sub>645</sub> = Absorbance at 645 nm wavelength
- A<sub>663</sub> = Absorbance at 663 nm wavelength

The results were expressed as chlorophyll content in mg/g of fresh weight.

## Results and Discussion

### Stomatal Conductance

**Table 2:** Effect of forchlorfenuron, prohexadione-calcium and brassinolide on leaf stomatal conductance of apricot

Treatment		Stomatal conductance ( $\text{m mol m}^{-2}\text{s}^{-1}$ )		
		2013	2014	Pooled
T <sub>1</sub>	CPPU (2.5 ppm)	12.4	13.0	12.7
T <sub>2</sub>	CPPU (5 ppm)	15.3	15.3	15.3
T <sub>3</sub>	CPPU (10 ppm)	16.3	16.3	16.3
T <sub>4</sub>	Pro-Ca (50 ppm)	13.1	13.5	13.3
T <sub>5</sub>	Pro-Ca (100 ppm)	16.9	17.3	17.1
T <sub>6</sub>	Pro-Ca (200 ppm)	18.2	18.1	18.2
T <sub>7</sub>	Brassinolide (0.5 ppm)	12.0	12.8	12.4
T <sub>8</sub>	Brassinolide (1 ppm)	13.9	14.9	14.4
T <sub>9</sub>	Brassinolide (2 ppm)	13.3	13.6	13.4
T <sub>10</sub>	Control	11.1	11.4	11.2
CD <sub>0.05</sub>		1.5	1.3	0.9

The perusal of data depicted in Table 2 indicates that the leaf stomatal conductance was significantly affected by different treatments. In the year 2013, the leaf stomatal conductance varied from 11.1 to 18.2  $\text{m mol m}^{-2}\text{s}^{-1}$ . It was observed significantly higher (18.2  $\text{m mol m}^{-2}\text{s}^{-1}$ ) in the trees subjected to the treatment with Pro-Ca at 200 ppm (T<sub>6</sub>) than those under

the remaining treatments except, T<sub>5</sub>. The leaf stomatal conductance was observed significantly lower in trees under control (11.1 m mol m<sup>-2</sup>s<sup>-1</sup>) than all other treatments except, T<sub>1</sub> (2.5 ppm CPPU) and T<sub>7</sub> (0.5 ppm Brassinolide).

In the year 2014, the leaf stomatal conductance was observed significantly higher (18.1 m mol m<sup>-2</sup>s<sup>-1</sup>) in the trees sprayed with Pro-Ca at 200 ppm (T<sub>6</sub>), than those under other treatments except, T<sub>5</sub>. The stomatal conductance was observed significantly lesser in control (11.4 m mol m<sup>-2</sup>s<sup>-1</sup>) in comparison to all other treatments.

The pooled data clearly revealed that the leaf stomatal conductance was significantly highest (18.2 m mol m<sup>-2</sup>s<sup>-1</sup>) in T<sub>6</sub> (200 ppm Pro-Ca) and significantly lowest (11.2 m mol m<sup>-2</sup>s<sup>-1</sup>) in control.

### Transpiration rate

The data on the leaf transpiration rate of apricot trees as influenced by different plant growth regulators have been given in Table 3.

The perusal of data depicted in Table 3 indicates that rate of transpiration was significantly affected by different treatments. In the year 2013, transpiration rate varied from 21.4 to 28.6 m mol m<sup>-2</sup>s<sup>-1</sup>. It was noticed maximum (28.6 m mol m<sup>-2</sup>s<sup>-1</sup>) in the trees given application of Pro-Ca at 200 ppm (T<sub>6</sub>), which was though significantly higher than other treatments, yet statistically at par with the treatment T<sub>5</sub> (100 ppm Pro-Ca). The minimum transpiration rate (21.4 m mol m<sup>-2</sup>s<sup>-1</sup>) was recorded in trees under control, which was significantly lesser than all other treatments except, T<sub>1</sub> and T<sub>7</sub>. In the year 2014, the values of transpiration rate varied from 23.3 to 31.1 m mol m<sup>-2</sup>s<sup>-1</sup>. It was recorded maximum (31.1 m mol m<sup>-2</sup>s<sup>-1</sup>) in the trees under the treatment T<sub>6</sub>, which was though significantly higher than other treatments, but statistically at par with the treatment T<sub>5</sub> (100 ppm Pro-Ca). The minimum transpiration rate (23.3 m mol m<sup>-2</sup>s<sup>-1</sup>) was recorded in trees under control, which was significantly lesser than all other treatments except, T<sub>7</sub>.

**Table 3:** Effect of forchlorfenuron, prohexadione-calcium and brassinolide on rate of transpiration of apricot

Treatment		Rate of transpiration (m mol m <sup>-2</sup> s <sup>-1</sup> )		
		2013	2014	Pooled
T <sub>1</sub>	CPPU (2.5 ppm)	22.9	25.4	24.2
T <sub>2</sub>	CPPU (5 ppm)	25.7	28.2	27.0
T <sub>3</sub>	CPPU (10 ppm)	26.4	28.9	27.7
T <sub>4</sub>	Pro-Ca (50 ppm)	23.8	26.3	25.1
T <sub>5</sub>	Pro-Ca (100 ppm)	27.4	29.9	28.7
T <sub>6</sub>	Pro-Ca (200 ppm)	28.6	31.1	29.9
T <sub>7</sub>	Brassinolide (0.5 ppm)	22.6	25.1	23.9
T <sub>8</sub>	Brassinolide(1 ppm)	24.1	26.6	25.4
T <sub>9</sub>	Brassinolide (2 ppm)	23.9	26.4	25.2
T <sub>10</sub>	Control	21.4	23.3	22.4
CD <sub>0.05</sub>		2.0	2.0	1.3

The pooled data further revealed that the rate of transpiration was significantly higher (29.9 m mol m<sup>-2</sup>s<sup>-1</sup>) in T<sub>6</sub> than all other treatments except, T<sub>5</sub> and it was significantly lowest (22.4 m mol m<sup>-2</sup>s<sup>-1</sup>) in control.

### Photosynthesis

The data on net photosynthetic rate of apricot trees as influenced by different plant growth regulator treatments are presented in Table 4.

The data depicted in Table 4 indicate that the net photosynthetic rate was significantly affected by different

treatments. In the year 2013, the net photosynthetic rate varied from 42.4 to 51.7 μ mol m<sup>-2</sup>s<sup>-1</sup>. It was observed significantly higher in the trees sprayed with Pro-Ca at 200 ppm (51.7 μ mol m<sup>-2</sup>s<sup>-1</sup>) than other treatments except, T<sub>3</sub> and T<sub>5</sub>. The net photosynthetic rate was recorded significantly lower (42.4 μ mol m<sup>-2</sup>s<sup>-1</sup>) in the trees under control than all other treatments.

**Table 4:** Effect of forchlorfenuron, prohexadione-calcium and brassinolide on net photosynthetic rate of apricot

Treatment		Net photosynthetic rate (μ mol m <sup>-2</sup> s <sup>-1</sup> )		
		2013	2014	Pooled
T <sub>1</sub>	CPPU (2.5 ppm)	45.8	46.6	46.2
T <sub>2</sub>	CPPU (5 ppm)	49.4	50.2	49.8
T <sub>3</sub>	CPPU (10 ppm)	50.2	51.0	50.6
T <sub>4</sub>	Pro-Ca (50 ppm)	46.8	47.6	47.2
T <sub>5</sub>	Pro-Ca (100 ppm)	50.7	51.5	51.1
T <sub>6</sub>	Pro-Ca (200 ppm)	51.7	52.5	52.1
T <sub>7</sub>	Brassinolide (0.5 ppm)	44.6	45.4	45.0
T <sub>8</sub>	Brassinolide(1 ppm)	48.3	49.1	48.7
T <sub>9</sub>	Brassinolide (2 ppm)	47.2	48.0	47.6
T <sub>10</sub>	Control	42.4	43.2	42.8
CD <sub>0.05</sub>		1.7	2.1	1.3

During the year 2014, net photosynthetic rate was recorded significantly higher (52.5 μ mol m<sup>-2</sup>s<sup>-1</sup>) in the trees given foliar application of Pro-Ca at 200 ppm (T<sub>6</sub>) than other treatments except, T<sub>3</sub> and T<sub>5</sub>. The minimum photosynthetic rate (43.2 μ mol m<sup>-2</sup>s<sup>-1</sup>) was recorded in trees under control, which was significantly lowest among all the treatments.

The pooled data clearly indicate that the net photosynthetic rate was significantly higher (52.1 μ mol m<sup>-2</sup>s<sup>-1</sup>) in T<sub>6</sub> (200 ppm Pro-Ca) in comparison to all other treatments except, T<sub>5</sub> (100 ppm Pro-Ca) and was significantly lowest (42.8 μ mol m<sup>-2</sup>s<sup>-1</sup>) in control among all the treatments.

### Total chlorophyll

The data on the leaf chlorophyll content of apricot trees as influenced by different treatments are presented in Table 5.

It is evident from the data (Table 5) that the leaf chlorophyll content was influenced significantly by different plant growth regulator treatments during both the years of study. During the year 2013, the maximum chlorophyll content (3.27 mg/g) was recorded in the treatment T<sub>6</sub> (200 ppm Pro-Ca), which was significantly superior to all the other treatments whereas, the chlorophyll content was recorded significantly lowest (2.56 mg/g) in trees under control.

**Table 5:** Effect of forchlorfenuron, prohexadione-calcium and brassinolide on leaf total chlorophyll content of apricot

Treatment		Total chlorophyll (mg/g fresh weight)		
		2013	2014	Pooled
T <sub>1</sub>	CPPU (2.5 ppm)	2.70	2.93	2.82
T <sub>2</sub>	CPPU (5 ppm)	2.96	3.19	3.08
T <sub>3</sub>	CPPU (10 ppm)	3.17	3.40	3.29
T <sub>4</sub>	Pro-Ca (50 ppm)	2.80	3.03	2.92
T <sub>5</sub>	Pro-Ca (100 ppm)	3.05	3.28	3.17
T <sub>6</sub>	Pro-Ca (200 ppm)	3.27	3.50	3.39
T <sub>7</sub>	Brassinolide (0.5 ppm)	2.64	2.87	2.76
T <sub>8</sub>	Brassinolide(1 ppm)	2.93	3.16	3.04
T <sub>9</sub>	Brassinolide (2 ppm)	3.11	3.34	3.22
T <sub>10</sub>	Control	2.56	2.79	2.68
CD <sub>0.05</sub>		0.06	0.06	0.04

In the year 2014, the values of leaf total chlorophyll content ranged from 2.79 to 3.50 mg/g under different treatments, wherein the maximum value pertained to the treatment T<sub>6</sub> (200 ppm Pro-Ca), which was significantly superior to all the other treatments. Its value was recorded significantly lowest in trees under control. Pooled data also revealed similar trend, where trees under T<sub>6</sub> registered significantly highest chlorophyll content and trees under control had significantly lowest leaf chlorophyll.

Stomatal conductance and transpiration rate depend upon stomatal aperture, which is regulated by endogenous hormone, abscisic acid (Levchenko *et al.*, 2005)<sup>[11]</sup>. However, cytokinins inhibit ABA-induced stomatal closure through the modulation of ethylene biosynthesis, and that ethylene inhibits the ABA-induced reduction of osmotic pressure in the guard cells (Tanaka *et al.*, 2006)<sup>[19]</sup>. Exogenous cytokinin decreased ABA accumulation and transpiration rate, therefore it appears that increased stomatal conductance with CPPU treatments could be due to increased stomatal aperture, in this study. Increase in stomatal conductance and transpiration rate with prohexadione-calcium treatments could be due to increase in the level of endogenous cytokinin (Bekheta *et al.*, 2009)<sup>[5]</sup>. As expected, increased stomatal conductance under different plant growth regulator treatments lead to an increase in photosynthetic rate during the study. In this study, increased leaf chlorophyll content in trees under different treatments resulted in a corresponding increase in the net photosynthetic rate, although other factors might also be involved in regulating photosynthesis. An increase in photosynthetic rates was found with the application of Pro-Ca in strawberry (Reekie *et al.*, 2005<sub>b</sub>) and apple (Sabatini *et al.*, 2003)<sup>[17]</sup>. In apple, leaves exhibited higher net photosynthesis and abrupt increase in CO<sub>2</sub> uptake when treated with Pro-Ca (Sabatini *et al.*, 2003)<sup>[17]</sup>. Cytokinins ability to increase the absorption of nutrients by the plants (Devlin, 1975)<sup>[9]</sup> might lead to increased concentration of photosynthesis in the shoot (Zahoor *et al.*, 2011)<sup>[20]</sup>. An elevated photosynthetic rate with the application of CPPU was observed by Gashaw *et al.* (2013)<sup>[10]</sup>. Cytokinin is known to stimulate the expression of photosynthetic enzymes like Rubisco and more generally, the development of functional chloroplasts (Chory *et al.*, 1994)<sup>[7]</sup>. Different plant growth regulator treatments significantly increased the leaf chlorophyll content (Table 7), which was however found highest under the treatment of Pro-Ca at 200 ppm followed by CPPU at 10 ppm and brassinolide at 2 ppm. Similar, increase in the leaf chlorophyll content with the application of Pro-Ca has been reported earlier in apple (Medjdoub *et al.*, 2007; Sabatini *et al.*, 2003)<sup>[13, 17]</sup>. Increased leaf chlorophyll pigmentation in CPPU treated trees could be due to the ability of cytokinin to checks the degradation of chlorophyll in leaves (Reis *et al.*, 1977)<sup>[16]</sup>. Another contributory factor in this regards could be cytokinin induced greater synthesis and translocation of assimilates and water (Devlin and Witham, 1983)<sup>[8]</sup>.

Brassinosteroids are actively involved in transcription and/or translation (Mandava, 1988)<sup>[12]</sup>, which may have increased chlorophyll synthesis during the course of present study. Homobrassinolides are associated with chlorophyll biosynthesis, mediated by the activation of specific genes (Ali *et al.*, 2006)<sup>[11]</sup>.

### Conclusion

Stomatal conductance, transpiration rate and net photosynthetic rate were observed significantly higher in the trees sprayed with Pro-Ca at 200 or 100 ppm in both the years

of study in comparison to all the remaining treatments and also the total chlorophyll content was observed to be highest in leaves from trees treated with Pro-Ca at 200 ppm at pit hardening and lowest in control, in both the years.

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