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Ameliorating thermo-tolerance in bell pepper (*Capsicum annuum* L. var. *grossum*) with plant growth regulators

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Abstract

Extreme temperatures have always been a serious threat to bell pepper production owing to its heat sensitivity. The present investigation was planned to assess the effect of foliar spray of growth regulators on morpho-physiological and yield parameters in bell pepper cv. Royal Wonder. Spraying bell pepper with Salicylic Acid (SA) and 24-Epibrassinolide (EBR) solutions significantly improved their tolerance to subsequent high temperatures. Exogenous application of three different concentrations of growth regulators *i.e* SA (0.10 mM, 0.20 mM and 0.50 mM) and EBR (0.05 µM, 0.10 µM and 0.20 µM) were applied at following stages; 30 days after transplantation (DAT), 60 DAT and 90 DAT. These treatments helped in mitigating the heat stress by improving growth parameters (plant height, plant spread and leaf area), photosynthetic efficiency, membrane thermostability, pollen viability and fruit set percent even at high temperature shows that application of PGRs definitely plays a role in initiating various mechanisms involved in overcoming high temperature limitations. However, foliar application of 0.20 mM SA (30 DAT) followed by 0.10 µM EBR (60 DAT) significantly enhanced the growth and yield parameters as compared to untreated control.

Keywords: Bell pepper, Epibrassinolide, High temperature, Salicylic acid, Yield.

Introduction

Bell pepper or sweet pepper is high valued and commercially important vegetable crops for its fruits. Its fruit is an excellent source of vitamins A, B_1, B_2, C (more than that obtained from tomato), D and E, besides having good antioxidants, flavonoids and phytochemicals (Maria *et al.*, 2010). The consumption of bell pepper has increased recently due to its occurrence in a wide variety of colours, shapes, sizes and its characteristic flavour. The plants of bell pepper flourish well in a warm climate. Their optimum temperature for proper growth ranges from 25-35°C. Since the reproductive phase is highly sensitive to heat shock than vegetative phase, slight alterations in the temperatures might leads to increased abscission of floral organs and can drastically affect the production. The prevalence of high temperature in the bell pepper growing areas results in low fruit set and ultimately causes yield losses. Due to lack of heat tolerant varieties and poor fruit setting of existing varieties during the hot dry season, growers fail to meet the demand of local market.

The adverse effects of heat stress can be mitigated by developing thermotolerant cultivar or improving the thermo-tolerance of existing cultivars. The more often preferred cultural practice is the use of plant growth regulators (PGRs) as it is quick and easy way of increasing the yield. The application of plant growth regulators (PGRs) plays an important role in plant to alleviate abiotic stresses and modify the growth and development of plants by inducing changes in cellular, physiological and morphological processes to enhance yield by improving fruit set, size and number of fruits. Salicylic acid (SA), a recognized plant hormone of phenolic nature, plays an important role in various physiological processes such as photosynthesis, flower induction, nutrient uptake, ethylene biosynthesis, stomatal conductance *etc*. It was also reported to be involved in fighting against biotic and abiotic stress. Another PGR, 24-Epibrassinolide (EBR), a brassinosteroid plays an important role in various physiological processes. They also help in alleviating the plant against high temperature stress. Keeping in view the losses incurred due to high temperature in bell pepper, the investigation was conducted to identify suitable dose and time of application of growth regulators for induction

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of thermo-tolerance in bell pepper under high temperature stress conditions.

Materials and Methods

Plant materials and treatment: The present trial was conducted in Vegetable Research Farm of Punjab Agricultural University, Ludhiana during 2015-16 and 2016-17. The experimental material comprises of bell pepper cultivar Royal Wonder obtained from Syngenta Seeds Private Limited, India. Seeds were sown in nursery in November 2015 and 2016 followed by transplanting in February 2016 and 2017. The experiment was laid out in randomized complete block design with three replications at a spacing of 90X30. There were 10 plants per treatment per replication and observations were recorded at 15 days interval from 5 representative plants after initiation of flowering (April). Foliar application of SA (0.1, 0.20 and 0.50 mM) and EBR (0.05, 0.10 and 0.20 µM) was done at following stages 30 days after transplantation (DAT) (vegetative stage), 60 DAT (reproductive stage) and 90 DAT (post reproductive stage). Untreated plants were taken as control. The standard field management practices were followed as recommended by Punjab Agricultural University, Ludhiana.

Observations recorded: The observations were recorded on growth parameters {plant height (cm), plant spread (cm) and leaf area (cm^2)}; flowering parameters {days to first flowering, pollen viability(%)}; leaf parameters {membrane thermostability (%), chlorophyll content (mg/g FW), carotenoid content (mg/g FW) and Hill reaction activity (DOD chl /mg h)} and yield parameters {Percent fruit set (%), fruit length (cm), fruit width (cm), pericarp thickness (mm), fruit chlorophyll content and total yield per plant (Kg)}. The leaf area was measured by using non-destructive method of De Swart *et al.* (2004) whereas membrane thermostability was estimated by method given by Liu and Huang (2000). The chlorophyll and carotenoid content were measured by using Hiscox and Israelstam (1979) method. Hill reaction activity is estimated by method given by Cherry (1973).

Statistical analysis: The data presented here represents the mean of two-year data and it was analyzed by using SAS software version 9.3.

Results and Discussion

The response of various traits to different treatments of plant growth regulators (mean value of two consecutive years) are given in table 1 and figures (1-3). Analysis of variance for experimental design revealed that all the studied parameters were influenced by both stage of spray and concentration of growth regulators. Significant differences among treatments were noted for most of the characters studied.

Growth parameters: Application of growth regulators significantly increased the growth parameters at all concentrations and growth stages (Figure 1). Treating the plants with 0.10 µM EBR at 30 and 60 DAT resulted

Table 1: Effect of PGRs and stage of spray on mean morpho-physiological and yield parameters of bell pepper cv. Royal Wonder

DAT	Treatment	Days to first flower	Pollen viability (%)	Membrane thermostability (%)	Fruit set (%)	Fruit Chl (mg/g FW)	Total yield (kg)
30	0.10 mM SA	35	53.45^{b-e}	57.04^{c}	34.31^{b-d}	0.12^{e-g}	2.13^{b}
	0.20 mM SA	33	54.78^{bc}	59.92^{bc}	38.06^{b}	0.10^{g}	2.36^{a}
	0.50 mM SA	34	52.67^{d-h}	58.75^{bc}	32.92^{c-e}	0.19^{b-f}	2.19^{b}
	0.05 µM EBR	35	53.21^{c-f}	59.73^{bc}	36.81^{bc}	0.18^{b-g}	1.88^{c}
	0.10 µM EBR	37	53.14^{c-g}	56.78^{c}	34.58^{b-d}	0.25^{ab}	2.28^{a}
	0.20 µM EBR	36	51.94^{e-h}	49.28^{d}	31.67^{d-f}	0.23^{a-c}	2.11^{b}
	Control	38	51.20^{h}	42.45^{e}	25.69^{hi}	0.11^{fg}	1.42^{f-i}
60	0.10 mM SA	35	55.03^{b}	44.76^{de}	28.47^{f-i}	0.18^{b-g}	1.47^{fg}
	0.20 mM SA	36	54.65^{bc}	45.20^{de}	30.97^{d-g}	0.20^{a-e}	1.69^{d}
	0.50 mM SA	37	54.41^{bc}	43.05^{e}	28.33^{f-i}	0.28^{a}	1.48^{f}
	0.05 µM EBR	36	55.03^{b}	44.83^{de}	30.83^{d-g}	0.18^{b-g}	1.42^{f-h}
	0.10 µM EBR	37	54.94^{b}	46.28^{de}	28.75^{e-h}	0.26^{ab}	1.59^{e}
	0.20 µM EBR	36	53.73^{b-d}	44.73^{de}	28.19^{f-i}	0.18^{b-g}	1.47^{fg}
	Control	37	51.31^{h}	42.92^{e}	25.28^{hi}	0.11^{fg}	1.42^{f-i}
90	0.10 mM SA	36	51.45^{h}	44.13^{e}	29.17^{e-h}	0.13^{d-g}	1.34^{ij}
	0.20 mM SA	38	51.61^{f-h}	44.17^{e}	27.22^{g-i}	0.11^{fg}	1.51^{ef}
	0.50 mM SA	37	51.45^{gh}	43.95^{e}	27.50^{f-i}	0.19^{b-f}	1.44^{f-i}
	0.05 µM EBR	36	51.63^{f-h}	43.72^{e}	27.78^{f-i}	0.16^{c-g}	1.24^{k}
	0.10 µM EBR	38	51.77^{f-h}	43.05^{e}	27.82^{f-i}	0.20^{a-d}	1.38^{g-j}
	0.20 µM EBR	39	51.60^{f-h}	43.27^{e}	25.83^{hi}	0.16^{c-g}	1.32^{jk}
	Control	40	51.27^{h}	42.56^{e}	24.44^{i}	0.11^{fg}	1.42^{f-i}

Least Squares-means with the different letters are significantly different ($P<0.05$)

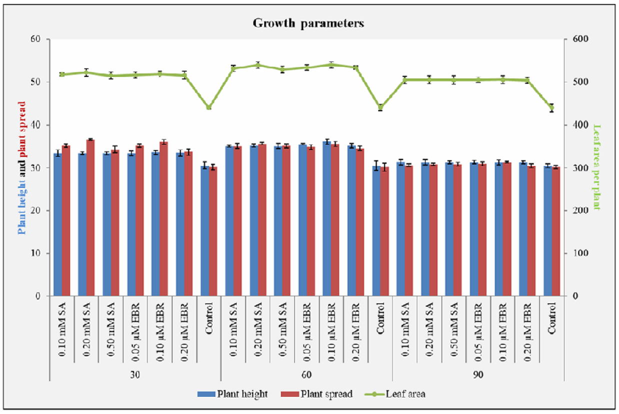

Figure 1: Effect of PGRs and the stage of spray on growth parameters of bell pepper cv. Royal Wonder

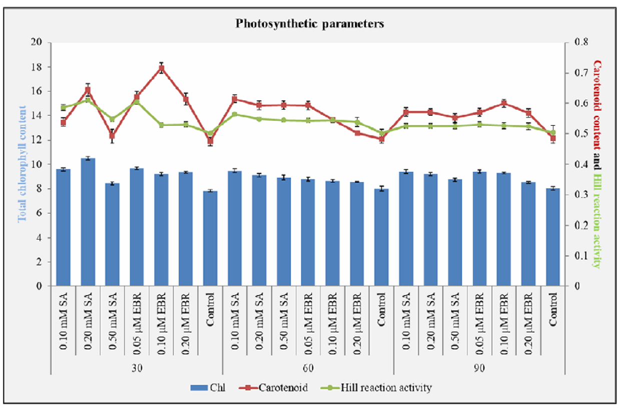

Figure 2: Effect of PGRs and the stage of spray on photosynthetic leaf parameters of bell pepper cv. Royal Wonder

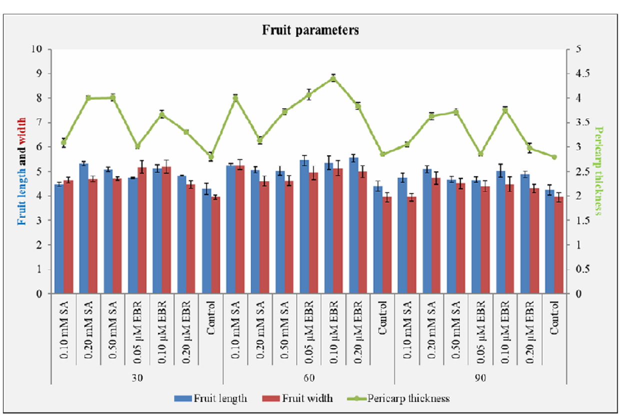

Figure 3: Effect of PGRs and the stage of spray on fruit parameters of bell pepper cv. Royal Wonder

in a maximum plant height and it increased by 9.74% and 18.17% as compared to unsprayed control. At 90 DAT, a maximum increase in plant height (2.75%) was recorded from the plants treated with 0.10 mM SA, although this increase was non-significant. Exogenous application of 0.20 mM SA done at 30 and 60 DAT, resulted in a maximum percentage increase of 21.18% and 17.87%, respectively, in the plant spread of treated plants in comparison to untreated control. At 90 DAT, 3.64% increase was noted in plant spread of 0.10 µM EBR treated plants as compared to unsprayed control plants. Our results corroborated with Ibrahim *et al.* (2019), who found that foliar application of SA helped in alleviating the harmful effects of high temperature by recuperating growth parameters of bell pepper. Wu *et al.* (2014) observed that under high temperature stress, growth and yield of eggplant was significantly reduced; however, EBR application improved plant growth. Beneficial results on plant growth with EBR application were also obtained by Peng *et al.* (2020) in stressed plants of pea. Heat stress reduces the leaf area expansion in stressed plants. Our data suggested that application of growth regulators alleviated the negative effects of heat shock on leaf area (Figure 1). A percentage increase of 18.72% (0.20 mM SA), 22.88% (0.10 µM EBR) and 14.87% (0.10 µM EBR) was recorded in plants when spray was done at 30, 60 and 90 DAT, respectively, as compared to unsprayed control. Similar observations were recorded by Kumari and Hemantranjan (2019) in heat stressed plants of wheat and reported the beneficial effect of EBR in increasing leaf area significantly.

Flower parameters: Data on the mean days to first flower recorded from the plants of variety Royal Wonder showed that exogenous application of growth regulators induced early floral initiation (Table 1). Treated as well as control plants of Royal Wonder showed flower initiation between 32 to 40 DAT. Amongst the treatments, foliar application of 0.20 mM SA reduced the days to first flowering in plants. Similar results were obtained by Ibrahim *et al.* (2019) in plants of bell pepper. Boldizsar *et al.* (2016) has studied the role of SA in regulation of flowering at genetic level and concluded that SA act via inhibiting the activity of flowering repressor gene thereby initiating flowering in the treated plants. Viability of pollen grains decreased on exposure to heat stress and ultimately effected its germination. The data on the pollen viability recorded from pollen grains of variety Royal Wonder at different growth stages indicates that both the growth regulators effectively improved the viability of pollen grains. Maximum viability of pollen grains (54.78%) was measured in 0.20 mM SA treated plants, when treatment was done at 30 DAT. According to the findings of Zhao *et al.* (2018), SA alleviates heat stress induced reduction in pollen viability by regulating ROS level in developing anthers of rice. Foliar application of 0.10 mM SA and 0.05 µM EBR done at 60 DAT helped the plants in attaining maximum viability of pollen grains (55.03%). At 90 DAT, application of 0.10 µM EBR was most effective in improving viability of pollen grains (51.77%) amongst all the treatments. Das *et al.* (2020) reported that the exogenous application of EBR showed maximum percent increase in the pollen viability of heat stressed plants of rice.

Leaf parameters: Application of both growth regulators showed an enhancement in membrane thermostability of all the treated plants when done at different growth stages (Table 1). Exogenous application of 0.20 mM SA helped in maintaining the maximum membrane thermostability (59.92% and 44.17%), when spray was done at 30 and 90 DAT. Foliar spray of SA might improve membrane integrity by reducing the activity of cell wall loosening enzymes. Our results corroborated with the findings of Jahan *et al.* (2019), who reported reduction of 27.84% in membrane injury of heat stressed seedlings of tomato when treated with SA. At 60 DAT, application of 0.10 µM EBR resulted in the plants with maximum membrane thermostability (46.28%). Similar observations were noted by Alam *et al.* (2018), who indicated that EBR is effective in alleviating cell membrane damage caused by high temperature stress. Treating the plants with SA and EBR, increased the total chlorophyll and carotenoid content of treated plants as compared to control plants (Figure 2). At 30 DAT, a significant increase of 34.23% was caused by 0.20 mM SA in total chlorophyll content of treated plants over unsprayed control. Foliar application of 0.10 mM SA done at 60 and 90 DAT recorded maximum mean chlorophyll content in treated plants. Our results are in agreement with Kousar *et al.* (2018), who observed significant increase in chlorophyll content in treated plants of wheat when compared to untreated control. The improvement might be associated with the protection of chlorophyll by plant growth regulators from heat-induced degradation or prevention of the inhibition of enzymes involved in chlorophyll biosynthesis. Plants treated with 0.10 µM EBR at 30 and 90 DAT showed the maximum mean carotenoid content (0.717 mg/g FW). At 60 DAT, maximum carotenoid content was recorded from the plants treated with 0.10 mM SA. Similar observations were noted by Agami (2013), who found that the treating the maize seeds with SA and EBR increased carotenoid content significantly.

Exogenous application of PGRs helped to improve the Hill reaction activity in plants under stressful environment (Figure 2). Maximum Hill reaction activity was attained by plants treated with 0.20 mM SA (21.53%), 0.10 mM SA (12.35%) and 0.05 µM EBR (3.59%), when spray was done at 30, 60 and 90 DAT, respectively. The direct effects of SA on photosynthetic apparatus and the indirect effects on photosynthesis through the limitation of CO_2 supply by regulating stomatal movements take an integral part of the plant immune responses. Effects of SA seem to be different in light or dark conditions. A subsequent amount of salicylates were retrieved in the middle of the night. In this aspect, the effects of high SA concentrations on photosynthetic apparatus and on chloroplast morphology in the dark can be especially important, because they can determine plant responses in the next light phase. Exogenous application of EBR also protects PSII against the high temperature driven overexcitation (Janda et al. 2014).

Fruit parameters: Yield traits of the bell pepper were positively enhanced by foliar application of growth regulator by alleviating the deleterious effects of heat stress. Application of SA and EBR (at all the concentrations) improved the fruit setting in treated plants (Table 1). Maximum fruit set percentage (38.06%) was noted in plants treated with 0.20 mM SA when done at 30 and 60 DAT. However, exogenous application of 0.10 mM SA done at 90 DAT showed maximum fruit setting percentage and lower the rate of flower abscission under elevated temperature. Our results are in agreement with Khedr (2018), who observed that SA was very effective in improving fruit setting and fruit retention in treated plants as compared to untreated ones. SA play an important role in mitigating the harmful effect of environmental stress that delay the fruit set and results in late maturity of the fruit. It improves photosynthetic ability of plants which may increase assimilate supply and can help in early flower formation, fruit and seed development. The data on fruit parameters recorded from the plants of variety Royal Wonder showed an improvement on application of growth regulators (SA and EBR) at all the growth stages (Figure 3). At 30 DAT, maximum fruit length, fruit width and pericarp thickness were recorded in the plants treated with 0.20 mM SA, 0.05 µM EBR and 0.20 µM EBR, respectively. Treating the plants with growth regulators at 60 DAT showed maximum enhancement in fruit length, fruit width and pericarp thickness of plants with foliar spray of 0.20 µM EBR, 0.10 mM SA and 0.10 µM EBR, respectively. An increment in the fruit length and width at 90 DAT was observed in the plants treated with 0.10 mM SA while maximum pericarp thickness was noted in 0.20 µM EBR treated plants. Our results are in accordance with the results obtained by Ibrahim et al. (2019), who reported an increase in fruit parameters of bell pepper treated with SA compared to untreated control. Beneficiary effects of EBR are also observed by Siri et al. (2020) in fruit parameters of chillies.

Fruit chlorophyll: Plant growth regulators were helpful in improving the fruit quality of bell pepper (Table 1). At 30 DAT, maximum fruit chlorophyll content (0.25 mg/g FW) was observed in the plants treated with 0.10 µM EBR with a significant increase of 127.27% compared to unsprayed control. When application of growth regulators was done at 60 and 90 DAT, maximum fruit chlorophyll (0.26 mg/g FW and 0.20 mg/g FW) was recorded in plants treated with 0.10 μM EBR and it significantly increased the quantity of fruit chlorophyll by 136.36% and 81.82%. Similar results were obtained by different researchers who worked to alleviate the harmful effects of stress in agricultural crops. Zhang et al. (2015) observed increased chlorophyll content in SA treated plants of cucumber compared to untreated control plants. In capsicum, ripening is initiated when fruit have stopped enlarging. Fruit that are ready to ripen are termed 'horticulturally mature'. If fruit are harvested before the breaker stage they do not ripen fully. During ripening, capsicums lose chlorophyll and synthesize carotenoids, the timing of which can be affected by ethylene. Our results suggested SA comprehends the degradation of chlorophyll content and increases the time period to reach maturity.

Total yield: The data on the total plant yield illustrates that the total yield increased in plants on application of growth regulators at all concentrations (Table 1). Maximum mean total fruit yield increased as compared to control plants by 66.20% (2.36 kg) and 19.01% (1.69 kg) when plants treated with 0.20 mM SA at 30 and 60 DAT, respectively. However, when spray was done at 90 DAT, application of 0.20 mM SA resulted in the plants with maximum total fruit yield (1.51 kg). Under high temperature stress, SA showed impressive results in enhancing growth and yield by playing an important role in plant metabolism. Ibrahim et al. (2019) reported that application of SA improved the yield contributing factors that resulted in significant increase in crop produce of bell pepper.

Conclusion

In conclusion, application of plant growth regulators to plants of bell pepper cv. Royal Wonder ameliorated the harmful effects of high temperature stress. This study analyzed the effect of different concentrations of growth regulators done at different growth stages. The results showed diversification in the concentrations of growth regulators responsible for improving the different traits at different growth stages. Moreover, Royal Wonder being thermosensitive variety, responded more positively when the application of growth regulators was done at vegetative phase than reproductive and post reproductive phase. However, both the growth regulators were effective in imparting thermotolerance in terms of improved growth parameters (plant height, plant spread and leaf area), flower parameters (earliness in days to first flower and increased viability of pollen grains), leaf parameters (photosynthetic efficiency, membrane thermostability) and fruit set percent even at high temperature ultimately resulting in improved total yield. Exogenous application of 0.20 mM SA at vegetative phase (30 DAT) followed by 0.10 μM EBR at reproductive phase (60 DAT) were most effective in alleviating the deleterious effects of high temperature stress and inducing thermotolerance in the plants of variety Royal Wonder.

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सारांश

अत्यधिक तापमान संवेदनशीलता के कारण शिमला मिर्च उत्पादन के लिए हमेशा चिंता का विषय रहा है। वर्तमान शोध में शिमला मिर्च की किस्म रॉयल वंडर में रूपात्मक और उपज मापदंडों पर वृद्धि नियामकों के छिड़काव के प्रभाव का आंकलन करने के लिए किया गया। सैलिसिलिक एसिड (एस.ए.) और 24–एपिब्रासिनोलाइड (ई.बी.आर.) का छिड़काव करने से शिमला मिर्च की उच्च तापमान के प्रति सहनशीलता में काफी सुधार हुआ। वृद्धि नियामकों के 3 अलग–अलग सांद्रता जैसे–एस.ए. (0.10 मिली मोलर, 0.20 मिली मोलर और 0.50 मिली मोलर) और ई.बी.आर. (0.05 माइक्रो मोलर, 0.10 माइक्रो मोलर और 20 माइक्रो मोलर) का पर्णीय छिड़काव निम्न चरणों–प्रत्यारोपण के 30 दिनों बाद (30 डी.ए.टी.), प्रत्यारोपण के 60 दिनों बाद (60 डी.ए.टी.) और प्रत्यारोपण के 90 दिनों बाद (90 डी.ए.टी.) में किया गया। इन उपचारों ने वृद्धि मापदंडों (पौधों की ऊँचाई, पौधों के प्रसार और पत्ती क्षेत्र), प्रकाश संलेषण (फोटोसिंथेटिक) दक्षता, झिल्ली ताप स्थिरिता (थर्मेस्थेटाबिलिटी), पराग व्यवहार्यता और फल बनने का प्रतिशत में सुधार करके उच्च तापमान के कारण होने वाले तनाव को कम करने में मदद मिली है। यह इस बात को दर्शाता है कि वृद्धि नियामकों का छिड़काव निश्चित् रूप से उच्च तापमान के नकारात्मक प्रभाव को नियन्त्रित करने वाली विभिन्न क्रियाओं को आरंभ करने में भूमिका निभाता है। 0.20 मिली मोलर एस.ए. (30 डी.ए.टी.) और 10 माइक्रो मोलर ई.बी.आर. (60 डी.ए.टी.) का छिड़काव अनुपचारित पौधों की तुलना एवं रूपात्मक और उपज मापदंडों में उच्च तापमान के कारण होने वाले तनाव के नकारात्मक प्रभाव को सुधारने में सबसे ज्यादा असरदायक रहा है।

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