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Effect of application of modified nourishing environment on the reproduction and yielding capacity of root celery

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We set the need of additional application of phytohormone at reproduction of root celery of Anita and Tsilitel varieties *in vitro* conditions using traditional nutrient medium *Murasige-Scuga* from our research within 2015–2017. For the control, the nutrient medium *Murasige-Scuga* (MS) was selected. To obtain a genetically identical material, the composition of nutrient medium with the concentration of plants growth regulator benzylaminopurine (BAP) 0.2 %, 0.3, and 0.5 % was investigated. We observed that prior the plants bedding-out from culture dishes into cassette, the Tsilitel variety have formed grater amount of callus tissue in comparison with Anita variety. We also established that application of *Murasige-Scuga*+6-BAP 0.2 % nutrient medium contribute the better growth of cultural based plants and seedlings, increasing the amount of leaves and plants height, which in its turn significantly increase the yield of multiplication material. An increase in 6-BAP 0.3 % concentration resulted in a significant reduction of these parameters, and an increase of up to 0.5 % did not contribute to plant growth. After planting the cassette seedlings into the open ground, the growth of plants at the first stages was slow, and as their adaption to the growing conditions, it was accelerated. In 30 days after plantation the biometric parameters of plants were higher due to their cultivation with addition of 6-BAP 0.2 to the nutrient medium. A similar trend was observed even in 60 days after sowing seedlings in the open ground. The researches have shown that the grater yield of studied varieties and the higher quality characteristics of their production ensured cultivation of explants at *Murasige-Scuga*+6-BAP 0.2 % medium.
Key words: root celery, variety, Murasige-Scuga nourishing environment, plants growth regulator, benzylaminopurine, yield.

Introduction

At the present stage, the use of genetic methods and breeding methods requires finding new approaches that would allow not only increasing yields and improving the quality of crops but also would be economically profitable in production and not harm the environment in the future. The biotechnology is estremely important for this purpose (Musienko, 2005; Butenko, 1991; Ivchenko, 2007). In recent years, the great development in our country and abroad has received the method of microcollonal reproduction with meristems in vitro culture (Bezugly, 2009; Blum, 2009; Abramchuk, 2009). The advantages of the method are that it allows taking explants from plants that are at different phases of development, which makes it possible significantly accelerate the reproduction of valuable breeding material (Madison, 2004, 2009; Bugaychenko, 2007).
The nourishing environment is a major factor in clonal micro propagation and it is recommended for most bividoms to use Murasige-Scuga, which contains plant-friendly plants for growth (Martoschuk, 2006). The method of culture of isolated cells and tissues is developed for many types of fruit, forest, decorative and other agricultural plants. In vitro culture only 30 kinds of vegetables were introduced, which is not enough compared to other crops. The technology of clonal micro propagation of potatoes, root crops is put on an industrial basis; selective genetic studies are carried out with other plants (Manzur, 2014; Markova, 1989; Butenko, 1991; Ivchenko, 2007). In recent years, the root celery provoke a great interest of Ukrainians thanks to its aromatic and flavours characteristics. The celery market in Ukraine requires more than 20 thousand tons of products, and 12 thousand tons is grown.

Methods

The research was carried out at the Uman National University of Horticultural. Studied varieties of root celery Anita and Tsilitel included in the State Register of plant varieties, suitable for cultivation in Ukraine, and the nourishing environment for the cultivation of plants in culture in vitro. In order to determine the optimum concentration of plant growth regulator in the nutrient medium (MS) for the production of a genetically identical material, its composition with the concentration of plant growth regulator benzylaminopurine (6-BAP) – 0.2%, 0.3, and 0.5% was investigated. Murasige-Scuga (MS) nutrient medium was used for control.
The clean off from the remnants of the substrate, the plants were planted out from a culture dish in a cassette of 1 plant in a cell with a substrate of 4×4 sm. Cartridges with planted plants were placed in a humid chamber with a humidity of 85%, a temperature of 20–220°C and a brightness of 5–10 calories. The plants were regularly watered with distilled water and fed once a week.
Seedlings of celery were grown for 60 days. Plants were planted in open ground in the first decade of May under the scheme of 45×20 sm, which corresponds to a density of 111 thousand pieces./ha. The rendence of plants in the next period of vegetation was commonly accepted for celery. Biometric measurements were performed on 10 typical labelled plants in repetitions of each variant of the experiment. The trial was laid in a four-time repetition.
The biometric and phenological observations conducted in dynamics, during which the beginning of the phase, when it entered the 10–15% of plants and the full phase, when it was observed in 75% of plants were observed. In the experiments, the simultaneous sowing of seeds and transplanting of regenerant plants on the nutrient medium were observed. In the open ground, plants were grown according to generally accepted technology.

Results

The method of root celery reproduction in vitro culture consists of use of the traditional Murasige-Scuga nutritional medium, which is supplemented by the addition of phytohormones at a certain concentration to induce the formation of leaves and shoots from the meristemic zones of fruit plants, which allows to increase the formation of the calus tissue and, consequently, the shedding of leaves in plants. The development of regeneration plants was carried out according to the type of direct organogenesis. During the first stage of reproduction, numerous small shoots were formed, which, after every 3–4 weeks, we were divided and transplanted for additional reproduction to a fresh medium (Fig. 1).

a) b) c)

Fig. 1. Regenerant plants development: a – creation of numeral small sprouts; b – transplantation for additional reproduction, c – creation of the root system of plants

During the last transplantation, the regenerated plants, with well-developed leaves, were planted for rooting on the nutritional medium, which included auxins. In regards to the planted crops of root celery, the phenological observations were carried out on the nutrient medium, which showed that seed germination occurred at the same time with the difference in 2–3 days. Plants-regenerants of the Tsilitel variety have form more calus tissues than in contrast to the Anita variety. With the purpose of acclimatization to the environment and subsequent cultivation, the plants of celery were planted from the cultured dishes in a greenhouse in the first decade of April, when the plants after rooting had 4–6 and 5–7 leaves and more developed roots.
Studies have shown that celery plants for cultivation in a culture vessel had different appearance and different sizes depending on the composition of the nutrient medium. Therefore, to determine the influence of the nutrient medium and the cultivation conditions on the examined varieties of the celery growth, we conducted biometric measurements and observations, starting with determination of the plants height prior their planting in the greenhouse (Fig. 2).
The obtained data affirm, that the plants of Anita and Tsilitel varieties have higher height for the expense of 6-BAP 0.2 medium – 8.9 and 9.4 cm accordingly, which is significantly lower comparing to the control per 3.1–3.2 cm (HIP_{05} = 0.3 cm). Application of a greater concentration of 6-BAP 0.3 % result in essential reduction of the plants height until 6.6 and 8.2 cm, while the difference with control was 0.9–1.9 cm. The increase in 6-BAP concentration for 0.5% did not contribute to the increasing of the intensity of plants growth and we observed the decrease up to 6.0-7.9 cm.

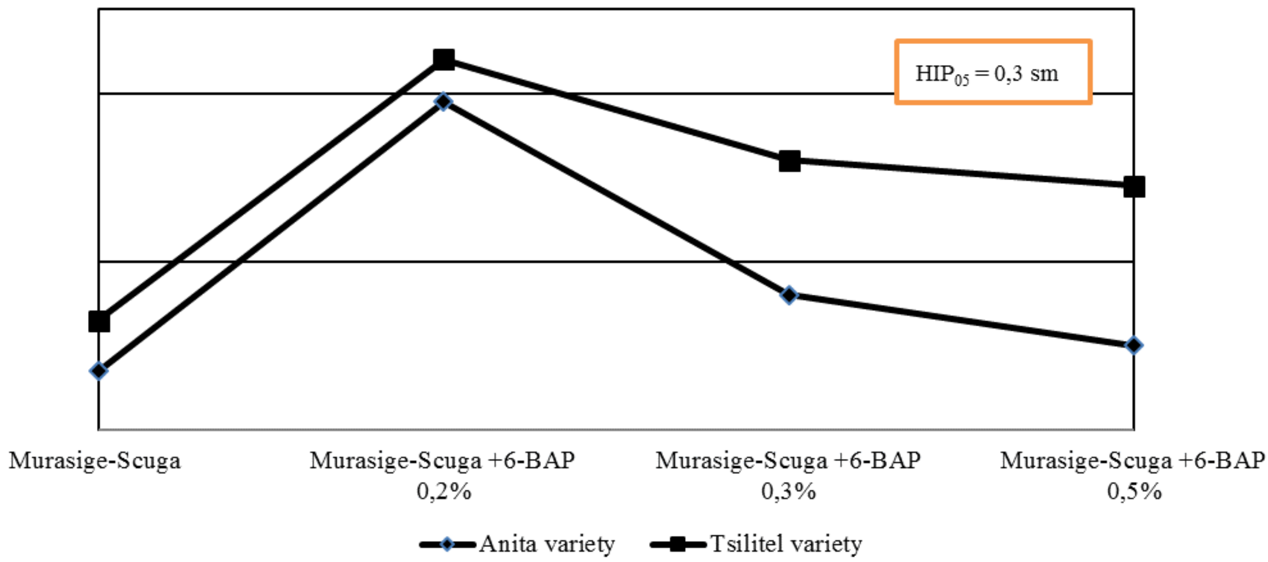


Fig. 2. The height of root celery plants prior their planting from the culture vessel into the greenhouse, sm (average for 2015–2017)

The plants growth on the first stages in the open ground was very slow and as their adaptation to the growing conditions, it accelerated (Figs 3, 4).

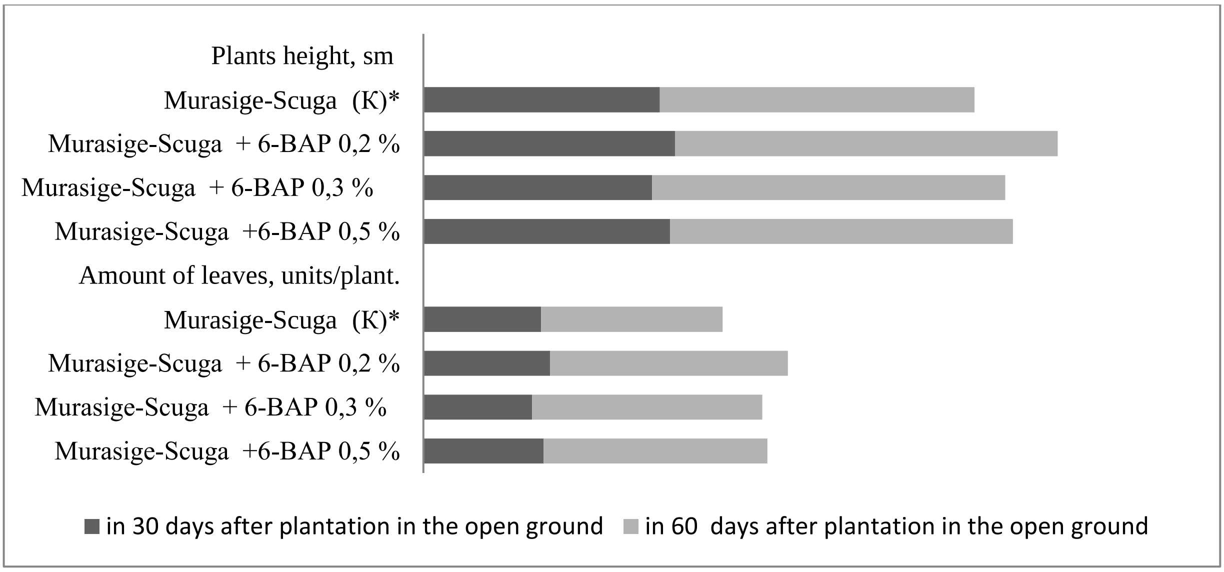
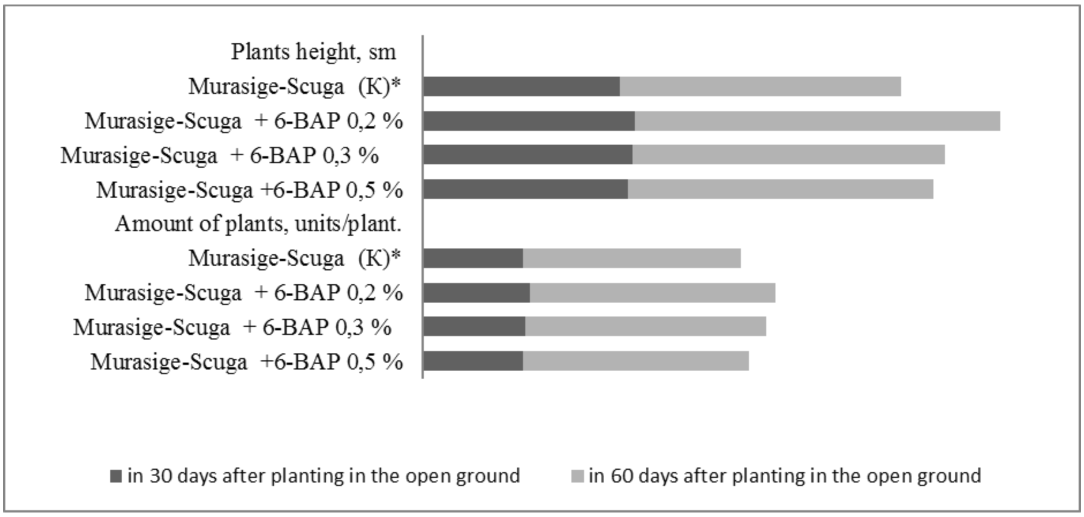


Fig. 3. Plants height of Anita variety root celery, cultivated *in vitro* conditions (average for 2015–2017)

The height of Anita and Tsilitel varieties plants of celery at the period of their intensive growth in 30 and 60 days after planting in the open ground depended on the growing technique of regenerant plants *in vitro* crop conditions. In this manner on the examined celery varieties the plants were higher in 30 days after planting when adding the 6-BAP phytohormone in 0.2 % concentration to the nutrient medium – 19.7 and 20,1 cm accordingly. The lower signs on the Anita variety registered by adding of 6-BAP 0.3 % to the nutrient medium – 17.9 cm, and on Tsilitel variety – 18.6 cm. The leaf coverage of plants was also higher upon application of 6-BAP 0.2 %. On Anita variety this index was at the level of 9.9 units/plant and on Tsilitel variety – 10.1 units/plant. The less amount of leaves per plant registered on Anita variety using 6-BAP 0.3 %; on the Tsilitel variety this index was lower in the control and for application of 6-BAP 0.5 % made 9.5 units/plant.

However in 60 days after planting in the open ground, the plants of Anita and Tsilitel varieties had higher height upon growing of regenerant plants with addition of 6-BAP 0.2 % to the nutrient medium – 29.9 and 34.6 cm correspondingly, the leaf coverage made 18.6 and 23.3 units/plant correspondingly. The less height and leaf coverage had plants of examined varieties upon growing of plants regenerants in the control, the Anita variety showings were at the level of 24.6 cm and 14.2 units/plant and the Tsilitel variety – 26.7 cm and 20.6 units/plant.



Pic. 4. Plants height of Tsilitel variety root celery, cultivated *in vitro* conditions (average for 2015–2017)

At the time of harvesting the increase in the total area of leaves on the celery varieties Anita and Tsilitel more intensively passed for adding of BAP 0.2 to the nutrient medium – 18,6 and 25,4 thous m^2/ha, correspondingly to the control – 16.1 and 20.7 thousand m^2/ha (table. 1). Depending on the growing method of regenerant plants the leaf index on Tsilitel variety was higher in comparison with Anita variety and made 2.0–2.3.

Table 1. Biometrical signs of the root celery grown in cultural conditions *in vitro* prior crop harvesting (average for 2015–2017)

Nutrient medium	Amount of leaves, per plant	Leaf area, cm^2	Leaves area, m^2/ha 10^3	Leaf index
		Anita		
Murasige-Scuga (control)	23.2	63.2	16.1	1.5
Murasige-Scuga + 6-BAP 0.2 %	25.6	66.1	18.6	1.8
Murasige-Scuga + 6-BAP 0.3 %	24.6	65.9	17.8	1.7
Murasige-Scuga +6-BAP 0.5 %	23.9	63.7	16.7	1.6
		Tsilitel		
Murasige-Scuga (control)	26.5	75.9	20.7	2.0
Murasige-Scuga + 6-BAP 0.2 %	29.3	78.7	23.9	2.3
Murasige-Scuga + 6-BAP 0.3 %	28.7	77.6	22.9	2.2
Murasige-Scuga +6-BAP 0.5 %	27.4	76.1	21.5	2.1

The crop yield and the quality of commercial yield are important signs, which characterize the influence of the different nutrient medium application in plans growing (Table 2, Fig. 5). Our researches showed that the higher yield of Anita variety was obtained for explants growing on *Murasige-Scuga*+6-BAP 0.2 % medium – 26.0 t/ha, which is 3.6 t/ha higher in comparison with the control. On Tsilitel variety the higher yield received on *Murasige-Scuga*+6-BAP 0.2 % medium – 24.8 t/ha.

Anita variety Tsilitel variety

Pic. 5. Edible roots of Anita and Tsilitel varieties *in vitro* culture on the nutrient medium *Murasige-Scuga*+6-BAP 0.2 %

Table 2. The commercial yield of root celery depending on the growing method of plants at *in vitro* culture, t/ha

Nutrient medium		Year 2015	2016	2017	Average for 3 years	± to the control
		Anita				
Murasige-Scuga (control)		19.2	21.1	27.0	22.4	0
Murasige-Scuga + 6-BAP 0.2 %		22.9	24.7	30.4	26.0	+3,6
Murasige-Scuga + 6-BAP 0.3 %		21.7	23.6	28.8	24.7	+2,3
Murasige-Scuga + 6-BAP 0.5 %		20.6	23.0	27.9	23.8	+1,4
	HIP_{05}	*1.1*	*1.0*	*1.3*	–	–
		Tsilitel				
Murasige-Scuga (control)		17.0	20.3	25.9	21.1	0
Murasige-Scuga+ 6-BAP 0.2 %		20.5	24.2	29.8	24.8	+3,7
Murasige-Scuga + 6-BAP 0.3 %		18.6	23.3	27.2	23.0	+1,9
Murasige-Scuga+ 6-BAP 0.5 %		17.8	23.5	26.5	22.6	+1,5
	HIP_{05}	*0.9*	*1.0*	*1.2*	–	

In the researches, the establishment of quality indices of edible root was carried out. Measurement of the length and diameter of the roots, the definition of their form index, showed that the Anita and Tsilitel varieties on these indicators were the best edible root for their growing with the addition of BAP 0.,2 to the nutrient medium. The Anita variety had a length of 6.1 cm, a diameter of 7.1 cm, and Tsilitel – 6.9 and 7.8 cm respectively (Table 3).
In the process of root celery growing on a nutrient medium in vitro also determined the chemical composition of the root crops, which is an important element of quality. The content of the dry soluble substance in the Anita variety was greater than the growth of regenerants in the nutrient medium with the addition of 6-BAP in the concentration of 0.2% – 18.5%, and less in the control – 16.8%.
The percentage of raw protein in the root of Anita variety was at the level of 1.0–1.5%. The content of ash in root crops was 0.,7%. The highest content of ascorbic acid was allocated to plants grown on a nutrient medium with the components Murasige-Scuga + 6-BAP 0.2% and Murasige-Scuga + 6-BAP 0.,3% – 25 mg/100 g.

Table 3. Quality signs of celery edible roots for the different plants growing at *in vitro* culture (average for 2015–2017)

Nutrient medium	Length of edible root, cm	Diameter of edible root, cm	Index of edible root form
	Anita		
Murasige-Scuga (control)	5.5	5.9	0.93
Murasige-Scuga + 6-BAP 0.2 %	6.1	7.1	0.86
Murasige-Scuga +6-BAP 0.3 %	5.9	6.0	0.98
Murasige-Scuga +6-BAP 0.5 %	5.8	6.2	0.94
	Tsilitel		
Murasige-Scuga (control)	5.6	6.0	0.93
Murasige-Scuga + 6-BAP 0.2 %	6.9	7.8	0.88
Murasige-Scuga + 6-BAP 0.3 %	6.0	7.2	0.83
Murasige-Scuga + 6-BAP 0.5 %	5.8	6.2	0.94

The content of carotene in root crops increased for plant cultivation -regenerators on the nutrient medium with the components Murasige-Scuga + 6-BAP 0.2% and Murasige-Scuga + 6-BAP 0.3% and was 0.10 mg/100 g, which is 0.03 mg/100 g more than in control. Thiamine was in the range of 0.49–0.53 mg/kg. According to the content of calcium, root crops, regeneration plants that were grown on a nutrient medium with 6-BAP added in the concentration of 0.,2% – 65 mg/100 g, which is 5 mg/100 g more than in the control, were distinguished.
The content of iron in the root crops was within the range of 1.0–1.9 mg/100 g (Table 4).

Table 4. The chemical content of commercial edible roots of celery depending on the nutrient medium at *in vitro* (average for 2015–2017)

Nutrient medium	Dry soluble substance, %	The mass fraction of sugars %	Raw protein, %	Ash, %	Ascorbic acid, mg/100 g	Carotin, mg/100 g	Thiamine (B_1), mg/kg	Riboflavin (B_2), mg/kg	Calcium, mg/100 g	Iron, mg/100 g
Anita										
Murasige-Scuga (control)	16.8	2.4	1.0	0.7	20	0.07	0.49	0.30	60	1.0
Murasige-Scuga + 6-BAP 0.2%	18.5	3.5	1.5	0.7	25	0.10	0.53	0.36	65	1.9
Murasige-Scuga + 6-BAP 0.3%	17.2	3.0	1.4	0.7	25	0.10	0.50	0.30	62	1.6
Murasige-Scuga + 6-BAP 0.5%	17.0	2.8	1.2	0.7	22	0.08	0.49	0.31	62	1.2
Tsilitel										
Murasige-Scuga (control)	17.4	1.1	1.0	0.6	29	0.10	0.46	0.30	60	1.1
Murasige-Scuga + 6-BAP 0.2%	19.2	1.7	1.6	0.6	33	0.10	0.50	0.35	66	1.5
Murasige-Scuga + 6-BAP 0.3%	18.8	1.5	1.4	0.6	33	0.10	0.50	0.32	64	1.5
*Murasige-Scug*a + 6-BAP 0.5%	17.9	1.2	1.4	0.6	30	0.10	0.48	0.32	63	1.2

The content of dry soluble matter in Tsilitel variety for plants-regenerants growing in the nutrient medium by adding it to the growth regulator of plants at different concentrations reached a level within the range of 17.4–19.2%. The higher this indicator was observed in plants grown on a nutrient medium with the addition of 6-BAP in it at a concentration of 0.2%–19.2%, which is 1.8 and 1.3% more than in the control and the medium added to it 6-BAP at a concentration of 0.5%. The mass fraction of sugars was at the level of 1.1–1.7%. The content of raw protein in the root crops increased for plants-regenerants growing on a nutrient medium with a concentration of 6-BAP 0.2%–1.6%, which is 0.6% more than in the control.

The content of ash and carotene in all variants of the experiment was one level and made 0.6% and 0.1 mg/100 g, respectively. In terms of ascorbic acid content, a method of plants-regenerants growing of Tsilitel variety was allocated on a nutrient medium with addition of 6-BAP at a concentration of 0.2 and 0.3% to 33 mg/100 g. In terms of thiamine content, medium was added with the addition of 6-BAP in the concentration of 0.2 and 0.3% – 0.5 mg/kg, which is 0.04 mg/kg more than in the control. The content of riboflavin increased for the cultivation of plant-regenerants of the Tsilitel variety on a nutrient medium with the addition of 6-BAP in a concentration of 0.2% – 0.35 mg/kg, which is 0–0.5 mg/kg more than in the control. The content of calcium and iron in the celery root was 60–66 mg/100 g and 1.1–1.5 mg/100 g, respectively.

Conclusion

It was established that the use of Murasige-Scuga + 6-BAP 0.2% of the nutrient medium contributed to the better growth of the seedlings, the increase in the amount of leaves and the height of the plant, which significantly increases the yield of multiplication material. It is proved that in order to increase the yield of celery root of Anita and Tsilitel varieties, it is expedient to grow regenerative plants on a nutrient medium of Murasige-Scuga + 6-BAP of 0.,2%. This allows additionally to receive 3.6–3.7 t/ha of commodity products with a diameter of the root crops of 7.7–7.9 cm, their height – 6.9 cm, the index of form 1.1–1.2 and with high quality. Thus, the higher content of ascorbic acid was 25–33 mg/100 g, carotene – 0.1 mg/100 g, calcium – 65–66 mg/100 g.

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