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Full Length Article

Breeding of New Varieties of Iron Stick Yam

Jianjun Li[1*], Xiaoya Lian[1], Meiling Ren[1], Jun Wang[2], Guolun Jia[2] and Shuwu Sun[3]
[1]*Engineering Technology Research Center of Nursing and Utilization of Genuine Chinese Crude Drugs, College of Life Science, Henan Normal University, Xinxiang, China*
[2]*Department of Food Engineering Quality Inspection Department, Luohe Food Vocational College, Luohe, Henan, China*
[3]*Henan Bai Liao Huai Medicine Science and Technology Development Co., Ltd., Jiaozuo, China*
[*]For correspondence: 043081@htu.cn

Abstract

To develop new varieties of iron stick yam with excellent comprehensive traits, seven new strains were screened through space mutation breeding to the bulbil of iron stick yam (*Dioscorea opposite* Thunb.), which were compared to the control group No.11, the common female parent of new strains. The results showed that the fresh weight, dry weight, and dry weight per plant of No. 6 and No.10 were higher than those of the control. The allantoin and water-soluble extract content of No.6 had significant advantages in contrast to the control, while No.10 and the control were similar. While No. 6 had a taste that was dry and soft, the new strain No.10 tasted dry, soft, sweet, and fragrant, which had obvious advantages. Compared to the control, the resistance of No. 6 was high, while the resistance of No.10 was good. The starch, reducing sugar, protein, and ash content of both No.6 and No.10 were higher than those of the control. Furthermore, the water content was lower and the nutrition quality was improved in contrast to the control. The new strain No.6 was found suitable for the promotion of new medicinal iron stick yam, while the new strain No.10 (known as "iron bar 06-1") for the promotion of new edible iron stick yam. This may solve the problem of degeneration in iron stick yam production, while improving quality and yield.

Keywords: Iron stick yam; New cultivar; Breeding; Medicinal ingredients; Nutrition quality

Introduction

Yam is the dried rhizome of *Dioscorea opposite* Thunb., which belongs to the family Dioscoreaceae. In medicinal terms, it exhibits efficacy in nourishing the spleen and stomach, while invigorating the lung and kidney (Committee of Pharmacopeia in Ministry of Health of the People's Republic of China, 2010). Yams with strong adaptability are widely planted across China, and are primarily produced in Henan (Institute of Botany of Chinese Academy of Sciences, 1983). The yam produced in Jiaozuo (the ancient Huaiqing Prefecture) possesses the best quality, and has the Chinese name of Huai Shan Yao. At present, iron stick yam and Taigu yam are the two main varieties that are planted in this region. Iron stick yam has a high medicinal value but low yield, while Taigu yam has a high yield, but low medicinal value (Yi *et al.*, 2011).

Long-term asexual reproduction in yam-producing areas has resulted in the degradation of yam varieties, while increasing serious plant diseases and insect pests, thereby reducing yam yields and quality. Original yam varieties can no longer meet the increasing demands of Chinese medicinal enterprises and farmers; hence, it is urgent to develop new varieties with high yields, good quality, and strong resistance. At present, the principal methods of obtaining new yam varieties are through introduction and directional breeding, such as "Southern medicine to North", "North medicine to South", and "wild form to tame type" etc., while sexual reproduction has not been fully studied as yet. Due to difficulties in the hybridization breeding of iron stick yam, researchers collected iron stick yam bulbils and sent them to space with the “Practice No.8” satellite in 2006 (Wang, 2006). Following their time in space, the iron stick yam bulbils were planted in Wuzhi. Seven new cultivars with good quality were generated following six years of breeding and propagation. Their yield, quality, and resistance were measured and compared with a control cultivar in the present study to screen for new varieties with the best properties; thus, providing a theoretical and technological basis for the identification and promotion of new yam varieties.

Materials and Methods

Plant Materials

Seven new iron stick yam cultivars (No.1, 2, 4, 6, 8, 9 and 10) were developed through spaceflight mutation and the control (No. 11), were grown in the same experimental filed

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on April 4, 2013 using conventional cultivation techniques. All the cultivars grew well and were identified as *Dioscorea opposite* Thunb. byan expert from Henan Normal University (Jianshe Road, Xinxiang, Henan, China). The rhizomes of the eight cultivars were harvested on November 25, 2013. Mold- and mildew-free samples were selected, rinsed with running water, peeled and sliced into 3 mm pieces , dried at 50ºC for 18 h, and finally crushed for later analysis.

Instruments and Reagents

An Agilent 1200 series HPLC System, equipped with a GI311 quaternary pump, automatic sampler, VWD detector, Agilent 1100 LC chemical workstation was purchased from Agilent Technologies Co., Ltd., KH-250DE CNC ultrasonic cleaner from Kunshan Hechuang Ultrasonic Instrument Co., Ltd., FA2204B electronic analytical balance from Shanghai Jinghai Instrument Co., Ltd., DHY-300 pulverizer from Beijing Donghua Medical Equipment Co., Ltd., ZRD-A5110 electric oven thermostat blast from Shanghai Zhicheng Analytical Instruments Co., Ltd., JP Roberval's balance from Shanghai Lineng Electronic Instruments Co., Ltd. The detailed description of instruments for nutrition quality analysis can be seen in reference (Fu, 2012). Allantoin standard (Batch No. 111501-200202) was purchased from the Chinese Food and Drug Inspection Institute (Beijing, China). Methanol was of chromatographic grade. Ultrapure water was used for the preparation of the reagents in this study. A detailed description of reagents for nutrition quality analysis has been given by Fu (2012).

Measurement of Yam Yield

Thirty plants of each cultivar were collected from three sites (10 plants from each site), where after the fresh weight of each plant was measured with a balance. Subsequently, a portion of the yam samples was dried at 50ºC to a constant weight, and weighed to calculate drying rate using the formula as follows: Drying rate = Weight of dried yam/Weight of fresh yam × 100%. Finally, the yam yield of each cultivar was calculated according to the planting density.

Evaluation of Yam Taste

Yam samples were rinsed with running water to remove any soil that was attached to their surface, and sliced into 10 cm pieces, before being steamed in a steamer for 25 min. Finally, the steamed samples of the seven new yam cultivars and the control were tasted by eight experts, and evaluated using the taste criterion of dry, floury, crisp, numb, sweet, fragrant, soft, and hard, etc.

Determination of Medicinal Ingredients in Yam

According to the method described in the Pharmacopoeia of the People’s Republic of China (Committee of Pharmacopeia in Ministry of Health of the People's Republic of China, 2010), 4 g of powder from each yam cultivar was weighed, sieved through a 20 mesh, and introduced into a 250 mL conical flask, to which 100 mL water was added. The flask was then sealed with a stopper, constantly shaken within the first 6 h, and then allowed to stand for another 18 h. Afterward, the solution was filtered, and 20 mL of the filtrate was transferred into an evaporating dish and dried to a constant weight from a water bath at 105ºC for 3 h. After cooling for 30 min in a dryer, the extract powder was precisely weighed.

The chromatographic conditions were set as follows: chromatographic column filled with octadecylsilane chemically bonded silica, Agilent ODS (4.6 mm × 250 mm, 5 μm); mobile phase: methanol-water (5:95); flow rate of 0.8 mL/min.; detection wavelength of 224 nm; column temperature of 30ºC; elution time of 7 min.; and sample volume of 10 μL. The HPLC was performed according to the methods described in the Pharmacopoeia of the People’s Republic of China (Committee of Pharmacopeia in Ministry of Health of the People's Republic of China, 2010).

Standard allantoin solutions were prepared by the following steps. Standard allantoin (18.5 mg) was dried to a constant weight, accurately weighed, and introduced into a 10 mL volumetric flask, after which it was dissolved with ultrapure water to the mark, and then mixed by shaking to obtain a 1.85 mg/mL standard stock solution of allantoin. Subsequently, 0.5, 1, 1.5, 2 and 2.5 mL of the stock solution were transferred into five 25 mL flasks. Ultrapure water was added to the marker of each flask and mixed by shaking to obtain standard allantoin solutions of 0.037, 0.074, 0.111, 0.148 and 0.185 mg/mL.

Iron stick yam sample solutions were prepared by the following steps. The yam powder, as prepared above, was sieved through an 80 mesh, and dried at 50ºC for 24 h. Afterward, 0.5 g of the sample of each yam cultivar was precisely weighed and dissolved in 25 mL of methanol, transferred into a flask, and treated by ultrasound twice (30 min each time, with an interval of 30 min). The solution was then centrifuged at 10,000 rpm for 15 min. Subsequently, the supernatant was filtered through a 0.45 μm organic microporous membrane and collected for later analysis (Bai *et al.,* 2003).

To plot the standard curve of allantoin, 10 μL from each of the standard allantoin solutions at 0.037, 0.074, 0.111, 0.148, and 0.185 mg/mL prepared above was loaded for HPLC. The standard curve of allantoin was obtained by the plotting peak area (mAU) on the Y-axis, and the concentration of standard allantoin (μg) on the X-axis. The regression equation was Y = 3 585.7X-1.819 9, R2 = 0.999 8, which confirmed a good linear relationship between the peak area and the concentration of the standard allantoin, ranging from 0.037 and 0.185 mg/mL. The allantoin content in the yam extract was then calculated according to the standard curve.

Identification of Yam Disease Resistance

The disease resistance of the seven new iron stick yam cultivars to *G. pestis* and *C. dioscoreae* was surveyed in the field in September, when leaf diseases were prevalent. Ten plants of each variety were selected, and all their aboveground leaves (1 m) were observed to record infection from *G. pestis* and *C. dioscoreae*. This field experiment was repeated twice. Finally, the disease index was calculated to identify their resistance to *G. pestis* and *C. dioscoreae*.

Analysis of Yam Nutritional Quality

Yam samples were prepared and their starch content was measured through acid hydrolysis according to GB/T 5009.9-2008 (for Starch in Foods), direct titration according to GB/T 5009.7-2008 (for Reducing Sugar in Foods), kjeldahl nitrogen method according to GB/5009.5-2010 (for Protein Content in Foods), combustion method according to GB 5009.4-2010 (for Ash Content in Foods) and drying according to GB 5009.3-2010 (for Water Content in Foods) as described by Ministry of Health of the People's Republic of China (2010).

Results

Comparison of Yield and Taste between the New Iron Stick Yam Cultivars and Control

SPSS software was employed for variance analysis of the fresh weight per plant of the seven new iron stick yam cultivars and the control. The dry weight per plant was then calculated according to the drying rate. The yam yield for each cultivar was calculated from the planting density. As shown in Table 1, the eight iron stick yam cultivars ranked in descending order of their fresh weight per plant were as follows: No. 6 (0.255 kg)>No. 10 (0.254 kg) > No. 4 (0.242 kg) > No. 9 (0.237 kg) > No. 1 (0.233 kg) > No. 11 (0.206 kg) > No. 2 (0.191 kg) > No. 8 (0.157 kg). Among them, the fresh weight per plant of No.6 (0.255 kg) and No.10 (0.254 kg) were greater than that of the other cultivars, while that of No.8 was the smallest. The fresh weight per plant had an insignificant difference between No. 6 and No. 10, a significant difference between No. 6 and No. 4, and an extremely significant difference between No. 6 and the other cultivars. These results verified that No. 6 and No. 10 were much better than control in terms of fresh weight per plant.

Eight iron stick yam cultivars ranked in descending order of their drying rate were as follows: No. 2 (32.641%) >No. 10 (32.230%) > No. 9 (28.223%) > No. 6 (25.174%) > No. 8 (25.122%) > No. 11 (control, 25.043%) > No. 1 (24.291%) > No. 4 (20.234%), in descending order of their dry weight per plant were as: No. 10 (0.082 kg) > No. 9 (0.067 kg) > No. 6 (0.064 kg) > No. 2 (0.062 kg) > No. 1 (0.056 kg) > No. 11 (control, 0.052 kg) > No. 4 (0.049 kg) > No. 8 (0.039 kg). These results suggested that the dry weight per plant of No. 10, No. 9, No. 6, No. 2 and No.1 were greater than that of the control, whereas No. 10, No. 9 and No. 6 were more competitive in their dry weight per plant.

The results of the taste evaluation revealed that No. 10, which tasted dry, floury, sweet, and fragrant, had the best eating quality; both No. 6, which tested dry, floury, and hard, and No. 9, which tasted dry and crisp, were not outstanding in their eating quality.

Comparison of Medicinal Ingredients between the New Iron Stick Yam Cultivars and Control

The extract content of the seven new iron stick yam cultivars and the control were measured, and the data were analyzed with SPSS. The HPLC fingerprints of the standard allantoin and yam samples are shown in Fig. 1 and 2, where the allantoin content of each cultivar was calculated based on the peak area in the HPLC fingerprints, and the data were analyzed using SPSS (Table 2).

As shown in Table 2, the extract content of the eight yam cultivars ranged from 11.87% to 20.49%, all of which exceeded the standard described in the 2010 version of the Pharmacopoeia of the People’s Republic of China (Committee of Pharmacopeia in Ministry of Health of the People's Republic of China, 2010). The highest extract content was found in No. 6 (up to 20.49%), while No.8 had the lowest extract content (only 11.87%). The significance test revealed that there was an extremely significant difference in extract content between No. 6 and the other cultivars. The extract content of No. 10 was higher than No.1, No.11 (control), No. 9, and No. 8, exhibiting an insignificant difference from that of No. 1, but a significant difference from that of No. 11 (control), and No. 9, and an extremely significant difference from that of No. 8. The extract content of No. 9 was lower than that of the control; with an insignificant difference between them. The results revealed that No. 6 had an extremely significant advantage, whereas No. 10 had a significant advantage in extract content over the control.

Table 2 shows that eight iron stick yam cultivars ranked in descending order of their allantoin content are as follows No. 6 (0.484%) > No. 4 (0.467%) > No. 10 (0.399%) > No. 11 (0.386%) > No. 9 (0.378%) > No. 8 (0.350%) > No. 2 (0.342) >No. 1 (0.325%). Among them, No. 6 had the highest allantoin content (0.484%), while No. 1 had the lowest allantoin content (0.325%). There was no significant difference in the allantoin content between No. 6 and No. 4, a significant difference between No. 6, and No. 10, No. 9 and No. 11 (control), and an extremely significant difference between No. 6 and the others. The allantoin content of No.10 was higher than that of No. 11 (control), No. 1, No. 2 and No. 8; however, the difference was not significant. In summary, No. 6 had a significant advantage in allantoin content over the control, whereas No. 10 did not.

Table 1: Comparison of fresh weight per plant and taste between new iron stick yam cultivars and control

Yam cultivars	Fresh weight per plant (kg)	Drying rate (%)	Dry weight per plant (kg)	Taste
No. 1	0.233±0.009 7^{bB}	24.291	0.056	Dry, floury, sweet and hard
No. 2	0.191±0.010 0^{dC}	32.641	0.062	Dry and floury
No. 4	0.242±0.004 6^{bA}	20.234	0.049	Dry, floury and sweet
No. 6	0.255±0.006 5^{aA}	25.174	0.064	Dry, floury and hard
No. 8	0.157±0.006 1^{eD}	25.122	0.039	Dry, floury and numb
No. 9	0.237±0.004 3^{bB}	28.223	0.067	Dry and crisp
No. 10	0.254±0.007 9^{aA}	32.230	0.082	Dry, floury, sweet and fragrant
No. 11	0.206±0.003 6^{cC}	25.043	0.052	Dry, sweet and fragrant

Note: The values followed by different lowercase letters are significantly different at the 0.05 level, and the values followed by different uppercase letters are extremely significantly different at the 0.01 level

Table 2: Comparison of extract content and allantoin content between the seven new yam cultivars and the control

Yam cultivars	Extract content%	Allantoin content%
No. 1	14.48+0.77cdBCD	0.325±0.034cC
No. 2	16.01+0.33bB	0.342±0.088cC
No. 4	15.54+0.11bcBC	0.467±0.016abAB
No. 6	20.49+0.58aA	0.484±0.023aA
No. 8	11.87+0.10eE	0.350±0.011cBC
No. 9	13.88+0.11dD	0.378±0.025cABC
No. 10	15.35+0.62bcBCD	0.399±0.011bcABC
No. 11	14.10+00.56dCD	0.386±0.003bcABC

Note: The values followed by different lowercase letters are significantly different at the 0.05 level, and the values followed by different uppercase letters are extremely significantly different at the 0.01 level

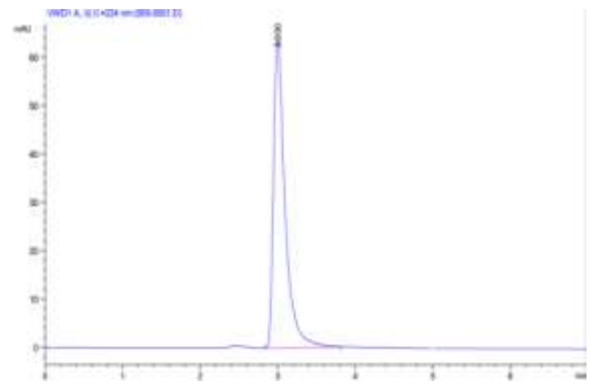

Fig. 1: HPLC fingerprint of allantoin standard

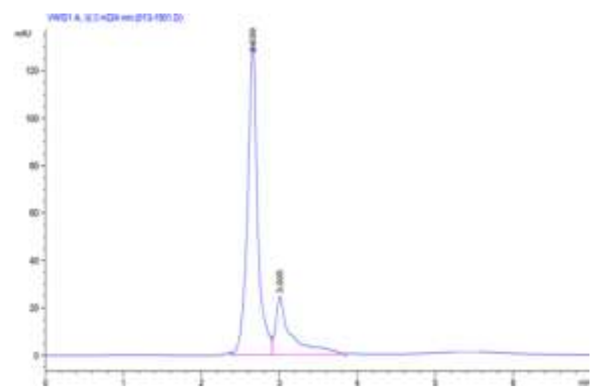

Fig. 2: HPLC fingerprint of yam sample

Disease Resistance of the Iron Stick Yam Cultivars

As shown in Table 3, No. 6 was highly resistant to *G. pestis*, while No. 1, No. 2, No. 4, No. 9 and No. 10 had an average resistance to *G. pestis*; the control (No. 11) was moderately susceptible to *G. pestis*, and No. 8 was highly susceptible to *G. pestis*. Both No. 6 and No. 10 were highly resistant to *C. dioscoreae*; No. 1, No. 2, No. 4, No. 9, and the control (No. 11) had an average resistance to *C. dioscoreae*, whereas No. 8 was highly susceptible to *C. dioscoreae*. It might be inferred that the new No. 6 variety had a high resistance to *G. pestis* and *C. dioscoreae*, which indicated that it was highly resistant to leaf diseases, while No. 10 was moderately resistant to *G. pestis* and highly resistant to *C. dioscoreae*, suggesting that it also had good resistance to leaf diseases.

Comparison of Nutritional Quality between Two New Iron Stick Yam Cultivars and Control

The content of nutritional ingredients in the three yam samples met the national standards of People's Republic of China for geographical indication product-Chinese yam (GB/T20351-2006) (Table 4). In detail, the content of starch, reducing sugar, protein, and ash in No. 6 and No. 10 were higher than that of the control, while the water content of No.6 and No. 10 was lower than that of control. These results revealed that No. 6 and No. 10 possessed improved nutritional quality than the control. Analysis of variance revealed that the starch content of No. 10 was extremely and significantly different than that of No. 6 and the control; the starch content of No.6 was higher than that of the control, and the difference between them was extremely significant. The reducing sugar content of No. 10 was higher than that of No. 6 and the control, with an insignificant difference between No. 10 and No. 6, and an extremely significant difference between No.10 and the control. The reducing sugar content of No. 6 was higher than that of the control, with an extremely significant difference between them. The protein content of No. 6 was higher than that of No. 10 and the control, with an extremely significant difference between No. 6, No. 10, and the control. The protein content of No. 10 was higher than that of the control, and the difference between them was extremely significant. The ash content of No. 10 was higher

Table 3: Resistance evaluation of the eight iron stick yam cultivars (*n*=20)

Variety	*G. pestis*			*C. dioscoreae*		
	Disease index	Resistance index	Resistance level	Disease index	Resistance index	Resistance level
No. 1	0.27	0.73	MR	0.26	0.74	MR
No. 2	0.31	0.69	MR	0.31	0.69	MR
No. 4	0.25	0.75	MR	0.24	0.76	MR
No. 6	0.20	0.80	HR	0.18	0.82	HR
No. 8	1	-	HS	1	-	HS
No. 9	0.28	0.72	MR	0.24	0.76	MR
No. 10	0.21	0.79	MR	0.17	0.83	HR
No. 11	0.44	0.56	MS	0.38	0.62	MR

Note: HR (highly resistant); MR (moderately resistant); MS (moderately susceptible); HS (highly susceptible)

Table 4: Comparison of nutrition quality between new cultivars No. 6, No. 10 and the control

Cultivars	Starch content%	Reducing sugar content%	Protein content%	Ash content%	Water content%
No. 6	71.68±0.11bB	2.19±0.05aA	16.74±0.08aA	2.95±0.09abA	76.21±0.11bB
No. 10	75.31±0.09aA	2.34±0.10aA	16.03±0.16bB	3.03±0.09aA	70.87±0.07cC
No. 11 (control)	70.22±0.24cC	1.89±0.09bB	14.71±0.08cC	2.85±0.07bA	76.52±0.10aA

Note: The values followed by different lowercase letters are significantly different at the 0.05 level, and the values followed by different uppercase letters are extremely significantly different at the 0.01 level

than that of No. 6 and the control, with an insignificant difference between No. 10 and No. 6, and a significant difference between No.10 and the control. The ash content of No. 6 was higher than that of the control; however, the difference was not significant. The water content of No. 6 and No. 10 was lower than that of the control, and the difference between No. 6, No. 10, and the control was significant. The water content of No. 6 was higher than that of No. 10, and the difference was extremely significant. In summary, both the two new cultivars, particularly No. 6 and No. 10, had advantages in nutritional quality over the control.

Discussion

As a medicinal and edible herb, yam is consumed much more as a food than as a medicine. Yield, taste, medicinal ingredients, disease resistance, and nutrition quality are important indices that are employed to evaluate yam varieties (Zhang *et al.,* 2007). Extract content comprises one of the test criteria of yam by pharmacopoeia, which reflects its general medicinal ingredient content (Ting *et al.,* 2011). Allantoin is one of the primary active ingredients of yam, which can soften cutin, promote wound healing, accelerate the growth of epithelial cells, as well as produce additional physiological effects such as anesthesia and analgesia. It also has bacteriostatic, antiphlogistic, and anti-corrosion properties. Hence, these criteria are often used as the basis for evaluating the quality of yam or yam preparations (Gu and Qin, 1990).

Through the comparative analysis of various indices between the seven new iron stick yam cultivars and the control, we discovered that the difference in fresh weight per plant was not significant between No. 6 and No. 10; however, it was extremely significant between both No. 6, No. 10, and the control; indicating that these two new cultivars had an obvious advantage in fresh weight per plant. The dry weight per plant of No. 6 was lower than that of No. 10, but higher than that of the control; thus, No. 10 had an obvious advantage in dry weight per plant. The taste assessment results revealed that No. 10 had the best in eating quality, while No. 6 was not outstanding.

The extract content of No.6 was highest among the eight iron stick yam cultivars tested, and significantly different from that of the control (No. 11), which indicated that No. 6 had an obvious advantage in extract content. The extract content of No. 10 was also higher than that of the control, and the difference between them was significant. The allantoin content of No. 6 was highest, which was significantly different from that of the control (No. 11), while the extract content of No. 10 was higher than that of the control; however, the difference between them was not significant. This suggested that No. 6 had a high content of medicinal ingredients, in contrast to No. 10 and the control.

The results of resistance identification revealed that No. 6 was highly resistant to *G. pestis* and *C. dioscoreae*, indicating that it had high a resistance to leaf diseases. No. 10 was moderately resistant to *G. pestis*, but highly resistant to *C. dioscoreae*, which indicated that it also had good resistance to leaf diseases.

An analysis of the nutritional quality of No. 6 and No. 10 revealed that their starch, reducing sugar, protein, and ash contents were higher than that of the control (No. 11), while their water content was less than that of the control, indicating that both No. 10 and No. 6 had an improved nutritional quality over the control.

The special space environment such as high vacuum, microgravity, high radiation and other factors change in the genes of iron stick yam the space environment altered the genes of iron stick yam, which enhanced the yield and quality of the new iron stick yam cultivars. The fresh weight per plant of No. 10 was 0.254 kg, which was second only to No. 6, and its drying rate was 32.230%, which was second only to No. 2, whereas the maximum dry weight per plant was 0.082 kg; The content of allantoin was 0.399% and the extract content was 15.35%, which was average and higher than the control. It had an average resistance to anthracnose and a high resistance to brown spot, which indicated a good resistance to leaf disease. The taste was also enhanced, having dry, sweet, and fragrant attributes. The content of each nutrient was starch (75.31%), original sugar (2.34%), and protein (16.03%), which was the highest, and higher than the control (No. 11). The ash content was 3.03%, while the water content was 70.87%. Therefore, it was determined to be suitable for promotion as a new variety of edible "iron rod" yam. Recently, No. 10 was approved as a new yam variety by the Henan Province Professional Pharmacy Committee, and is referred to as Iron 06-1 (Iron No. 1). The fresh weight per plant of No. 6 was up to 0.255 kg, with a moderate drying rate at 25.174%, and a dry weight per plant of 0.064 kg, which was second only to No. 10 and No. 9.

The allantoin content was 0.484% and the extract content was 20.49%, which was the highest, and significantly higher than that of the control. It had resistance to both anthracnose and brown spot, which confirmed an improved resistance against leaf disease, and the taste was good: dry and hard. The content of each nutrient was starch (71.68%), reducing sugar (2.19%), protein (16.74%), which was obviously higher than the control. The ash content was 2.95%, while the water content was 76.21%. Hence, it was determined to be appropriate for promotion as a new variety of medicinal "iron rod" yam.

Conclusion

The comprehensive indices of two new yams (No. 6 and No. 10) were both very high, which made them suitable for popularizing as new varieties. Overall, the new iron stick yam varieties we developed have significance for the healthy and sustainable development of the iron stick yam industry.

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