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Breeding of Kidney Bean (*Phaseolus vulgaris* L.) Cultivar Keyun1 and Exploration of Nitrogen Application and Planting Density

G.D. Yang[1#], Z.Y. Hu[1#], Z.Y. Hao[1], J.H. Li[1], B.S. Sun[1], X.X. Meng[2], Q. Wang[2]

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ABSTRACT

Background: Small white Kidney beans (*Phaseolus vulgaris* L.) is popular in the international market, they are widely cultivated in the high and cold areas of China. However, these areas have relatively short growth period, resulting in the low yield and quality of kidney beans, which limits the development of kidney bean. Keyun1 has since played an important role in improving kidney bean production in the country since 2020 because of its high yields, good upright and wide adaptability.

Methods: Keyun1 is a small grain white kidney bean cultivar that was developed by a systematic selection of excellent mutants in China in 2020.

Result: The average yield of Keyun1 was 2346.26 kg/ha, 34.42% higher than that of the control cultivar (British Red) in the national joint identification test of new kidney bean cultivars of China from 2016 to 2017. In 2018, Keyun1 was included in the national joint identification and production test of new kidney bean cultivars of China, the average yield per hectare was 2076.58 kg, which is 67.99% higher than that of the control cultivar (British Red). and exploration of nitrogen application and planting density showed the best nitrogen application that is 45 kg of active ingredient nitrogen per hectare and the best planting density is 15×10^4 -20×10^4 plants/ha, the greatest yield was 3198.17 kg/ha.

Key words: Grain yield, Kidney bean, Nitrogen fertilization, Planting density, Systematic selection.

INTRODUCTION

Kidney bean (*Phaseolus vulgaris* L.) belongs to the legume family Fabaceae. It is characterized by a wide adaptability and favouring a cold climate (Lin *et al*., 2009). Kidney bean has the characteristics of strong adaptability, grow in poor fertile soil and drought resistance (Yang *et al*., 2014). Grains are rich in crude protein, starch and other nutrients and the content of lysine in protein is high (Yao *et al*., 2012; Aiwa and Minuto, 2003; Hoover and Ratnayake, 2002), which is an important part of dietary structure. The kidney bean is the most exported miscellaneous bean cultivar in China and is sold to more than 60 countries and regions around the world. Previously, the export volume accounted for about 50% of the production volume. Asia, Europe and the Americas are the important export markets, for which net export is still maintained (Qu *et al*., 2021; Zhou and Zhang, 2018).The unique climate, geography and ecological environment of Heilongjiang Province are very suitable for the growth of kidney beans, especially Nehe, Yi'an, Hailun, Nenjiang and Bei2 an of Heilongjiang Province, which has the obvious advantages of production, with the annual about planting two hundred thousand hectares and an average unit yield of 1505.7 kg/ha. The annual export of kidney beans is 30×10^4 t, accounting for about 1/2 of the total export of kidney beans in China. The main types of kidney beans are small white kidney beans, small black kidney beans and red kidney beans (Wang *et al*., 2008;).The planting mode is generally done by large ridge cultivatio (Fig 1) which can resist drought and waterlogging. However, these areas are located in the alpine zone, with relatively short growth period, resulting in the low yield and quality of kidney beans, which limits the development of kidney bean. How to cultivate kidney bean cultivars with high resistance, high yield, high quality and excellent characteristics has become the main goal of breeders (Allen and Ort, 2001; Chai and Feng 2003). Therefore, in order to reduce production costs, growers require more upright cultivars which are better suited for direct harvest, which is of important practical significance. The project described here made use of local kidney bean resources with obvious early maturation and upright short vine, which were used in the breeding of new kidney bean cultivars with high yield, upright and disease resistant that are suitable for mechanized harvest as the breeding goal and the systematic selection of mutants resulted in the new kidney bean cultivar Keyun1. The objectives of this study

[1]Keshan Branch of Heilongjiang Academy of Agricultural Sciences, Shenjiang Road, Longsha District, Qiqihar-161005, China.
[2]Crop Resources Institute of Heilongjiang Academy of Agricultural Sciences 150086 Heilongjiang, China.
#These authors contributed equally to this work.

Corresponding Author: Q. Wang, Crop Resources Institute of Heilongjiang Academy of Agricultural Sciences 150086 Heilongjiang, China. Email: wangqianghlj@163.com

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were to state breeding process and characteristics of Keyun1 and evaluate an optimum planting density and nitrogen application to achieve higher yields, which provides cultivars and technical support for the development of kidney beans in Northern China.

MATERIALS AND METHODS

Materials sources

In June 2009, 50 individual small grain white kidney bean local resource plants were selected for early maturation and upright in Nenjiang, then they were breeded from May 2010 to December 2015 in experimental field of Keshan branch of Heilongjiang Academy of Agricultural Sciences (48°03′ 47″N, 125°87′ 57″E).

Breeding process

From 2010 to 2012, the offspring of a single plant were tested according to a random block group design, with two repetitions, 40 plants in one repetition (2 ridges, 0.65 m ridge spacing, 2 m long, double rows planting on the ridge). Prior to harvest, 10 plants were sampled from each plot for seed testing. From 2010 to 2011, plants that included upright short vine, lodging resistance, good pod load, bean seed size and color, freedom from diseases and midseason maturity were selected, from which excellent lines were selected in 2012. From 2013 to 2014, the superior lines selected in the previous year were planted separately and the product comparison test was carried out at the same time. In 2015, the yield was determined in an experiment using a random block group design, with 3 repetitions, 5 ridges (0.65 m ridge spacing, double rows planting on the ridge, 3 m long, 10 plant per meter). Anthracnose screening and Common bacterial blight screening were conducted by collaborators in Harbin and Qiqihar. Crude protein content, crude fat and crude starch content were determined by a near-infrared quality analyzer (perten DA7200, Swedish). Breeding process is shown in Fig 2.

Keyun1 was included in the national joint identification test of new kidney bean cultivars for two consecutive years from 2016 to 2017, the The control cultivar was British Red. The tests were conducted in Harbin, Qiqihar, Jinzhong, Hohhot, Bijie, Yulin, Qitai County, Huairen County and Gongzhuling City.In 2018, Keyun1 was included in the national joint identification production test of new kidney bean cultivars. The test sites were Qiqihar City, Hohhot, Bijie, Yulin, Urumqi and Changchun. The characteristics of the test sites of new kidney bean cultivars were shown in Table 1.

The regional test was designed in random groups with three repetitions (four rows, row spacing of 0.65 m and length of 5 m). The planting density was 20×10^4 plants/ha.

The planting area of production tests was 200 m^2 without repetition. The planting density was 20×10^4 plants/ha.

Exploration of nitrogen application and planting density

Trials materials and conditions

Agronomic trials were performed in an open field at Keshan (48°03′ 47″N, 125°87′ 57″E) (Heilongjiang Province, China) in 2017. The former crop was sorghum and the experimental soil was chernozem. The 0-20 cm deep soil had the following characteristics: organic matter 3.2%, pH 6.12, alkali hydrolyzed nitrogen 173 $mg \cdot kg^{-1}$, available phosphorus 28.8 $mg \cdot kg^{-1}$ and available potassium 307.2 $mg \cdot kg^{-1}$(Institute of soil, fertilizer and environmental resources, Heilongjiang Academy of Agricultural Sciences). The temperatures and rainfall during May to September were showed in Table 2.

Experimental design and crop management

A split zone design was adopted in the experiment with three replicates, the N application in the main plots, with Urea (46% N) was used as the nitrogen source and four nitrogen treatments were set up: 15 kg (N1), 30 kg (N2), 45 kg (N3) and 60 kg (N4) of active ingredient nitrogen per hectare. Nitrogen and superphosphate (P_2O_5 100 kg/ha) and potassium sulfate (K_2O 50 kg/ha) were applied once at the sowing stage. Split plot planting density five levels, including 10×10^4 (D1), 15×10^4 (D2), 20×10^4 (D3), 25×10^4 plants/ha (D4) and 30×10^4 plants/ha (D5). 1.3 m ridge spacing, 3 ridges (four rows planting on the ridge, with a large row spacing of 0.4 m and a small row spacing of 0.1 m), 5 m long, each experimental plot covered an area of 19.50 m^2 Manual seeding, sowing was conducted on May 16, 2017; and harvesting was conducted on September 3, 2017. At the mature stage, 15 plants were randomly selected from each plot, 100-seed weight, ,and seed number per pod were calculated. Grain yield and pods number per plot were estimated by manually harvesting 7.8 m^2 (the middle 8 rows by 3 m) area of each plot. Yields were adjusted to 14% moisture content.

Weeding after sowing and before emergence and spray 1500 ml of 96% metolachlor and 500 L of water per hectare. For bacterial blight, spray with 1000-1200 times of

Fig 1: The large ridge cultivation of kidney beans.

mesobiotic solution, spray once every 7-10 days, 2-3 times in total. For anthracnose, use 600 times 50% carbendazim wettable powder, spray once every 10 days, 2-3 times in total. Before the third instar of larvae, 900 ml of 4.5% beta cypermethrin was sprayed per hectare to control the larvae.

Analysis software and analysis method

Analysis of variance was performed with SPSS 16.0 software and data from each sampling data were analyzed separately. Means were tested by least significant difference at the P<0.05 level (Duncan 0.05).

RESULTS AND DISCUSSON

Basic agronomic characteristics of Keyun1 and the evaluation of disease resistance

Keyun1 is an early maturing cultivar with a growth period of approximately 90-95 days in the spring growing seasons and the plant height is about 77 cm. The blades are heart shaped, the main stem has 4 branches, the number of main stem nodes is 12 and the flowers are white (Fig 3a). The number of pods per plant is 20-25, the pods are round and stick shaped, the number of seeds per pod is 5~6 (Fig 3b), the seeds are oval and the seed coat is white (Fig 3c) and

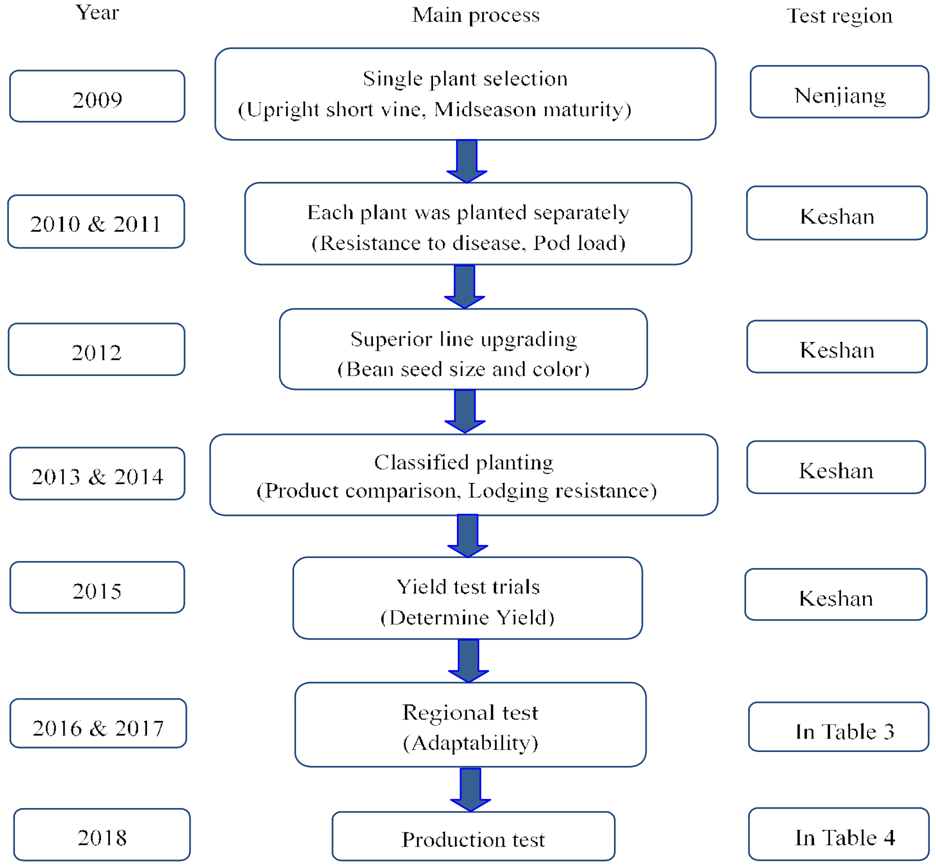

Fig 2: Timeline of the breeding process.

Table 1: The characteristics of the test sites of new kidney bean cultivars.

Test sites	Latitude (N)	Longitude (E)	Altitude (m)	Precipitation (mm)
Harbin	126°50′	45°49′	147.00	500.00
Qiqihar	123°40′	47°12′	160.00	415.00
Qitai	89°45′	43°59′	822.00	269.00
Hohhot	111°48′	40°48′	1051.00	400.00
Jinzhong	112°67′	37°55′	813.00	437.00
Yulin	109°45′	38°22′	1122.00	400.00
Huairen	113°16′	39°55′	1018.00	400.00
Bijie	106°17′	27°18′	1545.00	987.00
Gongzhuling	124°47′	43°31′	196.00	600.00

the 100-seed weight is 18.0-20.5g. Crude protein content was measured at 25.86%, crude fat at 1.60%, crude starch content at 46.68% and the natural incidence of field anthracnose and bacterial blight was less than 8%.

The results of the regional trials and production trials

The grain yield of Keyun1 was shown in Table 3. In 2016, a total of 8 regional test sites were set up, of which 7 were significantly higher than the control cultivar (The British Red). In 2017, there were 9 regional test site in total, of which 7 points were significantly higher than the control cultivar. In 2016 and 2017, the average yield per hectare was 2520.43 and 2191.43 kg, 35.71% and 33.28% higher than the control cultivar, respectively. The average yield over the two years was 2346.26 kg per hectare, 34.42% higher than that of the control cultivar.

Due to differences in climatic characteristics and geographical conditions, there was significant difference in yield at different test points. In 2016 and 2017, Qitai test site has the highest yield, the average yield per hectare was 3936.59 and 3806.69 kg. Jinzhong test site has the lowest yield, the average yield per hectare was 1139.43 and 1198.05 kg.

In 2018, Keyun1 was included in the national joint identification and production test of new kidney bean cultivars. The average yield per hectare was 2076.58 kg, which is 67.99% higher than that of the control cultivar (The British Red), as shown in Table 4.

Effect of N application and density on the yield

As indicated in Table 5, the pod number per square meter of N2D5, N3D4 and N3D5 was the highest. By the same N treatment, the 100-seeds weight under D1 and D2 was significantly higher than that of D3-D5 and the difference between D3 and D5 was significant. Under D1-D2, there was no significant difference in the 100-seeds weight among the different N treatments except for N1. Under the same density, the number of seeds per pod in the different N treatments had no significant difference. the pod number per square meter has no significant difference among different nitrogen treatments under the D1 density, all density

Table 2: The average temperature and total rainfall during the cropping cycles (May-Sept.)

Month	Date	Average temperature (°C)	Rainfall (mm)
May	1st to 10st	15.38	10.04
	11st to 20st	17.27	1.50
	21st to 31st	14.42	0.70
June	1st to 10st	15.10	18.70
	11st to 20st	20.83	62.60
	21st to 30st	24.04	11.00
July	1st to 10st	26.58	0.40
	11st to 20st	24.01	15.00
	21st to 31st	20.89	23.60
Auguest	1st to 10st	21.91	118.50
	11st to 20st	23.19	16.80
	21st to 31st	15.96	15.60
September	1st to 10st	15.70	21.70
	11st to 20st	13.26	27.20
	21st to 30st	10.78	13.70

Table 3: The results of the regional trials.

Year	Test regions	Yield of Keyun1 (kg/ha)	Yield increase than the control the British red (%)
2016	Harbin	1615.35±197.38d	68.00*
2016	Jinzhong	1139.43±258.81d	18.76
2016	Hohhot	3005.70±28.70b	25.57*
2016	Qiqihar	1326.45±153.76d	41.61*
2016	Bijie	3520.05±174.05ab	26.32*
2016	Yulin	3379.95±225.13b	45.27*
2016	Qitai	3936.59±150.26a	46.98*
2016	Huairen	2239.95±76.26c	13.13*
Average	8 regions	2520.43	35.71*
2017	Harbin	1715.38±150.41d	34.34*
2017	Jinzhong	1198.05±217.11e	102.82*
2017	Hohhot	2033.85±109.97cd	3.44
2017	Qiqihar	1114.54±164.40e	8.31
2017	Bijie	3236.67±96.13b	50.54*
2017	Yulin	2433.28±29.82c	6.57
2017	Qitai	3806.69±127.78a	44.56*
2017	Huairen	2160.04±103.06c	14.69*
2017	Gongzhuling	2024.39±130.90cd	34.29*
Average	9 regions	2191.43	33.28*
Overall average	17 regions	2346.26	34.42*

Note: Different letters indicate significant differences at p<0.05, "*" represent significant at the 0.05 level.

Table 4: The results of the production trials.

Year	Test regions	Yield (kg/ha)	Yield increase over British Red (%)
2018	Qiqihar	1099.95	210.87
	Bijie	3584.25	62.43
	Yulin	2937.00	19.83
	Urumqi	2335.20	65.99
	Changchun	903.00	-0.03
	Hohhot	1600.05	48.84
Average	6 regions	2076.58	67.99

Note: The production trial without repetition.

treatments under N3 treatment were significantly higher than other nitrogen treatments under the same density. The yield of the N3D2 and N3D3 combination were 3198.17 kg/ha and 3146.42 kg/ha, respectively, which was significantly higher than other combinations, followed by the N3D3 and N3D2 combination, the yield were 3063.90 kg/ha and 2980.15 kg/ha, respectively. The yield difference of N3D3 and N2D3 was not significant. The pod number per square meter was increased significantly with the increase in planting density.

Singh, Duran, Yamaguchi found that there were great differences in morphology among various races in the cultivated kidney bean gene bank (Singh *et al*., 1991;

Table 5: Effect of nitrogen and density on yield.

Density (Plants/ha)	Nitrogen application level (kg/ha) N1	N2	N3	N4
		Pod number per square meter		
D1	214.34±1.76i	221.67±3.18i	218.67±1.20i	216.67±2.19i
D2	264.00±6.24h	273.67±3.84gh	289.67±3.28f	266.67±2.96gh
D3	301.67±5.24e	315.00±1.73d	336.33±3.18bc	275.67±2.73g
D4	328.33±5.24c	333.00±5.69bc	341.00±3.46ab	291.67±2.85f
D5	337.67±3.84bc	350.00±1.53a	341.33±2.03ab	309.00±1.53de
		100-seed weight (g)		
D1	21.73±0.14a	21.39±0.14ab	21.32±0.27ab	21.11±0.36abc
D2	20.22±0.12cdef	20.45±0.29bcde	20.74±0.35bcd	20.56±0.29bcd
D3	19.73±0.62defg	20.11±0.33cdef	20.14±0.35cdef	19.45±0.42efgh
D4	18.71±0.56hij	19.28±0.07fghi	19.05±0.02ghij	18.59±0.30hij
D5	18.34±0.18ij	18.97±0.29ghij	18.65±0.25hij	18.08±0.13j
		Seed number per pod		
D1	5.60±0.12b	5.82±0.06a	5.86±0.03a	5.64±0.08b
D2	5.26±0.07d	5.47±0.10bc	5.52±0.05b	5.30±0.06cd
D3	4.99±0.05e	5.22±0.02d	5.18±0.03d	4.78±0.07f
D4	4.36±0.05ghi	4.48±0.04g	4.39±0.03gh	4.21±0.03hij
D5	4.15±0.08j	4.18±0.04ij	4.18±0.05ij	3.95±0.05k
		Grain yield (kg/ha)		
D1	2094.80±25.68j	2283.30±35.54gh	2405.70±29.02f	2506.65±36.84ef
D2	2139.75±43.36ij	2980.15±69.37c	3198.17±11.59a	2711.72±20.90d
D3	2390.42±29.93fg	3063.90±43.50bc	3146.42±26.67ab	2544.28±43.54e
D4	2409.28±20.55f	2705.70±43.95d	2656.80±57.70d	2227.00±60.56hi
D5	2121.45±38.37ij	2402.25±24.02f	2223.15±30.29hi	2073.30±11.93j

Note: Different letters indicate significant differences at p<0.05 Level of significance.

(a) (b) (c)

Fig 3: Keyun1 (a); Seeds, (b); Harvested plants and (c); Plants in the field at the mature stage.

Durán*et al*., 2005; Kondo *et al*., 1998). R, J. Redden, Singh and McClean found that the phenotype of wild-type kidney beans changed greatly during the formation of cultivated kidney beans by using morphological marker analysis (Mcclean *et al*., 2004; Redden *et al*., 2009; Markert *et al*., 1959). Because the phenotypic traits are greatly affected by the external environment and micro effect alleles, the phenotypic traits are quite different in different climates, therefore, screening for excellent cultivars and lines from the aspects of suitable regions and phenotypic traits is still one of the main strategies of kidney bean breeding in China (Zhao, 2021). The northern China has a cold climate and short growth period,at present, most of kidney bean varieties are poorly resistant to low temperatures. It is necessary to screen and identify resources to develop excellent germplasm resources for the local natural conditions. We selected six target characteristics for assessment of growth habits, number of pods per plant, number of seeds per plant, 100-seeds weight, disease index and lodging resistance. For early maturation and upright short vine, we maintain the typicality of the original cultivar and optimized for yield and constituent elements, variety resistance. The selected lines were determined according to the yield measurement results. The selected lines were still planted and compared with the original varieties in the same plant nursery. On the other hand, the superior lines that had made new breakthroughs in agronomic traits and typicality of varieties were confirmed through plant line comparison and upgraded to the product comparison nursery in time. In order to determine the better yield stability, adaptability and stress resistance of varieties, we carried out a nationwide remote identification test, which revealed that the comprehensive characteristics of the varieties were vastly superior. Thus, these varieties was passed the identification test of the professional technical Committee of Edible Beans of the China Crop Association and can be promoted.

An appropriate planting density can maintain material production capacity (Kalaji *et al*., 2017). The study showed that the planting density has an obvious regulatory effect on the yield of kidney beans in alpine areas. Under the condition of D1 density, although the number of seeds per pod and 100-seeds weight of kidney bean increased, it could not make up for the yield loss caused by the decrease of pod number, indicating that reduction of pod number per unit area was the main reason for the low yield of D1 density treatment. However, high density intensified the competition between plant individuals for light, water and fertilizer, resulting in the decline of individual photosynthetic capacity and finally form yield. The results showed that the suitable planting density of Keyun1 was 15×10^4-20×10^4 plants/ha. Once this density is exceeded, the yield will decrease.

Nitrogen has significant effects on plant growth and crop yield (Ronga *et al*., 2015; Mosisa and Habtamu, 2007). The ability of legume nodule to provide nitrogen to itself in the early stage is limited, so it can only rely on the application of nitrogen fertilizer in the field to meet the growth and development (Qiao, 2014), The results showed that N2 and N3 treatments were the best under the same density and the yield and number of pods per unit area increased significantly compared with other treatments. Under N1 treatment, the nitrogen supply was insufficient, resulting in weak and small vegetative body, which was not conducive to the increase of the number of branches, thus reducing the number of pods per unit area and the number of seeds per unit area. In N4 treatment, due to the excessive vegetative growth in the later stage, kidney beans growntoo high, caused lodging and disease and the short growth period in high latitudes, the number of empty and shriveled seeds increased, resulting in the decline of the number of pods per unit area and number of seeds per pod, resulting in the yield lower than that of N2 and N3.

Under different planting densities and nitrogen fertilizer treatments, the yield of N3D2 and N3D3 was the highest, followed by the combination of N2D2 and N2D3, but the yield difference between N3D3 and N2D3 was not significant, pure nitrogen 45 kg/ha and 15×10^4-20×10^4 plants/ha combination could obtain higher yield. Among the yield components of kidney beans, the number of pods per unit area and the number of seeds per pod vary greatly and the variation of 100-seeds weight was small, indicating that nitrogen fertilizer and planting density mainly affect the yield through the number of pods per unit area.

CONCLUSION

Keyun1 is a small grain white kidney bean cultivar with extremely early maturation, high yield and good upright, which is a popular choice for direct harvest. The suitable area for planting is the early maturing regions in northwest China. The combination of 45 kg/ha of pure nitrogen and 15×10^4-20×10^4 plants/ha could obtain higher yield. The findings of this study offer useful cultivar and management information for improving kidney bean production in alpine regions of China.

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Conflict of Interest

The authors declare no conflict of interest.

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