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Proximation of minerals in different genotypes of rice lines in agroclimatic region of north plain

Anil Kumar, Bhudeo Rana Yashu, Uday Pratap Singh and Sanjay Kumar

Abstract

Rice is important staple cereal crop on which population of India so identification of nutrient rich is necessary to feed those population who heavily dependent on it. Biofortification of rice with multiple micronutrients could be best strategy to combat the nutrient deficiencies in developing countries. We determined the content of nutrient in dehusked polished rice grains in popularly grown lines of rice crop. Nutrients content in different lines, Nitrogen ranges from 27.94 to 16.82 mg g⁻¹, Potassium ranges from 1.24 to 0.54 mg g⁻¹, where as the content of potassium and phosphorous ranges from 0.33 – 0.06 and 0.57-.0.03 respectively.

Keywords: Proximation, different genotypes, rice lines, agroclimatic region

Introduction

Rice (*Oryza sativa* L.) is an important component of the basic human diet at global level. It is considered as one of the most important sources of nutrients for millions of people around the world. Rice is the staple food for almost two-third of the population. Moreover, India ranks first in the world in area of rice cultivation with 43.97 million hectare and second in production with 112.32 million tons. Unfortunately, populations of many of the main rice consuming countries suffer from nutrient-deficiency-related diseases, with an insufficient intake of important essential elements in their diet. Moreover, in some cases, diet is also an important source of toxic elements ^[1].

Research efforts focused on development of high-yielding varieties and adoption of modern production technologies resulted in enhanced production leading to self-sufficiency in the country. Rice is a primary source of carbohydrate and it is also a good source of protein, but it is not a complete protein, which means that it does not contain all of the essential amino acids in sufficient amounts, along with yield, grain and nutritional quality has also become a primary consideration in rice breeding programs; not only in India but also in various, rice growing countries across the world. Rice can be considered to be the most important cereal which supplies good amount of minerals, vitamins, essential fatty acids and dietary fiber besides energy. It has good digestibility, biological value and good quality protein due to the presence of higher amount of lysine. Milled rice has been shown to contain about 78% carbohydrate. Many reports on variability in protein content in rice are available. Usually, the average value of total crude protein content is taken as 7%, and rice having more than 10% total crude protein is considered to be of high protein type ^[2]. Milled rice is referred to as “polished” or “whitened” and there are various degrees or fractions of polishing-white rice implies between 8 and 10% bran removal. In general, the more rice bran is removed from the grain during polishing, the more vitamins and minerals are lost. Protein loss due to milling is estimated at between 10 and 15 percent ^[3].

In order to increase the essential mineral nutrient in rice; two main strategies may be followed:

- Selection of genotypes with high mineral content
- Biofortification.
- Nutritional quationification.

Biofortification differs from ordinary fortification because it focuses on improving plant products nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed ^[4]. As such, biofortification is seen as an upcoming strategy for dealing with deficiencies of micronutrients in the developing world. In the case of iron, WHO estimated that biofortification could help curing the 2 billion people suffering from iron deficiency; induced anemia ^[5]. The grain has large genetic variability in micronutrient

concentration. Hence, rice has been included in biofortification program which implicitly targets low income households who have limited access to commercially marketed fortified foods that are more readily available in urban areas. In all crops studied, it is possible to combine the high micronutrient density trait with high yield economically. Predictive benefit-cost analyses show biofortification to be important for controlling micronutrient deficiencies. Getting consumers to accept biofortified crops will be a challenge, but with the advent of good seed systems, the development of markets and products, and demand creation, this can become a reality [7].

Above mentioned facts clearly indicate that besides enhancing the growth and yield of rice, enrichment of the grains with nutrients holds immense importance to address more important nutrient malnutrition related global health problem.

We should focus on producing better food, not only more food, because health cannot come from pharmacy.

1. Quantifying mineral nutrients status in polished grains of rice lines.
 2. Quantifying the availability of nitrogen protein in polished grains of rice lines.
- Identifying high essential nutrients and high protein containing genotypes.

Material Methods

1. Sample collection and preparation

Seeds of 52 lines of rice were procured from National Bureau of Plant Genetic Resources, Seeds were dehusked by using palm dehusker. To obtain polished grains, dehusked grains were gently rubbed the between sand papers till aleurone layer was completely removed.

Table 1: Rice lines used in this study

S.N.	Line	Character	S.N.	Line	Character
1.	467349	Indigenous collection	27.	98858	Indigenous collection
2.	383441	Indigenous collection	28.	328439	Indigenous collection
3.	282418	Indigenous collection	29.	554782	Indigenous collection
4.	577324	Indigenous collection	30.	580290	Indigenous collection
5.	426076	Indigenous collection	31.	578465	Indigenous collection
6.	577587	Indigenous collection	32.	577282	Indigenous collection
7.	580272	Indigenous collection	33.	313140	Indigenous collection
8.	580270	Indigenous collection	34.	577109	Indigenous collection
9.	580439	Indigenous collection	35.	311862	Indigenous collection
10.	450026	Indigenous collection	36.	145594	Indigenous collection
11.	85722	Indigenous collection	37.	337528	Indigenous collection
12.	331166	Indigenous collection	38.	361732	Indigenous collection
13.	580185	Indigenous collection	39.	466824	Indigenous collection
14.	580344	Indigenous collection	40.	282212	Indigenous collection
15.	298479	Indigenous collection	41.	578030	Indigenous collection
16.	578485	Indigenous collection	42.	578118	Indigenous collection
17.	335396	Indigenous collection	43.	298559	Indigenous collection
18.	298552	Indigenous collection	44.	337569	Indigenous collection
19.	580440	Indigenous collection	45.	496926 [#]	Exotic collection
20.	464906	Indigenous collection	46.	577568	Indigenous collection
21.	384176	Indigenous collection	47.	350107	Indigenous collection
22.	580254	Indigenous collection	48.	282450	Indigenous collection
23.	578478	Indigenous collection	49.	298572	Indigenous collection
24.	413609	Indigenous collection	50.	416700	Indigenous collection
25.	466532	Indigenous collection	51.	278774	Indigenous collection
26.	282386	Indigenous collection	52.	377560	Indigenous collection

[#] 496926 (other lines are indigenous lines).

Seeds were dehusked by using palm dehusker. To obtain polished grains, dehusked grains were gently rubbed the between sand papers till aleurone layer was completely removed. Estimation Total nitrogen content in grains was determined by nitrogen analyzer (Pelicon, Model KEL 20L). Polished grains (100 mg) were taken in a Kjeldahl digestion tubes containing 3 g of catalyst mixture and to this 10 mL of concentrated sulfuric acid was added. Tubes were put in the digestion block, fitted with manifolds and scrubber. The temperature was gradually raised to 350° C. The digestion continued till the solution became colour less. After completion, samples were brought to room temperature. Distillation of digested samples was done by auto distillation system (Pelicon Distil EM). Kjeldahl tubes containing digested plant samples were fitted in the assembly. Sufficient amount (60-65 mL), 40% NaOH was added to it till the colour of the solution became brown. At the collection end a conical flask containing 24 mL 4% boric acid and 0.5 mL mixed indicator was put. The sample was allowed to steam distilled

to 9 minutes. The boric acid solution of the conical flask was titrated by 0.1 N HCL with the help of micro titration unit. At the end point light pink colour appeared.

The amount of nitrogen in the sample was calculated as

$$N \text{ (mg g}^{-1} \text{ dry weight)} = \frac{14 \times \text{Titrant value} \times \text{normality of acid} \times 1000}{\text{Sample weight (g)} \times 100}$$

Sample digestion for estimation of P, K and NaPolished rice grains were digested using di-acid digestion method. It consisted of concentrated H₂SO₄ and perchloric acid (60%). 100 mg of polished rice grains were taken in Kjelhdahl flasks containing 2.0 mL of concentrated H₂SO₄. The samples were heated for 1-2 minutes and then 2.0 mL of perchloric acid (60%) was added to the samples. Then the samples were heated directly in the digestion block till it became colourless. Samples were cooled and volume was raised up to 100 mL

with double glass distilled water and used for the estimation of different mineral elements viz. K, Na and P. A blank was prepared by taking same amount of H₂SO₄ and perchloric acid without samples and treating in the same manner. Phosphorus in plant samples (polished rice grains) was determined by method as described by [8]. Estimation A 15 mL of the digested stock solution was transferred to a 50 mL test tube. 5 mL of vandate- molybdate solution was added to it and finally volume was made up to 25 mL. It was mixed well and the colour intensity was read at 420 nm by setting blank as zero with the help of spectrophotometer (Systronics Viciscan 167). Standard curve for phosphorus was prepared by taking 2, 4, 6, 8 and 10 mL of 100 mg KH₂PO₄ L⁻¹ stock solution. Colour development was done in the same manner as that of the test solution. Phosphorus content was calculated from the standard curve. P content in samples was expressed as mg g⁻¹ weight of grains. Potassium contents were estimated in the digested plant samples by flame photometer (Systronics Flame Photometer-128). 2 ppm, 5 ppm and 10 ppm K solutions were used as standard solutions for estimation of potassium content. The reading was obtained in ppm and then expressed as mg g⁻¹ dry weight of the sample. Sodium contents were estimated in the digested plant samples by

flame photometer (Systronics Flame Photometer-128). 2 ppm, 5 ppm and 10 ppm Na solutions were used as standard solutions for estimation of potassium content. The reading was obtained in ppm and then expressed as mg g⁻¹ dry weight of the sample. Nitrogen proteins were estimated by following formulae [9].

$$\text{Nitrogen protein (mg g}^{-1}\text{ dry weight)} = \text{N content} \times 6.25$$

4. Statistical analysis

For determining the significance of difference among the genotypes means, and to draw the valid conclusions, the data obtained by various observations were subjected to statistical analysis by Duncan’s multiple range test (DMRT) by using software version SAS 9.2. Correlation between analyzed parameters were also calculated and significance of correlation was determined at degree of freedom n-2 (n= number of observations).

5. Categorization of lines

Categorization of lines under high, medium and low mineral nutrient contents in grains was on the basis of relative amounts. It was calculated using formula as given below:

$$X = \frac{\text{Highest value of a nutrient in a line} - \text{lowest value of the nutrient in a line}}{3}$$

Lines under high mineral nutrient category (a) = Line with highest nutrient Content - X

Lines under low mineral nutrient category (b) = Line with lowest nutrient Content + X

Lines under medium mineral nutrient category = lines between a and b

6. Chemicals and glass wares and plastic wares

All chemicals used were of analytical reagent grade. Glass wares of ASGI make unless stated otherwise. Plastic wares were of TORSONS make.

Result and Discussion

The present experiment entitled “Evaluation of mineral nutrients in polished grains of rice (*Oryza sativa* L.) lines” was conducted in polished grains of 52 lines of rice. The experiment was performed in Tissue Analysis Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The grains of different rice lines were collected from NBPGR, New Delhi.

The observations regarding macro nutrients (N, P, K) in polished grains of rice and micronutrients (Na, Fe, Zn) and nitrogen protein were made in polished grains of rice. The findings regarding to the observation are presented in this chapter.

Mineral nutrients

1. N content (mg g⁻¹ dry weight)

Data regarding N content (mg g⁻¹ dry weight) are presented in Table 4.1. The Duncan’s Multiple Range Test (DMRT) indicated the differences among mean values of the 52 lines of rice. Line 467349 contained the highest N and line 377560 the lowest in polished grains. These lines were also classified in high, medium and low N contents as illustrated in Table 4.2. Lines 467349> 383441> 282418> 577324> 426076> 577587 contained high N in polished grains. Lines 580272> 580270> 580439> 450026> 85722> 331166> 580185> 580344> 298479> 578485> 335396> 298552> 580440> 464906> 384176 contained medium N and lines 580254> 578478> 413609>466532> 282386> 98858> 328439> 554782> 580290> 578465> 577282> 313140> 577109> 311862> 145594> 337528> 361732> 466824> 282212> 578030> 578118> 298559> 337569> 496926> 577568> 350107> 282450> 298572> 416700> 278774> 377560 contained low N in polished grains

Table 2: Nitrogen content (mg g⁻¹ dry weight) in polished grains of 52 lines of rice.

S. No.	Line with high N	N content (mg g ⁻¹ dry weight)	S. No.	Line with medium N	N content (mg g ⁻¹ dry weight)	S. No.	Line with low N	N content (mg g ⁻¹ dry weight)
1.	467349	27.94 ^{a*}	1.	580272	23.30 ^{bcde}	1.	580254	20.23 ^{ghijklmnopq}
2.	383441	26.23 ^a	2.	580270	22.99 ^{cdef}	2.	578478	19.31 ^{ghijklmnopqr}
3.	282418	25.97 ^{ab}	3.	580439	22.64 ^{defg}	3.	413609	19.95 ^{ghijklmnopqr}
4.	577324	25.36 ^{abc}	4.	450026	22.45 ^{defgh}	4.	466532	19.94 ^{hijklmnopqr}
5.	426076	24.78 ^{abcd}	5.	85722	21.66 ^{efghi}	5.	282386	19.80 ^{hijklmnopqr}

6.	577587	24.52 ^{bcd} e	6.	331166	21.48 ^{fg} hij	6.	98858	19.52 ^{hijklm} nopqrs
			7.	580185	21.42 ^{fg} hijk	7.	328439	19.50 ^{ijklm} nopqrs
			8.	580344	21.31 ^{fg} hijkl	8.	554782	19.31 ^{ijklm} nopqrs
			9.	298479	21.10 ^{fg} hijkl	9.	580290	19.14 ^{ijklm} nopqrs
			10.	578485	21.04 ^{fg} hijklm	10.	578465	19.06 ^{ijklm} nopqrs
			11.	335396	21.00 ^{fg} hijklmn	11.	577282	18.99 ^{ijklm} nopqrs
			12.	298552	20.76 ^{fg} hijklmn	12.	313140	18.72 ^{ijklm} nopqrs
			13.	580440	20.69 ^{fg} hijklmno	13.	577109	18.73 ^{ijklm} nopqrs
			14.	464906	20.54 ^{fg} hijklmnop	14.	311862	18.70 ^{ijklm} nopqrs
			15.	384176	20.50 ^{fg} hijklmnop	15.	145594	18.78 ^{ijklm} nopqrs
						16.	337528	18.51 ^{klm} nopqrs
						17.	361732	18.94 ^{klm} nopqrs
						18.	466824	18.59 ^{klm} nopqrs
						19.	282212	18.48 ^{klm} nopqrs
						20.	578030	18.32 ^{klm} nopqrs
						21.	578118	18.30 ^{lm} nopqrs
						22.	298559	18.15 ^m nopqrs
						23.	337569	18.04 ^m nopqrs
						24.	496926 [#]	18.02 ^{no} pqrs
						25.	577568	17.90 ^{no} pqrs
						26.	350107	17.71 ^o pqrs
						27.	282450	17.74 ^o pqrs
						28.	298572	17.65 ^p qrs
						29.	416700	17.48 ^r s
						30.	278774	17.29 ^r s
						31.	377560	16.82 ^s

[#] 496926 (other lines are indigenous lines).

*Indigenous lines with different letter differ significantly with each other.

2. P content (mg g⁻¹ dry weight)

Data regarding P content (mg g⁻¹ dry weight) are presented in Table 4.3. The Duncan’s Multiple Range Test (DMRT) indicated the differences among mean values of the 52 lines of rice. Line 383441 contained the highest P and line 496926 the lowest. These lines were also classified in high, medium and low P content categories (Table 4). The lines 383441> 577324> 298552 were grouped under high P content category. Lines 426076> 282418> 464906> 328439>298479>

577587> 554782> 331166> 578030> 282450> 337528> 335396> 580440> 282386> 577568> 580344> 361732 were classified as medium P content category lines and lines 577109> 350107> 578478> 578465> 466824> 578485> 450026> 580439> 282212> 337569> 413609> 98858> 577282> 384176> 298572> 578118> 311862> 580290> 467349> 580272> 377560> 85722> 278774> 580185> 580270> 580254> 313140> 298559> 416700> 145594> 466532> 496926 under low P category lines.

Table 3: Phosphorus content (mg g⁻¹ dry weight) in polished grains of 52 lines of rice

S. No.	Lines with high P	P content (mg g ⁻¹ dry weight)	S. No.	Lines with medium P	P content (mg g ⁻¹ dry weight)	S. No.	Lines with low P	P content (mg g ⁻¹ dry weight)
1.	383441	1.24 ^{a*}	1.	426076	0.92 ^c	1.	577109	0.76 ^{hijklm}
2.	577324	1.06 ^b	2.	282418	0.87 ^{cd}	2.	350107	0.75 ^{ijklmn}
3.	298552	1.02 ^b	3.	464906	0.86 ^{cd}	3.	578478	0.75 ^{ijklmn}
			4.	328439	0.85 ^{cd}	4.	578465	0.75 ^{ijklmn}
			5.	298479	0.84 ^{def}	5.	466824	0.73 ^{ijklmno}
			6.	577587	0.84 ^{defg}	6.	578485	0.73 ^{ijklmno}
			7.	554782	0.82 ^{defgh}	7.	450026	0.73 ^{ijklmnop}
			8.	331166	0.82 ^{defgh}	8.	580439	0.72 ^{klmnopq}
			9.	578030	0.81 ^{defghi}	9.	282212	0.70 ^{lmnopqr}
			10.	282450	0.80 ^{efghij}	10.	337569	0.70 ^{lmnopqr}
			11.	337528	0.79 ^{fghij}	11.	413609	0.70 ^m nopqrs
			12.	335396	0.78 ^{fghij}	12.	98858	0.70 ^m nopqrst
			13.	580440	0.78 ^{ghijk}	13.	577282	0.69 ^{no} pqrst
			14.	282386	0.77 ^{hijklm}	14.	384176	0.69 ^{no} pqrst
			15.	577568	0.77 ^{hijkl}	15.	298572	0.68 ^{op} qrstuv
			16.	580344	0.77 ^{hijklm}	16.	578118	0.67 ^{op} qrstuvw
			17.	361732	0.77 ^{hijklm}	17.	311862	0.67 ^p qrstuvw
						18.	580290	0.66 ^{rst} vwxy
						19.	467349	0.65 ^{rst} vwxy
						20.	580272	0.65 ^{rst} vwxy
						21.	377560	0.65 ^{rst} vwxy
						22.	85722	0.64 ^{rst} vwxy
						23.	278774	0.64 st vwxy
						24.	580185	0.63 ^t vwxyz
						25.	580270	0.63 ^{uv} wxyz

						26.	580254	0.63 ^{u v w x y z}
						27.	313140	0.61 ^{v w x y z}
						28.	298559	0.61 ^{w x y z}
						29.	416700	0.60 ^{x y z}
						30.	145594	0.59 ^{a y z}
						31.	466532	0.57 ^{a z}
						32.	496926	0.54 ^a

*Indigenous lines with different letter differ significantly with each other.

4. K content (mg g⁻¹ dry weight)

Data regarding K content (mg g⁻¹ dry weight) are presented in Table 4.4. The Duncan’s Multiple Range Test (DMRT) indicates the differences among mean values of the 52 lines of rice. Line 282386 contained the highest K and line 85722 the lowest. The collections were also classified in high, medium and low K content categories (Table4.5). Lines 282386> 467349> 580270> 383441> 413609> 145594> 466824> 282450> 337569> 580440> 298572> 98858> 466532>

578465> 311862> 282212> 577282> 384176> 578118 contained high K. Lines 298552> 337528> 577324> 426076> 577568> 578478> 298479> 5775887 contained medium amount of K and lines 298559> 496926> 377560> 313140> 416700> 282418> 580272> 580185> 580254> 361732> 464906> 331166> 578485> 577109> 450026> 335396> 554782> 350107> 580439> 580344> 580290> 578030> 328439> 278774> 85722 contained low K in polished grains.

Table 4: K content (mg g⁻¹ dry weight) in polished grains of 52 lines of rice.

S. No.	Lines with high K	K content (mg g ⁻¹ dry weight)	S. No.	Lines with medium K	K content (mg g ⁻¹ dry weight)	S. No.	Lines with low K	K content (mg g ⁻¹ dry weight)
1.	282386	0.33 ^{a*}	1.	298552	0.24 ^{d e f g}	1.	298559	0.15 ^{i j}
2.	467349	0.32 ^{a b}	2.	337528	0.23 ^{e f g h}	2.	496926 [#]	0.14 ^{j k}
3.	580270	0.31 ^{a b}	3.	577324	0.22 ^{f g h}	3.	377560	0.13 ^{j k l}
4.	383441	0.30 ^{a b c}	4.	426076	0.22 ^{g h}	4.	313140	0.13 ^{j k l}
5.	413609	0.30 ^{a b c}	5.	577568	0.21 ^{g h}	5.	416700	0.12 ^{j k l m}
6.	145594	0.30 ^{a b c}	6.	578478	0.21 ^{g h}	6.	282418	0.12 ^{j k l m n}
7.	466824	0.29 ^{a b c d}	7.	298479	0.21 ^{g h}	7.	580272	0.11 ^{j k l m n o}
8.	282450	0.29 ^{a b c d}	8.	577587	0.18 ^{h i}	8.	580185	0.11 ^{j k l m n o}
9.	337569	0.28 ^{a b c d e}				9.	580254	0.10 ^{j k l m n o}
10.	580440	0.28 ^{a b c d e f}				10.	361732	0.10 ^{j k l m n o}
11.	298572	0.27 ^{b c d e f}				11.	464906	0.09 ^{k l m n o}
12.	98858	0.26 ^{c d e f g}				12.	331166	0.09 ^{l m n o}
13.	466532	0.26 ^{c d e f g}				13.	578485	0.09 ^{l m n o}
14.	578465	0.26 ^{c d e f g}				14.	577109	0.08 ^{l m n o}
15.	311862	0.25 ^{c d e f g}				15.	450026	0.08 ^{l m n o}
16.	282212	0.25 ^{c d e f g}				16.	335396	0.08 ^{l m n o}
17.	577282	0.25 ^{c d e f g}				17.	554782	0.08 ^{l m n o}
18.	384176	0.25 ^{c d e f g}				18.	350107	0.08 ^{l m n o}
19.	578118	0.25 ^{c d e f g}				19.	580439	0.07 ^{m n}
						20.	580344	0.07 ^{m n}
						21.	580290	0.07 ^{m n}
						22.	578030	0.07 ^{m n}
						23.	328439	0.07 ^{m n}
						24.	278774	0.06 ^o
						25.	85722	0.06 ^o

496926 (other lines are indigenous lines).

*Indigenous lines with different letter differ significantly with each other.

5. Na content (mg g⁻¹ dry weight)

Data regarding Na content (mg g⁻¹ dry weight) are presented in (Table 5). The Duncan’s Multiple Range Test (DMRT) indicated the difference among mean values of the 52 lines of rice. Line 383441 contained the highest Na and line 85722 contained the lowest Na in polished grains. Lines were also classified in high, medium and low Na content categories (Table 5). Lines 383441> 426076> 57758> 298552 contained high Na in polished grains. Lines 282418> 337528> 298479>

337569> 98858> 466532> 578465> 577324> 577282> 413609 contained medium amount of Na while lines 311862> 578478> 578030> 464906> 282386> 466824> 577568> 2822125> 580440> 298572> 282450> 450026> 331166> 580270> 335396> 467349> 361732> 577109> 145594> 328439> 580344> 578118> 554782> 578485> 350107> 384176> 377560> 298559> 278774> 580439> 580272> 416700> 580185> 496926> 313140> 580254> 580290> 85722 contained low Na in polished grains.

Table 5: Data regarding Na content (mg g⁻¹ dry weight) are presented

S. No.	Lines with high Na	Na content (mg g ⁻¹ dry weight)	S. No.	Lines with medium Na	Na content (mg g ⁻¹ dry weight)	S. No.	Lines with low Na	Na content (mg g ⁻¹ dry weight)
1.	383441	0.57 ^{a*}	1.	282418	0.36 ^{c d}	1.	311862	0.20 ^{e f g h}
2.	426076	0.46 ^b	2.	337528	0.30 ^{d e}	2.	578478	0.20 ^{e f g h}
3.	577587	0.44 ^{b c}	3.	298479	0.30 ^{d e}	3.	578030	0.20 ^{e f g h}

4.	298552	0.43 ^{bc}	4.	337569	0.30 ^{de}	4.	464906	0.20 ^{efghi}
			5.	98858	0.25 ^{ef}	5.	282386	0.20 ^{efghi}
			6.	466532	0.25 ^{ef}	6.	466824	0.20 ^{efghi}
			7.	578465	0.24 ^{ef}	7.	577568	0.19 ^{efghij}
			8.	577324	0.22 ^{efg}	8.	282212	0.19 ^{efghij}
			9.	577282	0.22 ^{efg}	9.	580440	0.19 ^{efghij}
			10.	413609	0.21 ^{efg}	10.	298572	0.19 ^{efghij}
						11.	282450	0.19 ^{efghij}
						12.	450026	0.19 ^{efghij}
						13.	331166	0.19 ^{efghij}
						14.	580270	0.19 ^{efghij}
						15.	335396	0.18 ^{efghij}
						16.	467349	0.18 ^{efghij}
						17.	361732	0.18 ^{efghij}
						18.	577109	0.17 ^{efghij}
						19.	145594	0.17 ^{efghij}
						20.	328439	0.16 ^{efghijk}
						21.	580344	0.16 ^{efghijk}
						22.	578118	0.16 ^{efghijk}
						23.	554782	0.16 ^{efghijk}
						24.	578485	0.15 ^{efghijk}
						25.	350107	0.15 ^{efghijk}
						26.	384176	0.12 ^{efghijkl}
						27.	377560	0.12 ^{efghijkl}
						28.	298559	0.11 ^{efghijkl}
						29.	278774	0.10 ^{efghijkl}
						30.	580439	0.09 ^{efghijkl}
						31.	580272	0.09 ^{efghijkl}
						32.	416700	0.09 ^{efghijkl}
						33.	580185	0.09 ^{efghijkl}
						34.	496926 [#]	0.09 ^{efghijkl}
						35.	313140	0.09 ^{efghijkl}
						36.	580254	0.06 ^{efghijkl}
						37.	580290	0.03 ^{efghijkl}
						38.	85722	0.03 ^{efghijkl}

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