

Short Communication: Selection of doubled haploid lines of rainfed lowland rice in preliminary yield trial

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Abstract. Akbar MR, Purwoko BS, Dewi IS, Suwarno WB, Sugiyanta. 2019. Short Communication: Selection of doubled haploid lines of rainfed lowland rice in preliminary yield trial. *Biodiversitas* 20: 2796-2801. Yield trial is an important step in rice breeding program. This research was aimed at evaluating agronomic characters and selecting the best doubled haploid rainfed rice lines for next advanced yield trial. An experiment was conducted in Indramayu from March to August 2017. The research was arranged in randomized complete block design (RCBD) with three replications. Materials used were fifty-eight doubled haploid lines and two check varieties namely Ciherang and Inpari 18. The results revealed that all observed characters had broad genetic variability and high heritability. The number of productive tillers and the number of filled grains per panicle had a positive correlation and direct effect on productivity. Based on index selection, thirty lines were selected to have good agronomic characters and high yield. These lines had characteristics of plant height (85.7-124.7 cm), number of productive tillers (8.6-14.8 tillers), day to harvest (104.0-117.3 days), number of filled grains per panicle (136.7-264.7 grains), number of total grains per panicle (152.0-305.7 grains), weight of 1000 grain (21.5-31.3 g), and productivity (4.1-6.8 tons ha⁻¹).

Keywords: Good agronomic trait, high yield potential, selection index

INTRODUCTION

Rice (*Oryza sativa*) is a staple food in Indonesia. Indonesia's population reaches 261.9 million in 2017 with a growth of 1.49% every year (BPS-Statistics Indonesia 2018). Rice consumption is predicted to increase in the future. The effort to increase rice production needs to be done to maintain national food stability. One of problems is the decline of irrigated lowland rice area due to the high conversion. The main source of national rice production comes from irrigated lowland rice so that the decline of the land will have a major impact on national rice production. As an alternative to overcome the problem is by utilizing land which can be planted with rice.

Rainfed lowland area has the potential to be developed despite of having low water availability. The main source of irrigation of the land comes from rainwater. Indonesia has 3.4 million hectares of rainfed rice area (Ministry of Agriculture Republic of Indonesia 2018) with range of productivity 3.0-3.5 tons ha⁻¹ (Widiyantoro and Toha 2010). Productivity of rainfed lowland rice may decrease dramatically due to poor land conditions and drought. Breeding for high yielding rainfed rice varieties and tolerant to drought can become alternative to solve the problems.

Rainfed rice breeding is expected to obtain a pure line to release new varieties. In conventional breeding, it takes

four to five years to obtain pure lines. On the other hand, rice anther culture can produce a pure line as fully homozygous line only need one generation or only takes one year, so it can increase efficiency of the selection process as well as saving costs, time, and labor (Dewi and Purwoko 2012; Mishra and Rao 2016; Purwoko 2017).

Anther culture is in vitro culture technique using anther to produce haploid plants. In rice species, there is spontaneous doubling of chromosome, so it makes breeding using anther culture easier and faster. The application of anther culture techniques in rice breeding is started with the selection of crossing parents, crossbreeding of the parents, planting the donor explant, in vitro anther culture of donor plant, callus induction, green plant regeneration, rooting, acclimatization, doubled haploid (DH) plant characterization, DH plant seed propagation, and desirable character selection (Dewi and Purwoko 2012).

Selection can be done directly on the main character, which is productivity (Acquaah 2007). However, the character is controlled by many genes (Singh et al. 2000) so the mechanism is very complex (Islam et al. 2017). Selection index is a selection method based on several characters simultaneously (Rajamani 2016). The advantages of this method are the standardization of each character so that characters have the same degree (Akter et al. 2010). The selection index value is obtained by setting

the weighting of each character according to its economic value (Smith 1936; Singh and Chaudhary 2007). Genotypes with the largest value will be selected for further evaluation.

Breeding for rice varieties needs several steps to obtain desirable lines. The first step is the characterization of doubled haploid lines and the next step is evaluation of its yield potential. The previous research was done by Gunarsih et al. (2016). It had obtained rainfed rice lines by using anther culture. These lines need to be evaluated further in preliminary yield trial. The preliminary yield trial is conducted to evaluate yield potential at initial stage. This trial uses small plots due to a large number of tested lines. Selection will be carried out from the preliminary yield trial and evaluation will be continued further in advanced yield trial. This research was aimed at evaluating agronomic characters and selecting the best doubled haploid rainfed rice lines for next advanced yield trial.

MATERIALS AND METHODS

Plant materials

The genetic materials consisted of 58 doubled haploid lines. They were derived from anther culture of F1. Two checks varieties, i.e. Ciherang and Inpari 18 were included. The combination of cross breeding populations (F1s), were: 1) CG-7 = Inpago 8 x B12825E-T1-25, 2) CG-8 = Inpago 8 x IR8770514-11-SKI-12, 3) CG-9 = Inpago 8 x IR83140-11-B, 4) CG-10 = B1111430D-MR-1-PN-3-MR-2-Si-3-PN x B12825E-T1-25, 5) CG-11 = B1111430D-MR-1-1-PN-3-MR-2-Si-3-PN x IR87705-14-11-SKI-12, 6) CG-12 = B1111430D-MR-1-1-PN-3-MR-2-Si-3-PN x IR8314011-B.

Procedures

The experiment was conducted in Indramayu, Indonesia during March-August 2017 using randomized complete block design with three replications. It consisted of 58 doubled haploid lines and two check varieties (Ciherang and Inpari 18) as treatments. The experimental unit was a 1 m x 4 m plot. The seedlings were planted 21 days after sowing with plant spacing of 25 cm x 25 cm. Observations were done on plant height, number of productive tillers, days to 50% flowering, days to harvest, panicle length, number of filled grain per panicle, number of total grains per panicle, percentage of filled grain per panicle, 1000-grain weight, and productivity. The other characters were considered for selection, i.e. panicle exertion and awn existence (IRRI 2013).

Data analysis

Data were analyzed using F test variance. The estimation of genetic parameters includes estimation of genotypic variance, environmental variance, and phenotypic variance. Furthermore, phenotypic correlation (Singh and Chaudhary 2007) was analyzed to see the correlation among characters towards productivity. Path analysis was done to see the direct effect on character which has correlation with productivity. Selected

characters will be standardized (Walpole 1982) to be used in selection using weighted selection indices (Falconer and Mackay 1996) based on the value of principal component analysis. The data was analyzed using STAR IRRI and R Studio software.

The value of the variances were obtained through:

$$\sigma_e^2 = \text{MS error}, \sigma_g^2 = (\text{MS genotype} - \text{MS error})/r, \sigma_p^2 = \sigma_g^2 + (\sigma_e^2/r)$$

MS = mean of square, σ_g^2 = genotypic variance, σ_p^2 = phenotypic variance, σ_e^2 = environmental variance. Grouping of broad sense heritability was done according to Stanfield (1983): high ($0.50 < h_{bs}^2 < 1.00$), moderate ($0.20 < h_{bs}^2 < 0.50$), and low ($h_{bs}^2 < 0.20$).

Prediction of broad-sense heritability (h_{bs}^2) based on entry means was calculated following Acquah (2007). The selected agronomic important traits (X) were weighted using the value of principal component analysis (PC). Determination of the selection index were conducted based on Falconer and Mackay (1996): $I = b_1X_1 + b_2X_2 + b_3X_3 + \dots + b_nX_n$.

RESULTS AND DISCUSSION

Agronomic characters variability

In general, the observed agronomic characters had low standard deviation values and wide range values (Table 1). A wide range value indicated a high variability of these characters (Singh and Chaudhary 2007). The results of the variance analysis showed that the genotype had a significant effect on all observed characters (Table 1). The coefficient of variance on all characters ranged from 1.25-16.65%. The coefficient of variance showed heterogeneity in the population (Mattjik and Sumertajaya 2013). Bowman (2001) stated that the lower the coefficient of variance obtained in the data, the higher the degree of accuracy.

Variance component and heritability value

Genetic variability (GV) has an important role in conducting selection activities in a population. Broad genetic variability means that selection of these characters is effective. All observed characters had broad categories of genetic variability (Table 2). Broad or narrow genetic variability is determined by GV (σ_g^2) and standard deviation of GV ($\sigma\sigma_g^2$). In this study, all traits had broad genetic variability, because of $\sigma_g^2 > 2\sigma\sigma_g^2$. These findings were similar to Akinwale et al. (2011) and Sadeghi (2011).

Heritability described the proportion of genetic variance to phenotype variance (Falconer and Mackay 1996). The estimated value of character heritability ranged from 0.52 (percentage of filled grain per panicle) to 0.97 (flowering age and harvesting age) (Table 2). Grouping heritability values according to Stanfield (1983), all characters had high category of heritability values. High heritability values were also reported by researchers on plant height and number of productive tillers (Sadeghi 2011), flowering age, harvest age, panicle length, and 1000 weighted grain (Seyoum et al. 2012), and productivity (Ullah et al. 2011).

Characters with high heritability value indicated that genetic factors were more dominant or genetic factors contribute more than environmental factors (Begum et al. 2015). Effective selection can be performed on characters which had broad genetic variability and high heritability.

Relationship among agronomic characters and path analysis

Correlation analysis is very important to determine the relationship among characters and important character which correlates to yield (Hastini et al. 2019). The results of the correlation analysis among agronomic characters are presented in Table 3. The results showed that productivity trait had a positive and significant correlation towards the number of productive tillers ($r = 0.52^{**}$; $p < 0.01$), number of filled grain per panicle ($r = 0.42^{**}$; $p < 0.01$), and percentage of filled grain per panicle ($r = 0.41^{**}$). The same results were obtained on the number of productive tillers (Kole et al. 2008), number of filled grain per panicle and percentage of filled grain per panicle (Akbar et al. 2018). On the other hand, productivity trait had a negative and significant correlation with day to flowering ($r = -0.45^{**}$), day to harvesting ($r = -0.52^{**}$), and number of

empty grain per panicle ($r = -0.30^*$). The same observations were obtained by Soyoum et al. (2012) on flowering age and harvesting age.

Path analysis is used to determine the direct and indirect effect on the character that correlates with productivity. The results of path analysis (Table 4) showed that the number of productive tillers (0.46) had the highest positive direct effect on productivity followed by the number of filled grains per panicle (0.39). The number of productive tillers showed the highest positive direct effect and had a positive correlation with productivity ($r = 0.52^{**}$) (Table 3). In addition, the number of filled grains per panicle showed a positive direct effect and had a positive correlation with productivity ($r = 0.42^{**}$) (Table 3). These same results were also found on the number of productive tillers (Soyoum et al. 2012; Agahi et al. 2007) and number of filled grains per panicle (Hairmansis et al. 2010). The residual effect of the model was 0.664 indicating that contribution of component characters on productivity was 0.336. The rest variability was influenced by other factors such as environmental factor and other characters not observed in this study.

Table 1. Means, population range, and analysis of variance of agronomic traits of 58 doubled haploid lines of rainfed rice

Character	Mean \pm S.D.	Population range	Mean of check		Mean of square	Coefficient of variance (%)
			Ciherang	Inpari 18		
Plant height	103.2 \pm 10.8	85.7 - 124.7	98.6	93.6	353.2 ^{**}	3.49
Number of productive tillers	11.2 \pm 1.5	8.6 - 14.8	12.0	9.0	7.1 ^{**}	14.18
Day to 50% flowering	81.6 \pm 3.8	75.0 - 93.0	82.3	75.0	43.6 ^{**}	1.46
Day to harvesting	103.1 \pm 4.7	97.0 - 113.0	101.0	97.7	68.7 ^{**}	1.25
Panicle length	26.6 \pm 1.6	23.3 - 29.3	24.3	26.7	7.5 ^{**}	5.93
Number of filled grains	173.8 \pm 27.9	127.0 - 264.7	139.7	157.3	2379.5 ^{**}	18.26
Number of unfilled grains	39.3 \pm 18.5	13.3 - 109.0	16.7	66.3	0.1 ^{**}	16.62
Number of total grains	213.0 \pm 34.3	152.0 - 305.7	156.3	223.7	3587.1 ^{**}	16.51
Percentage of filled grains	82.1 \pm 7.1	57.8 - 93.5	89.7	73.0	154.3 ^{**}	10.45
Weight of 1000 grains	25.2 \pm 2.1	20.7 - 31.3	25.5	29.9	14.0 ^{**}	6.1
Productivity	4.5 \pm 0.8	3.0 - 6.8	4.0	3.6	2.1 ^{**}	16.65

Note: S.D.= standard deviation, ^{**} = significantly different at α 0.01

Table 2. Component of variance and broad-sense heritability of agronomic traits of doubled haploid lines of rainfed rice

Character	σ^2_g	σ^2_p	$2\sigma\sigma^2_g$	Genetic variability criteria	h^2_{bs}	
					Value	Criteria
Plant height	113.42	117.75	42.66	Broad	0.96	High
Number of productive tillers	1.53	2.36	0.88	Broad	0.65	High
Day to 50% flowering	14.05	14.52	5.26	Broad	0.97	High
Day to harvesting	22.28	22.92	8.30	Broad	0.97	High
Panicle length	1.69	2.51	0.93	Broad	0.67	High
Number of filled grains	457.64	793.18	300.02	Broad	0.58	High
Number of unfilled grains	202.08	349.50	132.17	Broad	0.58	High
Number of total grains	783.16	1195.71	445.93	Broad	0.65	High
Percentage of filled grains	26.90	51.43	19.67	Broad	0.52	High
Weight of 1000 grains	3.89	4.68	1.71	Broad	0.83	High
Productivity	0.52	0.71	0.26	Broad	0.73	High

Note: σ^2_g : genotypic variance, σ^2_p : Phenotypic variance, $2\sigma\sigma^2_g$: 2x standard deviation of genotypic variance, h^2_{bs} : broad-sense heritability

Table 3. Pearson correlation coefficient of agronomic character of doubled haploid lines of rainfed rice

	PH	NPT	FD	HA	PL	NFG	NUG	NTG	PFG	WOG
NPT	-0.22									
DF	0.16	-0.31**								
DH	0.01	-0.37**	0.87**							
PL	0.47**	-0.13	0.38**	0.29**						
NFG	0.17	-0.07	-0.28*	-0.25	0.25					
NUG	0.07	-0.2	0.5**	0.5**	0.54**	0.05				
NTG	0.17	-0.16	0.04	0.07	0.49**	0.84**	0.58**			
PFG	0.00**	0.17	-0.55**	-0.57**	-0.45**	0.21	-0.95**	-0.34**		
WOD	0.15	-0.06	-0.04	-0.11	0.15	-0.08	0.01	-0.07	-0.01	
PRD	0.12	0.52**	-0.45**	-0.52**	-0.04	0.42**	-0.30*	0.19	0.41**	0.18

Note: PH: plant height, NPT: number of productive tillers, FD: day to flowering, HA: day to harvesting, PL: panicle length, NFG: number of filled grains per panicle, NUG: number of unfilled grains per panicle, NTG: number of total grains per panicle, PFG: percentage of filled grains per panicle, WOG: weight of 1000 grains, PRD: productivity, ** : significant at α 0.01; * : significant at α 0.05

Table 4. Path coefficient of several characters towards yield on doubled haploid lines of rainfed rice

	Direct effect	Indirect effect					Correlation with yield
		NPT	FD	HA	NFG	NUG	
NPT	0.46	-0.03	0.09	-0.03	0.00	0.03	0.52**
FD	0.10	-0.14	-0.22	-0.11	0.01	-0.10	-0.45**
HA	-0.25	-0.17	0.09	-0.10	0.01	-0.11	-0.52**
NFG	0.39	-0.03	-0.03	0.06	0.00	0.04	0.42**
NUG	0.02	-0.09	0.05	-0.12	0.02	-0.18	-0.30*
PFG	0.19	0.08	-0.06	0.14	0.08	-0.02	0.41**

Note: NPT: number of productive tillers, FD: day to flowering, HA: day to harvesting, NFG: number of filled grains per panicle, NUG: number of unfilled grains per panicle, PFG: percentage of filled grains per panicle, residual effect: 0.664

Table 5. Principal component analysis of agronomic character of doubled haploid lines of rainfed rice

Character	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Plant height	-0.20	0.05	-0.54	0.19	0.52	0.00
Number of productive tillers	0.16	-0.28	0.19	0.57	-0.05	-0.08
Day to 50% flowering	-0.03	0.50	0.01	0.19	0.06	0.39
Day to harvesting	-0.04	0.50	0.11	0.06	0.01	0.41
Panicle length	-0.27	0.21	-0.18	0.45	0.01	-0.50
Number of filled grains	-0.39	-0.24	0.18	-0.02	0.12	0.13
Number of unfilled grains	-0.27	0.32	0.18	0.21	-0.28	-0.23
Number of total grains	-0.47	-0.02	0.24	0.10	-0.05	-0.01
Percentage of filled grains	0.39	-0.16	0.20	0.15	0.19	-0.21
Weight of 1000 grains	-0.03	-0.05	-0.50	0.11	-0.72	0.25
Productivity	-0.08	-0.43	-0.08	0.38	-0.06	0.35
Standard deviation	2.01	1.77	1.27	1.14	0.97	0.76
Proportion of variance	0.31	0.24	0.12	0.10	0.07	0.04
Cumulative of variance	0.31	0.55	0.68	0.78	0.85	0.89
Eigen value	4.05	3.15	1.62	1.29	0.93	0.57

Principal Component Analysis

The results of principal component analysis (PC) showed that there were four PC with eigenvalues >1. Mattjik and Sumertajaya (2013) explained that PC with eigenvalues >1 can become representative model to be used

as a selection index because it can explain high variability. Kumar et al. (2016) stated that the selection should consider the PC value of the main character. Productivity character was selected as the main character because of breeding main objective to obtain lines with high productivity. The PC2 model was selected because it had a high productivity value (0.43). The other main characters were the number of productive tillers (0.28) and number of filled grains per panicle (0.24). The three characters had same direction as indicated by the value of the vector. Anshori et al. (2019) also obtained productivity and number of productive tillers as important selection character in rice.

Selection of doubled haploid lines

Understanding the relationship between yield and yield component is very important. Selection will give the best results if it uses several agronomic characters as character selection. Characters were chosen based on broad genetic variability, high heritability value, positive correlation towards productivity, and had direct effect. Characters that met these criteria were number of productive tillers (NPT) and number of filled grain per panicle (NFG). Productivity (PRD) character was chosen due to the main character. The weighted selection index was used to obtain an index that can be rated from the highest value. The weighting used PC2 values because the productivity character had the highest value (Table 5).

The character of productivity had an economic value greater than the other characters so that the weighting of the character of productivity must be greater than the weighting of other characters. Sabouri et al. (2008) explained that the character of production had three times greater weight than other characters. Based on this, weight of productivity was made three times greater. This is in accordance with the level of character's importance that productivity had the greatest weighted value compared to other characters. Index model according to the formula is as follow: Index = (3*0.43 PRD) + (0.28 NPT) + (0.24 JGI).

Table 6. Weighted selection index of 58 doubled haploid lines of rainfed rice

Rank	Genotype	NPT	NFG	PRD	Index
1	CG-9-81-1-1	12.5	212.0	6.8	4.07
2	CG-9-62-1-1	13.2	191.7	6.3	3.15
3	CG-12-30-1-2	10.9	251.3	6.0	2.82
4	CG-8-93-1-1	11.8	168.0	6.1	2.42
5	CG-9-68-1-4	14.3	161.7	5.6	2.14
6	CG-12-85-1-2	12.3	207.7	5.4	1.83
7	CG-9-81-1-2	11.5	230.3	5.3	1.66
8	CG-9-5-1-1	14.8	154.7	5.3	1.65
9	CG-8-9-1-3	11.6	160.9	5.6	1.52
10	CG-9-68-1-5	14.7	162.3	5.1	1.40
11	CG-11-35-1-1	12.4	161.7	5.3	1.32
12	CG-8-18-1-2	11.1	173.0	5.4	1.31
13	CG-9-2-1-5	12.6	183.3	5.1	1.21
14	CG-12-85-1-3	10.9	197.0	5.1	1.08
15	CG-9-53-1-1	12.0	173.7	5.1	1.07
16	CG-9-2-1-6	13.4	167.7	5.0	1.05
17	CG-11-69-1-1	11.0	196.7	5.0	0.93
18	CG-9-53-1-2	12.7	181.3	4.9	0.92
19	CG-12-71-1-1	11.4	201.3	4.9	0.85
20	CG-9-68-1-3	12.4	168.0	4.9	0.80
21	CG-9-46-1-1	10.8	188.3	5.0	0.78
22	CG-8-92-1-1	12.5	143.0	5.1	0.78
23	CG-8-18-1-1	11.6	139.3	5.2	0.73
24	CG-9-2-1-7	12.6	191.7	4.7	0.69
25	CG-8-9-1-4	10.9	192.0	4.9	0.61
26	CG-9-53-1-3	12.4	165.3	4.8	0.59
27	CG-12-30-2-1	10.9	161.3	4.9	0.47
28	CG-10-78-1-1	11.6	202.7	4.6	0.40
29	CG-12-73-1-1	11.5	216.0	4.4	0.29
30	CG-8-9-1-2	11.6	167.7	4.7	0.28
31	CG-12-58-1-1	8.6	264.7	4.5	0.27
32	CG-9-53-1-4	11.2	170.7	4.7	0.27
33	CG-7-72-1-7	8.6	194.3	4.8	0.15
34	CG-8-35-1-2	13.0	198.3	4.2	0.01
35	CG-8-9-1-5	11.1	197.0	4.4	-0.06
36	CG-7-72-1-1	9.0	208.3	4.3	-0.51
37	CG-9-27-1-1	12.3	127.0	4.2	-0.71
38	CG-12-30-1-3	10.2	172.3	4.1	-0.78
39	CG-9-26-1-6	9.2	161.3	4.3	-0.83
40	CG-8-92-1-2	11.3	136.7	4.1	-0.89
41	Ciherang	12.0	139.7	4.0	-0.92
42	CG-9-27-1-2	10.8	144.0	4.0	-1.07
43	CG-8-97-1-1	9.4	175.3	4.0	-1.09
44	CG-7-72-1-5	8.9	168.0	4.1	-1.18
45	CG-7-72-1-6	10.0	164.3	3.9	-1.21
46	CG-8-97-1-2	9.8	166.0	3.9	-1.33
47	CG-7-72-1-2	9.0	179.7	3.8	-1.39
48	CG-12-53-1-1	12.2	151.7	3.6	-1.40
49	CG-9-26-1-2	9.6	149.0	3.7	-1.79
50	CG-9-26-1-4	9.2	176.3	3.5	-1.93
51	Inpari 18	9.0	157.3	3.6	-1.95
52	CG-9-107-1-1	12.7	128.7	3.3	-2.00
53	CG-12-53-1-3	11.9	186.7	3.0	-2.03
54	CG-7-72-1-3	9.1	161.0	3.5	-2.10
55	CG-8-115-1-1	10.0	137.3	3.4	-2.28
56	CG-9-26-1-1	9.4	143.0	3.4	-2.33
57	CG-9-26-1-5	9.0	149.3	3.4	-2.34
58	CG-9-26-1-3	10.1	154.0	3.2	-2.38
59	CG-7-72-1-4	10.2	136.7	3.2	-2.46
60	CG-11-69-1-2	10.2	155.3	3.1	-2.54

Note: NPT= number of productive tillers, NFG= number of filled grain per panicle, PRD= productivity

From the formula, the selection index was calculated. Then, the index value will be sorted from the highest index to the lowest index. The lines with the highest index and above the check variety will be selected. The index value indicated that the CG-9-81-1-1 line had the highest index value of 4.07, while the CG-11-69-1-2 line had the lowest index value of -2.54 (Table 6). Based on the weighted selection index, 30 doubled haploid lines were selected to have higher index than Ciherang variety. Other agronomic characters were used as consideration for selection such as awn existence in grains. The following lines were not selected because they had awn on the grains: CG-9-68-1-4, CG-9-68-1-5, CG-9-68-1-3, CG-9-46-1-1, CG-9-2-1-7, CG-12-30-2-1, CG-10-78-1-1, CG-12-73-1-1, CG-7-72-1-1, and CG-9-27-1-1. Lines with awn will tend to be difficult to harvest and be dislike by farmers.

Based on index selection, it was concluded that there were thirty selected lines to be evaluated further in next yield trial. These lines had the characteristics of plant height (85.7-124.7 cm), number of productive tillers (8.6-14.8 tillers), day to harvest (104.0-117.3 days), number of filled grains per panicle (136.7-264.7 grains), number of total grains per panicle (152.0-305.7 grains), 1000 grain weight (21.5-31.3 g), and productivity (4.1-6.8 tons ha⁻¹).

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