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Determination of Plant Traits of Dwarf Fresh Bean Lines with Ayşe Kadın' Characteristics and Gene Loci for The Resistance to Anthracnose (Colletotrichum lindemuthianum (Sacc. & Magnus) Lambs. Scrib.) Disease

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Abstract

In this study, single line (pure-line) selection method was applied on local populations of dwarf fresh beans with the characteristics of Aysekadın type which were collected from Çarsamba, Terme, Tekkeköy towns of Çarsamba plain and Ladik region in 2003. Observantion gardens were set up in 2005 and consecutively pre-yield, local yield and regional yield tests were conducted in the years starting 2005 to 2009. In these lines, phenological observations and morphological measurements were determined in accordance to UPOV criteria and rates of their yields were examined. As a result of all assesments, 7 lines (KO, TK15, T26, Ç31, TK7, TK1 and T23) were approved to comfort with our purpose, selected as candidates for registration and were also examined for resistance to anthracnose disease by means of STS and SCAR molecular markers closely linked to anthracnose resistance *Co*- genes. While, resistance gene loci markers for Co-2, Co-4, Co-4[2] genes were present in all of the 7 lines, Co-1 gene locus marker was observed in only one of the line (KO) and 5 lines were positive for the marker of Co-6/co-8 gene loci. Two of the lines T26 and TK15 were registered under the variety names of "Karabacak" and "Zeynebim" respectively and given certification for production permits in 2009.

Key Words: Anthracnose, breeding, STS and SCAR molecular markers, *Phaseolus vulgaris*, pure-line selection, UPOV criteria.

INTRODUCTION

Although the origin of domestication for common bean (*Phaseolus vulgaris* L.) is America, it has spreaded throughout the world after the discovery of the Continent. The common bean, is a vital nutrition in Turkey, and cultivated almost 250-years. The comon bean is grown easily in both seaside and internal regions of the country as well as at the height of over 1000 m [19]. Turkey ranks in the 3rd place after China and Indonesia in fresh bean production (603,653 tons/year) [7]. Fresh beans are widely grown in the fields in the Middle Blacksea Region. Samsun is primarily a notable center with production of around 63.336 tons per year [10]. There are several different types of local fresh bean populations particularly in Samsun province, Carsamba plain and Ladik town [14,18]. These local populations indicate affluency in terms of genetic variations and awaits to be evaluated. Determination and preservation of the genetic variations of local populations in terms of their resistance against abiotic and biotic stresses guarantee the security of nutrition in the future of mankind. Although beans are easily grown in every part of the world, they come across different problems. Of the most important problem is anthracnose disease resulted from *Collethotrichum lindemuthianum* fungus, which is a widespread treat in Turkey as well as in the world. Anthracnose leads to immature seedlings death, drying in green parts of mature plants, spotting in beans and a great amount of economic losses [11, 1]. Five races of antrachnose disease were determined in middle Blacksea region, and inoculations of dwarf fresh bean lines and commercial varieties with these races between 2005 and 2007, have proven their susceptibility [15].

In the hope to benefit from the vast genetic variations in local dwarf fresh bean populations and to develop new varieties for local farmers, single line selection method has been applied to dwarf fresh "Aysekadın" type local

bean populations collected from Çarsamba plain and Ladik town. Several registration candidate lines for certification were selected over 7 years period through phenological, morphological measurements and yield tests. Also to determine these lines as potential gene sources for anthracnose resistance in breeding programs planned hereafter, gene profiles of the selected lines were screened by several resistance loci linked molecular markers.

MATERIALS and METHODS

Plant material and characterization

Total of 250 fresh bean populations were collected from Ladik town and Terme, Çarsamba, Tekeköy regions of Çarsamba plain between 2002 and 2003. Observation gardens were set up in 2003 and fourty one plants were selected with the characteristics of Ayse Kadın type by single-line selection method in 2004. Pre-yield tests for single plants in 2005, I and II yield tests in 2006-2007, and local yield experiments in Amasya were conducted in 2008-2009. Studies were conducted by Agricultural Research Institute of Blacksea Region-Samsun. Seeding was carried out_on 15 May 2008 and 13 May 2009. Regional yield tests were set up with 7 lines (KO, TK15, T26, Ç31, TK7, TK1 and T23) and 4 varieties (Volare, Gina, Balkız, Karayse) in random blocks repeated four times. Seeding was carried out in 4 rows per parcel through the intervals of seed by 70 X 20 cm (Row inter(RI) X Row on (RO)) and 5m long of parcel. Six kg CAN fertilizers per decares were added. Harvests were started in the second week of July and carried out 4 times until the last week of August in 2008-2009. The characterization of lines were detected in accordance to UPOV criteria. These characteristics were; duration of initial germination (day), duration of initial flourishing (day), 50% duration of flourishing (day), colour of flowers, colour of pods, stringy of pods, flesh shape of pods, spotting of pods, clarity of seeds in pods, shape of braktes, length of pods(cm), width of pods (mm), thickness of pod flesh (mm), length of brakte (mm), length of beak (mm), weight of 100 grain (gr), height of plant (cm), the number of branches of plant (unit), the number of pods per branch (unit), the number of seeds in pod (unit) and the yield (kg/da). The statistical analysis programme of "SAS" was applied to rate the yields [16].

Molecular characterization

Molecular markers were screened in 7 dwarf fresh bean lines (KO, TK15, T26, ÇC31, TK7, TK1 and T23) for resistant genes against anthracnose disease. Samples of 50 mg leaves collected from plants grown in greenhouse and DNA were isolated through Maxwell 16 Tissue DNA isolation kit (Promega) on automated Maxwell 16 machine. Total of six resistance gene loci and allelic forms were screened using primers and PCR reaction conditions as mentioned in the literatures [9]. The name of the STS/SCAR markers and corresponding resistance genes with expected marker band sizes (in paranthesis) were as follows; H20 (Co-2, 450 bp), C08 (Co-4, 910 bp), Y20 (Co-4^3, 830 bp), SAS13 (Co-4^2, 950 bp), SZ04 (Co-6, 567 bp), SB12 (Co-3/Co-9, 350 bp) ve SeactMcca (Co-1, 80 bp). Analysis of PCR products except with SeactMcca primers were carried out in 2% agarose gel electrophoresis and bands were visualised under UV illumination following ethidium bromide staining. Scoring of the results were performed by presence (+)/ absence (-) of the expected band size. The PCR results with SeactMcca primers were analysed using 6% polyacrylamide gel electrophoresis and visualised following silver nitrate staining. Due to the co-dominant nature of SeactMcca marker, as indicated in the literature [20], 80 bp bands scored as resistant and 79 bp bands were recorded as susceptible Co-1 gene alleles. Molecular studies were carried out in the Department of Molecular Biology and Genetics of Bogazici University.

RESULTS

Phenological observations

The results of the phenological observations were given in Table 1. Observations were taken in two consecutive years of 2008 and 2009. For the year 2008, 6-8 days for the initial germination of seeds, 33-41 days for the initial flourishing, and 38-46 days for 50% flourishing were determined. Similarly in the year 2009. 6-8 days for the initial germination, 38-42 days for the initial flourishing and 43-46 days for %50 flourishing were observed. The colour of flowers displayed by dwarf fresh bean lines with characterisitics of "Aysekadın" type and 4 control varieties were white, purple or dark purple (Table 1).

Table 1. Phenological traits and flower color of dwarf fresh bean lines with the characteristics of Aysekadın type (2008-2009)

Dwarf fresh bean lines and control varieties	Duration of initial germination (day)		Duration of initial flourishing (day)		% 50 duration of fourishing		Color of flower	
	2008	2009	2008	2009	2008	2009	2008	2009
1.Ç31	6	6	35	40	41	44	D.Purple	D.Purple
2.T26	6	6	35	40	41	43	D.Purple	D.Purple
3.TK7	6	6	34	38	40	43	Purple	Purple
4.TK15	6	6	34	39	40	43	Purple	Purple
5.Volare (CV)	6	6	34	39	40	44	Purple	Purple
6.Gina (CV)	6	6	33	39	38	43	White	White
7.T23	8	7	36	40	44	43	Purple	Purple
8.Karaayşe (CV)	8	7	34	39	40	43	D.Purple	D.Purple
9.Balkız (CV)	7	6	36	39	40	43	Purple	Purple
10.KO	8	8	41	42	46	46	Purple	Purple
11.TK1	8	8	41	41	46	44	Purple	Purple

Ç:Çarşamba, T: Terme, TK: Tekkekoy (towns located in Çarşamba plain), CV: Control variety, D:Dark

Morphological measurements

Stringy which is accepted as an unwanted characteristic in beans was not observed in any of the lines. In both measurements at 2008 and at 2009, pod colors were observed to be either light green or green. Spotting on pods were not determined except KO line, pod flesh shapes were narrow eliptic (NE), the clarity of seed in pods were either light (L), normal (N) or definite (D) and shape of braktes were narrow long (NL) (Table 2).

Average mean values for the measurements at 2008-2009 were 11,30 cm, 12,18 mm, 7,01 mm, 4,56mm and 6,71 mm for pod lengths, pod widths, pod flesh thickness, brakte lengths and beak lengths respectively. (Table 3). Above average mean values were obtained for TK7 line for pod length and pod width measurements for two years whereas, values for pod flesh thickness, brakte length and beak length were highest in lines KO, Ç31 and TK1 respectively (Table 3). Among the control varieties, pod width, brakte length and beak length in average

Table 2. Morphological traits of dwarf fresh bean lines with the characteristics of Aysekadın type (2008-2009)

Dwarf fresh bean lines and control varieties	Color of pod		spotting of pod		Flesh shape of pod		Clarity seed in pod		Shape of brakte	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
1.Ç31	G	G	NE	NE	NE	NE	D	D	NL	NL
2.T26	LG	LG	NE	NE	NE	NE	N	N	NL	NL
3.TK7	LG	LG	NE	NE	NE	NE	L	L	NL	NL
4.TK15	LG	LG	NE	NE	NE	NE	L	L	NL	NL
5.Volare (CV)	LG	LG	NE	NE	NE	NE	L	L	NL	NL
6.Gina (CV)	LG	LG	NE	NE	NE	NE	L	L	NL	NL
7.T23	G	G	NE	NE	NE	NE	N	N	-	NL
8.Karaayşe (CV)	LG	LG	NE	NE	NE	NE	N	N	NL	NL
9.Balkız (CV)	LG	LG	NE	NE	NE	NE	N	N	NL	NL
10.KO	LG	LG	E	E	NE	NE	N	N	NL	NL
11.TK1	LG	LG	NE	NE	NE	NE	D	D	NL	NL

Ç:Çarşamba, T:Terme, TK:Tekkeköy (towns located in Çarşamba plain), CV: Control variety, G:Green, LG:Light Green, E:Existent, NE:Nonexistent, L:Light, N:Normal,, D:Definite, NE:Narrow Eliptic, NL:Narrow Long

Table 3. Measurements of morphological characters in dwarf fresh bean lines with the characteristics of Aysekadın type.(2008-2009)

Dwarf fresh bean lines and control varieties	Length of pod (cm)		Width of pod flesh (mm)		Thickness of pod flesh (mm)		Length of brakte (mm)		Length of beak (mm)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
1.Ç31	10,74	10,89	11,74	12,78	8,17	6,55	5,30	5.04	5,53	6,30
2.T26	8,35	13,35	12,43	12,73	7,95	7,77	4,69	4,84	5,91	6,28
3.TK7	12,57	12,61	13,08	12,51	7,09	6,52	3,89	4,32	5,31	6,90
4.TK15	11,07	11,77	11,64	12,60	7,02	6,77	3,14	4,19	8,31	7,52
5.,KO	11,75	10,50	13,18	11.45	8,12	7.86	4,95	4,14	7,94	7,12
6.TK1	11,63	11,60	11,43	11,49	6,20	5,64	4,91	4,86	8,30	7,56
7.T23	9,99	11,49	11,21	12,37	6,44	6,11	5,07	-	5,29	5,76
Mean value	10,87	11,74	12,10	12,27	7,28	6,74	4,56	4,56	6,65	6,77
Average mean	11,30		12,18		7,01		4,56		6,71	
8.Volare (CV)	12,81	13,54	**13,93**	**14,15**	7,00	5,91	**4,82**	**5,29**	**7,80**	**8,57**
9.Gina (CV)	13,00	12,43	14,40	12,53	**7,78**	**7,12**	4,97	4,99	7,79	8,46
10.Karaayşe (CV)	10,30	10,16	11,68	12,53	6,65	5,95	4,60	4,64	5,96	4,90
11.Balkız (CV)	**12,95**	**13,56**	13,73	14,18	5,30	5,68	4,75	4,97	8,00	8,02

Ç:Çarşamba, T:Terme, TK: Tekkeköy (towns located in Çarşamba plain), CV: Control variety

mean values were highest for Volare while Balkız and Gina have shown the highest pod length and pod flesh thickness in average during 2008 and 2009.

Plant characteristics and yield (kg/da)

Plant characteristics of 7 lines and and 4 commercial control varieties including plant heigth, number of branches/plant, number of pods/branches, number of seeds/pods and 100 grain weigths were given in Table 4. Line T23 has shown the highest values in all characteristics measured except the number of seeds/pods for year 2008 and 2009. Seed numbers per pod were highest in the line TK7. Among the seven lines, the shortest plants were observed in Ç31 lines, the lowest branches were observed in TK7 lines, the lowest pods/branches were obtained in TK1 lines, the lowest seed numbers in a pod were observed in KO lines and the lowest weigth of 100 grains were obtained in TK15 lines (Table 4). Among the commercial varieties, while Volare and Balkiz have shared the similar values for all characteristics, Karaayse was the shortest variety with the highest number of pods in branches. Gina variety as being the tallest plants among the control commercial varieties, has given the lowest weigth of 100 grains. Although in terms of mean values for 7 lines, most of the measured plant characteristics were in aggrement with the values obtained from the control varieties in two years, values for weigth of 100 grains were always higher in lines compare to control varieties.

The results of regional yield test of 7 lines with Aysekadin types and yield rates of each lines in year 2008 to 2009 were given in Table 5. Yield evaluations of 2008 and combined yields (2008 and 2009) have shown that the top three highest yields belonged to lines T26 with 1750.0 kg/da, Ç31 with 1722.5 kg/da and TK7 with 1415.0 kg/da respectively and the lowest yields were obtained by line KO. However in year 2009, top two rates have exchanged places between line T26 and Ç31 and the highest yield was obtained from Ç21 with 1990.0 kg/da. (Table 5).

Table 5. Rates of regional yield of lines with the characteristics of Aysekadın in 2008-2009 (kg/da)

Dwarf fresh bean lines and control varieties	Yield rates of 2008 (kg/da)	Yield rates of 2009 (kg/da)	Combined analysis of yield rates of 2008-2009 (kg/da)
1.Ç31	**1722,5 ab**	**1990,0 a**	**1856,2 a**
2.T26	**1750,0 a**	**1615,0 b**	**1682,5 ab**
3.TK7	**1415,0 abc**	**1517,5 ab**	**1466,2 bc**
4.TK15	1182,5 abcd	1382,5 bc	1282,5 cd
5.TK1	633,2 d	390,0 ef	595,3 fg
6.KO	**698,7 d**	**557,5 f**	**544,3 g**
7.T23	1069,2 cd	890,0 de	979,6 de
8.Volare (CV)	1127,0 bcd	1020,0 cd	1073,5 de
9.Gina (CV)	1159,7 abcd	965,0 cde	1062,3 de
10.Karaayşe (CV)	897,0 d	922,5 de	909,7 ef
11.Balkız (CV)	740,7 d	732,5 def	736,6 efg
Means	1126,8	1089,3	1108,1
% CV			15.23

* Means followed by the same letter are not significantly different at P<0,01

T:Terme, Ç:Çarşamba, TK: Tekkekoy (towns located in Çarşamba plain), CV:Control variety

Molecular characterization

Our previous inoculation studies on the seven dwarf fresh bean lines (KO, TK15, T26,Ç31,TK7,TK1 and T23) with 5 races of *C. lindemuthianum* pathogen of middle Blacksea region have shown that all of the lines were susceptible to the 5 races (2175, 4071, 3303, 3321, and 3071). Race numbers of the pathogens have also indicated that only half of them have lost their virulence for *Co-2, Co-4, Co-6/co-8* resistance genes while still being virulant for the other Co-gene loci. [15] Therefore it was vital for us to understand the availability of

Table 6. Lines and resistance gene loci analysed by molecular markers.

Resistance genes (*)	TK15	Ç31	T26	TK1	KO	T23	TK7
Co-1	-	-	-	-	+	-	-
Co-2	+	+	+	+	+	+	+
Co-4^3	-	-	-	-	-	-	-
Co-4	+	+	+	+	+	+	+
Co-4^2	+	+	+	+	+	+	+
Co-6/co-8	-	+	-	+	+	+	+
Co-3/Co-9	-	-	-	-	-	-	-

Table 4. Plant characteristics in terms of heigth, number of branches, pod numbers/branches, seed numbers/pods and weight of 100 grains in dwarf fresh bean lines with the characteristics of Aysekadın type (2008-2009)

Dwarf fresh bean lines and control varieties	Height of plant (cm)		The Number ofbranches of plant (Unit)		The number of pods per branch (Unit)		The number of seeds in pod (Unit)		Weight of 100 grain (gr)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
1.Ç31	30,48	31.12	3,61	3,56	12,83	12,50	2,89	2,77	42,39	43,00
2.T26	40,94	40,00	3,59	3,65	15,24	15,10	2,78	2,65	40,01	40,15
3.TK7	39,13	40,03	3,04	3,10	15,08	15,10	3,54	3,50	39,06	40,05
4.TK15	32,80	33,25	3,22	3,30	14,77	14,30	3,23	3,29	33,10	33,45
5.KO	33,08	33,67	3,85	3,45	14,93	14,53	2,59	2,30	4138,	41,45
6.TK1	32,38	33,83	3,75	3,23	12,53	12,15	2,66	2,75	41,80	41,35
7.T23	48,88	47,75	4,65	4,57	17,33	17,10	3,42	3,30	43,94	44,04
8.Volare (CV)	35,53	35,00	2,77	2,84	14,07	14,00	3,23	3,30	36,44	35,89
9.Gina (CV)	45,54	45,64	3,49	3,51	17,14	17,04	3,45	3,50	28,27	28,33
10.Karaayşe (CV)	27,78	26,47	3,85	3,10	25,13	24,83	3,13	3,00	31,72	31,94
11.Balkız (CV)	35,00	37,82	2,85	2,91	14,00	14,11	3,35	3,79	37,50	37,58

T:Terme, Ç:Çarşamba, TK: Tekkeköy (towns located in Çarşamba plain), CV: Control variety

resistance gene profiles on the 7 dwarf fresh bean lines against those other Co-genes. Screening of the lines with seven different molecular markers covering the gene loci of *Co-1, Co-2, Co-4, Co-4^2, Co-4^3,Co-6/co-8 and Co-3/Co-9* have shown that all the lines contained resistance markers for *Co-2, Co-4, Co-4^2* and *Co-6/co-8* (except TK15 and T26) (Table 6). These results confirmed our previous inoculation results and predicted loss of virulence pattern in the races. Although the Mesoamerican race resistance genes of *Co-3/Co-9* and *Co-4^3* markers were absent in all the lines, presence of Andean race resistance gene of *Co-1* in at least one of the line (KO line) was very encouraging to assign this line as potential *Co-1* resistance donor in future breeding programs.

DISCUSSION

Research on morphological studies in dwarf bean varieties by Balkaya and Yanmaz [3] have catagorised these varieties as early flourishing groups if they have shown 50% duration of flourishing quality between 36-45 days, moderate, if they were flourishing in 41-51days and late, if they were over 52 days. Similarly our results with Aysekadin type dwarf bean lines in Table 1 also indicated that except KO and TK1 lines, all others were considered as early flourishing group.

Breeding of local bean varieties through pure line selection method highly dictated by the farmers and consumers demand on certain physiological and morphological qualities of the bean. Moreover, fresh or dry consumption preferences of the beans also change the demands on the selection criteria. Selection criteria focus more on the pod length (11-14cm), stringless trait, spotless pods with indefinite seeds in middle Blacksea region where beans are preferably consumed fresh [2]. However, in eastern Blacksea region where the consumption of beans occur both as fresh and dry (mature seeds), charateristics such as as pod length, pod width, thickness of pod flesh, brakte length, beak length and plant height were considered in more detail for good quality seeds [17]. In this study, middle Blacksea local genotypes with the characteristics of "Aysekadin" type have been processed for certification by determination of their phenological, morphological and agronomic qualities over 7 years through selection out of 41 single plants. With respect to determined qualities, selected seven lines were very well in range of all desired features suitable for fresh consumption as dwarf lines and the results have also shown high similarity with the studies on local populations drived from the same region [2,3].

Physiological maturity is known to be a very variable quality in plants which is highly controlled by genotype and environmental factors [6]. Although throughout the whole growth period of selected 7 lines and control varieties were exposed to natural environmental conditions in the field, measurements in height, number of branches, number of pod/branches and weigth of 100 grains values did not show significant change within the same lines over two years (2008-2009), most of the variation has been observed among the lines mostly due to differences in the genotypes. For example, as shown in Table 4, variation of plant height between T23 line and Ç31 line was around 18 cm in average of two years measurements and similarly almost 1,54 units difference was observed in the number of branch/plant between T23 and TK7 lines. Similar to the findings in this study, other researches have also observed more profound effect by genotype differences in regarding physiological and morphological characteristics rather than environmental factors for at least short time spans such as 2 years [21]. Regional yield test experiments were performed in the inner parts of the middle Blacksea region, mostly south of Carsamba plain (Amasya area). In this area climate deviates significantly more warmer terrestrial –like climate with less rain in comparison to Carsamba plain where receives heavy rain throughout the year with mild climate. Therefore, among the seven lines tested for yield performance in regional tests results have shown significant differences between lines, while top three performance shared by Ç31, T26 and TK7 lines with no significant difference ($p<0,01$) among themselves, the rest of the lines and particularly TK1 and KO have rated with lowest yields (Table 5). Previously it was stated that in bean cultivars flourishing duration and yield reach maximum levels at temperatures around 28^0C, whereas, drastic decrease of 14%-30% in yield might have been observed when the temperatures reach 34^0C [13]. Not suprizingly due to the climatic conditions of the regional test area, those lines such as TK1 and KO which were moderate group for 50% flourishing duration, have shown almost 1 week difference in flourishing and this delayed period has most probably reflected on the yield performances. Top three yield performer lines Ç31, T26 and TK7 were early in flourishing duration and this had positively reflected on their yields and thus were higher than any other lines previously developed from middle Blacksea region [5,4, 19]. Stable and good yield performances of T26 and TK15 lines as local types over the years and morphological as well as plant characteristics suitable for consumption both as fresh and dry beans by the locals were resulted in certification of them in 2009 under the names of "Karabacak" and "Zeynebim" respectively [23].

The resistance of common bean to *C. lindemuthianum* is controlled by single, duplicate or complementary race specific 13 genes (*Co-1 to Co-13*) [12;8]. Pyramiding specific genes for resistance in a single plant insures durable resistance and is a main strategy in the breeding programs. The parallel usage of Co-gene markers and inoculation by race testing allows determination of the clustering rate of specific genes for resistance fast and

efficiently [20]. To understand the Co- gene profile for anthracnose resistance and potential of dwarf bean lines as resistance gene donors in future breeding programs, lines were screened with STS/SCAR molecular markers linked to *Co-1, Co-2, Co-4, Co-4^2, Co-4^3,Co-6/co-8 and Co-3/Co-9* genes. Majority of the common beans in Turkey are Andean orijinated beans (Nueva Granada and Chile) [12, 22] and thus under treat of virulence from Andean race pathogens. Therefore, presence of Andean originated resistance genes in cultivars is a major requirement for the battle against anthracnose problem. Marker screening on the 7 dwarf bean lines resulted in realisation that not only Mesoamerican originated Co-2, Co-4, Co-4^2 ve Co-6/co-8 genes were present in almost all lines (Table 6), but the only Andean originated Co-1 gene was missing in all lines except line KO. Although this results were encouraging to understand that KO line can be a potential Co-1 resistance donor in the future, results also alarmed us for the immediate need to start using the lines as susceptible parents in hybrid breeding programs to transfer Co-1 gene resistance from available foreign bean sources. Such hybrid breeding program has already been started at 2008, and seeds of first backcross population have been obtained (S.Y. Madakbaş pers. comm.).

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