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ASSESSMENT AND EXPLOITATION OF GENETIC VARIATION FOR RESISTANCE TO *PUCCINIA RECONDITA* FOR STABILIZING WHEAT PRODUCTION

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ABSTRACT

Adult plant responses to infection with pathotype 77-5 and a mixture of important pathotypes of *Puccinia recondita* were determined in the series of near-isogenic lines/stocks each carrying a known leaf rust resistance gene and certain lines each with a newly described stem or stripe rust resistance genes. Near isogenic lines/stocks with single leaf rust resistance genes *Lr18, Lr23, Lr36, Lr38, Lr41, Lr42, Lr44, Lr45,* besides *Lr9, Lr14b, Lr14ab, Lr19, Lr21, Lr22a, Lr24, Lr25, Lr28, Lr29, Lr30, Lr32, Lr34, Lr35* and *Lr37* that were reported to be effective in earlier studies, exhibited partial to complete resistance. Most of these genes, either effective throughout life or only in adult plants, were derived from alien sources and have not so far been exploited in Indian wheat breeding programmes. Leaf rust resistance in a near-isogenic line with stem rust resistance gene (*Sr39*) indicated additional leaf rust resistance that is likely to be linked with this stem rust resistance gene. Leaf rust resistance in two of the near-isogenic lines with stripe rust resistance gene *Yr24* backcrossed to cv. Meering was partly due to Meering. Higher resistance to leaf rust in these lines than in Meering could be attributed to additional gene(s) that may be linked with the stripe rust resistance gene. Pathotype 77-5 is a very virulent strain and attacks most of the genes for resistance to leaf rust originating from *T. aestivum*. Identification of leaf rust resistances, particularly against pathotype 77-5 provided useful and diverse sources for strategic use in future breeding programmes.

Seedling tests of the newly described resistance genes to a number of leaf rust pathotypes identified *Lr41, Lr42, Lr44* and *Lr45* as additional genes that confer over-all resistance to leaf rust.

A number of genetically enhanced germplasm lines with different resistance genes were developed. Two of the wheats Vaishali and Vidisha with alien resistances of *Thinopyrum ponticum (Lr24/Sr24)* have recently been released and notified. Commercial exploitation of these resistances was the first attempt in the Indian subcontinent.

Key words : *Triticum aestivum,* leaf rust, genetic diversity, adult plant resistance, over-all resistance, genetically enhanced germplasm.

Leaf rust is the most widespread disease of wheat and cultivars possessing

Present address : Adviser, Hybrid Wheat Programme, Monsanto Enterprises Pvt. Ltd. 1016-1018, Vishal Towers, Janakpuri District Centre, Janakpuri, New Delhi 110 058 inadequate levels of resistance are not officially recommended for cultivation in any part of the country. Resistance to leaf rust is more often neutralized with the evolution of new pathogenic races soon after new resistant cultivars carrying specific resistance are extensively grown. Therefore, a constant flow of resistance genes becomes imperative for the continuous process of resistance breeding. Several Indian wheats possess *Lr23* and *Lr26* individually and in combination with other genes. *Lr23* was probably derived from Gabo (*Lr10* + *Lr23*) which was used extensively in the earlier breeding programme. *Lr26* is carried by the 1RS-1BL wheat-rye translocation and was used in early 80's. Extensive use of these genes resulted in evolution of several pathotypes that possess virulence for these genes. At present there are eight pathotypes that possess virulence for *Lr26* and nine that posses virulence for *Lr23*, with different avirulence/virulence gene combinations. More recently, pathotypes 77-5, 104-2 and 104-3, with combined virulence for *Lr23* and *Lr26*, have appeared resulting in susceptibility of most Indian wheats to leaf rust. Combined virulence for *Lr23* + *Lr26* increased from 8.3% in 1992 and to 93.6% in 1996 (S.K. Nayar *et al.* unpublished report). This high frequency has emphasised the urgent need to identify resistance to these pathotypes. Pathotype 77-5 is most virulent among them. This communication describes sources of resistance in adult plants to this pathotype and to a mixture of important pathotypes, for strategic use in resistance breeding. Seedling tests of the lines carrying newly described genes have identified over-all resistance genes to leaf rust. Commercial exploitation of these resistances is also described.

MATERIALS AND METHODS

Host

Near-isogenic lines/stocks each with known single genes for leaf-rust resistance (*Lr*) up to *Lr36*, mainly in the background of Thatcher wheat were studied. Also the expanded series of lines with rust resistance genes described by 1993 [1], that included near-isogenic lines each with single *Lr* gene up to *Lr45*, near-isogenic lines/stocks each with single *Sr* gene (*Sr39* to *Sr43*) and two near-isogenic lines each with *Yr24* backcrossing into cv. Meering were studied.

Pathogen

Newly evolved leaf-rust pathotype 77-5 with virulence/avirulence for resistance genes in the differential set *Lr1, Lr2a, Lr2b. Lr2c, Lr3, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr20, Lr23, Lr26/Lr18, Lr19, Lr24*, and a mixture of important pathotypes, were used separately for adult plant tests. In seedling tests, pathotypes 12-2, 77-2, 77-3, 77-4, 77-5, 104, 104-B and 104-2 were used individually.

Seedling tests

Seedlings of near-isogenic lines for newly described leaf rust resistance genes, stem rust resistance genes and stripe rust resistance genes were raised in 4" pots in green house. Ten days old seedlings were inoculated with an array of leaf rust pathotypes. After inoculation seedlings were incubated at high humidity for 24hr and kept in a glasshouse at a temperature range of 8°C to 25°C. Reactions were classified after 12 days of inoculation, according to Stakman *et al.* [2].

Adult plant tests

The seeds were sown in one meter long rows. The row to row distance was 30 cm. Every 15th row was planted with a mixture of highly susceptible varieties to serve as spreader to produce inoculum. The nurseries were surrounded by three rows of spreader, followed by two rows of oat to check possible contamination from the adjoining rust inoculated fields. A set of lines each with different leaf rust resistance gene up to *Lr36* was planted on November 24, 1995. The second set of newly obtained material was sown at two sites. One along with the material sown on November 24, 1995 and the second in the late sown conditions on December 28, 1995, in a widely isolated nuersery. Spreader rows were inoculated with spore suspension of pathotype 77-5 at 7 days intervals using a hypodermic syringe. Lines exhibiting resistance to 77-5 were tested in the 1996-97 crop season with a mixture that included pathotypes 12-2, 77-2, 77-5 and 104-2. Fields were irrigated adequately to ensure sufficient humidity for the spread of the pathogen. Reactions were recorded as severity (percentage of infection) and response (pustule type) according to Loegering [3]. Growth stage of the newly obtained material was also recorded. No natural infection to rusts appeared at the IARI Farm in these tests.

RESULTS AND DISCUSSION

Seedling Tests

Seedling reactions of near-isogenic lines carrying newly described leaf rust resistance genes with a number of pathotypes are given Table 1. Lines carrying *Lr37* and *Lr38* were susceptible. Lines carrying *Lr41, Lr42, Lr44* and *Lr45* produced high resistance with very low infection types. Lines carrying stem rust resistance gene *Sr39, Sr40, Sr43* were susceptible to most of the pathotypes except that pathotype 12-2 was avirulent on lines with *Sr39* and *Sr40,* which needs retesting. Meering and its two derivates carrying *Yr24* were susceptible to all pathotypes, indicating lack of seedling resistance to leaf rust.

Adult Plant Tests

Adult plant responses of near-isogenic lines/stocks with known genes for leaf rust resistance up to *Lr36* with leaf rust pathotype 77-5 and a mixture consisting of pathotypes 12-2, 77-2, 77-5 and 104-2 are given in Table 2. The lines carrying non-alien genes *Lr14b, Lr14ab, Lr23, Lr30* and *Lr34* showed partial resistance. Also a score of

Table 1. Infection types produced on the near-isogenic lines carrying newly described rust resistance genes against leaf rust pathotypes

Stock		Pathotype							
		12-2	77-2	77-3	77-4	77-5	104	104B	104-2
Tc*8/VPMI RL 6081	*Lr37/ Lr38/ Yr17*	3+	3+	33+	33+	3+	3+	33+	3+
Tc*6/T-7 RL 6097	*Lr38*	3+	3+	33+	33+	33+	3+	3+	3+
TAM107*3/*T. tauschii*	*Lr41*	;	0;	-	;	;	0;	0;	0;
Century*3/*T. tauschii*	*Lr42*	;	;1-	;	;	;1-	;	;1-	;
Tc*6/*T. spelta*	*Lr44*	;1-	.1-	;	;	;1-	;	;1-	;
Tc*4/ST-1 RL 6144	*Lr45*	;1-	;1-	;	;	;1-	;1-	;1-	;
Tc*4/ST-1 RL 5711	*Sr39*	0;	3+	33+	33+	3+	33+	3+	3+
Tc*4/ST-1 RL 6087	*Sr40*	0;	3+	3+	3+	3+	33+	3+	3+
Norin40 KS 23-9	*Sr43*	3+	3+	3+	3+	3+	3+	3-3	3+
Norin40 KS 24-1	*Sr43*	3+	3+	33+	33+	3+	3-	33+	-
Meering	-	3+	3+	33+	3+	3+	3-	33+	3+
Meering + *Yr24* [K733 derivative]		3+	-	33+	3+	-	-	3+	3+
Meering + *Yr24* [K733 derivative]		3+	3+	33+	33+	33+	-	33+	3+

Resistance = IT 0;, ;, ;1-, 3-
Susceptible = IT 33+, 3+
- = Not tested.

70Y was observed at anthesis on the near-isogenic line carrying *Lr10* (Tc + Lr10); 'Y' type of infection is used to record when there are higher infection types towards the leaf tip. However, the terminal response score of 70S recorded on this line is considered as fully susceptible. McIntosh *et al.* [4] reported that *Lr10* is not widely effective on its own but may play a role in gene combination in most parts of the world except Australia. DL1056-1, developed in the IARI programme, possesses *Lr10* + *Lr26* (unpublished) and its resistance in these tests is possibly due to additional resistance factors, because neither of these genes individually produce any resistance. Resistance due to interaction of *Lr10* and *Lr26* is unlikely because seedlings of a stock Fed 4*/Kavkaz carrying *Lr10* and *Lr26* showed seedling susceptibility [5].

Thatcher near-isogenic lines with *Lr14b*, *Lr14ab* and *Lr30* were identified as novel adult plant resistance sources in India [6] and in Australia [4]. Resistance

Table 2. Adult plant respdnses on near-isogenic lines/varieties with designated genes for resistance to leaf rust tested with pathotypes of *Puccinia recondita*

Near-isogenic line/Variety	Source	Pathotype		Remarks
		1995-96 II reading 77-5	1996-97 Mixture (12-2,77-2, 77-5,104-2)	
Tc^6+*Lr1*	*T. aestivum*	90S	-	S
Tc^6+*Lr2a*	*T. aestivum*	80S	-	S
Tc^6+*Lr2b*	*T. aestivum*	90S	-	S
Tc^6+*Lr2c*	*T. aestivum*	60S	-	S
Tc^6+*Lr3*	*T. aestivum*	90S	-	S
Transfer (*Lr9*)	*T. umbellulatum*	0	0	F
Tc^6+*Lr10*	*T. aestivum*	80S	-	S
Tc^6+*Lr11*	*T. aestivum*	80S	-	S
Tc^6+*Lr12*	*T. aestivum*	70S	-	S
Tc^6+*Lr13*	*T. aestivum*	90S	-	S
Tc^6+*Lr14a*	*T. aestivum*	80S	90S	S
Tc^6+*Lr14b*	*T. aestivum*	10MR	20MRMS	R
Tc^6+*Lr14ab*	*T. aestivum*	30MR	40MRMS	R
Tc^6+*Lr15*	*T. aestivum*	80S	-	S
Tc^6+*Lr16*	*T. aestivum*	80S	-	S
Tc^6+*Lr17*	*T. aestivum*	80S	-	S
Tc^6+*Lr18*	*T. timopheevii*	60MR	40MR	R
Tc^6+*Lr19*	*Th. ponticum*	0	-	F

(*Table 2 contd.*)

Thew *Lr20*	*T. aestivum*	80S	-	S
Tc^6+*Lr21*	*T. tauschii*	40RMR	30MR	R
Tc^6+*Lr22a*	*T. tauschii*	30RMR	20MR	R
Tc^6+*Lr23*	*T. aestivum*	60MRMS	40MRMS	PR
Gabo (*Lr23*)	*T. aestivum*	50MS	40MS	PR
TR 380.27*4/3Ag3(*Lr24*)	*Th. ponticum*	TR	o	R
Transec *Lr25*	*S cereale*	-	0	F
Kavkaz *Lr26*	*S. cereale*	80S	-	S
Gatcher *Lr27+Lr31*	*T. aestivum*	70S	-	S
CS 2A/2M/4/2 *Lr28*	*T. speltoides*	0	TR	F
CS 7D/Ag #11 *Lr29*	*Th. ponticum*	20RMR	10MRMS	R
Tc^6+*Lr30*	*T. aestivum*	30MS	20MRMS	PR
Tc^6+*Lr32*	*T. tauschii*	40R	30MR	R
Tc^6+*Lr33* RL 6057	*T. aestivum*	70S	40MS	S
Tc^6+*Lr34* RL 6058	*T. aestivum*	20MR	30MRMS	PR
Tc^6+*Lr35*	*T. speltoides*	5R	-	R
Lr36	*T. speltoides*	10MS	20MRMS	PR

0/F = No infection/Free, R = Resistance, TR = Trace Resistance, MR = Moderate Resistance, MS = Moderate Susceptible, S = Susceptible, PR = Partial resistance; - = Not tested.

responses on all the three lines to pathotype 77-5 identified these lines as useful for future use in the wheat breeding programmes. Adult plant resistance in Thatcher lines carrying *Lr14b* and *Lr14ab* has already been transferred into popular cultivars Kalyansona [7] and Sonalika. These are useful pre-breeding stocks because Thatcher near-isogenic lines were tall, agronomically poor, late and red seeded, and are difficult to exploit in breeding programmes. Near-isogenic line with *Lr23* (Tc + *Lr23*) and Gabo (*Lr10, Lr23*) exhibited partial resistance response (40MRMS-60MRMS and 40MS-50MS) respectively, which is interesting because *Lr23* was reported to be ineffective [8]. Pathotype 77-5 possesses virulence for *Lr23* in seedlings. Partial adult plant resistance provided by this gene to 77-5 seems to be possible due to the expression of its resistance at higher temperatures in field conditions. Further tests would be needed for confirmation of this. The results are important because several Indian wheats possess *Lr23* [9, 10]. Resistance of *Lr34* to pathotype 77-5 and a mixture of important pathotypes re-established the usefulness of this gene known to be associated with durability. *Lr34* was identified in some Indian wheats [9, 11].

Of the alien genes that were reported to confer resistance at all the growth stages, *Lr9, Lr19, Lr24, Lr25, Lr28* and *Lr29* [8] continue to produce high levels of resistance but *Lr26* became ineffective with the appearance of new pathotypes including 77-5 [12]. It may be pointed out that alien sources, other than *Lr26* in the 1RS-1BL translocation, have not been exploited to the required extent to test their durability, perhaps due to reports that alien chromatin is often associated with depression in yield [13]. Sawhney and Sharma [7] attempted a backcross breeding programme in which it was demonstrated that, with limited backcrosses and selection for desirable rust resistant plants in each self and backcrossed generation, it became possible to produce lines which yield higher or comparable to the recurrent parent, Kalyansona and Sonalika. The stocks thus developed and used in breeding wheats, have produced several improved cultivars/germplasm lines. Two of the wheats with resistance of *Thinopyrum ponticum (Lr24/Sr24)* have been recently released and notified. These varieties were developed by using Kalyansona backcross line with *Lr24/Sr24* as one of the parents. The variety Vaishali (DL 784-3) was notified in 1994 for timely planting in high fertility, irrigated conditions for eastern India [14]. The other wheat DL 788-2 (Vidisha) was notified for late planting, high fertility, irrigated conditions for central India in 1996 [15]. Exploitation of resistance from *Thinopyrum ponticum* for the development of cultivars was the first attempt in India using this source.

The four other genes *Lr19* (source : *Thinopyrum ponticum*), *Lr25* (source : *Secale cereale*), *Lr26* (source : *T. speltoides*) and *Lr29* (source : *Thinopyrum ponticum*), all originated from alien species related to wheat, showed complete resistance to pathotype 77-5. A reaction of 20RMR was however observed in a line carrying *Lr29* in the 1995-96 tests. Development of Kalyansona backcross derivatives with *Lr28* and *Lr29* [7] and Sonalika backcross derivatives with *Lr19, Lr29* provided useful stocks for potential use in breeding programmes for development of genetically diverse rust resistant cultivars.

Different levels of adult plant resistance in lines with *Lr21, Lr22a, Lr32, Lr35,* confirmed their earlier performance [8, 16, 17], showing that they continued to be effective even with pathotype 77-5. The tests also identified *Lr18* and *Lr36* as new sources conferring resistance to this pathotype. *Lr18* (Tc + *Lr18*), derived from *T. timopheevii,* is reported to be a useful source [4] but has not been used in crop improvement. Resistance of *Lr18* to the newly evolved and most virulent pathotype emphasised the potential importance of this source for imparting resistance to leaf-rust in India. It is, however susceptible at the seedling stage to series of other Indian leaf rust pathotypes.

The gene *Lr21* from *T. tauschii (Aegilops squarrosa)* has been reported to be effective in India (8, 18). It is available in the Kalyansona [7] and the Sonalika (unpublished) backcross derivatives. Kalyansona backcross derivatives carrying

Lr21/Sr21 resistances were used in different cross combinations and have produced promising germplasm lines DL1012- 2, DL1013-6, DL1017-1, DL1063-1, DL1079-1 [19]. This is the first successful use of *Lr21/Sr21* resistances leading to the development of cultivars. DL1013-6 was identified to possess a unique combination of *Lr21* + *Lr23* [19]. McIntosh *et al.* [4] reported that *Lr21* has potential for use in breeding but remained so far unexploited.

An adult plant resistance gene, *Lr22a* was also derived from *T. tauschii*. It was reported [8, 18] that Tc + *Lr22a* conferred a high level of adult plant resistance to a mixture of races and to individual races. Sawhney [12] reported this gene to confer reduced level of resistance in routine tests with mixture of races. Resistance of *Lr22a* to 77-5 identifies another potentially important gene for use in Indian breeding programmes and it is likely to impart an adequate level of resistance with the current racial composition of the pathogen population.

Lr32 (source : *T. tauschii*) is a newly identified gene that conferred resistance at all stages of plant growth [16]. It produced high levels of resistance with a maximum infection intensity of 40R with pathotype 77-5, though a response of 30MR was observed with a mixture of pathotypes. This identifies Tc + *Lr32* as a stock that can confer adequate level of resistance even when used singly. Resistance conferred by this gene was reported to be widely stable [4].

Two of the near-isogenic lines (RL 6082, RL 6083) carrying *Lr35* (source : *T. speltoides*) exhibited uniformly susceptible reactions (IT3) in seedlings and the resistance response (TR-40MR) with six genetically diverse pathotypes in adult plant stage [17]. Adult plant resistance response of the line carrying *Lr35* to 77-5 confirms the earlier report that *Lr35* is a race-non-specific adult plant resistance gene that may provide durability [17].

Lr36 (source : *T. speltoides*) was tested for the first time. The terminal score of 10MS and 20MRMS with individual and a mixture of pathotypes, respectively, provided resistance for use in breeding programmes.

Table 3 lists adult plant responses on a set of 14 near-isogenic lines/stocks comprising newly designated leaf-rust resistance genes (*Lr37-Lr45*), near-isogenic lines with stem rust resistance genes (*Sr39-Sr43*), two Meering derivatives each carrying stripe rust resistance gene *Yr24* and Meering, the background wheat, to 77-5 and a mixture consisting of 12-2, 77-2, 77-5 and 104-2 pathotypes.

Near-isogenic lines with newly designated leaf-rust resistance genes *Lr37* (source: *T. ventricosum*), *Lr41* and *Lr42* (source : *T. tauschii*), *Lr44* (source : *T. spelta*) and *Lr45* (source : Japanese wheat-rye translocation) were observed to produce varying levels

Table 3. Adult plant responses on near-isogenic lines/varieties with newly designated leaf, stem and stripe rust resistance genses tested with pathotypes of *Puccinia recondita*

Near-isogenic line/Variety	Source	Pathotype 1995-96 Site 1 77-5 *(24.11.95)	Pathotype 1995-96 Site 2 77-5 *(28.12.95)	Pathotype 1996-97 mixture (12-2,77-2, 77-5,104-2)	Remarks
Leaf rust resistance genes					
Tc^6+*Lr37/Sr38/Yr17*	*T. ventricosum*	40MR	30MR/MS	40MSS	R.VL
Tc^6+*Lr38*	*T. intermedium*	90S	80S	30MSS	S.VL
TAM107^3+*Lr41*	*T. tauschii*	TR	TR	-	R.EL
Century^3+*Lr42*	*T. tauschii*	F	TR	0	R.EL
Tc^6+*Lr44*	*T. spelta*	60R	40R	40MRMS	R.VL
Tc^6+*Lr45*	Japanese wheat-rye translocation	TR	TR	5RMR	R.VL
Stem rust resistance genes					
Mq^8+*Sr39*	T. speltoides	TR	TR		R.VL
Mq^8+*Sr40*	*T. timopheevii*	80S	80S	50MSS	S.VL
KS23-9 *Sr43*	Norin 40	30S	50S	30MSS	S.L
KS24-1 *Sr43*	Norin 40	100S	-		S.L
KS24-2 *Sr43*	Norin 40	100S	90S		S.L
Stripe rust resistance genes					
Meering		30MRMS	10MS	40MRMS	PR.N
Meering + *Yr24* (K733 derivative)	*T. durum*	20R	TR	40MSS	R.N
Meering + *Yr24* (*K733* derivative)	T. durum	5MR	5MR	30R	R.N

EL = Extremely late maturity, VL = Very late maturity, L = Late maturity, N = Normal maturity, R = Resistant, S = Susceptible, PR = Partial resistant, *Sowing date

of resistance response. *Lr37* is reported to be linked with stem rust resistance gene *Sr38* and stripe rust resistance gene *Yr17*. These linked resistances have been extensively used in Australian wheat breeding programmes where rust resistance due to these genes is still effective. Cultivars Sunbri [4], Sunstate and Trident carrying these resistances have been developed/released. Kloppers and Pretorius [20] reported that adult plant leaf-rust resistance in the line carrying *Lr37* is expressed through decreased rate of uredinal appearance, fewer uredina and smaller uredinium size indicating this source as slow rusting. *Lr37* was susceptible in seedlings and produces resistance only at adult stage and was reported as an adult plant resistance gene [21].

Lr38 (source : *T. intermedium*) showed partial resistance response (30 MSS) with a mixture of pathotypes and complete susceptible response with 77-5. This indicated that perhaps *Lr38* has resistance in adult plants against certain components of the mixture comprising four pathotypes used in tests. *Lr38* was however, susceptible to these pathotypes in seedlings tests.

Of the near-isogenic lines with stem rust resistance genes, Mq^8 + *Sr39* (source : *T. speltoides*) exhibited high level of field resistance to leaf rust. This suggested the possibility that the stem rust resistance gene is linked with an adult plant leaf rust resistance gene that is highly effective to 77-5 and is likely to confer a good level of field resistance, the lines being susceptible in seedlings. It may be pointed out that Marquis is susceptible to the Indian population of leaf rusts. *Sr39* has also been reported to confer complete resistance against all the Indian pathotypes of stem rust in seedling stage (S.K. Nayar *et al.* unpublished report). This could be another source that may, therefore play a significant role in breeding for simultaneous resistance to leaf and stem rusts.

It can be seen that among the newly described sources conferring resistance to leaf rust, a number of lines were very late to extremely late in maturity (Table 3) which is likely to limit the direct use of these sources in breeding programmes as this factor will restrict recovery of desirable genotypes of suitable maturity groups to fit in the contemporary cropping systems. Therefore, transfer of these genes into the background of popular but currently susceptible wheats, through backcross procedure, should be of great value in providing readily usable sources of diverse resistances. Certain rust resistance genes that were assessed to be effective both earlier and in current studies when transferred into the formerly popular wheats Kalyansona [7] and Sonalika have served as useful pre-breeding stocks. These stocks therefore offer wider opportunities in mobilising alien genetic diversity of rust resistance in sustaining future crop improvement efforts. The strategic use of these diverse resistances shall prevent losses, extend effective life of the deployed resistances and thus help in achieving stability of wheat yields.

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