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Thinopyrum ponticum-derived leaf rust gene *Lr24* continued to be effective against all the occurring leaf rust pathotypes in India

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Introduction

Brown rust caused by Puccinia triticina is one of the devastating rust diseases targets wheat worldwide. The upper surface of the leaves occupied with orange-brown uredinia and form uredinio spores (Wegulo and Emmanuel, 2012). The infectious urediospores of *P*. triticina can travel to hundreds of miles by wind hence it can cause endemic outbreak (Bolton et al., 2008). The infected plants before the flowering stage results in less grain filling period and so kernel size reduced there by decreases yield by 14- 50% depending upon environmental conditions (Wegulo and Emmanuel, 2012). Tomar et al. (2014) reported that there are 49 Pathotypes of leaf rust pathogen in India. Among them, P.triticina race 77-1, 77-5, 77-9 and 104-2 are the most virulent path types with more frequency in India(Pramod Prasad et.al 2020).

To date there are 79 leaf rust resistance genes which were reported in wheat (Riaz et al., 2016). Almost more than 30 years, *Lr*24 continue to provide resistance to leaf rust in India. 'Amigo' wheat had an Agropyron (Thinopyrum ponticum)-derived segment with stem rust and leaf rust resistance gene, Sr24/Lr24 respectively (The et al., 1991). The leaf rust resistance gene Lr24/Sr24 was originally detected in a bread wheat line possessing spontaneous translocation involving chromosome 3D in agent in the stock Agent derived from Agropyron elongatum (now Lophopyrum elongatum) selected from across of this line cv. Triumph is the origin (Gough and Merkle, 1971). The gene present in the genotype Agent is a spontaneous wheat- translocation involving 3 Ag and 3DL chromosomes. In Agent, Lr24 is tightly linked with stem rust resistance gene Sr24 and with a gene for red seed colour(McIntosh,1992). The initial impediment to use of Lr24/Sr24 from Agent was in association with red grain colour but RA McIntosh and M. Partridge (un published 1977) were able to recover white-seeded recombinants. One of the white seeded recombinant lines Tr 380-4^{*}7/3 Ag#14 and Darf Kite were proved to be a valuable genetic stocks for wheat improvement, were used to develop BC lines of popular Indian cultivars carrying Lr24/Sr24 at Wellington. The resistance gene Lr24tightly linked to Sr24 showed complete

resistance to all leaf rust Pathotypes in India. It showed both seedling resistance as well as adult plant resistance (APR) (Tomar et al., 2014).

There were some apprehensions raised on effectiveness of *Lr24* in India, hence the study was initiated to confirm the effectiveness of *Lr24* gene through introgressed lines with *Lr24* which needs to be validated against the occurring leaf rust path types. About 20 genetic stocks (NIL's/BC lines) in the background of popular Indian wheat cultivars were developed with introgression of *Lr24* gene in India at ICAR-IARI, RS, Wellington (Tomar *et al.*, 2014).

Material and Methods

Plant and Fungal material

Twenty Near Isogenic Lines (NILs)/BC lines introgressed with Lr24 gene were developed by backcross breeding using Tr380-14*7/3/Ag#14 and *Darf kite* as donor parents at ICAR- IARI, Regional Station, Wellington, Tamil Nadu. Out of which 16 stocks all carrying Lr24 and their corresponding recurrent parents (listed in Table 1& 2) were used for this study

DNA isolation and molecular validation of *Lr*24 gene

The leaf samples were collected from the NILs at 15 days old seedlings and thei 2

DNA was isolated using CTAB method (Murray and Thompson 1980). Two markers, Sr24#12 (Mago *et al.*, 2005) and SCS73₇₁₉ (Prabhu *et al.*, 2004) were used to molecularly validate the NILs for the presence of Lr24 gene along with the recurrent parents and donor parent Tr380-14*7/3/Ag#14.

The DNA samples were amplified with gene specific marker Sr24#12 and SCAR marker SCS73719. The PCR reactions were carried out with 2X Dream Taq PCR master mix (Thermo Fisher Scientific) and 0.4pm forward and reverse primers. For Sr24#12, initial denaturation was kept at 94°C for 5mins, and 35 cycles of 94°C for 30s, 55°C for 30s and 72°C for 1min, and the final extension at 72°C for 10min and for SCS73₇₁₉ same condition with annealing temperature of 51°C was carried out. The PCR products were resolved with 1.2% agarose gel and documented with gel documentation system (Syngene, Gene Genius Match GGM/D2/F2-1).

Seedling Resistance Test

The seedling reaction of molecularly validated 16 NIL's (Lr24) and their corresponding recurrent parents were done with 6 different leaf rust Pathotypes viz., 12-5, 77-2, 77-5, 77-9, 104-2 and 106 during 2019 and again 16 different of NIL's sets against three predominantly occurring leaf rust pathotypes 77-5, 77-9 and 104-2 and stem rust pathotypes 15-1, 40-1 and 40A

during 2020 at ICAR. IIWBR. Flowerdale, Shimla. Lines were inoculated at 14 days after sowing with leaf rust Pathotypes and incubated in humid chambers with diffused light for 48 hours. After 48 hours they were kept at glass house and maintained for development. symptom Symptoms appeared ten days after inoculation and seedling reactions were recorded (Nayar et al., 1994).

Adult plant resistance

The near isogenic lines with their recurrent parents were sown in field at ICAR-IARI, Regional Station, Wellington, Tamil Nadu with spreader rows around the field and artificial inoculations were also given. The adult plant reaction in the field conditions was recorded as per modified Cobb's scale(Peterson *et al* 1948) during Kharif 2019 and Rabi 2019-20.

Results

Genotypic validation of Lr24

The 16 NIL's and their corresponding recurrent parents DNA were isolated and amplified with Sr24#12 and $SCS73_{719}$ during 2019. All markers the introgressed lines were amplified with 500bp positive band for Sr24#12 (Fig. 1A and 1B) and 650bp positive band for SCS73₇₁₉ (Fig. 2A and 2B) but there is amplification in corresponding no

recurrent parents. Further reconfirmation was done during 2020 and all the introgressed lines were amplified with 500bp positive band for Sr24#12(Fig.3 & 4)

Phenotypic validation at seedling stage(SRT)

The infection type developed by the NIL's and corresponding recurrent parents were recorded and given in Table-1(2019) and Table-2(2020). The recurrent parents developed an infectious urediospores with score ranging from 1 to 3+ against different pathotype, where as NIL's with Lr24 showed resistant response. The results revealed and confirmed that the Lr24 gene had seedling resistant response to all the occurring leaf rust Pathotypes in India. The Lr24 linked stem rust gene Sr24 recorded susceptible reaction

Phenotypic validation at adult plant stage

In the field the recurrent parents showed susceptible response with severity ranging from 80-100S score, while lines carrying Lr24 showed resistant response. The scores were tabulated in Table 1&2.

Table 1: Phenotypic validation of Seedling(SRT) and Adult plant resistance response of *Lr*24/Sr24 in NILs/back crossed lines, recurrent parent and donors against leaf rust pathotypes during 2019

S. No	Variety	SRT Score	SRT Score (IIWBR, Shimla)						Field Score	
		(IARI, RS, Wellington mixed races)	12-5	77-2	77-5	77-9	104-2	106	(IARI, RS, Wellington)	
1	HW 2001A	;	-	;	2	2	2	;-	0	
2	Sonalika	3+	3+	3+	3+	3+	3+	0;	100s	
3	HW 2002	0	-	;-	;	-	;	0;	0	
4	Kalyansona	3+	-	3+	3+	3+	3+	0;	100s	
5	HW 2003	0	;	;-	2-3	0;	;-	0;	0	
6	NI 5439	2+	-	-	3+	;-	-	-	80s	
7	HW 2004	0	0;	-	-	1	-	-	0	
8	C 306	3+	1	3+	2	3+	3+	0;	60s	
9	HW 2006	0	;	1	-	-	;	0;	0	
10	LOK 1	3+	-	-	-	-	-	-	100s	
11	HW 2007	0	-	2	-	-	-	-	0	
12	HD 2329	3+	0;	-	0;	3+	0;	0;	80s	
13	HW 2008	0	-	-	-	-	-	0;	0	
14	HD 2285	3+	-	-	-	-	-	-	80s	
15	HW 2014	0	;	;-	;-	;	;-	-	0	
16	WL 711	3+	-	-	-	-	3+	0;	100s	
17	HW 2015	;	0;	-	;-	;-	0;	0;	0	
18	HUW 234	3+	;	3+	3	3+	3+	0;	100s	
19	HW 2016	0	0;	;	;-	;-	;	0;	0	
20	PBW 226	3	;	-	3+	3+	;	0;	80s	
21	HW 2017	0	0;	;-	0;	;-	;-	;-	0	
22	HD 2402	3+	;	-	-	-	-	0;	80s	
23	HW 2018	0	0;	;-	0;	;-	;	0;	0	
24	HI 1077	3	0;	3+	;	3+	-	0;	80s	
25	HW 2019	;	0;	0;	;-	;-	;-	0;	0	
26	WH 542	3	3	0;	3+	3+	3+	0;	80s	
27	HW 2020	0	0;	;-	0;	0;	0;	0;	0	
28	HS 240	3+	0;	0;	2	3+	3+	0;	100s	
29	HW 2022	0;	;-	;-	0;	;	2	;1	0	
30	WH 147	3+	1-2	3+	3+	3+	3-3+	0;	80s	
31	Darf Kite (Donor)	0	0;	0;	0;	-	0;	0;	0	

Table 2: Phenotypic validation of Seedling(SRT) and Adult plant resistance response of *Lr*24/Sr24 in NILs/back crossed lines, recurrent parent and donors against predominant leaf and stem rust pathotypes during 2020

S.No.	Wheat Lines		SRT Score (IARI, RS, Wellington) mixed races leaf	SRT Score IIWBR, Flowerdale Shimla Leaf rust pathotypes			SRT Score IIWBR, Flowerdale Shimla Stem rust pathotypes			Adult plant response
			rust pathotypes*	77-5	77-9	104-2	15-1	40-1	40A	under natural epiphytotic conditions at IARI, RS, Wellington
1.	HW 2002	K.sona (<i>Lr24/Sr24</i>)	0	;	;	0;	2-	3-	2-	0
2.	HW 2002A	K.sona (Lr24/Sr24)	0	;	;	0;	2=	3-	2-	0
3.	Kalyansona	Recurrent Parent	3+	3+	3+	3+	2-	3+	3+	100S
4.	HW 2003	NI5439(Lr24/Sr24)	0;	;	;	;	;	2=	;1	0
5.	NI 5439	Recurrent Parent	2+	;	3+	0;	2=	3+	3+	80S
6.	HW 2004	C306(<i>Lr24</i> /Sr24)	0	12	;	;	;	3-	2-	0
7.	C 306	Recurrent Parent	3+	3+	3+	3+	3+	3+	3+	80S
8.	HW2007	HD 2329(Lr24/Sr24)	0	;1	;1	;1	0;	2=	2-	0
9.	HD 2329	Recurrent Parent	3+	3+	;1	3+	3+	33+	3+	100S
10.	HW 2008	HD 2285(Lr24/Sr24)	0;	;1	;	;	;	2=	2-	0
11.	HD 2285	Recurrent Parent	3+	3+	;1	3+	;1	2=	3+	80S
12.	HW 2010	J24(<i>Lr24/Sr24</i>)	0;	;	;	;	;	2=	2-	0
13.	J24	Recurrent Parent	3+	3+	3+	3+	3+	33+	3+	100S
14.	HW 2011	HD2009(Lr24/Sr24)	0	0;	;1	0;	2-	2=	12-	0
15.	HD 2009	Recurrent Parent	2+	3+	3+	3+	2-	3-	2-	80S
16.	HW 2012	UP 262(Lr24/Sr24)	0;	;	;	;	0;	2=	2-	0
17.	UP 262	Recurrent Parent	2+	3+	;1	3+	2-	33+	3	80S
18.	HW 2015	HUW234(Lr24/Sr24)	;	;1	;	0;	2-	2-	2-	0
19.	HUW 234	Recurrent Parent	3+	3+	3+	3+	2=	2=	3+	100S
20.	HW 2016	PBW226(<i>Lr24</i> /Sr24)	0	;	;	;	0;	2=	2-	0
21.	PBW 226	Recurrent Parent	3	;1	12	3+	0;	0;	;	80S
22.	HW 2017	HD2402(Lr24/Sr24)	0;	;	;	;	;	2=	2	0
23.	HD 2402	Recurrent Parent	3+	3+	;1	3+	0;	2=	2	80S
24.	HW 2018	HI1077(Lr24/Sr24)	0	0;	;-	0;	;	2=	2-	0
25.	HI 1077	Recurrent Parent	3	3+	3+	3+	;	2=	2	80S
26.	HW 2019	WH 542(<i>Lr24</i> / <i>Sr24</i>)	;	;1	0;	0;	0;	2=	12	0
27.	WH542	Recurrent Parent	3+	3+	;1	3	0;	2=	;	80S
28.	HW 2020	HS240(<i>Lr24</i> / <i>Sr24</i>)	0	;1	;1	;-	2-	2=	2-	0
29.	HS 240#	Recurrent Parent	3+	0;	;-	0;	2=	0;	2-	80S
30.	HW 2022	WH147(<i>Lr24</i> / <i>Sr24</i>)	0;	;-	;-	0;	;	2	;1	0
31.	WH 147	Recurrent Parent	3+	3+	3+	3+	0;	3+	2-	100S
32.	Agent	Lr24/Sr24		;	;	;	2=	3+	2-	0
33.	Tr380-14#	Lr24/Sr24	0;	3+	3+	;1	0;	2-	2	0

*Predominant leaf rust races occurring at Wellington are 77-1, 77-5, 77-9 and less frequent ones are 12-4, 12-8, 20, 77-6, 104-1, 162 & 1R31(*Mehtaensis 40 (2) July 20, IIWBR, Shimla*) # The resistance response to leaf rust pathotypes could be due to wrong seed supply of source seeds

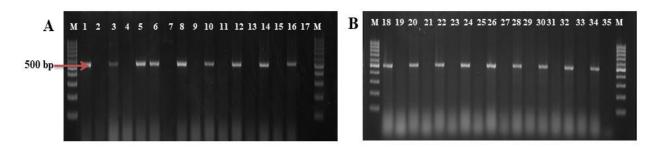
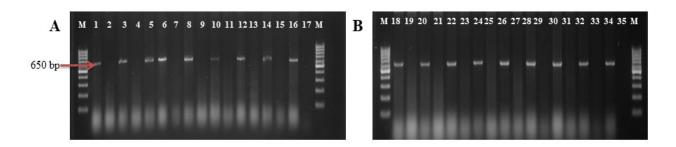


Fig 1: Molecular validation of *Lr*24 gene in NIL's with *Sr*24#12marker(2019)



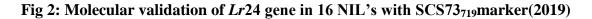


Fig. 1 and Fig. 2: M- 100bp Ladder, 1- Dargkite, 2- Sonalika, 3- HW 2001A, 4- Kalyansona, 5- HW 2002, 6-HW 2002A, 7- NI5439, 8- HW 2003, 9- C306, 10- HW 2004, 11- WH147, 12- HW 2005, 13- LOK1, 14- HW 2006, 15- HD2329, 16- HW 2007, 17- NTC, 18- Darfkite, 19- HD 2285, 20- HW 2008, 21- WL711, 22- HW 2014, 23- HUW234, 24- HW 2015, 25- PBW226, 26- HW 2016, 27- HD2402, 28- HW 2017, 29- HI1077, 30- HW 2018, 31- WH542, 32- HW 2019, 33- HS 240, 34- HW 2020, 35- NTC

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

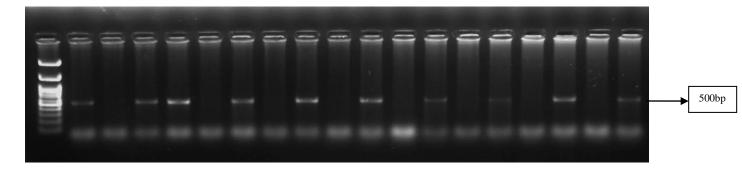


Fig 3: Molecular validation of *Lr*24 gene in NIL's with *Sr*24#12marker re-confirmed during 2020

M-100bp Ladder, 1.Tr380-4 (Donor), 2.Kalyansona , 3.HW 2002 (Kalyansona * Lr24/Sr24), 4. HW 2002A (Kalyansona * Lr24/Sr24), 5. NI 5439, 6. HW 2003 (NI 5439 * Lr24/Sr24), 7. C 306, 8. HW 2004 (C 306 * Lr24/Sr24), 9. HD 2329, 10. HW 2007 (HD 2329* Lr24/Sr24), 11. HD 228, 12. HW 2008 (HD 2285 * Lr24/Sr24), 13. J24, 14. HW 2010 (J24*Lr24/Sr24)

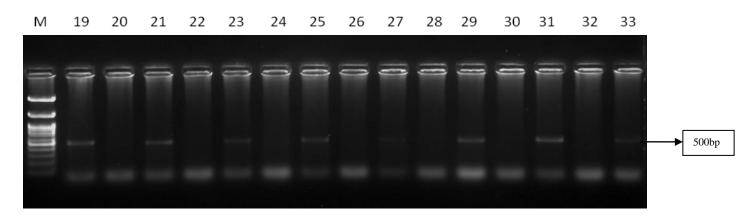


Fig 4: Molecular validation of *Lr*24 gene in NIL's with *Sr*24#12marker re-confirmed during 2020

M- 100bp ladder, 19. Tr380-4, 20. HUW 234, 21. HW 2015(HUW 234* *Lr24/Sr24*), 22. PBW 226, 23. HW 2016 (PBW 226**Lr24/Sr24*), 24. HD 2402, 25. HW 2017 (HD 2402**Lr24/Sr24*), 26. HI 1077, 27. HW 2018 (HI 1077**Lr24/Sr24*), 28.WH 542, 29.HW 2019(WH 542 * *Lr24/Sr24*), 30. HS 240, 31. HW 2020 (HS 240**Lr24/Sr24*), 32. WH 147, 33. HW 2022 (WH 147**Lr24/Sr24*)

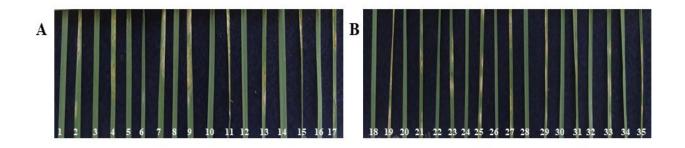


Fig 3: Seedling resistance pattern in NIL's

A: 1- Darfkite, 2- Sonalika, 3- HW 2002, 4- Kalyansona, 5- HW 2002, 6- HW 2002A, 7- NI5439, 8- HW 2003, 9-C306, 10- HW 2004, 11- WH147, 12- HW 2005, 13- LOK1, 14- HW 2006, 15- HD2329, 16- HW 2007, 17-Agralocal; **B:** 18- Drafkite, 19- HD2285, 20- HW 2008, 21- WL711, 22- HW 2014, 23- HUW234, 24- HW 2015, 25- PBW226, 26- HW 2016, 27- HD2402, 28- HW 2017, 29- HI1077, 30- HW 2018, 31- WH542, 32- HW 2019, 33- HS240, 34- HW2020, 35- WH147

Discussion

Among the several leaf rust resistant genes incorporated in wheat cultivars, Lr24 is one of the important genes conferring high level of resistance in India. In the recent past, efforts have been made to understand seedling and adult plant resistance provided by Lr24

gene through transcriptome analysis (Manjunatha, 2015). In the present study, molecular validation of Lr24 gene using two different markers (Sr24#12 and $SCS73_{719}$) showed that all the 16 introgressed lines carries Lr24 gene. It confirms that the breeding approaches followed to develop near isogenic lines

were efficient, systematic and successful. Seedling resistance test carried out at ICAR- IIWBR, RS, Flowerdale, Shimla for the 16 NIL's carrying *Lr24* against 6 different pathotypes (12-5, 77-2, 77-5, 77-9, 104-2 and 106) showed complete resistance reaction and also seedling resistance test done at ICAR- IARI, RS, Wellington using mixed pathotypes collected from field. The adult plant response under field conditions at Wellington showed complete resistance reaction in all 16 NIL's.

Pathotypes virulent on *Lr24* have been reported from North America (Browder, 1973), Canada (Kolmer, 1991), South America (Singh, 1991) and South Africa (Pretorius et al., 1990) However, Lr24 still continues to be highly effective in seedling as well as in adult stage to Indian pathotypes of *P.recondita* and virulence for Lr24 occur in low frequencies in most geographical areas(Huerta-Espino, 1992). Bread wheat cultivars carrying Lr24/Sr24 are widely grown in Australia, North America and South Africa. In India the cultivars viz., DL784-3 (Vidisha), HW 2004 (Amar), DL788-2 (Vaishali), HW 2045 (Kausambi), HD 2781 (Aditya), HI 1500 (Amrita), MP4010, Raj4037, HD2851

(Pusa Vishesh), HD 2833 (Tripti), HI 1531, COW(W)-1, HD 2888 (Pusa Wheat), **AKAW3722** (Vimal), AKAW4627 5207(Pusa and HW Navagiri) all carrying Lr24/Sr24 have been released in recent years in India for commercial cultivation. The deployment of this effective gene complex in Indian released cultivars for the last more than a decade, widely across India played pivotal role in checkmating the brown rust (Tomar.et.al, 2014).

However the linked stem rust gene Sr24 is not effective in India and a virulent pathotype 40-1 was reported (Bhardwaj et al., 1990) although the donor TR 380- $14^*7/3Ag\#14$ exhibited a high degree of adult plant resistance to stem rust indicating the presence of some additional factors for resistance. Field evaluation of same 16 NIL's carrying *Lr*24 showed resistance type of reaction; however their corresponding recurrent parents were sowing susceptibility score ranging from 60S to 100S. From this study it is confirmed that Lr24 gene continued to provide resistance both in seedling as well as adult plant stage against all the occurring the leaf rust Pathotypes in India.

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Germplasm Maintenance:

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ICAR-IARI, RS, Wellington

At ICAR-IARI, RS, Wellington large number(>5000) of wheat (dicoccum, durum related and aestivum) and wheat species(wild spp) including primary, secondary, tertiary gene pools, leaf, stem, stripe rusts gene sources, synthetics, non host resistance sources including, barley, oats and constituted rust resistant back crossed wheat lines are continuously maintained. They were screened against all three rusts, powdery mildew, FHB etc., during 2017 both Kharif and rabi seasons. The elite stocks and effective gene sources were used for developing multiple disease resistant wheat varieties and stocks

S.No	Stocks	No.Lines
1	Lr, Sr, Yr, Pm, FHB resistance gene source stocks	446
2	Indian Released wheat varieties	427
3	Dicoccum	23
4	Durum	68
5	Aestivum parental stocks	711
6	Barley	233
7	Rye	32
8	Oats	24
9	Triticale	57
10	Synthetics	123
11	CIMMYT advance line/gene stocks	668
12	Constituted NIL/back crossed rust/pm resistant lines	820
13	Wild spp. Primary, secondary	1650
	and tertiary gene pool including	
	Agropyron	
	Total	5282

Demonstration of ICAR-IARI released Indian mustard varieties in nontraditional areas of Tamilnadu and Karnataka

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During the rabi 2016-17, ICAR-IARI Regional Station, Wellington conducted demonstration of three IARI released Indian mustard varieties (Pusa mustard 25, Pusa mustard 28 and Pusa mustard 30) at selected places of Tamilnadu and Karnataka (Table). The seed germination and crop establishment was proper at all the four places but due to consequent drought (which was very severe during 2016-17, for second consecutive years, over the entire peninsula region) the crop suffered heavily leading to complete loss of crop at two places i.e Hosur, Krishnagiri dist., TN and D.Naganahalli village, Tumakuru dist., Karnataka. However, by providing irrigation at critical stages of the crop, the performance of variety Pusa mustard 25 at Kodaikanal was very impressive with a seed yield of 4 kg/20 m² plot (2000 kg/ha) underlying the genetic potential of this variety and its suitability for growing in high altitude places like Kodaikanal. At another place, Thambatty village, Nilgiri, TN, variety Pusa mustard 30 expressed well but the yield recorded was low due to lack of thinning and crowding effect.

Sl.	Place	Variety	Date of	Seed	Remarks
No.		grown	sowing	yield	
				(kg)	
1	Thambatty village,	PM 30	02-12-2016	$6.5/60 \text{ m}^2$	Good crop but thinning
	Nilgiri dist., TN.				was not proper
2	Hosur, Krishnagiri	PM 25 &	23-11-2016	Not	Crop suffered due to
	dist., TN.	PM 30		reported	severe drought
3	Mannnavanur,	PM 25	14-12-2016	$4/20 \text{ m}^2$	Very good expression
	Kodaikanal, Dindigul				
	dist., TN.				
4	D. Naganahalli	PM 25,	05-12-2016	Not	Crop suffered due to
	village, Tumakuru	PM 28 &		reported	severe drought
	dist., Karnataka.	PM 30			_

Table: Performance of IARI released Indian mustard varieties in non-traditional areas of Tamilnadu and Karnataka





Thambatty, Ooty (DOS:02-12-2016)



Hosur, Krishnagiri (DOS:23-11-2016)

D. Naganahalli, Tumakuru, Karnataka (DOS:05-12-2016)



Mannavanur, Kodaikanal (DOS:14-12-2016)

Students Visit

Nearly 268 students of under graduate B.Sc(Ag), B.Sc(Horti), B.Sc(Botany) and post graduate M.Sc(Ag) from Acharya N G

Ranga Agricultural University, Naira, AP, Kerala Agricultural University, Vellanikara, Thirussur, conventional University colleges, Kerala, constituent colleges of Tamil Nadu Agricultural University (RVS Padmavathy College of Horticulture, Sempatti, Dindugul, TN, College of Agriculture, Vazhavachanur, Thiruvannamalai, TN, SRS Institute of Agricultural Technology, Vedansanthur, Dindugul, TN) and students of KV, Wellington had exposure visit to ICAR-IARI, RS, Wellington during Kharif and Rabi 2017. They have been explained about the research activities under taken at this station, role of this station in rust control programme and techniques involved in wheat improvement mainly of germplasm maintenance, crossing/ hybridization techniques, field phenotyping/rust scoring, field selection, seed production, AICWIP trials, lab techniques and SRT, glass house activities and summer nursery activities etc.,.





