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**Attention: Considering practical two crop cycles per annum at Wellington, data collection and compilation it has been decided to bring out NWN two issues in a year. Hence from January 2023 the NWN will be published as half yearly**

# Gene Stewardship in developing improved Indian bread wheat cultivars and genetic stocks with low terminal disease value-*A compendium –Part-II*: Introgression of Stem rust resistance genes

**Sivasamy M[1*], P.Jayaprakash[1], V.K.Vikas[1], Nisha.R[1], P.Shajitha[1],Vinod[2], S.M.S.Tomar[2], Niharikha Mallick[2], Rajbir Yadav[2], S.C.Bhardwaj[3], O.P.Gangwar[3], C.Uma Maheswari[1], P.Nallathambi[1], Sindhu A[1], Vijaishree S[1], K.Sivan[1],C. Arun Kumar[1], John Peter[1], Geetha M[1],V.Balaji[1] and M. Gokulakrishna[1]**

[1]ICAR-Indian Agricultural Research Institute Regional Station, Wellington, Tamil Nadu -643 231, India
[2]ICAR-Division of Genetics, Indian Agricultural Research Institute, Pusa, New Delhi-12. India
[3]ICAR - Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla-171001, India

***Corresponding author:**
iariwheatsiva@gmail.com

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**Introduction:**

Stem rust on wheat caused by the basidiomycete *Puccinia graminis* f.sp. *tritici* (Pgt) Eriks. & E. Henn., has historically posed a significant threat to wheat production worldwide as it can lead to substantial losses in both grain yield and quality (Singh et al., 2015). Stem rust, also referred to as black rust has an alternate host known as common barberry (*Berberis vulgaris*), (Lind, 1915; Stakman, 1923; Hermansen, 1968). However, the removal of common barberry has resulted in reduced severity of stem rust in Europe during the twentieth century. Stem rust is more prevalent in regions where wheat plants are exposed to warmer environments during the later stages of crop growth (Bhawani et al., 2022). During an epidemic, stem rust has the potential to completely devastate a seemingly healthy crop, leading to 100% yield losses. Early infections can result in failure to produce grains, and the panicles may be reduced to chaff.

The first detailed reports of wheat stem rusts were given independently by Italian scientists Fontana and Tozzetti in 1767 (Fontana, 1932; Tozzetti, 1952) and the causal organism was named *Puccinia graminis* in 1797 by Persoon (Schuman and Leonard, 2000). Stakman and Piemeisel (1917) showed that the stem rust pathogen had various forms or races only after the two devastating stem rust epidemics in North America in 1904 and 1916. The *P. graminis tritici* causing stem rust has been demonstrated to exhibit a very large host range and it is also able to survive in uredinal form on several grasses (Prasada, 1948; Joshi and Manchanda, 1963; Bahadur *et al*., 1973 and Pathak *et al*., 1979). Wheat stem rust epidemics occurred regularly from 1900-1955 in North America (Kolmer, 2001). Heavy epidemics in different years such as 1916, 1935, 1937, 1950-54 and others caused massive yield losses in wheat. Though stem rust has been successfully controlled worldwide by the use of highly resistant wheat cultivars and also by eradicating the secondary host of *P. graminis*, berberry (*Berberis vulgare*), it has been a major problem historically mainly in Africa, Australia, New Zealand, Europe, the Americas (both North and South), the Middle East of Asia (Saari and Prescott, 1985). The last major stem rust

epidemic occurred in Ethiopia in 1993 and 1994 (Shank, 1994).

The earliest record of stem rust epiphytotic in India has been reported in 1827 from Central India. Later severe stem rust epiphytotic was also recorded during 1956-57 from Pusa (Bihar), in Rajasthan during 1973-74, in Narmada valley (MP) during 1978-79.

In India, stem rust poses a significant threat affecting approximately 7 million hectares in the Central and Peninsular regions (Bhardwaj et al., 2019). Unlike in some other regions, alternate hosts do not play a role in the perpetuation of wheat rust pathogens under Indian conditions. This is primarily due to the non-synchronization of vulnerable tender barberry leaves with the availability of basidiospores, rendering alternate hosts inconsequential in the recurrence of stem rust in India (Mehta 1940; Nagarajan and Joshi 1985; Bhardwaj 2017).In India, teliospores of *P. graminis* f. sp. *tritici* are abundant in the plains region, but Berberis species, the alternate hosts, are found only in the hills. Moreover, the hot summers and rains that follow the wheat harvest create unfavorable conditions for the survival of the obligate parasite rust in the absence of wheat. Therefore, self-sown wheat plants and summer crops, particularly in the hills, are believed to be the primary sources for the survival and perpetuation of wheat rust pathogens in India, particularly in the form of urediospores (Prasad et al., 2018).

The presence of *Puccinia* pathogens in India has spurred significant research endeavors and interventions aimed at thwarting their destructive potential. Over the years, systematic breeding programs have been implemented to develop rust-resistant wheat varieties, with the utilization of genetic diversity playing a pivotal role in mitigating rust epidemics successfully (Bhardwaj et al., 2019).

In recent times, the re-emergence of the stem rust (SR) race "Ug99" in East Africa has raised serious concerns and posed a significant threat to global wheat production, despite being under control for more than three decades (Bartos et al., 1996; Singh et al., 2006). This resurgence has been especially alarming as it targets the stem rust resistance gene *Sr31*, rendering many wheat cultivars susceptible to the disease (Pretorius et al., 2000; Singh et al., 2011). Recognizing the urgency of the situation, Dr. Norman Borlaug took the lead in advocating for a joint effort to confront this threat, leading to the establishment of the Borlaug Global Rust Initiative (BGRI), formerly known as the Global Rust Initiative. The BGRI framework has played a crucial role in carefully monitoring the evolution and migration route of the "Ug99" group of races, thus providing early warning signals to all stakeholders in the event of an epidemic.

Since the initial detection of the original Ug99 isolate, the pathogen group has proven to be a highly dynamic and widespread threat to wheat crops. A total of 13 races belonging to the Ug99 group have been identified, with their presence spanning across several countries, including Uganda, Kenya, South Africa, Ethiopia, Sudan, Yemen, Iran, Tanzania, Zimbabwe, Eritrea, Mozambique, Rwanda, and Egypt (Singh et al., 2011a; http://rusttracker.cimmyt.org). The continuous emergence of new races within the Ug99 lineage poses a persistent challenge, rendering once-effective Sr (stem rust) genes ineffective against the evolving pathogen strains. This unsettling reality was confirmed by Bhavani et al. (2010), who found that newly evolved Ug99 strains, with added virulence to *Sr24* and *Sr36* resistance genes, resulted in susceptibility in over half of the TTKSK-resistant

wheat lines. Among the identified Ug99 races, TTKSF, TTKSF+Sr9h, and PTKST have been detected in both Zimbabwe and South Africa, while TTKSP has been observed solely in South Africa (Terefe et al., 2016).

The widespread distribution and frequent emergence of new races within the Ug99 group underscore the urgency for continuous surveillance and innovative strategies to combat stem rust in affected regions. Understanding the dynamics of these pathogen populations and their spatial distribution is crucial for formulating effective control measures to safeguard global wheat production and food security. By gaining insights into the challenges posed by Ug99, we can better prepare and develop sustainable approaches to mitigate the impact of this virulent pathogen on wheat crops in the affected regions.

Resistance genes that conferred low reactions to race TTKSK in seedling tests and in the field nursery at Njoro include *Sr13, Sr22, Sr24, Sr25, Sr26, Sr27, Sr28, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr40, Sr44* and *Sr Tmp* (Jin *et al*., 2007). Singh *et al.* (2005) has reported that certain genes show intermediate value for the race Ug99 such as *Sr*22, *Sr*24, *Sr*25, *Sr*26, *Sr*36 and *Sr*Tmp among the effective genes. But also it was found that both *Sr*24 and *Sr*36 that were resistant to Ug99 earlier are no longer effective against the new variants of Ug99 when present alone (Jin *et al*., 2008,2009).

In India's pursuit of rust resistance, the strategic deployment of resistance genes, such as *Sr31* in conjunction with *Sr2, Sr24, Sr5*, and *Sr8*, has proven highly effective in protecting wheat against stem rust (Bhardwaj et al., 2019). The concerted effort to leverage diverse resistance sources and deploy resistant varieties has not only contributed to the stability of wheat production in India but has also reaffirmed the nation's unwavering commitment to safeguarding its agricultural productivity against the ever-evolving threats posed by rust pathogens. India stands at the forefront of global efforts to combat wheat rust diseases and ensure food security by continuously pushing the boundaries of wheat breeding and implementing modern approaches.

The success of India's breeding endeavors to develop disease-resistant wheat varieties is well-documented, with detailed accounts of remarkable achievements in this field (Tomar et al., 2014). Furthermore, the integration of marker-assisted backcross breeding has become an integral part of Indian wheat breeding programs, enhancing the efficiency and precision of incorporating rust resistance (Bhardwaj et al., 2016).

In this context, a meticulously planned wheat improvement programme was initiated to introgress & pyramid effective stem rust resistance genes into popular Indian bread wheat cultivars (**Table 2**) at IARI RS Wellington since 1990'. The stem rust genes and the donor sources used are listed below in **Table 1**. This write up deals with the current status of stem rust in India, the factors influencing the prevalence, recurrence of the disease and the proactive measures undertaken to control and manage rust diseases, the strategies employed in breeding for resistance and the role of genetic diversity in developing rust-resistant wheat cultivars. Furthermore, we examine the challenges posed by the evolving nature of rust pathogens and the ongoing measures to ensure preparedness and surveillance against potential rust outbreaks.

**Table 1: Stem rust genes & donor sources used in the back-cross breeding programme (*Triticum aestivum*) and its adult plant response to rust diseases at Wellington**

| Stock | Gene(s) | Reaction to | | | |
|---|---|---|---|---|---|
| | | Stem rust | Leaf rust | Stripe rust | Powdery mildew |
| 1.Lok-1 | ***Sr2*** *+Lr27+ Yr30+* (Pseudo Black Chaff) **APR** | 70S | 80S | 80S | 4 |
| 2. | *Sr22* | 20S | 80S | 60S | 4 |
| 3. Tr380-147/3Ag#14 | *Sr24 Lr24* | 15R MR | F | 5MR | 2+ |
| 4. DARF6/3Ag3/Kite | *Sr24 Sr26 Lr24* | 10R MR-20R MR | F | 10MS | 3 |
| 5. Sunstar6/C80-1 | *Sr25 Lr19* | 10R MR-30R MR | F | F | 4 |
| 6. Cook6/C 80-1 | *Sr25/Lr19, Sr36/Pm6* | F | F | F | 1 |
| 7.DARF6/3Ag3/Kite | *Sr26* | F | F | 20S | 2 |
| 8. Kalyanasona4/*Sr27* | *Sr27* | F-Tr | 80S | 90S | 3 |
| 9. WH 542 | *Sr31 Lr26 Yr9 Pm8* | 10R MR | 80S | F | 3 |
| 10. Abe | *Sr36* | 15R MR | F | 40S | 1 |
| 10A. Cook | *Lr19/Sr25,* ***Sr36****/Pm6* | F | F | F | 1 |
| 11.Thatcher8/VPM 1, RL 6081 | *Sr38* | 20R – MR MS | F | 15MS | 4 |
| 11A. EC 381198 | *Sr 38* | F | F | F | 4 |
| 12. Thatcher+ Lr 35 | *Sr39* | F | F | F | 2 |

**Table 2: Recurrent parents in the Back-Cross programme (*Triticum aestivum*) & adult plant response to rusts & powdery mildew diseases**

| Stock | Gene(s) | Reaction to | | | |
|---|---|---|---|---|---|
| | | Stem rust | Leaf rust | Stripe rust | Powdery mildew |
| 1. **C 306** | *Lr34+* | 90S | 90S | F | 3 |
| 2. HD 2009 | | 40S | 60S | 100S | 3 |
| 3. HD 2285 | | 30MS | 100S | 30S | 3 |
| 4. HD 2329 | *Lr34+* | 80S | 90S | 90S | 3 |
| 5. HD 2402 | *Sr2+* | 30S | 100S | F | 3 |
| 6. HD 2687 | *Sr31 Lr26 Yr9 Pm8* | 15R MR | 80S | F | 3 |
| 7.HD 2733 | *Sr31 Lr26 Yr9 Pm8* | F | 60S | F | 3 |
| 8.HD 2877 | *Sr31 Lr26 Yr9 Pm8* | F | 60S | F | 3 |
| 9. HI 1077 | | 30MS S | 50S | 40S | 3 |
| 10. HS 240 | *Sr31 Lr26 Yr9 Pm8* | 5R MR | 70S | F | 3 |
| 11. HUW 234 | | 20MS S | 100S | F | 3 |
| 12. J 24 | | 90S | 100S | 100S | 3 |

| | | | | | |
|---|---|---|---|---|---|
| 13. Kalyansona | | 80S | 90S | 90S | 3 |
| 14. Lok-1 | *Lr34, Sr2+* | 70S | 80S | 80S | 3 |
| 15. NI 5439 | *Lr34* | 90S | 90S | 100S | 3 |
| 16. PBW 226 | | 20S | 90S | F | 3 |
| 17. Sonalika | *Sr2+* | 60S | 80S | 60S | 3 |
| 18. UP 262 | | 50S | 50S | 50S | 3 |
| 19.VL 421 | | 60S | 90S | 80S | 3 |
| 20. WH 147 | | 90S | 90S | 90S | 3 |
| 21. WH 542 | *Sr31 Lr26 Yr9 Pm8* | 10R MR | 80S | F | 3 |
| 22. WL 711 | | 100S | 100S | 90S | 3 |
| 23. HI 977 | | F | 60S | 40S | 2 |
| 24. HP 1205 | | 60SS | 80SS | 90S | 3 |
| 25. PBN 51 | *Sr31 Lr26 Yr9 Pm8* | 20MR | 40S | S | 2 |
| 26. PBW 343 | *Sr31+, Yr24* | 20MR | 60S | 5S | 3 |
| 25. Raj 3077 | *Lr23, Sr2+* | 5MR | 60SS | 60SS | 1 |
| 26. HW 3070 | *Sr24+, Sr31 Lr26 Yr9 Pm8* | F | F | 5S | 2 |
| 27.GW 273 | *Sr2+* | | | | |
| 28.Lal Bahadur | | 20S | 80S | 20S | |
| 29.NIAW 34 | *Sr2+* | | | | |
| 30.UP 2425 | *Sr2+* | | | | |
| 31.HD 2189 | *Sr2+* | 20S | 60S | 20S | 3 |
| 32.PBW 502 | *Sr31 Lr26 Yr9 Pm8* | 20MR | 40S | 5S | 3 |
| 33. UP 2338 | *Sr31 Lr26 Yr9 Pm8* | 20MR | 60S | F | 2 |

**Stem Rust genes:**

### *Sr2 (Pseudo Black Chaff/Pbc)*-derived from *T.aestivum*-APR gene

The adult plant resistance gene *Sr2* located in 3BS chromosomeshows recessive inheritance and closely associated with *Lr27* and *Yr30* (Singh and McIntosh 1984). *Sr2* was originally introgressed from cultivated emmer (*T. dicoccum*) over 80 years ago by McFadden (1930) in developing the bread wheat lines Hope and H-44. Hare and McIntosh (1979) determined the stem rust resistance in the cultivar Hope was largely controlled by a single gene (*Sr2*) located on the short arm of chromosome 3B.

*Sr2* is widely occurring in many wheats in Australia, Canada, Kenya, USA, Mexico and Indian subcontinent (Luig, 1983; Roelfs, 1988) which include some of the varieties like Songlen (additionally carrying *Sr5, Sr6, Sr8a* and *Sr36*), Bluebird series include Nuri70 (additionally carrying *Sr5, Sr6* and *Sr8a*), Lerma Rojo64 (+ *Sr6, Sr7b* and *Sr9e*). The variety Pavon (+*Sr8a, Sr9g* and *Sr30*) and Sonalika carried *Sr2* (McIntosh, 1988). *Sr2* is the most important stem rust resistant gene to be deployed in modern plant breeding in wheat (McIntosh, 1988; Rajaram *et al.,* 1988 and Roelfs, 1988). This could be attributed to its non-hypersensitive and non-race specific resistance (APR) and in combination with other genes offers durable resistance for the stem rust worldwide (Hare

and McIntosh, 1979). In Indian wheats, however this gene alone is not effective to the pathotypes of stem rust prevailing in the Nilgiris (Wellington) and in association with other genes through additive gene action confers high degree of resistance. Several Indian wheat cultivars carry this gene *Sr2* deployed unintentionally and its tight linkage to a phenotypical marker - Pseudo black chaff (*Pbc*) offers better scope for the breeders to easily introgress the gene (**Fig.1**). Through planned breeding programme a variety HW 5207 (CoW3) carrying *Sr2* (**Fig.2**) pyramided with *Sr24/Lr24* and *Yr15* developed by the authors showing lesser intensity of *Pbc* has been released as state release for cultivation in Tamil Nadu.

*Figure 1: Pbc*- Phenotypical marker associated with *Sr2+*

Almost more than 50 different stem rust resistance genes are now catalogued. Several of which are incorporated in wheat from alien relatives of wheat (McIntosh, 1998). Of these only *Sr2* has been characterised as an APR with a slow rusting phenotype (Hare and McIntosh, 1979). Stem rust resistance conferred by the *Sr2* gene located on the short arm of chromosome 3B is an important disease resistance gene in many wheat breeding programs around the world (Hayden *et al*., 2004). For more than 50 years, this adult plant resistance gene has provided effective broad-spectrum resistance to wheat stem rust caused by the fungal pathogen *Puccinia graminis Pers.* f. sp.*tritici*. *Sr2* gene is race – non-specific and is expressed in both seedling and adult plants. *Sr2* plays an important role in wheat production throughout the world as reflected by the presence in many wheat cultivars (McIntosh, 1988; McIntosh *et al*., 1995; Rajaram *et al*., 1988; Roelfs, 1988). Wheat with *Sr2* was moderately susceptible to race 15B during the epidemics of the 1950s; however, with this exception the gene has provided durable resistance since being introduced into common wheat.

Stem rust resistance has been stable after 40 years of utilization of the genes derived from the cultivar Hope, and losses due to stem rust have been negligible since the late '60s'. The genetic nature of this adult plant resistance is not completely known, but the *Sr2* gene is recognized as a major component (Sunderwith and Roelfs, 1980).

Combination of *Sr2* with other unknown slow rusting resistance genes possibly originating from Thatcher and Chris, commonly known as the "*Sr2*-complex" which actually

consists of *Sr2* plus 4-5 minor genes pyramided into three to four gene combinations (Singh *et al*., 2008) provided the foundation for durable resistance to stem rust in germplasm from the University of Minnesota in the United States and the Sydney University in Australia (McIntosh, 1988; Rajaram*et al*., 1988).

Seedling leaf rust resistance gene *Lr27* specifically co-segregated with *Sr2* suggesting that a single gene may confer race specific leaf rust and non-race specific, adult plant stem rust resistance in wheat (Spielmeyer *et al.,* 2009). *Sr2* is reported to be tightly linked to the leaf rust resistance gene *Lr27*, and partial APR stripe rust resistance gene *Yr30* and powdery mildew (Singh and McIntosh, 1984; Singh *et al*., 2000b). Wheat plants with inactivated *Lr27* alleles from mutagenesis appear to have lost *Sr2* possibly indicating pleiotrophism (Spielmeyer *et al*., 2009).

The genetic association of phenotypic markers with low rust response allows indirect selection of resistance in breeding programs. Hare and McIntosh (1979) reported that such linkages have been used to select for resistance to rust diseases of wheat. One such example is pseudo-black chaff (*Pbc*) and seedling chlorosis (Brown, 1997) are linked with *Sr2* (Singh, 1992). *Pbc* is a dark pigmentation usually present on the lower most internodes and on the glumes (**Fig. 1**). The *Pbc* phenotype facilitates the selection of the breeding lines carrying *Sr2* but high levels of *Pbc* expression (especially on glumes) are thought to reduce yield and farmer acceptance in some circumstances (Sheen *et al*., 1968). Attempts to break the linkage between *Sr2* and *Pbc* have been failed (Kota *et al*., 2006).

Singh *et al.* (2011b) has reported in his earlier studies that *Sr*2 alone will not confer adequate level of resistance to *Ug*99. Kingbird, a new advanced line, is at present the best known source of APR gene *Sr2* in semi dwarf wheat with maximum score recorded to be 5 MR-MS during the same period. Because these wheat are susceptible as seedlings with race Ug99, their resistance is speculated to be based on multiple additive genes where *Sr2* is an important component.

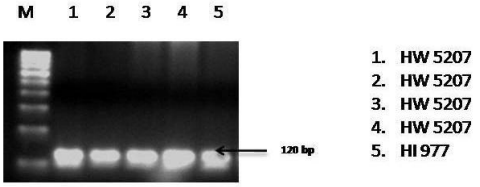
A *Sr2* – carrying cultivar, Sonalika, released in the mid – 1960s in the Indian subcontinent and subsequently grown on millions of hectares, remained resistant to stem rust. Singh *et al.* (2011b) has reported in his earlier studies that *Sr2* alone will not confer adequate level of resistance to *Ug*99. Later on wheat lines that displayed *Pbc*, Singh *et al*. (2008) observed varying degree of disease severity in Kenya ranging from traces to about 60-70% compared to 100% severity for highly susceptible lines. Reaction types varying from MR to S (Moderately resistant to Susceptible) on the same internodes of *Sr2* bearing plants clearly indicated that *Sr2* did confer at least some resistance. Resistance gene *Sr25* located on *A. elonagatum* translocation together with leaf rust resistance gene *Lr19* on chromosome 7DL conferred high level of resistance only in some genetic background especially when the APR gene *Sr2* was also present. This translocation is also known to enhance yield potential (Singh *et al*., 1998).

As part of the on-going evaluation of wheat cultivars at field sites in Kenya it is evident that genotypes combining *Sr2* and other seedling resistance genes exhibit enhanced levels of adult plant resistance relative to the effects attributed to the seedling resistance genes alone (Njau *et al*., 2010). These evaluations managed by CIMMYT in Kenya and elsewhere, enabled identification of wheat cultivars carrying APR genes additional to

*Sr2*(Singh *et al*., 2008; Njau *et al*., 2010). *Sr2* is recessively inherited, making it difficult to detect in segregating populations, especially in the presence of other rust-resistance genes (Brown, 1993; McIntosh *et al*., 1995). Hence, use of molecular markers will aid in the selection of this gene

.

**Figure 2: Confirmation *Sr2* in HW 5207 using SSR marker- *gwm 533***




### *Sr14 (T. turgidum)*

The *T. turgidum*-derived gene *Sr14* was first transferred to hexaploid cultivar Steinwedel which resulted in another cultivar Khapstein (Waterhouse, 1933). This gene shows low infection type and low environmental variability but it appears to enhance the distinct necrosis which is very characteristics of this gene (Knott, 1989). The Khapli emmer *Sr13* along with *Sr14* is the reference stock along with the source stock Yuma in USA. Although this gene was not widely exploited worldwide but its combined effect with other genes has been lately realized by the breeders especially for durum wheat improvement. The efforts are on at IARI, RS, Wellington to take up this gene in the gene pyramiding programme.

### *Sr22 (T.monococcum)*

The temperature sensitive stem rust gene *Sr22* with chromosomal location 7A (Kerber and Dyck, 1973) is more effective at lower temperatures. The *monococcum*-derived gene *Sr22* present in the stock RL5244 often found in the wild einkorns (The, 1973). The use of this gene in agriculture was limited until recent time because of the linkage drag of larger segment of *Sr22* transfer which resulted in yield penalties. However, the authors are currently using this stock, *Co 1213 HSBVN 163313* (Bariana and Lagudah, 2012, Personal communications) with reduced segment in their gene pyramiding programme.

### *Sr24/Lr24 (Agropyron elongatum=Thinopyrum ponticum)*

This gene complex has already been discussed elaborately under *Lr24.* Although it is been deployed in a number of cultivars worldwide, the virulence for *Sr24* has been reported in South Africa (Le Roux and Rijkenberg, 1987b) and in India (Bhardwaj *et al.,* 1990) compelling to use this gene complex with other effective stem rust genes to harness the effectiveness of *Lr24* in India. Number of back crossed and NIL lines carrying *Sr24/Lr24* has been developed at Wellington (See under *Lr24*)

Smith *et al.* (1968) first determined *Sr24* to reside on the 3DL chromosome, within a spontaneous translocation from the 3Ag

chromosome of *Agropyron elongatum*. The leaf rust resistance gene *Lr24*, also found within the *A. elongatum* translocation was found to be linked to *Sr24* in all the recombinant types. Thus the selection of genotypes with the molecular marker for *Sr24* gives an additional advantage of selection for *Lr24* also. In 1973, Sears developed more recombinant lines, successfully introducing a much smaller *A. elongatum* translocation segment containing *Sr24/Lr24* into the 3DL chromosome. This truncated segment broke the linkage between *Sr24/Lr24* and red grain colour observed in Agent, allowing the subsequent introgression of *Sr24/Lr24* into white – grained wheat.

Artificial mutation studies suggested that the avirulence gene corresponding to *Sr24* rarely mutates to virulence (Luig, 1983). *Sr24* offers resistance to most races of stem rust, including the virulent race Ug99 (TTKSK) now established in East Africa and Ethiopia. In South Africa previous experience has shown the devastating effect of *Sr24* virulence on cultivars protected by this gene alone (Le Roux and Rijkenberg, 1987). Also, *Sr24* individually is not effective against a more recent variant of Ug99, designated as TTKST.

Until recently, *Sr24/Lr24* conditioned resistance in both seedling and adult plants to stem rust and leaf rust worldwide. Kumar *et al*. (2011) reported that gene *Lr24* shows promise as it stands resistance to all pathotypes of leaf rust prevailing in Nilgiris, the rust source area of India. Because of the widespread effectiveness of *Sr24/Lr24* in controlling stem and leaf rust, it has been exploited extensively. *Sr24* also serves as a universal resistance tester in pathogen variability surveys worldwide (Lombard, 1986; Martens, 1985 and Roelfs *et al*., 1983). The authors have developed nearly 16 NIL's/BIL's in the back-ground of popular Indian bread wheat cultivars listed as under which have been confirmed molecularly with SSR marker *Sr24#12* and gene specific marker $SCS73_{719}$ (**Fig 3 & 4**).

**Table 3: Phenotypic validation of Seedling response of *Lr*24/Sr24 in NILs/back crossed lines, recurrent parent and donors against predominant stem rust pathotypes**

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

**Fig 3: Molecular validation of *Sr*24 gene in NIL's with *Sr*24#12marker**

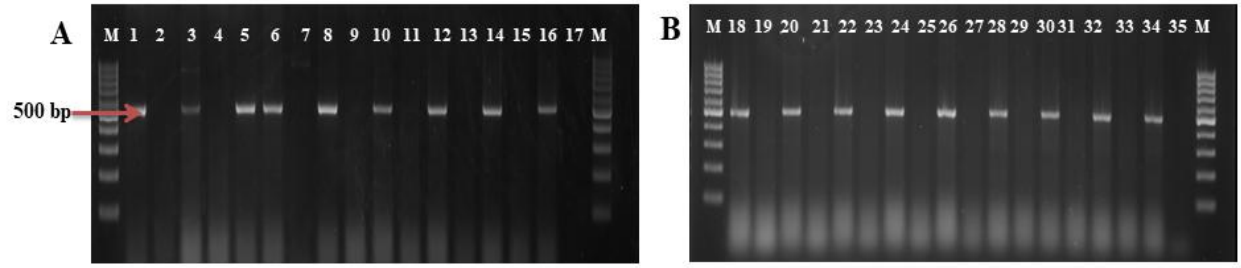



**Fig 4: Molecular validation of *Sr*24 gene in 16 NIL's with $SCS73_{719}$marker**

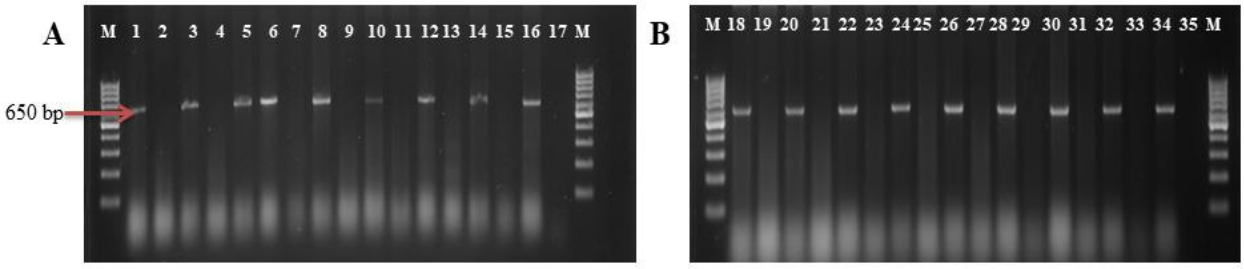



**Fig. 3 and Fig. 4**: M- 100bp Ladder, 1- Darfkite, 2- Sonalika, 3- HW 2001A, 4- Kalyansona, 5- HW 2002, 6-HW 2002A, 7- NI5439, 8- HW 2003, 9- C306, 10- HW 2004, 11- WH147, 12- HW 2005, 13- LOK1, 14- HW 2006, 15- HD2329, 16- HW 2007, 17- NTC, 18- Darfkite, 19- HD 2285, 20- HW 2008, 21- WL711, 22- HW 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

### *Sr25/Lr19 (Agropyron elongatum=Thinopyrum ponticum)*

Very few cultivars carrying these linked genes have been released for commercial use in the world. *Sr25* in combination with *Sr36* and *Sr6* has exhibited a high degree of resistance in Australian cultivar Cook which indicated that *Sr25* could be useful in combination with other genes (Luig, 1983). The line Cook6/C80-1 exhibited immune reaction to stem rust pathotypes prevailing in the Nilgiris as compared to Sunstar6/C 80-1 and its derivatives. Both these lines carry *Sr25*. Prabhu *et al.,* (1998) confirmed this line (Sunstar6/C 80-1) carry *Lr24/Sr24* not *Sr25.* Nearly 96 BC lines has been constituted and molecularly confirmed and published (Sivasamy et al., 2009) (see under *Lr19*). The wheat lines carrying this gene complex already listed in *Lr19/Sr25* in previous issue of NWN 10(1) (See under *Lr19*)

Link:
https://iari.res.in/files/Publication/Nilgiri_Wheat_News/Nilgiri_Wheat_News_02012023.pdf

### *Sr26 (Agropyron elongatum=Thinopyrum ponticum)*

Knott (1961 and 1968) used irradiation for transferring stem rust resistance gene *Sr26* from long arm of chromosome 6 from *Agropyron elongatum* to the long arm of wheat chromosome 6A. The use of *Sr26* has

contributed immensely towards cultivar improvement. Martin (1971) for the first time, in Australia, transferred *Sr26* to a variety named Eagle. Subsequently the spectacular resistance imparted by this gene has been extensively used in several Australian cultivars grown widely, which competed satisfactorily with contemporary cultivars although it does cause a reduction in yield (The *et al.*, 1988; McIntosh *et al.*, 1995). The gene *Sr26* continues to be very effective in Indian also and this effective alien stem rust resistance has been introgressed into five well adapted but stem rust susceptible Indian bread wheat cultivars through a judicious backcrossing (**Table 4**). The gene *Sr26* is dominant and produces a typical infection type which has served as a good indicator for selection of genotypes in each segregating generation for making subsequent backcrosses. The gene exhibits low infection type and no virulence has been identified anywhere in the world (Huerta-Espino, 1992). Additionally, the presence of *Sr26* gene can also be confirmed using the microsatellite marker Sr26#43 (Mago et al., 2005) (**Fig. 5 & 5A**)

**Table 4: Wheat genotypes pyramided with *Sr26* developed at IARI, RS, Wellington (Potential source for resistance against Ug99 and its variants)**

| Recurrent Parent cultivar | Introgressed line | Genes Incorporated/Pyramided | Pedigree of improved line | Reaction to Indian Spectrum of stem rust pathogen at Wellington |
|---|---|---|---|---|
| C 306 | HW 2023 | *Sr24Sr26Lr24* | C 3067// DARF6/ 3AG3/Kite | 15R MR |
| Kalyansona | HW 2021 | *Sr24Sr26Lr24* | Kalyansona7// DARF6/ 3AG3/Kite | 20R MR |
| Lok-1 | HW 2094 | *Sr24Sr26Lr24* | Lok-16// DARF6/ 3AG3/Kite | 10R MR |
| NI 5439 | HW 2026 | *Sr24Sr26Lr24* | NI 54397// DARF 6/ 3AG3/Kite | 20R MR |
| Sonalika | HW 2027 | *Sr24/ Lr24, Sr26* | Sonalika7// DARF 6 /3AG3/Kite | 5R MR |
| WH 147 | HW 2022 | *Sr24/Lr24, Sr26* | WH 1477// DARF 6 / 3AG3/Kite | 20R MR |
| Kalyansona | HW 2088 | *Sr26,Lr28* | Kalyansona3//CS 2A/2M 4/2/Kite | 10R MR |
| Lok-1 | HW 2096 | *Sr26,Lr28* | Lok-13//CS 2A/2M 4/2/Kite | 10R MR |
| WH-147 | HW 2099 | *Sr26,Lr28* | WH-1473//CS 2A/2M 4/2/Kite | 20R MR |
| Kalyansona | HW 2089 | *Sr26,Lr32* | Kalyansona 3//C86-8 /Kalyansona(F4)/Kite | 10R MR |
| NI 5439 | HW 2090 | *Sr26,Lr32* | NI 54393//C 86-8/ Kalyansona(F4)/Kite | 20R M |

Individually not effective to Ug99 but effective in combination with *Sr26 orSr27* and other effective stem rust genes

**Figure 5: Marker Assisted selection of *Sr26* gene using STS marker – *Sr26#43* in different bread wheat backgrounds**

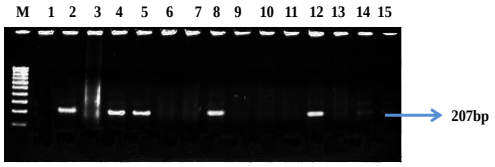



**Fig 1 Amplification of STS marker *Sr26#43* specific to *Sr26* gene of wheat (M-100bp ladder, Lane 1-WH 147 (Susceptible parent), Lane 2-Darf kite (Donor), Lane 3-HW 2021(*Sr26*), Lane 4-HW 2096(*Sr26*), Lane 5- 15-WH 147x HW 2021(*Sr26*) backcrossed segregating lines**

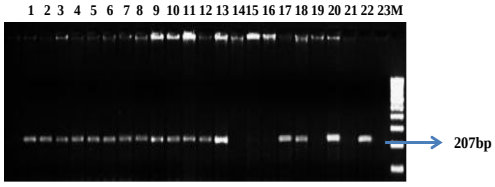



**Fig 2 Amplification of STS marker *Sr26#43* specific to *Sr26* gene of wheat; (M-100bp ladder, Lane 1-HW 2023(*Sr26*), Lane 2-22 Lok-1 x HW 2023 (*Sr26*) backcross derived lines, Lane 23-Lok-1(susceptible parent )**

**Figure 5A: Marker Assisted selection of *Sr26* gene using STS marker – *Sr26#43* in different bread wheat backgrounds**

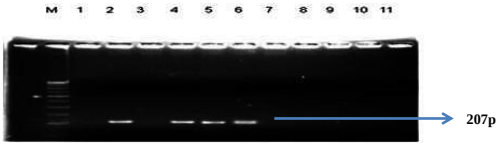



**Fig 3 Amplification of STS marker *Sr26#43* specific to *Sr26* gene of wheat (M-100bp ladder, Lane 1-WH 147(susceptible parent), Lane 2 - Darf kite (Donor), Lane 3-11-WH 147 x Darf kite *Sr26* segregating lines**

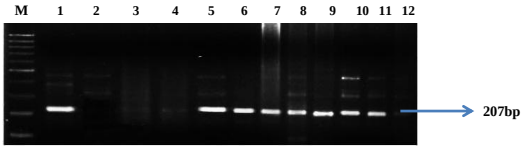



**Fig 4 Amplification of STS marker *Sr26#43* specific to *Sr26* gene of wheat (M-100bp ladder, Lane1-HW 2096(Donor), Lane 2- HD2329 (Susceptible parent), Lane 3-12-HD 2329 x *Lr24* x HW 2096 with *Sr26* (segregating lines).**

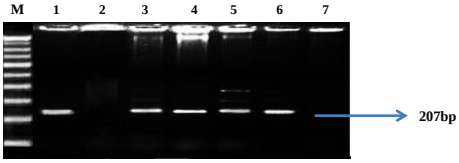



**Fig 5 Amplification of STS marker *Sr26#43* specific to *Sr26* gene of wheat (M-100bp ladder, Lane1-HW 2094(Donor), Lane 2- J24(Susceptible parent), Lane 3-7 J24x HW 2094(*Sr26* Segregating lines).**

## *Sr27 (Secale cereale; Imperial rye)*

The gene *Sr27* was transferred, by using irradiation treatment, from *Secale cereale* (Imperial rye) chromosome 3R to chromosome 3A of Chinese Spring wheat (Acosta, 1962). Rao (1978) confirmed that *Sr27* is derived from short arm of rye chromosome 3R. Sawhney and Goel (1981) reported that *Sr27* is effective in seedling stage against 19 pathotypes of stem rust in India which included the pathotypes commonly occurring in the Nilgiris. The line carrying *Sr27* exhibited very high degree of resistance at the adult stage in Wellington. Virulence for *Sr27* is rare. Harder *et al.,* (1972) isolated an east African culture virulent on a Pembina line with *Sr27*. Initially, *Sr27* was very effective in Australia but later on isolates of stem rust from triticale variety Coorong were virulent on wheat seedlings with *Sr27*. Cultivar Satu was recommended in Australia as a replacement for Coorong, later mutant of the Coorong pathotype evolved (McIntosh, 1983). Despite not being employed for commercial purposes, the gene *Sr27* has not found practical application in breeding. We have transferred *Sr27* in the genetic background of Indian wheat varieties like Kalyansona, C306 and Lok-1. The successful transfer and pyramiding of the effective stem rust genes *Sr26, Sr27* in the adapted Indian bread wheat cultivars, already carrying other linked leaf rust genes *Lr19, Lr24, Lr28* and *Lr32* which are expected to confer resistance to occurring stem rust pathotypes in India and also Ug99, developed through

backcross programme at IARI, Regional Station, Wellington are listed in **Table 5** .

**Table 5: Wheat genotypes pyramided with *Sr27* developed at IARI, RS, Wellington (Potential source for resistance against Ug99 and its variants)**

| Parent cultivar | Introgressed line | Genes Incorporated | Pedigree of improved line | Reaction to Indian Spectrum of stem rust pathogen at Wellington |
|---|---|---|---|---|
| C 306 | HW 2091 | *Sr27,Sr24/Lr24* | C 3063//TR 380-14 7/3Ag#14/KS *Sr27* | F |
| Kalyansona | HW 2025 | *Sr27,Sr24/Lr24* | Kalyansona3//TR380-147/3Ag#14/KS *Sr27* | F-TR |
| Lok-1 | HW 2095 | *Sr27Sr24/Lr24* | Lok-13//TR 380-14 7/3Ag#14/KS *Sr27* | F-TR |
| C 306 | HW 2093 | *Sr27,Lr28* | C 3063//CS 2A/2M 4/2/KS *Sr27* | F |
| Kalyansona | HW 2024 | *Sr27,Lr28* | Kalyansona//CS 2A/2M 4/2/KS *Sr27* | F-TR |

Individually not effective to Ug99 but effective in combination with *Sr26 orSr27* and other effective stem rust genes

### *Sr30*

Knott and McIntosh (1978) identified *Sr30*, a recessive gene which is located on long arm of 5D chromosome. The Webster gene *Sr30* believed to carry morphogenic resistance to stem rust is the only non-alien gene conferring moderate resistance to stem rust in India. *Sr30* is reported to be effective to 12 cultures of Indian stem rust pathotypes at seedling stage; however the most prevalent pathotypes viz., 12,40A and 117A-1 exhibited virulence on *Sr30* (Sawhney and Goel, 1981). Virulence(s) to *Sr30* have been reported in several countries (Huerta-Espino, 1992). Commercial cultivars with *Sr30* were released in Australia but soon virulent pathotypes increased. Genotype likes Lerma Rojo 64A when introduced in Indian was initially resistant to stem rust but later, virulent pathotypes developed.

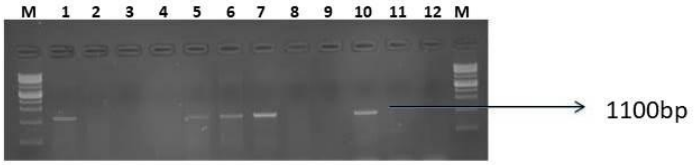
### *Sr31/Lr26/Yr9/Pm8 (Secale cereale* cv. Petkus Rye)

A *Secale cereale* cv. Petkus derivative was a spontaneous translocation (see *Lr26*) isolated from Germany in the 1930s (Zeller, 1973). *Sr31* has been profitably exploited in CIMMYT wheat breeding programme and continues to occur at high frequencies. It is present in many European, Chinese and USA wheats. Widespread occurrence in Indian subcontinent can be noticed among the recently released cultivars, viz., Pak. 81, Sarhad 82, CPAN 1922, HUM 206, CPAN 3004, UP 2338, WH 542, PBW 343, HD 2687. The value of *Sr31* as a source of protection against stem rust is difficult to determine (McIntosh *et al.*, 1995). However, use of the gene *Sr31* may reflect the broad agronomic adaptability worldwide rather than the unique contribution of stem rust resistance. The global importance of 1BL. 1RS in wheat breeding programme has been well documented (Rajaram *et al*., 1988; Villareal, 1991 and Kazman *et al.*, 1998). These are potential problems of bread making characteristics associated with *Sr31* which has restricted its use in Australia. The gene *Sr31* exhibits low infection type in seedling stage and shows moderately resistant to moderately susceptible reaction to stem rust pathotypes in the Nilgiris. The authors observed that the gene *Sr31* in combination with the gene *Sr25* and *Sr24* have shown enhanced

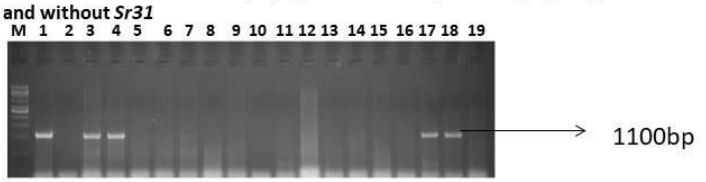
resistance to stem rust in the genetic backgrounds of many Indian bread wheats. However the lines carrying Sr31 showing enhanced susceptibility to wheat powdery mildew (WPM)

In India and its neighboring countries, the absence of pathotypes within the Ug99 lineage, notorious for their devastating impact on wheat crops, comes as a relief (Prasad et al 2019). However, India remains steadfast in its proactive approach towards combating potential threats. Extensive pathotype analyses have underscored the effectiveness of the resistance gene *Sr31* against the stem rust pathogen, bolstering the country's readiness to protect its wheat crops. India has established a robust system, encompassing ongoing rust surveillance, pathotype monitoring, and strategic deployment of resistant varieties, to ensure continuous vigilance and swift response to any potential rust outbreak that may endanger wheat production. Under the BGRI initiatives the rust survey and surveillance programme was initiated during 2009 as per Shimla declaration and since then the Ug99 quickset (**Table 6**) is being regularly raised at Wellington a 'Hotspot' for rust diseases to effectively monitor the spread of Ug99 into India.

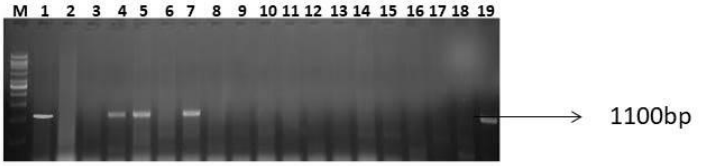
**Figure 6: Marker Assisted selection of *Sr31* gene using STS marker – *IAG95* in different bread wheat backgrounds**




**M: 1KB ladder, Lane 1: COW(W)1 (Positive control), 2-12:Segregating lines with and without *Sr31***




**M: 1KB ladder, Lane 1: COW(W)1 (Positive control), 2-19:Segregating lines with and without *Sr31***




**M: 1KB ladder, Lane 1: COW(W)1(Positive control), 2-19:Segregating lines with and without *Sr31***

**Table 6: Ug 99 quick set reactions/seedling response to isolates of stem rust at IARI RS Wellington**

| Differential Line | Observed Reaction at Wellington quick set | Ug99 Reaction |
|---|---|---|
| Morrocco | 3+ | 3+ |
| LMPG | ;1 and 2 | 3+ |
| MACS 2496 | ; and ;2 | 3+ |
| Bacanora – WH 542 | - | 3+ |
| PBW 343 | ; and 2 | 3+ |
| *Sr31*/LMPG | ;2 | 3+ |
| *Sr24* (Tr 380-14) | ;1 and 2 | 3+ |
| *Sr36* (Cook)-2 | ; and 2 | 3+ |
| *Sr36* (Cook) | ;1 | 3+ |
| *Sr36* (LMPG | ;1 and ;2 | 3+ |
| Rye | ; | 3+ |

### *Sr32 (Aegilops speltoides)*

Sears (1973) used homoeologous recombination to introgress gene *Sr32* imparting resistance to stem rust from the group 2 *Aegilops speltoides* chromosome 2S# 1 to wheat chromosomes (McIntosh, 1991) 2A (C 82.1), 2B (C 82.2) and 2D (C 82.3). Although C 82.2 (*Sr32*) is a normal translocation (McIntosh *et al.*, 1995), the reasons for non-utilization of this gene are not known. No virulence on *Sr32* has been found anywhere in the world. The gene *Sr32* exhibited a very high degree of adult plant resistance to stem rust pathotypes at Wellington. However seedling reaction to 40A and 40-1 pathotypes was low (IT; 1+). Patil and Deokar (1996) reported that *Sr32* conferred effective seedling resistance to 18 Indian stem rust pathotypes. Stem rust resistant reactions obtained world over indicate that *Sr32* may be a useful gene for the improvement of wheat cultivars (McIntosh *et al*., 1995).

### *Sr33/Lr21(Aegilops squarrosa)*

The gene *Sr33* has been transferred from *Aegilops squarrosa* (Kerber and Dyck, 1979) and has been located on chromosome 1DS of wheat. It has exhibited moderate resistance to Indian pathotypes of stem rust in the adult plant stage. No virulence on *Sr33* has been reported in the survey made by Huerta-Espino (1992). This gene has not yet been utilized commercially anywhere in the world. The gene *Sr33* is linked to the genes *Lr21*, *Rg2* and *Gli-D1* (McIntosh *et al*., 1995). Genes with moderate intensity of infection like *Sr33* may be quite useful in wheat breeding.

### *Sr36/Pm6 (Triticum timopheevii)*

In 1954, Allard and Shands, as well as Nyquist in 1957, successfully moved the stem rust resistance gene *Sr36* from *Triticum timopheevii* to the 2BS chromosome of common wheat, it was later mapped on the short arm of chromosome 2B (Gyarfas, 1978; McIntosh and Luig, 1973). This gene, Sr36, offers significant protection against various stem rust pathotypes found in India, displaying robust resistance. Moreover, it has demonstrated strong adult plant resistance against prevalent pathotypes in the Nilgiris region. Timgalen *(Sr36),* an Australian wheat cultivar was used as one of the parent in the development of cvs. HW 657 and HW 888. The

variety, HW 657 was released as a commercial cultivars and exhibited stem rust resistance in peninsular India, while HW 888 showed resistance to stem rust over a period 20 years at multilocations in India. Both these genotypes presumably carry *Sr36* gene (Kochumadhavan, unpublished). The stem rust resistance conferred by *Sr36* has been very valuable in Australia and many cultivars like Mengavi, Mendos, Timgalen and Cook were released. However, pathotypes of stem rust virulent on *Sr36* were isolated in Australia. Pathogenic variations in most of the major regions have also been reported (Huerta-Espino, 1992).

*Sr36* was originally transferred into two hard red spring wheat lines, CI12632 and CI12633 (Allard and Shands, 1954; Dyck, 1992; Tsilo *et al*., 2008). Powdery mildew resistance gene *Pm6* is also found to be tightly linked to be tightly linked to this gene (Sivasamy *et al*., 2009). This gene complex occurs in a high frequency in the US soft winter wheat (Jin and Singh, 2006) and in some Australian wheat varieties (Bariana *et al*., 2001).

To some races of stem rust, *Sr36* conditions unusual (mixed) type of infection (Ashagari and Rowell, 1980) which can make it difficult to distinguish cultivars carrying this gene. The *Sr36* gene is one of the 18 stem rust resistance genes that provided a major source of resistance to TTKS (Singh *et al*., 2005a; Wanyera *et al*., 2006).

The *Sr36* gene displays an almost immune response to the TTKSK and TTKST races of *Puccinia graminis* f. sp. *tritici* at both the early growth stage and the adult plant stage, as observed in studies by Jin et al. in 2008 and 2007a. Despite the presence of virulent races that can overcome *Sr36's* resistance, this gene remains highly valuable for its effectiveness against Ug99 and its wide distribution in adapted germplasm. However, there have been instances of susceptible pustules appearing in Kenya during 2007 on wheat lines carrying this gene, indicating the continued evolution of Ug99, a finding that was subsequently confirmed. Given the existence of *Sr36*-virulent races, the optimal approach is to combine this gene with other Sr genes, as suggested by Knott in 1988 and confirmed by the presence of such races (Knott, 1989).

The authors initially constituted a line carrying only *Sr36/Pm6* in the back-ground of HUW 234 through the cross HUW 234// Cook6/C 80-1 christened as HW 4444. This was later used as donor to pyramid this gene with other rust resistance genes to enhance the resistance for stem rust and developed nearly 100 back-crossed lines in the back-ground of popular Indian bread wheat cultivars as listed in **Table 7**.

**Table 7: Popular Indian bread wheat cultivars pyramided with *T.timopheevii*-derived effective linked stem rust & Pm gene *Sr36/Pm6* at IARI RS wellington**

| Sl. No | Backcross line/ cultivars | | Rust resistance genes it carries | Adult plant response to | | | |
|---|---|---|---|---|---|---|---|
| | | | | Stem rust | Leaf rust | Stripe rust | Powdery mildew |
| | **Cook6/C 80-1** | | ***Sr25 Sr36 Lr19 Pm6*** | **F** | **F** | **F** | **1** |
| 1 | C 3062//Cook6/C 80-1 | HW 3601 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | C 306 | | | 90S | 90S | F | 3 |
| 2 | GW 273 2//Cook 6/C 80-1 | HW 3602 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | GW 273 | | | | | | |
| 3 | HD 20093//Cook6/ C 80-1 | HW 3603 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |

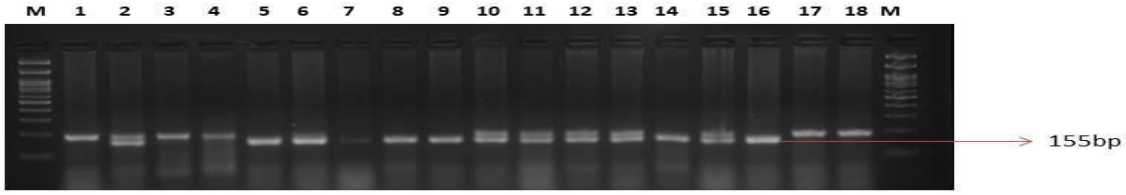
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | HD 2009 | | | 40S | 60S | 100S | 3 |
| 4 | HD 21893//Cook6/ C 80-1 | HW 3604 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | HD 2189 | | | | | | |
| 5 | HD 22853//Cook6/ C 80-1 | HW 3605 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | HD 2285 | | | 30MS | 100S | 30S | 3 |
| 6 | HD 23293//Cook6 /C 80-1 | HW 3606 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | HD 2329 | | | 80S | 90S | 80S | 3 |
| 7 | HD 24023//Cook6/ C 80-1 | HW 3607 | *Sr25 Sr31 Sr36 Lr19 Lr26 Yr9 Pm6 Pm8* | F | F | F | 1 |
| | HD 2402 | | | 30S | 100S | F | 3 |
| 8 | HD 26873//Cook6 / C 80-1 | HW 3608 | *Sr25 Sr31 Sr36 Lr19 Lr26 Yr9 Pm6 Pm8* | F | F | F | 1 |
| | HD 2687 | | *Sr31 Lr26 Yr9 Pm8* | 10R MR | 80S | F | 3 |
| 9 | HD 27333//Cook6 / C 80-1 | HW 3609 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | HD 2733 | | | | | | |
| 10 | HD 28773//Cook6 / C 80-1 | HW 3610 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | HD 2877 | | | | 80S | | |
| 11 | HI 9773//Cook6 / C 80-1 | HW 3611 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | HI 977 | | | | | | |
| 12 | HI 10773//Cook6 / C 80-1 | HW 3612 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | HI 1077 | | | 30MS S | 50S | 40MS | 3 |
| 13 | HP 12053//Cook6 / C 80-1 | HW 3613 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | HP 1205 | | | | | | |
| 14 | HS 2403//Cook6 / C 80-1 | HW 3614 | *Sr31 Lr26 Yr9 Pm8 +Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | HS 240 | | *Sr31 Lr26 Yr9 Pm8* | 5R MR | 70S | F | 3 |
| 15 | HUW 2343//Cook 6 /C 80-1 | HW 3615 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | HUW 234 | | | 20MS S | 100S | F | 3 |
| 16 | J 243//Cook6/C 80-1 | HW 3616 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | J 24 | | | 90S | 100S | 100S | 3 |
| 17 | Kalyansona3//Cook6/C 80-1 | HW 3617 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | Kalyansona | | | 80S | 80S | 90S | 3 |
| 18 | Lal Bahadur3// Cook6/C 80-1 | HW 3618 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | Lal Bahadur | | | | 80S | | 3 |
| 19 | Lok-13//Cook6/C 80-1 | HW 3619 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | Lok-1 | | | 70S | 80S | 80S | 3 |
| 20 | MACS 24963//Cook 6/C 80-1 | HW 3620 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | MACS 2496 | | | | 90S | | 3 |
| 21 | NI 54393//Cook6/ C 80-1 | HW 3621 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | NI 5439 | | | 90S | 90S | 100S | 3 |
| 22 | NI 54393//Cook6/ C 80-1 | HW 3621A | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | NI 5439 | | | 90S | 90S | 100S | 3 |
| 23 | NIAW343//Cook6/ C 80-1 | HW 3622 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | NIAW 34 | | | | | | |
| 24 | PBN 513//Cook6 /C 80-1 | HW 3623 | *Sr25 Sr36 Lr19 Pm6* | | | | |
| | PBN 51 | | | | | | |
| 25 | PBW 2263//Cook6 /C 80-1 | HW 3624 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | PBW 226 | | | 20S | 90S | F | 3 |
| 26 | PBW 3433//Cook6 /C 80-1 | HW 3625 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | PBW 343 | | | | 80S | | 3 |
| 27 | PBW 5023//Cook6 /C 80-1 | HW 3626 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | PBW 502 | | | | | | 3 |
| 28 | Raj 30773//Cook6 /C 80-1 | HW 3627 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | Raj 3077 | | | | | | 3 |
| 29 | Raj 30773//Cook6 /C 80-1 | HW 3627 A | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | Raj 3077 | | | | | | 3 |
| 30 | UP 2623//Cook6/C 80-1 | HW 3628 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | UP 262 | | | 50S | 50S | 50S | 3 |
| 31 | UP 23383//Cook 6/C 80-1 | HW 3629 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | UP 2338 | | | 70S | 80S | 80S | 3 |
| 32 | UP 24253//Cook 6/C 80-1 | HW 3630 | *Sr25 Sr36 Lr19 Pm6* | F | F | | 1 |
| | UP 2425 | | | | | | |
| 33 | WH 1473//Cook6 /C 80-1 | HW 3631 | *Sr25 Sr36 Lr19 Pm6* | F | F | F | 1 |
| | WH 147 | | | 90S | 90S | 90S | 3 |

| | **RL 6144// HW 4444** | | ***Sr 36 Lr 45 Pm 6*** | **F** | **F** | **F** | **1** |
|---|---|---|---|---|---|---|---|
| 34 | C3063//RL 6144// HW 4444 | HW 3637 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | C 306 | | | 90S | 90S | F | 3 |
| 35 | GW 2733//RL 6144 // HW 4444 | HW 3638 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | GW 273 | | | | | | |
| 36 | HD 21893//RL 6144 // HW 4444 | HW 3639 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | HD 2189 | | | | | | |
| 37 | HD 22853//RL 6144 // HW 4444 | HW 3640 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | HD 2285 | | | 30MS | 100S | 30S | 3 |
| 38 | HD 23293//RL 6144 // HW 4444 | HW 3641 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | HD 2329 | | | 80S | 90S | 90S | 3 |
| 39 | HD 2402 3//RL 6144 // HW 4444 | HW 3642 | *Sr 36 Lr 45 Pm 6* | TR | F | F | 3 |
| | HD 2402 | | | 30S | 100S | F | 3 |
| 40 | HD 26873//RL 6144 // HW 4444 | HW 3643 | *Sr 31 Lr 26 Yr 9 Pm 8 + Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | HD 2687 | | *Sr31 Lr26 Yr9 Pm8* | 15R MR | 80S | F | 3 |
| 41 | HD 27333//RL 6144 // HW 4444 | HW 3644 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | HD 2733 | | | | | | |
| 42 | HD 28773//RL 6144 // HW 4444 | HW 3645 | *Sr 36 Lr 45 Pm 6* | 5 MR | F | F | 3 |
| | HD 2877 | | | 5MR | 40SS | F | 3 |
| 43 | HI 9773//RL 6144 // HW 4444 | HW 3646 | *Sr 36 Lr 45 Pm 6* | F | F | F | 3 |
| | HI 977 | | | F | 60S | 40S | 2 |
| 44 | HI 10773//RL 6144 // HW 4444 | HW 3647 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | HI 1077 | | | 30MS S | 50S | 40S | 3 |
| 45 | HP 12053//RL 6144 // HW 4444 | HW 3648 | *Sr 36 Lr 45 Pm 6* | 15R MR | F | F | 3 |
| | HP 1205 | | | 60 SS | 80SS | 90S | 3 |
| 46 | HS 2403//RL 6144 // HW 4444 | HW 3649 | *Sr 31 Lr 26 Yr 9 Pm 8 + Sr 36 Lr 45 Pm 6* | 5R MR | F | F | 3 |
| | HS 240 | | *Sr31 Lr26 Yr9 Pm8* | 5R MR | 70S | F | 3 |
| 47 | HUW 2343//RL 6144// HW 4444 | HW 3650 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | HUW 234 | | | | | | |
| 48 | J 243//RL 6144 // HW 4444 | HW 3651 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | J24 | | | 90S | 100S | 100S | 3 |
| 49 | Kalyasona3//RL 6144 // HW 4444 | HW 3652 | *Sr 36 Lr 45 Pm 6* | 15R MR | F | F | 3 |
| | Kalyansona | | | 80S | 90S | 90S | 3 |
| 50 | LalBahadur3//RL 6144 // HW 4444 | HW 3653 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | LalBahadur | | | | | | |
| 51 | Lok 13//RL 6144 // HW 4444 | HW 3654 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | Lok-1 | | | 70S | 80S | 80S | 3 |
| 52 | MACS 24963//RL 6144 // HW 4444 | HW 3655 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | MACS 2496 | | | | | | |
| 53 | NI 54393//RL 6144 // HW 4444 | HW 3656 | *Sr 36 Lr 45 Pm 6* | 15R MR | F | F | 3 |
| | NI 5439 | | | 90S | 90S | 100S | 3 |
| 54 | NIAW 343//RL 6144 // HW 4444 | HW 3657 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | NIAW 34 | | | 90S | 90S | 90S | 3 |
| 55 | PBN 513//RL 6144 // HW 4444 | HW 3658 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 2 |
| | PBN 51 | | | 20MR | 40S | S | 2 |
| 56 | PBW 2263//RL 6144 // HW 4444 | HW 3659 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | PBW 226 | | | 20S | 90S | F | 3 |
| 57 | PBW 3433//RL 6144 // HW 4444 | HW 3660 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | PBW 343 | | | 20MR | 60S | 5S | 3 |
| 58 | PBW 5023//RL 6144 // HW 4444 | HW 3661 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | PBW 502 | | | | | | |
| 59 | Raj 30773// RL 6144// HW 4444 | HW 3662 | *Sr 36 Lr 45 Pm 6* | 5 MR | F | F | 1 |
| | Raj 3077 | | | 5MR | 60SS | 60SS | 1 |
| 60 | Raj 30773// RL 6144// HW 4444 | HW 3662 A | *Sr 36 Lr 45 Pm 6* | 5 MR | F | F | 1 |
| | Raj 3077 | | | 5MR | 60SS | 60SS | 1 |
| 61 | UP 23383//RL 6144 // HW 4444 | HW 3663 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | UP2338 | | | | | | |
| 62 | UP 24253//RL 6144 // HW 4444 | HW 3664 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | UP 2425 | | | | | | |
| 63 | WH 1473//RL 6144 // HW 4444 | HW 3665 | *Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | WH 147 | | | 90S | 90S | 90S | 3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 64 | WH 5423//RL 6144 // HW 4444 | HW 3666 | *Sr 31 Lr 26 Yr 9 Pm 8 + Sr 36 Lr 45 Pm 6* | 10R MR | F | F | 3 |
| | WH 542 | | *Sr31 Lr26 Yr9 Pm8* | 10R MR | 80S | F | 3 |
| 65 | Yr 103//RL 6144 // HW 4444 | HW 3667 | *Sr 36 Lr 45 Pm 6* | | F | F | |
| | Yr 10 | | | | | | |
| | **TR380-147/3Ag# 14// HW 4444** | | ***Lr 24 Sr 24 Sr 36 Pm 6*** | **F** | **F** | **F** | **1** |
| 66 | C3063 **//**TR380-147/3Ag# 14// HW 4444 | HW 3668 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | C 306 | | | 90S | 90S | F | 3 |
| 67 | GW 2733**//**TR380-147/3Ag# 14// HW 4444 | HW 3669 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | GW 273 | | | | | | |
| 68 | HD 20093 **//**TR380-147/3Ag# 14// HW 4444 | HW 3670 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2009 | | | | | | |
| 69 | HD 21893**//**TR380-147/3Ag# 14// HW 4444 | HW 3671 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2189 | | | | | | |
| 70 | HD 22853**//**TR380-147/3Ag# 14// HW 4444 | HW 3672 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2285 | | | 30MS | 100S | 30S | 3 |
| 71 | HD 23293//RL 6144 // HW 4444 | HW 3673 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2329 | | | 80S | 90S | 90S | 3 |
| 72 | HD 2402 3 **//**TR380-147/3Ag# 14// HW 4444 | HW 3674 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2402 | | | 30S | 100S | F | 3 |
| 73 | HD 26873 **//**TR380-147/3Ag# 14// HW 4444 | HW 3675 | *Sr 31 Lr 26 Yr 9 Pm 8 + Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2687 | | *Sr31 Lr26 Yr9 Pm8* | 15R MR | 80S | F | 3 |
| 74 | HD 27333**//**TR380-147/3Ag# 14// HW 4444 | HW 3676 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2733 | | | | | | |
| 75 | HD 28773 **//**TR380-147/3Ag# 14// HW 4444 | HW 3677 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HD 2877 | | | 5MR | 40SS | F | 3 |
| 76 | HI 9773 **//**TR380-147/3Ag# 14// HW 4444 | HW 3678 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HI 977 | | | F | 60S | 40S | 2 |
| 77 | HI 10773 **//**TR380-147/3Ag# 14// HW 4444 | HW 3679 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HI 1077 | | | 30MS S | 50S | 40S | 3 |
| 78 | HP 12053**//**TR380-147/3Ag# 14// HW 4444 | HW 3680 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HP 1205 | | | 60 SS | 80SS | 90S | 3 |
| 79 | HS 2403**//**TR380-147/3Ag# 14// HW 4444 | HW 3681 | *Sr 31 Lr 26 Yr 9 Pm 8 + Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HS 240 | | *Sr31 Lr26 Yr9 Pm8* | 5R MR | 70S | F | 3 |
| 80 | HUW 2343 **//**TR380-147/3Ag# 14// HW 4444 | HW 3682 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | HUW 234 | | | | | | |
| 81 | J 243 **//**TR380-147/3Ag# 14// HW 4444 | HW 3683 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | J24 | | | 90S | 100S | 100S | 3 |
| 82 | Kalyasona3 **//**TR380-147/3Ag# 14// HW 4444 | HW 3684 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | Kalyansona | | | 80S | 90S | 90S | 3 |
| 83 | Kalyasona3 **//**TR380-147/3Ag# 14// HW 4444 | HW 3685 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | Kalyansona | | | 80S | 90S | 90S | 3 |
| 84 | LalBahadur3 **//**TR380-147/3Ag# 14// HW 4444 | HW 3686 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | LalBahadur | | | | | | |
| 85 | Lok 13 **//**TR380-147/3Ag# 14// HW 4444 | HW 3687 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | Lok-1 | | | 70S | 80S | 80S | 3 |
| 86 | MACS 24963 **//**TR380-147/3Ag# 14// HW 4444 | HW 3688 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | MACS 2496 | | | | | | |
| 87 | NI 54393**//**TR380-147/3Ag# 14// HW 4444 | HW 3689 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | NI 5439 | | | 90S | 90S | 100S | 3 |
| 88 | NIAW 343 **//**TR380-147/3Ag# 14// HW 4444 | HW 3690 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | NIAW 34 | | | 90S | 90S | 90S | 3 |
| 89 | PBN 513 **//**TR380-147/3Ag# 14// HW 4444 | HW 3691 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | PBN 51 | | | 20MR | 40S | S | 2 |
| 90 | PBW 2263 **//**TR380-147/3Ag# 14// HW 4444 | HW 3692 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | PBW 226 | | | 20S | 90S | F | 3 |
| 91 | PBW 3433 **//**TR380-147/3Ag# 14// HW 4444 | HW 3693 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | PBW 343 | | | 20MR | 60S | 5S | 3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 92 | PBW 5023 **//**TR380-147/3Ag# 14// HW 4444 | HW 3694 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | PBW 502 | | | | | | |
| 93 | Raj 30773**//**TR380-147/3Ag# 14// HW 4444 | HW 3695 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | Raj 3077 | | | 5MR | 60SS | 60SS | 1 |
| 94 | Raj 30773**//**TR380-147/3Ag# 14// HW 4444 | HW 3695 A | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | Raj 3077 | | | 5MR | 60SS | 60SS | 1 |
| 95 | UP 2623 **//**TR380-147/3Ag# 14// HW 4444 | HW 3696 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | UP262 | | | | | | |
| 96 | UP 23383 **//**TR380-147/3Ag# 14// HW 4444 | HW 3697 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | UP2338 | | | | | | |
| 97 | UP 24253 **//**TR380-147/3Ag# 14// HW 4444 | HW 3698 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | UP 2425 | | | | | | |
| 98 | WH 1473 **//**TR380-147/3Ag# 14// HW 4444 | HW 3699 | *Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| | WH 147 | | | 90S | 90S | 90S | 3 |
| 99 | WH 5423 **//**TR380-147/3Ag# 14// HW 4444 | HW 3700 | *Sr 31 Lr 26 Yr 9 Pm 8 + Lr 24 Sr 24Sr 36 Pm 6* | F | F | F | 1 |
| 100 | **HUW 234// Cook 6/C 80-1** | **HW 4444** | ***Sr36/Pm6*** | **F** | **80S** | **60S** | **1** |

**Figure 7: Marker Assisted selection of *Sr36/Pm6* gene using molecular marker *Stm 773-2* in different bread wheat backgrounds**




M- 100bp ladder; **1-8**: PBW 502// (*Lr45,Sr36*); **9-10** : PBW 343 //(*Lr45,Sr36*); **11-16** : PBW 343//(*Lr47,Sr36*); **17**-PBW 502(Recurrent parent); **18**-PBW 343 (Recurrent parent); **19,20**- PBW 226 //(*Lr19,Sr36*); **21, 22** – COOK (*Sr36* donor)

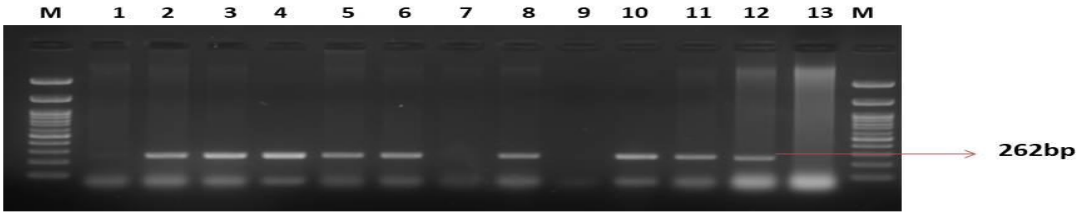
### *Sr38/Lr37/Yr17 (Aegilops ventricosa=Triticum ventricosa)*

The stem rust resistance gene *Sr38* has been found to be completely linked with leaf rust resistance gene *Lr37* and stripe rust resistance gene *Yr17* (Bariana and McIntosh, 1993). The gene *Sr38* exhibited a moderate degree of resistance at adult stage to stem rust pathotypes prevailing in the Nilgiris. The gene has not been used widely in Agriculture, though a few varieties in Australia carry these linked genes. In India, the authors have also introgressed this useful linkage in several genetic backgrounds (see *Lr37*) where *Sr38* exhibited moderate resistance to stem rust. The gene *Sr38* showed 1+ to 2C infection type to Indian stem rust pathotype 40A and 40-1(V.C. Sinha, Personal communication). Also, *Sr38* offers a notable advantage by conferring resistance to another significant threat: blast disease. Blast, caused by the fungus *Magnaporthe oryzae* pathotype Triticum, affects a wide range of cereals, including wheat. The presence of *Sr38* in wheat varieties

provides a valuable line of defense against blast, contributing to the overall protection of wheat crops (Singh et al., 2019;Cruz et al., 2016). The wheat lines carrying this gene complex already listed in *Lr37* in previous issue of NWN 10(1)(See under *Lr37*)

Link: https://iari.res.in/files/Publication/Nilgiri_Wheat_News/Nilgiri_Wheat_News_02012023.pdf

**Figure 8: Molecular confirmation of the presence of *Lr37+Sr38+Yr17* gene through *VENTRIUP/ LN2* marker in the background of popular Indian bread wheat cultivars**




M- 100BP LADDER, **1**- Lok-1 (Negative control); **2**-VPM (*Lr37* donor); **3**-HW 4022(HD 2285); **4**-HW 4023(HD 2329); **5**HW 4024(HUW 234); **6,7**- HW 4025(KS); **8**-HW 4028(PBW 226); **9,10** -HW 4029(Sonalika); **11**-HW 4030(WH147); **12**- HW 4031(WH 542)

**NB:**

The authors take this opportunity to pay our respectful homage to Dr. M.K.Menon and Shri S.Bojan. We place on record and recognize their immense contribution in initiating meticulously planned backcross program at IARI Regional Station, Wellington and their role in developing NILs/BILs carrying rust resistance genes in popular Indian bread wheat cultivars.

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## "Enhancing wheat defense against multiple diseases by transferring *2NvS* segment of *Aegilops ventricosa* carrying linked gene *Lr37-Sr38-Yr17* through Marker-Assisted Selection"

**Nisha.R[1]*, P.Shajitha[1], Sivasamy M[1], P.Jayaprakash[1], V.K.Vikas[1], Vinod[2], S.M.S.Tomar[2], Niharikha Mallick[2], Rajbir Yadav[2], S.C.Bhardwaj[3], O.P.Gangwar[3], Vijaishree S[1] , John Peter[1], K.Sivan1, C. Arun Kumar[1], Sanjeth V[1], V.Balaji[1] and M. Gokulakrishna[1]**

[1]ICAR-Indian Agricultural Research Institute Regional Station, Wellington, Tamil Nadu -643 231, India
[2]ICAR-Division of Genetics, Indian Agricultural Research Institute, Pusa, New Delhi-12. India
[3]ICAR - Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla-171001, India
***Corresponding author:**
rebekahnisha@gmail.com

**Abstract:**

Rust diseases, caused by fungal pathogens, pose a significant threat to wheat production worldwide, including India. The incorporation of rust resistance genes through conventional breeding has proven effective in combating these pathogens, but the process can be time-consuming. Marker-assisted selection (MAS) offers a rapid and precise method for introgressing specific resistance genes into elite wheat varieties. In this study, we aimed to transfer the *Lr37-Sr38-Yr17* rust resistance gene cluster derived from *Aegilops ventricosa* into select popular elite Indian bread wheat cultivars using MAS. The seedling and adult plant stages of backcross populations and advanced lines were phenotyped to evaluate their resistance against commonly occurring rust pathotypes. The lines carrying the resistance gene cluster exhibited high levels of resistance to leaf and stem rust, along with desirable agronomic traits resulting in increase yields when compared to their recurrent parents. Moreover, these lines carrying *Triticum ventricosa*-derived translocation of segment **2NvS** carrying *Lr37/Sr38/Yr17+* expected to show effective resistance against wheat blast caused by *Magnaporthe oryzae Triticum(MoT)*, another devastating disease. Subsequently, some of these lines were further utilized to transfer the *Lr37* gene cluster into recent wheat varieties, specifically targeting the North Eastern Plains Zone (NEPZ), a region more prone to wheat blast. The advance lines in the BC3F4 stage are being extensively tested at multiple locations to validate their resistance performance against rusts. The marker-assisted transfer of the *Lr37-Sr38-Yr17* gene cluster into elite Indian wheat backgrounds represents a significant advancement in rust resistance breeding and holds promise for enhancing wheat productivity

and resilience against these destructive diseases. The promising lines may either be released as cultivars or utilized as genetic stocks to develop multiple disease resistance wheat varieties.

Key words: *Triticum aestivum*, *Lr37/Sr38/Yr17*, 2NvS, Leaf, Stem, Stripe rusts, wheat blast, *Puccinia*, *Magnoporthe oryzae* & MAS

**Introduction:**

Bread wheat (*Triticum aestivum* L.) is a staple cereal crop that ensures food and nutritional security worldwide and serves as one of the major food crop in India. Despite its significance, wheat production faces various production constraints, including both biotic and abiotic stresses. Among the biotic stresses, rust diseases caused by fungi pose a substantial threat to wheat crops globally, including India (Bhardwaj et al., 2019). The three major rust diseases affecting wheat are leaf (brown) rust (*Puccinia triticina* Eriks.), stem (black) rust (*P. graminis* f. sp. *tritici* Eriks. & E. Henn), and stripe (yellow) rust (P. *striiformis* Westend.). These rusts have been responsible for significant yield losses, with reports of up to 7-30% loss due to leaf rust and up to 100% loss due to stripe and stem rust, especially after the emergence of the devastating Ug99 race (Hawkesford et al., 2013; Singh et al., 2011; Leonard and Szabo, 2005).

Traditional control of rust pathogens through chemical fungicides is costly, often inefficient on a large scale and environmentally not safe. To address these challenges and minimize crop losses caused by rusts, breeding for resistant wheat varieties and its release has proven to be cost effective and promising alternative. This approach has resulted in significant improvements in wheat yield over recent years, as breeders have incorporated rust resistance genes from wild relatives of wheat, which harbor a wide range of genetic diversity (Wulff and Moscou, 2014).

In 1983, G. Doussinault introduced the 2NS segment from *Ae. ventricosa* into the wheat cultivar VPM1 in an effort to transfer a gene for eyespot resistance caused by the fungus *Pseudocercosporella herpotrichoides*. Surprisingly, this segment was found to confer resistance to three rust diseases: leaf rust (*Lr37*) stem rust (*Sr38*) and stripe rust (*Yr17*) (Bariana and McIntosh, 1994). This gene cluster, known as *Lr37+Sr38+Yr17*, has since been widely utilized for developing rust-resistant wheat varieties.

Despite occasional reports of virulence for *Lr37* in different countries, it remains effective against a wide range of rust races and has shown synergy with other resistance genes (Park and McIntosh, 1994; Kolmer et al., 2008).Moreover, recent findings indicate that the 2NS segment from *Ae. ventricosa* also confers resistance against nematode diseases (Williamson et al., 2013) and, most notably, provides resistance against the devastating wheat blast disease caused by the fungus *Magnaporthe oryzae* Triticum pathotype (MoT) (Singh et al., 2019;Cruz et al., 2016; Cruz and Valent, 2017).

To introgress these valuable resistance linked genes into desirable wheat backgrounds, conventional breeding approaches can be time-consuming. However, the advent of molecular markers specific to particular resistance loci has revolutionized the process (Chao, 2006). In this study, our primary objective was to introduce the 2NvS translocation from *Ae. ventricosa,* carryingthe *Lr37-Sr38-Yr17* gene cluster, into eleven Indian wheat backgrounds. We employed both conventional and marker-

assisted backcross selection to achieve this goal. Since 2002 we have already transferred this segment into several Indian well adapted popular bread wheat cultivars

Recognizing the vulnerability of the North Eastern Plains Zone (NEPZ) to wheat blast and the presence of the aforementioned gene cluster in varieties that are no longer cultivated, we took a step further by integrating the *Lr37* gene cluster into more recent wheat varieties. The key motivation behind this endeavor was to bolster the rust resistance of these Indian wheat varieties and contribute to the development of superior, rust-resistant cultivars that can sustainably improve wheat production in the region. Furthermore, an additional goal was to investigate the potential of these new wheat varieties introgressed with *Lr37* gene cluster to develop resistance against emerging pathotypes of wheat blast. This would subsequently fortify their ability to withstand this destructive disease, thus increasing their overall resilience.

**Materials and Methods**

***Crossing Program and Plant Materials***

The crossing program was conducted at ICAR-Indian Agricultural Research Station, Wellington, and The Nilgiris, India (11 º N latitude and 77 º E longitude). Popular bread wheat genotypes such as HD 2285, HD 2329, HUW234, Kalyansona, LOK-1, PBW 226, Sonalika, WH 542, WH147 and HD 2687 were selected as recurrent parents. The donor parent for transferring the gene cluster *Lr37-Sr38-Yr17* was VPML-1 (RL 6081). After three backcrosses and subsequent selfing, the resulting homozygous and stable lines (HW 4022, HW 4023, HW 4024, HW 4025, HW 4026, HW 4028, HW 4029, HW 4030, HW 4031 and HW 4033) were constituted. This was followed by careful selection of the resulting hybrid plants based on molecular markers *VENTRIUP-LN2* specific to the *Lr37* gene cluster. These markers allowed for precise identification of the presence of the *Lr37* gene cluster in the resulting lines. This advancement involved integrating established lines carrying the *Lr37* gene cluster into recent wheat varieties, namely DBW 39, HD 2733, HW 2045, HD 2967, and PBW 343. These varieties were chosen based on their relevance and suitability for cultivation in the NEPZ region.

***Glasshouse Evaluation***

Seedling response to leaf rust was evaluated at the Greenhouse of the Indian Institute of Wheat and Barley Research, Flowerdale, Shimla. Eight-day-old seedlings were inoculated with virulent races of leaf and stem rust using urediniospores. On the 14th day after inoculation, plants were evaluated using a 0-to-4 scale following the method described by Stakman et al (1962). ITs ranging from 0 to 22+ were considered low, indicating the presence of host plant resistance, while ITs 3 to 4 were categorized as high, indicating host susceptibility.

***Field Screening***

Field screening at IARI RS Wellington for rust resistance was performed under field conditions under natural infections and additionally ensured by raising spreader rows sprayed with rust inoculums containing occurring pathotypes. The scoring for level of infection was done when rust symptoms were fully developed, approximately at the early dough stage. The scoring was categorized into different levels, including Immune (0), Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), and Susceptible (S), based on

rust severity and symptom appearance (Peterson et al., 1948; Zadoks et al.,1974).

***DNA Isolation & PCR Analysis***

DNA was isolated from 7-day-old seedling leaves using a modified CTAB method. PCR analysis was performed in 10µL reaction volumes with genomic DNA and specific reagents with two pairs of primers, including the *VENTRIUP-LN2* primers developed by Helguera et al. (2003) to detect the 2NS fragment from *T. ventricosa*. Amplification products were separated on a standard agarose gel.

***Marker Assisted Selection***

In this study, Marker-assisted selection (MAS) was implemented at each backcross generation using molecular markers linked to the *Lr37*+ gene. During the BC3F4 generation, seedlings were tested using markers to identify individuals carrying the resistance genes from the recurrent parents. The selected positive plants from the BC3F4 generation were then selfed to establish the BC3F8 population.

**Results and Discussions**

***Phenotyping of backcross population and advance lines***

Phenotyping of the backcross population and advanced lines involved evaluating the stable advance lines for their response to various pathotypes at the seedling stage under controlled conditions. The seedling reactions exhibited variability depending on the different races used for inoculation, with infection types (ITs) ranging from 0 to 3+. The lines carrying the resistance gene cluster displayed resistant infection types (ITs) ranging from 0 to 1 to leaf and stem rust pathotypes (**Table 1**). In the adult plant stage, individual plants carrying the resistance genes exhibited resistance, in contrast to the susceptible recurrent parents to leaf and stem rusts. To ensure the successful introgression of the *Lr37*+ gene cluster, phenotypic selection was carried out at each backcross and selfing generation.

***Marker assisted backcross selection***

Marker-assisted selection (MAS) has become an indispensable component of traditional plant breeding practices, where markers are now commonly employed for foreground selection, facilitating the successful introgression of individual genes or quantitative trait loci (QTLs) (Gupta et al., 2010). In our study, both the recipient and donor parents were carefully assessed for the presence or absence of the *Lr37*+ gene, utilizing the specific molecular marker *VENTRIUP/LN2* located on chromosome arm 2NS. To distinguish between plants homozygous for the 2N-allele (indicative of possessing the *Lr37*+ gene) and those lacking the gene, we employed the 2NS-specific marker. The presence of the *Lr37*+ gene was indicated by the amplification of a 262bp DNA fragment, while the absence of the gene resulted in the amplification of a null allele, serving as a reliable indicator for selecting the desired resistant wheat genotypes (**Figure 1 &2**).

Through a series of meticulously planned and executed crosses, the genetic material from the established lines was systematically integrated into the genetic makeup of DBW 39, HD 2733, HW 2045, HD 2967, and PBW 343. The gene introgressed lines are in BC3F4 stage and MAS was followed in each generation. This process aimed to equip these varieties with enhanced rust and blast resistance traits while preserving their desirable

agricultural characteristics. By utilizing this marker-assisted backcross breeding approach, the study aimed to contribute to the development of wheat varieties with heightened resistance against rust and blast diseases, thus bolstering their suitability for cultivation in the challenging NEPZ environment.

Since the early 1990s, the 2NS translocation carrying linked genes *Lr37+Sr38+Yr17* has been extensively utilized in breeding programs conducted by CIMMYT (International Maize and Wheat Improvement Center) and USDA (United States Department of Agriculture) to improve bread wheat varieties(Gao et al.,2021). This translocation has been associated with beneficial alleles that have a positive impact on yield. Reports from CIMMYT suggest a consistent yield advantage for lines containing the 2NvS segment, with an approximate yield enhancement of 1.7% under optimal conditions (Huelgera et al., 2003).

Further, recent studies by Gao et al. (2021) have supported the positive effects of the 2NvS segment on grain yield. They reported the presence of the 2NvS segment in central US winter wheat breeding lines, which exhibited a positive effect on grain yield over the study period. Additionally, they suggested that the 2NvS segment is also associated with increased resistance to crop lodging, making it a desirable trait for wheat breeding programs. The constituted lines were also showing better yield traits in comparison to the respective recurrent parents.

As the sole confirmed source of wheat blast resistance in field experiments conducted across diverse environments, the 2NS translocation holds immense importance and should be actively incorporated into wheat breeding programs, especially in countries where wheat blast is prevalent. Indeed, wheat varieties containing the 2NvS translocation have been employed in various countries affected by wheat blast to counter the severe consequences of this disease. Notable instances include CD116 in Brazil, Urubo, INIAF Okinawa, and INIAF Tropical in Bolivia, Caninde#1 in Paraguay, and BARI Gom 33 in Bangladesh (Islam et al., 2020).

Its wide adoption and deployment in various countries highlight its significance in addressing the challenges posed by wheat blast infection.The choice to focus on varieties for the NEPZ in India is strategic, considering the higher susceptibility of this region to wheat blast. The inclusion of 2NS segment, which has shown potential cross-resistance against wheat blast, holds promise for imparting enhanced resistance to blast in these wheat lines. The utilization of marker-assisted backcross breeding allows for precise and efficient selection of lines carrying the *Lr37* gene cluster, accelerating the process of introgression and ensuring the retention of desirable agronomic traits from the recurrent parents.

***Conclusion***

In conclusion, the successful transfer of the *Lr37*+ gene to elite Indian wheat backgrounds using MAS has proven to be highly effective in conferring resistance to stem, leaf and yellow rusts. The newly constituted lines not only demonstrated robust resistance to these rust diseases but also exhibited improved agronomic traits compared to their susceptible recurrent parents. Moreover, the incorporation of the linked genes *Lr37+Sr38+Yr17* cluster may also offer potential resistance against wheat blast, a devastating and emerging disease. These rust-resistant lines hold great promise for

sustainable wheat production, offering a potential solution to the challenges posed by rust diseases in wheat-growing regions. Additionally, the potential cross-resistance against wheat blast adds further value to these newly developed lines, providing an extra layer of protection against another serious threat to wheat crops. The incorporation of the *Lr37+* gene cluster into recent wheat varieties through marker-assisted backcross breeding represents a promising approach to enhance rust and blast resistance in varieties targeted for the NEPZ. These advanced lines hold significant potential in contributing to disease-resistant wheat varieties, thereby bolstering food security and agricultural sustainability in the region. Rigorous field testing and evaluation will be crucial to select the most promising lines for further testing and releasing, with the ultimate goal of providing farmers with improved, high-yielding, and disease-resistant wheat varieties for enhanced productivity and resilience in the face of ever-changing pathogen pressures.

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**Figure 1**

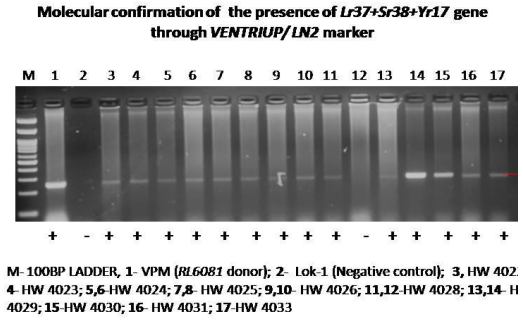
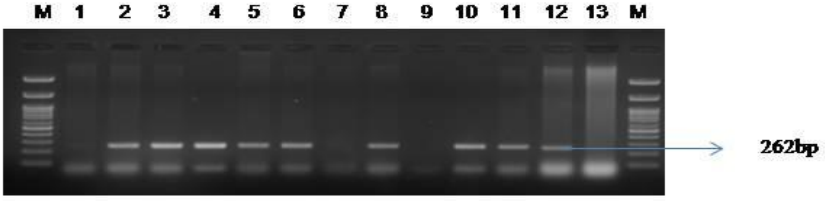
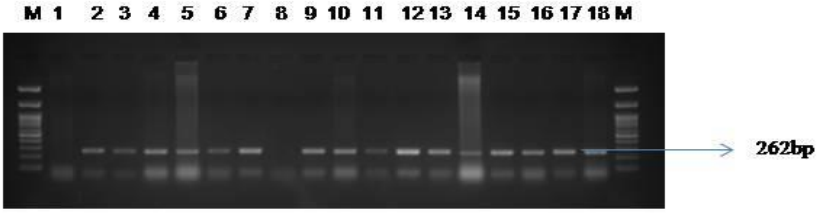


**Figure 2**

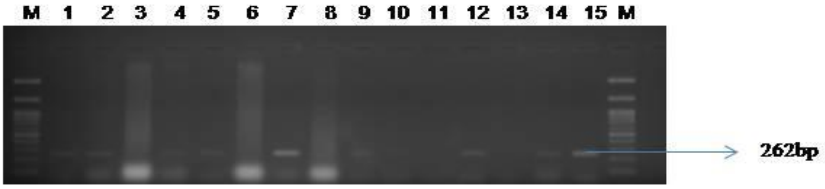
**Molecular confirmation of *Lr37/Sr38/Yr17* using Ventriup/LN2 marker in NEPZ varieties**




M-100bp ladder; 1-HD 2733 (RP), 2-RL6081 3-13 – BC3F2 lines in the background of HD 2733




M-100bp ladder; PBW343 3(RP), 2-RL6081; 3-18 – BC3F2 lines in the background of PBW343




M-100bp ladder; HW 2045 (RP), 2-RL6081 3-15 – BC3F2 lines in the background of HW2045

**Table 1: Response to rust diseases in the seedling and adult plant stage and marker analysis data of the donor, recurrent parent and the constituted lines carrying** *Lr37+Sr38+Yr17*

| SNo | Parent/ Cross | Seedling response leaf rust pathotypes | | | | | | Seedling response stem rust pathotype | | | | Field response | | Marker Confirmation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12-5 | 77-1 | 77-5 | 77-8 | 77-9 | 104-2 | 11 | 40A | 40-2 | 117-6 | Leaf rust | Stem rust | *VENTRIUP/LN2* |
| 1. | HD 2285(RP) | ; | 3 | 3+ | ;- | 3+ | 3+ | 12 | 2- | 0; | ;- | 80S | 60S | - |
| **2.** | **HW 4022** | **;-** | **;-** | **0** | **0** | **0** | **0** | **0** | **0** | **1;** | **0** | | **F** | **+** |
| 3. | HD 2329(RP) | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | ; | ;- | 80S | 80S | - |
| **4.** | **HW 4023** | **0** | **0** | **0** | **0;** | **0** | **0** | **;** | **0** | **0** | **0** | **F** | **F** | **+** |
| 5. | HUW234(RP) | ; | ;- | 3+ | 3+ | 3+ | 3+ | 2= | 2C | ;- | ;- | 80S | 60S | - |
| **6.** | **HW 4024** | **0** | **;-** | **0** | **0** | **;-** | **0;** | **0** | **0** | **0** | **0** | **F** | **F** | **+** |
| 7. | KALYANSONA(RP) | 33+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 0; | 3+ | 60S | 60S | - |
| **8.** | **HW 4025** | **0** | **0** | **0** | **0;** | **0** | **0** | **0** | **0** | **0** | **0** | **F** | **F** | **+** |
| 9. | LOK-1(RP) | 33+ | 3+ | 3+ | 3+ | 3+ | 3+ | 2- | 0; | ;- | 2- | 80S | 80S | - |
| **10.** | **HW 4026** | **0** | **0** | **;-** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **60S** | **60S** | **+** |
| 11. | PBW 226(RP) | ;1 | ;1 | 23 | ; | 3+ | 33+ | 2= | 0; | ;- | ;- | 40S | F | - |
| **12.** | **HW 4028** | **;** | **;1** | **0** | **;** | **0** | **;** | **2** | **0** | **0;** | **0** | **F** | **F** | **+** |
| 13. | SONALIKA(RP) | 33+ | 3+ | 3+ | 0; | 12 | 3+ | ; | ;- | 0; | ;- | 60S | F | - |
| **14.** | **HW 4029** | **0** | **0** | **0** | **0** | **0** | **0** | **;** | **;** | **0** | **;-** | **20S** | **F** | **+** |
| 15. | WH542(RP) | 23 | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | ;- | 33+ | 80S | 80S | - |
| **16.** | **HW 4030** | **0** | **0** | **0** | **0** | **0** | **1;** | **;-** | **0** | **0** | **0** | **F** | **F** | **+** |
| 17. | WH147(RP) | 12 | ;1 | 3 | 0; | 3+ | 3+ | ;- | 2= | ; | ;- | 40S | F | - |
| **18.** | **HW 4031** | **0;** | **;-** | **0;** | **0** | **;-** | **;-** | **;-** | **;-** | **0** | **;-** | **40S** | **20S** | **+** |
| 19. | HW2687(RP) | ;- | ;1 | 33+ | ;- | 3+ | 3+ | 12 | 12 | 0; | ;- | 40S | 20S | - |
| **20.** | **HW 4033** | **;-** | **;-** | **0;** | **;-** | **;-** | **;-** | **;** | **;** | **;-** | **;-** | **F** | **F** | **+** |
| 21. | RL6081 (Positive control) | 1 | 2 | ;- | ;1 | ;- | ;1 | 0; | 3+ | 0; | 0; | F | 60S | + |

*RP-Recurrent parent, S-Susceptible; F- Free

# "Empowering *Dicoccum wheat*-an ancient wheat through-Transferring the *Pm6/Sr36* gene for enhanced PM resistance"

**Sivasamy M[1]*, V.K.Vikas[1], P.Jayaprakash[1], Shailendra Jha[2], Niharikha Mallick[2], Rajbir Yadav[2], Nisha. R[1], P.Shajitha[1], Vijaishree S[1], C. Arun Kumar[1], K.Sivan[1], V.Balaji[1] and M. Gokulakrishna[1]**

[1]ICAR-Indian Agricultural Research Institute Regional Station, Wellington, Tamil Nadu -643 231, India

[2]ICAR-Division of Genetics, Indian Agricultural Research Institute, Pusa, New Delhi-12. India

[3]ICAR - Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla-171001, India

*** Corresponding author:**
iariwheatsiva@gmail.com

Emmer, also known locally as Khapli wheat (*Triticum turgidum* ssp. *dicoccum*), is an ancient annual crop characterized by large elongated grains and brittle ears. It belongs to the tetraploid wild species *Triticum turgidum* ssp. *dicoccum*, which resulted from a natural hybridization event between two wild diploid grass species. The BBAA genome composition of emmer wheat is likely a result of spontaneous interspecific hybridization and the selection of desirable morphological traits (Damania and Yang, 1998).The origins of Emmer wheat can be traced back to the Abyssinia center of origin, and historical evidence suggests that it may have been introduced to India by Arabian traders in the Western Ghat region. Currently, major cultivation areas for Emmer wheat in India include northern Karnataka, southern Maharashtra, Coastal Gujarat in the Saurashtra region, and parts of Tamil Nadu and Andhra Pradesh, where it is known by various names like Popathiya, Khapli, Jave and Samba (Hanchinal et al., 2005).

Emmer wheat holds great historical significance as one of the world's oldest crops and has been a staple food for millennia. Its cultivation is predominantly found in rural, marginal areas where other crops may not thrive. This is due to its ability to adapt to poor and stony soils, tolerate both low and high temperatures, and resist common cereal diseases (Zohary and Hopf, 1993).

Differing from commercially available bread and durum wheat, Emmer wheat or dicoccum wheat possesses distinct physical characteristics, nutritional properties, and cultivation practices. Over the last decade, there has been a significant increase in the cultivation of dicoccum wheat, driven by rising market demand and increased public awareness of its health benefits. Dicoccum-based food products have been found to have low digestibility and a low glycemic value, making them suitable for individuals with diabetes (Bhuvaneshwari, 1999; Yenagi et al., 2001).

The nutritional profile of Emmer wheat is impressive, with over 16% dietary fiber and varying levels of protein and carbohydrates. The grain quality of conventional dicoccum wheat varieties is superior, with better flavor, texture, and taste. Additionally, coarse semolina products made from dicoccum wheat are highly nutritious and suitable for various culinary applications. Ready-to-eat dicoccum wheat products like madeli have an extended shelf life, and its lower glycemic index makes it an ideal choice for therapeutic diets, especially for

diabetic patients (Singh et al., 2015). Dicoccum as functional food and its rich nutritional profile and health benefits have led to increased market demand, making it an essential component of the Indian agricultural landscape and an important dietary staple for many individuals. The *dicoccum* due to its nutritional & therapeutic properties gradually emerging as 'future wheat'

Cultivation of emmer wheat, locally known as Samba or Khapli wheat (*Triticum turgidum* ssp. *dicoccum*), in the southern hills of Nilgiri district in Tamil Nadu presents a unique opportunity for farmers to grow this ancient and nutritious crop. The Nilgiri Hills, situated in the Western Ghats of southern India, boast distinct climatic conditions compared to the plains and lowland regions. Emmer wheat thrives in cool to temperate climates, making the Nilgiris' ideal for its growth.

The cultivation of emmer wheat in the Southern hill zone (Nilgiri district) is not without its challenges and one of the prominent obstacles faced by farmers is the incidence of wheat powdery mildew (WPM). This common and destructive disease is caused by the obligate biotrophic fungal pathogen *Blumeriagraminis* f. sp. *tritici* (Bgt). WPM is favored by the high humidity (85-100%) and temperatures ranging from 15°C to 22°C (Caffier et al., 2014) that prevail in the Nilgiri region. Consequently, the disease can spread rapidly under the cool and humid climate prevalent in these areas. Powdery mildew's impact on emmer wheat yield can be significant, affecting various stages of the crop's growth, including seedling emergence, plant development and grain filling. Infected plants exhibit stunted growth, reduced tillering, and the development of white powdery patches on leaves, stems, and ears. These powdery patches contain fungal spores and severe infections can lead to premature senescence and reduced photosynthetic capacity, ultimately resulting in decreased grain yields (Cowger and Brown, 2019). In severe cases, powdery mildew can cause substantial yield losses, ranging from 10% to 40%, and in extreme situations, even up to 50% (Gao et al., 2018). Such losses can have significant economic implications for farmers and the local agricultural community, especially in a region where *dicoccum* is one of the staple food crops and play a vital role in sustaining livelihoods.

Despite the challenges posed by powdery mildew, the cultivation of emmer wheat in this zone holds promise for the region's farmers. By adopting suitable management practices and selecting appropriate emmer wheat varieties, farmers can mitigate the impact of powdery mildew on yield and cultivate a valuable crop that is not only nutritious but also environmentally friendly. One of the effective strategies to manage WPM is planting emmer wheat varieties with host resistance to powdery mildew that can be an effective long-term solution. Resistant cultivars can reduce the disease incidence and limit the severity of infections. In India, the significance of Powdery mildew disease in wheat has grown notably, particularly in the North Western Plains zone, Northern Hills zone and Southern Hills zone (Singh et al., 2009).

In this context, the search for and development of resistant wheat cultivars has become a crucial aspect of disease management strategies. These resistant varieties offer a sustainable solution to combat WPM and can play a vital role in safeguarding wheat production against the detrimental effects of this devastating fungal pathogen.

In recent years, advances in biotechnology and plant breeding have opened up exciting possibilities to enhance crop resistance against various pathogens. One such approach involves the transfer of beneficial genes from related species with natural resistance to WPM in the target crop. In this case, a promising candidate is *Triticum timopheevi*, a wild relative known to possess the *Pm6/Sr36* gene located on 2B chromosome (McIntosh and Gyárfás 1971), which confers high level of resistance against powdery mildew caused by Bgt (Sivasamy et al., 2017).

The *Pm6/Sr36* gene has demonstrated its efficacy in conferring durable resistance to powdery mildew in modern wheat varieties. The introduction of this gene into emmer wheat offers a promising solution to combat the challenges posed by powdery mildew, leading to improved yield and crop sustainability.

The successful transfer of the *Pm6/Sr36* gene to emmer wheat demands meticulous planning and the utilization of advanced biotechnological tools. This article presents the successful transfer of the *Pm6/Sr36* gene to emmer wheat, detailing the employed methods in gene transfer and addressing the challenges like currently this gene complex is available in hexaploid wheat and opportunities in adapting this trait to the emmer wheat cultivar. The incorporation of this gene holds immense promise in bolstering the resilience of this ancient crop against powdery mildew, ensuring its continuous cultivation as a valuable food resource. In this pursuit, researchers and plant breeders must prudently select appropriate breeding techniques to facilitate successful gene transfer while preserving the genetic integrity of emmer wheat particularly its *'dicoccum'* milling quality(for rawa etc.,.)

HW1098 is a semi-dwarf dicoccum wheat variety developed through irradiation at IARI-RS, Wellington (Sivasamy et al., 2014). This variety has a maturity period of 105-110 days and exhibits resistance to black and brown rust diseases. Overall, HW1098 offers promising traits that is well-suited for cultivation in specific regions, providing farmers with the potential for improved yields and disease resistance in their wheat crops.

Unfortunately, the current variety of wheat under consideration is significantly vulnerable to powdery mildew, limiting its ability to achieve its full yield potential. To address this issue and develop a wheat variety with improved resistance to powdery mildew, a breeding program was initiated at IARI, RS, Wellington. The goal of this program was to transfer the effective *Pm6/Sr36* gene, derived from *T. timopheevi* and present in the advanced line HW2436-2, to the dicoccum variety HW 1098.

Wellington, being a hotspot for various foliar diseases of wheat, including rusts and powdery mildew, presented an ideal environment for the selection of powdery mildew-resistant plants (Vikas et al., 2020). The initial cross breeding resulted in F1 plants that exhibited complete resistance to powdery mildew, a promising trait for further development. However, a significant challenge arose even after subsequent BC generations involving dicoccum and F1 sterile pentaploid as all the BC1F1, BC2F1 etc carrying *Pm6/Sr36* and conferring resistance to WPM but plants obtained from these crosses were relatively sterile and not fully fertile, which hindered their use in subsequent breeding efforts.To overcome this obstacle, a series of backcrosses were carried out, with the F1s being

backcrossed thrice with the HW 1098 wheat variety. This repeated backcrossing process helped in restoring fertility in the subsequent BC population. However, even after restoration, a considerable number of plants from the BC2 population remained either fully or partially sterile, making it essential to identify and select only the fertile plants for further breeding (Figure-A).

The selection process was then refined, and only those resistant plants from the BC2 population that were fertile were backcrossed and advanced to the succeeding generation (BC3F3-F4). This rigorous selection and advancement process ensured that only the most promising plants, with both resistance to powdery mildew and fertility, were carried forward in the breeding program.

To expedite the breeding process and facilitate the identification of plants with the desired gene, a molecular marker, *Xstm 773-2* (Tsilo et al., 2008) closely linked to the *Sr36* gene, was utilized. *Sr36* is tightly linked to the *Pm6* gene. The presence of the *Pm6* gene in the selected plants was confirmed using this specific STM marker, ensuring that the targeted trait was accurately identified and passed on to subsequent generations. The marker amplified a 155bp allele for the presence of the gene (Figure B). Being a co-dominant marker, it also aided in the identification of heterozygotes.

By combining traditional breeding methods with molecular marker-assisted selection, the breeding program in Wellington made significant progress in developing a wheat variety with enhanced resistance to powdery mildew. The use of molecular markers provided a valuable tool for efficiently identifying and tracking the presence of the *Pm6*/*Sr36* gene in the breeding population, enabling the development of wheat varieties that are better equipped to withstand the challenges posed by this devastating foliar disease. Notably, prior to this, efforts were made to transfer the *Sr36/Pm6* gene to durum wheat through marker assisted backcross breeding (Prasad et al., 2014). However, this represents the first successful report of transferring the *Pm6/Sr36* gene to dicoccum wheat, a significant achievement in expanding the range of wheat varieties with enhanced powdery mildew resistance.This transfer of the beneficial gene holds promise for enhancing the powdery mildew resistance of the dicoccum wheat variety, ultimately leading to improved crop yields and increased productivity.

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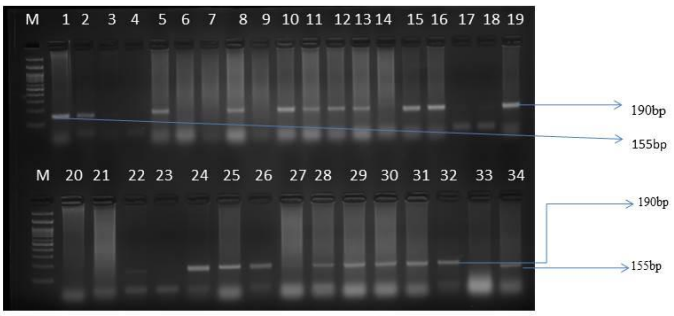
Figure A

Sterile spike of F1s | PM Resistant & Susceptible plant | PM Susceptible plant

Figure B

**Confirmation of *Sr36/Pm6* gene in HW1098 x HW2436-2 (BC3F2) using STM marker Xstm 773-2**




M-Marker; 1- Cook, 2-HW2436-2 (Donor), 3- Lok-1 (Negative line), 4-HW1098 (Recurrent parent), 5-33 –BC3F2s

# Developing Herbicide-Tolerant wheat (HTW) with multiple disease resistance for sustainable wheat production- An ICAR-IARI initiative

**Sivasamy M[1]*, Nisha.R[1], P.Shajitha[1], Vijaishree S[1], P.Jayaprakash[1], V.K.Vikas[1],C. Arun Kumar[1], V.Balaji[1], M. Gokulakrishna[1] and Madhan P[1]**

[1]ICAR-Indian Agricultural Research Institute Regional Station, Wellington, Tamil Nadu -643 231, India

*** Corresponding author:**
iariwheatsiva@gmail.com

**Introduction**

Wheat (*Triticum aestivum L*.) holds a prominent position as one of the most important cereal crops globally, playing a crucial role since human civilization as a staple food source. With a history dating back thousands of years, wheat has become an indispensable component of diets in various regions worldwide. This introduction sheds light on the importance of wheat cultivation, its global distribution, and its significance in India, while also exploring the challenges posed by weeds as biotic stress in wheat cultivation and its potential solution specially targeting herbicide tolerance.

According to estimates, weed infestations can cause up to 40-80% yield losses in wheat fields, depending on the severity of the weed pressure and the effectiveness of weed management practices (Korres et al., 2018). In wheat cultivation, weed infestation emerges as a major limiting factor for achieving the crop's full genetic yield potential (*Chhokar et al., 2012*).

In many parts of the world, manual weed control methods, such as hand weeding, have been traditionally employed to manage weed infestations in wheat fields. However, these labor-intensive methods are time-consuming, costly, and may not be sufficient to control weeds effectively, especially in large-scale agricultural settings. As a result, herbicides have become a crucial tool for weed management in wheat cultivation. The use of herbicides in wheat weed control has significantly improved weed management practices and crop yields. Herbicides provide a more efficient and cost-effective approach to control weeds, reducing labor dependency and ensuring proper weed control.

To address the challenges in weed control, herbicide tolerance in wheat has emerged as a promising solution. Herbicide-tolerant wheat varieties, such as Clearfield® wheat, have been developed to withstand specific herbicides' applications, enabling targeted weed control while preserving the wheat crop. Clearfield® wheat is engineered to tolerate certain herbicides, like *imazamox* and *imazapyr*. Herbicide tolerance allows for a more precise and environmentally friendly approach to weed management, promoting optimal crop growth and ensuring food security (Shewry & Hey, 2015). This approach not only improves overall crop yields but also minimizes the dependency on manual labor, addressing the challenges of labor scarcity in the Indian agricultural landscape (Rahman et al., 2018).

In our ongoing research, we are working on developing advanced wheat lines that have resistance to rusts through the deliberate introduction and combining of specific rust resistance genes. We are also incorporating genes that provide resistance to multiple diseases using traditional and marker-assisted breeding methods. Additionally, we are

introducing ALS gene-mediated herbicide tolerance into these wheat lines. The ALS gene, obtained from an Australian Spring wheat line (BCL0618), is being used as the source to make the advanced wheat lines resistant to the herbicide *Imazethapyr*. Through this comprehensive approach, our goal is to improve crop resilience, productivity, and sustainability in wheat farming.

**Wheat and Weed in India: Challenges and Common Management Strategies**

Weeds pose a significant challenge in crop production as they compete with crop plants for essential resources such as moisture, nutrients, light, and space, ultimately depriving the crop of vital inputs.

This competition becomes particularly critical when crop plants and weeds grow in close proximity, leading to overlap in their root or shoot systems. In the rice-wheat system, where sufficient soil moisture is available after rice harvesting, weeds tend to emerge earlier than wheat or concurrently with the wheat crop, intensifying the competition and causing substantial yield losses primarily attributed to a reduction in tillering (Chhokar et al., 2012).

Studies have shown that the average yield losses caused by weeds in different wheat growing zones range from 20% to 32% (Chhokar et al., 2012). Notably, the North Western Plains Zone (NWPZ), Northern Hills Zone (NHZ), and North Eastern Plains Zone (NEPZ) exhibit higher yield losses compared to the Peninsular Zone (PZ) and Central Zone.

The wheat production in India faces significant challenges due to weed infestation, causing substantial yield losses ranging from 15% to 70% (Malik et al., 2018; Chhokar and Sharma, 2014).

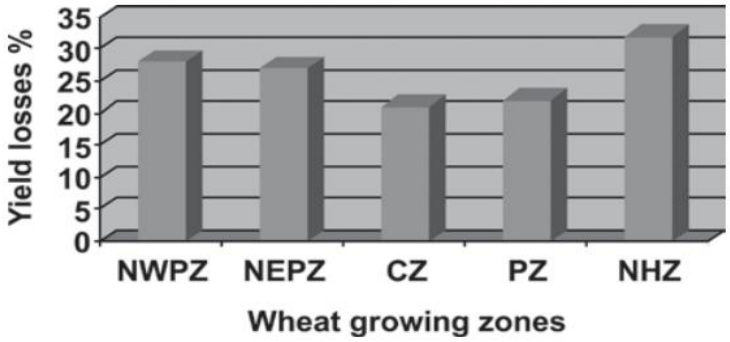



**Fig 1.** Yield losses in different wheat growing zones due to weeds (Mongia *et al*., 2005)

The prevalent weed species in Indian wheat fields include wild oat *(Avena fatua),* wild mustard *(Sinapis arvensis), Phalaris minor, and Chenopodium album* (Jha and Sharma, 2016; Singh et al., 2012). Farmers employ various weed management strategies, such as cultural practices, mechanical methods, and chemical weed control (Singh et al., 2014).

This widespread adoption of herbicides in wheat cultivation has resulted in a substantial reduction in the labor requirements for weeding operations, allowing farmers to divert their focus to other critical farming activities.

Numerous studies have highlighted the significant benefits of herbicide use in wheat weed control. For instance, researchers have observed that herbicides have led to a remarkable increase in weed control efficiency, resulting in higher crop yields and reduced economic losses due to weed competition (Pannu & Singh, 2016). The ability of herbicides to selectively target weeds without harming the wheat crop has been instrumental in achieving effective weed control, leading to improved overall productivity.

The most common herbicides used in wheat weed control include groups such as ACCase inhibitors (e.g., clodinafop, fenoxaprop), ALS inhibitors (e.g., sulfosulfuron, metsulfuron), and synthetic auxins (e.g., 2,4-D) (Dhima et al., 2006). These herbicides offer a diverse range of modes of action, providing flexibility in weed management and reducing the risk of herbicide resistance development.

The development of herbicide tolerance in wheat has emerged as a promising solution to combat weed infestations effectively. Herbicide-tolerant wheat varieties possess genetic traits that allow them to withstand the application of specific herbicides, providing farmers with a powerful tool for weed control while ensuring minimal damage to the wheat crop (Sarkar et al., 2010). This trait confers a competitive advantage to the wheat crop against herbicide-sensitive weed species, enabling efficient weed management without compromising wheat productivity.

**Clear field wheat**

Chemical families like IMIs, sulfonylureas, riazolopyrimidines, pyrimidinylthiobenzoates, and sulfanilamide-carbonyl-thiazolidinones have been used to control harmful weeds by inhibiting the ***acetolacto synthase*** enzyme, ultimately causing the weeds to die (Devine et al., 1993; Pang et al., 2003; McCourt et al., 2006; Yu and Powles, 2014).

In 2010, agricultural research institutes in Chile partnered with BASF (Badische Anilin und Soda Fabrik) to utilize traditional plant breeding methods like mutagenesis, plant selection, and backcrossing with elite cultivars. This collaboration led to the successful development of multiple wheat cultivars resistant to IMI herbicides, which are now known as "Clearfield crops" (Newhouse et al., 1992). The first Clearfield wheat variety with herbicide tolerance was introduced in the United States in 2001. Two experimental breeding lines, "Above" and "AP502 CL," were carefully selected and released to seed producers.

BASF played a crucial role in the development of these herbicide-tolerant wheat varieties, working closely with breeders from the Texas Agricultural Experiment Station and Colorado State University (Johnson et al., 2002).

**Herbicide tolerance origin and mechanism**

The origin of herbicide tolerance and the first discovery of ***ALS (Acetolactate Synthase)*** inhibition mechanism can be traced back to the development of the first sulfonylurea herbicides in the 1970s. The sulfonylurea herbicides were initially synthesized as synthetic auxins but were later found to have potent herbicidal properties due to their ability to inhibit ALS, an essential enzyme in the biosynthesis of branched-chain amino acids (Chaleff & Mauvais, 1984). The discovery of ALS inhibition as the mechanism of action for these herbicides marked a significant breakthrough in herbicide

research and revolutionized weed control strategies in agriculture.

As for rice herbicide tolerance to the ALS inhibition mechanism, it has been explored and studied extensively. Rice, like many other crops, can also be made tolerant to ALS-inhibiting herbicides through genetic modifications. Researchers have successfully developed herbicide-tolerant rice varieties by introducing specific ALS gene variants or mutations that confer tolerance to ALS-inhibiting herbicides, including sulfonylurea and imidazolinone herbicides (Matzrafi et al., 2010). The genetic modification of rice to be tolerant to ALS-inhibiting herbicides allows for effective weed control while ensuring minimal damage to the rice crop. The modified ALS enzyme in herbicide-tolerant rice varieties is less susceptible to inhibition by ALS-inhibiting herbicides, enabling the rice plants to continue their normal growth and development in the presence of these herbicides (Yu et al., 2018).

**Mechanism**

The biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine) in plants is attributed to the ALS (Acetolactate Synthase) gene (Matzrafi et al., 2010). Imazethapyr, classified as a Group 2 ALS-inhibiting herbicide, disrupts the activity of this enzyme, leading to the cessation of amino acid production in susceptible plants (Foes et al., 1999).

In herbicide-sensitive plants, Imazethapyr binds to the ALS enzyme's active site, inhibiting its function. This inhibition prevents the conversion of pyruvate and 2-ketobutyrate into acetolactate, which is a crucial step in the biosynthesis of branched-chain amino acids (Matzrafi et al., 2010). Consequently, the accumulation of toxic intermediates occurs, leading to the plant's growth arrest and eventual death.

However, in herbicide-tolerant wheat varieties, the ALS gene has undergone specific modifications, resulting in altered ALS enzyme structures (Petit et al., 2010). These modifications reduce the affinity of the enzyme for Imazethapyr, making it less susceptible to inhibition by the herbicide (Foes et al., 1999). Consequently, the ALS enzyme in herbicide-tolerant wheat varieties can continue to catalyze the biosynthesis of branched-chain amino acids, even in the presence of Imazethapyr. As a result, the wheat crop remains unaffected, while the susceptible weed species are controlled efficiently by the herbicide (Petit et al., 2010).

The molecular study confirmed that Pantera (Clearfield®) released for commercial cultivation in Chile by BASF carries a mutation Ser-Asn627 conferring resistance to imazamox in two out of three ***acetolactate synthase (ALS)*** genes (*imi1* and *imi2*), located in wheat on chromosomes 6B and 6D, respectively. However, the last gene (*imi3*) located on chromosome 6A does not carry any mutation conferring resistance. As a result, photosynthetic activity and chlorophyll content were reduced after imazamox treatment (Francisco Jimenez et.al 2016)

**Advancements in Herbicide Tolerance Research in Wheat at CIMMYT: Current Status and Methods**

CIMMYT (International Maize and Wheat Improvement Center) has been at the forefront of research to enhance herbicide tolerance in wheat, aiming to improve crop productivity and weed management (ElRamlawy et al., 2020).

CIMMYT's research efforts have made significant strides in developing herbicide-tolerant wheat varieties. Several promising lines have been identified, showing enhanced tolerance to commonly used herbicides, such as Group 2 ALS-inhibitors and others, that target critical enzymes in weed growth pathways.

To achieve herbicide tolerance, CIMMYT employs a comprehensive approach that integrates modern biotechnological tools, gene editing techniques (such as CRISPR-Cas9), and traditional breeding methods (ElRamlawy et al., 2020). This multifaceted strategy enables researchers to identify and manipulate key genes responsible for herbicide tolerance in wheat. Through extensive screening and selection processes, CIMMYT evaluates the genetic variability in wheat germplasm to identify plants with natural tolerance to specific herbicides, which serve as valuable genetic resources in developing new herbicide-tolerant varieties.

Moreover, targeted gene editing techniques are employed to introduce or modify specific genes associated with herbicide tolerance, expediting the development of tolerant wheat varieties with precision and efficiency (ElRamlawy et al., 2020). These advancements hold significant promise for sustainable weed management and improved wheat yields, contributing to food security in wheat-growing regions.

**IARI Wellington and the development of first herbicide tolerant wheat variety in India through IARI initiatives**

Developing herbicide-tolerant wheat varieties is crucial to enhance weed management practices and improve crop productivity. In our research conducted at the Indian Agricultural Research Institute, Wellington, since kharif 2018 we aimed to introduce herbicide tolerance into wheat lines possessing resistance genes for rusts. Recurrent parents like **HW 2436-1, HW 2436-2, Hw 2436-3a, HW36-4, HD 3086, HD 3059, WH1124, PBW343, DBW39, HD2733, and PBW723** were selected for their rust resistance traits. The donor Australian Spring wheat line (BCL0618) harboring the ALS gene(s), responsible for herbicide (Imazethapyr) tolerance, was used as donor for introgression through a backcross method. At every stage the Imazethapyr was sprayed with 125g/ha at 25$^{th}$ DAS

**Selection of Recurrent Parents:** Wheat lines HW 2436-1, Hw 2436-2, Hw 2436-3a, HW36-4, HD 3086, HD 3059, WH1124, PBW343, DBW39, HD2733, and PBW723, known for their rust resistance, were chosen as recurrent parents for the herbicide tolerance introgression.

**Donor for herbicide tolerance:** The Australian Spring wheat line (BCL0618), possessing the herbicide tolerance ALS gene, was used as the donor. Backcrossing was carried out by crossing the recurrent parents with BCL0618, followed by successive backcrosses to the recurrent parent.

**Selection of F1, F2, F3, and F4 Generations:** The F1, F2, F3, and F4 generations were obtained from the backcrossed plants. Among these generations, BC1F3 and BC1F4 showed promising herbicide tolerance to imazethapyr.

**Development of Backcross Lines**: The BC1F1, BC2F1, and BC3F1 generations displayed high parental traits but relatively low herbicide tolerance due to the presence of heterozygous genes, resulting in segregation for herbicide

tolerance. Therefore, these lines were either selfed or further BC effected on a much stunted plants with reduced tillers to develop BC1F2, BC2F2, and Bc3F2 generations to enhance herbicide tolerance stability.

**Results and Next Steps**

The research progress shows promising results in developing herbicide-tolerant wheat lines by introgressing the ALS gene from the donor Australian Spring wheat line. The BC1F3, BC2F2, and BC3F2 generations have shown higher stability for herbicide tolerance. As we move forward, the next generation, BC3F2, is expected to exhibit even better tolerance to the herbicide imazethapyr. The advance HTW lines are compared with unweeded plot and hand weeded ones to asses the efficacy of HTW. The common weeds present in the fields include *Biden pilosa, Oxalis stricta, Trifolium repens, Veronica persica, Nocandra pjysalodes, Phalaris minor, Elymus repens, Cyperus rotundus and Eleusine indica* and the herbicide *imazethapyr* could fairly suppress the weed growth with HTW lines showing high level of tolerance to herbicide spray. This ongoing work is a significant step towards creating improved wheat varieties with enhanced weed management capabilities and higher crop productivity.


**Wheat line HW 5207 don't carry ALS totally killed when sprayed with 150gm/ha of**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# "Building climate-resilient wheat: Unveiling the solid stem trait"

**Shajitha.P[1*], Nisha.R[1], Sivasamy M[1], P.Jayaprakash[1], V.K.Vikas[1], Niharikha Mallick[2], Rajbir Yadav[2], Vijaishree S[1], Sanjeth V[1], Akileshwaran. K[1], Suganya. C[1],Balaji V[1], M. Gokulakrishna[1], Geetha M[1], Arun Kumar[1] and C. John Peter[1]**

[1]ICAR-Indian Agricultural Research Institute Regional Station, Wellington, Tamil Nadu -643 231, India
[2]ICAR-Division of Genetics, Indian Agricultural Research Institute, Pusa, New Delhi-12. India
[3]ICAR - Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla-171001, India

***-Corresponding author:** shaji.sathi@gmail.com

**Introduction:**

As the global population is projected to reach 9.7 billion by 2050, ensuring food security becomes an increasingly pressing challenge. This mandate is compounded by factors such as climate change, limited arable land, and environmental concerns (United Nations, 2019). Notably, the rise of each degree-Celsius in temperature leads to a 6.0 ± 2.9% reduction in global wheat yields (Zhao et al., 2017). Projected climate change impacts for India in the mid-term (2012-2039) indicate a 4.5 to 9% yield reduction, roughly equating to a 1.5% GDP decline annually (Kumar et al., 2019).The Indo-Gangetic Plains (IGP)/ North Western plain wheat belt in India is particularly vulnerable due to its classification as a climate change hotspot. Escalating temperatures in this region pose a substantial threat to local food security, resulting in significant crop losses (Zachariah et al., 2021; Lavania, 2021). In this context, the imperative to develop resilient crop varieties capable of thriving under adverse conditions becomes evident.

Wheat (Triticum aestivum), a crucial global staple, faces challenges from lodging in conventional varieties characterized by hollow stems. This leads to reduced yields and compromised grain quality (Berry et al., 2020; Cox et al., 2019). The solid pith, consisting of undifferentiated parenchyma cells, plays a critical role in enhancing drought and heat tolerance. It acts as a reservoir for water and water-soluble carbohydrates, enabling plant survival in moisture-limited environments (Ford et al., 1969; Saint et al., 2010).

Addressing stem lodging is paramount as it not only exposes crops to disease but also diminishes light penetration and airflow, impacting overall productivity (Peterson et al., 1948; Joshi et al., 1982). Introducing the solid stem trait into wheat cultivars offers the promise of mitigating lodging risks and increasing crop resilience. Recent advancements in wheat breeding have led to the development of solid-stemmed varieties with robust pith tissue, leading to improved stem strength, lodging resistance, and enhanced water-soluble carbohydrate utilization during drought stress (De Paepe & Van Damme, 2018; Liu et al., 2020; Zhang et al., 2015).

This breakthrough innovation holds transformative potential for sustainable agriculture, addressing lodging challenges, and enhancing overall crop vigor (Patil et al., 2021). It even has implications for bioethanol production (Krasileva et al., 2017). In this manuscript, we delve into the promise of solid-stemmed wheat, spotlighting its role in advancing sustainable agriculture, boosting food production and fortifying resilience against

future agricultural demands and environmental pressures. Recognizing the significance of this advancement lays the foundation for a more resilient and productive agricultural landscape poised to tackle emerging challenges.

## Materials & Methods

### Plant materials

A concerted effort was undertaken to introduce the solid stem trait identified from CoW(W)1 (Thermo tolerant wheat) & DBW 39 (Unpublished data) into modern Indian wheat cultivars. These cultivars were equipped with rust-resistant genes targeting leaf rust, stem rust, yellow rust, and powdery mildew across various genetic backgrounds. The targeted regions encompassed the North Western and North Eastern plain zones, including HD 2687, HD 2967, HD 2733, HD 2877, PBW 502, PBW 343, COW(W-1), HW 5207, and HD 2833. The breeding initiative was conducted at the ICAR-Indian Agricultural Research Institute (IARI), Regional Station in Wellington, Tamil Nadu, India, situated at 11°02'47.5''N; 76°46'26.1''E, and an altitude of 1850 meters above mean sea level (AMSL).

To ensure the stable inheritance of the solid stem trait in the progeny, the backcross breeding method was employed. Selected solid stem lines underwent multiple rounds of backcrossing with elite parental lines to enhance genetic makeup and stability. Backcross breeding (BC3) and hybridization techniques were employed to amalgamate disease resistance and the solid stem trait within a single plant. The germplasm underwent an initial screening process to identify plants harboring the solid stem trait and displaying resistance against leaf rust, stem rust, yellow rust, and powdery mildew. Rigorous controlled disease inoculation was conducted to gauge the levels of resistance. Furthermore, a thorough screening procedure for the solid stem trait was executed for advance lines TNAU Coimbatore to identify plants featuring robust stem structures capable of withstanding adverse weather conditions.

### Field Evaluation

Pyramided lines' reaction to rust diseases was assessed using Peterson's modified Cobb scale (Peterson et al., 1948). Evaluation occurred at key growth stages (Zadoks et al., 1974): Z-60 (Beginning of anthesis), Z-73 (Early milk stage), and Z-85 to 87 (Soft dough stage). Disease severity and area coverage were scored (5%-100%). Host response categories from Loegering (1959) were used: 0 - No infection, R - Resistant (necrosis, possible uredia), MR - Moderately resistant (small uredia, necrosis), MX - Intermediate (variable uredia), MS - Moderately susceptible (medium uredia), S - Susceptible (large uredia).

### Solid Stem Evaluation

To assess stem solidity, ten plants per plot were sampled. The middle of five consecutive internodes was evaluated using a 5-grade scale (DePauw and Read, 1982): grade 1, hollow stem (0% pith) to grade 5, fully filled stem (100% pith) showed in Figure1. Cumulative pith content was calculated by summing values from all internode cuts. Field trials evaluated line performance under varied conditions, offering insights into agro-climatic adaptability.

### Molecular analysis

The DNA extraction process involved the collection of leaf tissues from 7-10 days old seedlings or 3 to 4-week-old plants. Genomic DNA was isolated utilizing the CTAB method as

described by Murray and Thompson (1980). The quantification of the isolated DNA was performed using a spectrophotometer, while the purity of the DNA samples was assessed using a Nanodrop machine. Subsequently, the DNA was appropriately diluted in TE buffer, achieving a final concentration of around 25 ng/µl, in preparation for PCR amplification.

For Marker Assisted Selection (MAS), a precise approach was employed, utilizing specific microsatellite markers linked to a range of rust resistance genes. These markers encompassed Gb, associated with *Lr19* (Prins et al., 2001), Sr24#12 connected to *Sr24/Lr24*, and Ventriup/LN2 linked to Lr37/Sr38/Yr17 (Helguera et al., 2003). Complementing these, additional markers for stem rust resistance, such as stm773 linked to *Sr36* (Tsilo et al., 2008), and gwm 533 linked to *Sr2* (Spielmeyer et al., 2003), were effectively integrated into the analysis. Moreover, the investigation included specific gene markers tailored for enhancing yellow rust resistance, precisely targeting *Yr10(E1)* and *Yr15(Barc 8)*. **In pursuit of the second objective, the study harnessed a specific marker, GWM 247 (Cook et al., 2004), closely linked to the solid stem trait.** This comprehensive approach allowed for the precise selection and enhancement of key traits, contributing to the overall advancement of crop resilience and productivity.

**Marker Assisted Selection**

**Results**

A total of 137 lines were meticulously developed, each encompassing distinct combinations of rust-resistant genes for leaf rust, stem rust, yellow rust, and powdery mildew across diverse genetic backgrounds, including HD 2687, HD 2967, HD 2733, HD 2877, PBW 502, PBW 343, HW 5207, and HD 2833. As part of a secondary endeavor, the solid stem trait from CoW(W)1 & DBW 39 was additionally introduced into elite parental lines across varied agro-climatic zones, aimed at producing climate-resilient wheat varieties. In this study, in the segregating populations it was observed that the wheat lines with the solid pith range 3-5 scale was moderate resistant for stem rust or black rust caused by *Puccinia graminis* while the lines with resistant genes was highly resistant for black rust **(Figure 2)** .

The presence of specific genes was discerned through molecular analyses. The *Lr19* gene was detected via 130 bp amplification, *Sr24* gene presence was indicated by a 500 bp fragment, and the *Lr37*+ gene was confirmed by a 262 bp DNA fragment **(Figure 3).** The Sr36 gene, closely linked to PM6, exhibited a 155 bp band size (**Figure 4)**. *Yr10* gene confirmed depicts the band size at 750 bp **(Figure 5)**. The solid stem trait, detectable at 175 bp, was distinguished from its absence at 180/190 bp **(Figure 6).**

Among the 137 lines, 125 were identified to possess both the solid stem trait and a diverse array of rust-resistant genes. The strategic integration of rust-resistant genes and the solid stem trait was achieved through meticulous backcross breeding and hybridization techniques, facilitating the successful transfer of desired traits from donor plants CoW(W)-1 and DBW 39 into the newly developed lines.

In the context of this study, a field trial was conducted at TNAU, Coimbatore, assessing solid-stemmed lines for terminal heat tolerance a crucial attribute for climatic resilience. Encouragingly, solid pith lines exhibiting assessment scores of 3-5 demonstrated

commendable robustness in the face of terminal heat stress **(Figure 3).** This result underscores the potential of the introduced solid stem trait in enhancing crop resilience under challenging environmental conditions, paving the way for more climate-resilient wheat varieties.

**Discussion**

The solid-stemmed trait initially gained recognition for its efficacy in deterring sawfly (Hymenoptera: Cephidae) infestations in wheat crops (Lamb, 1989). Gradually, this trait has evolved from its sole role in sawfly resistance to offer a range of advantages. These include augmented stem strength, increased lodging resistance, and heightened adaptability to diverse environmental challenges, including enhanced water-soluble carbohydrate remobilization under drought stress (De Paepe & Van Damme, 2018; Liu et al., 2020). Despite its limited exploration by Indian breeders due to minimal stem sawfly and borer threats, as well as lower lodging incidence (Bainsla et al., 2020), the solid-stem trait is now gaining prominence. This innovation holds transformative potential for sustainable agriculture by addressing lodging issues and enhancing overall crop vigor (McLean et al., 2019; Patil et al., 2021; Nilsen et al., 2020).

Solid-stemmed wheat presents a compelling solution to counteract the lodging challenges encountered by conventional wheat varieties with hollow stems (Berry et al., 2020; Cox et al., 2019). Stem lodging not only diminishes grain yields and quality but also elevates disease susceptibility, disrupts airflow, and restricts light penetration within the crop canopy (Peterson et al., 1948; Joshi et al., 1982). Additionally, solid stems serve as a physical barrier against fungal pathogens, curbing their penetration and spread. Our study observed moderately resistant stem rust in solid-stemmed lines, a finding consistent with Mundt et al. (2002), showcasing the mechanical resistance of solid-stemmed wheat against pathogens. Furthermore, solid stems minimize potential entry points for pathogens, as demonstrated by Liu et al. (2013) in maize. The reduced moisture retention in solid stems inhibits fungal growth, supported by Xue et al. (2017) in wheat. Elevated chemical defenses in solid-stemmed rice, as observed by Zheng et al. (2020), add to their antifungal properties. Comparative studies by Zheng et al. (2018) identified decreased fungal colonization and disease severity in solid-stemmed barley, underscoring their role in pathogen resistance.

The backcross breeding method was employed to ensure the inheritance of the solid stem trait in the progeny, followed by hybridization techniques to combine disease resistance and the solid stem trait in a single plant (Patil et al., 2021). Integrating molecular insights on transcription factors and genomic regions into targeted crosses can accelerate yield gains by combining improved sink capacity with lodging resistance (Alvarez et al., 2021; Barrero et al., 2020; Kim et al., 2020; Zhao et al., 2021).

Field trials evaluating 137 developed lines across various environments offered insights into their adaptability to specific agro-climatic regions (Chen et al., 2020). This study, conducted at TNAU, Coimbatore, assessed terminal heat tolerance in solid-stemmed lines, with lines scoring 3-5 displaying promising heat tolerance potential. Integrating solid-stem traits in wheat breeding could enhance resilience to terminal heat stress, vital in the context of climate change and rising temperatures.

Further research into the genetic basis of heat tolerance in these lines may facilitate the development of heat-resilient wheat varieties, ensuring food security in challenging environments (Stone & Nicolas, 1995). Out of these lines, 125 possessed both the solid stem trait and a diverse set of rust-resistant genes, rendering them potential candidates for cultivation in rust-prone regions (Kumar et al., 2021). In this study, field screening confirmed resistance to leaf rust, stem rust, yellow rust, and powdery mildew, coupled with the presence of the solid stem trait achieved through strategic backcross breeding and hybridization (Rasheed et al., 2014; Patil et al., 2021). Additionally, solid stem trait screening facilitated the identification of sturdy-stemmed plants.

The successful development of solid-stemmed wheat cultivars offers promising prospects for sustainable agriculture (McLean et al., 2019). These cultivars exhibit reinforced structural integrity, reduced lodging risk, and improved agronomic performance (De Paepe & Van Damme, 2018). Moreover, their capacity for water-soluble carbohydrate remobilization under drought stress contributes to drought tolerance and resilience, extending advantages to water-limited environments (Zhang et al., 2015). Elevating the water-soluble carbohydrate (WSC) content present in straw provides a multi-fold advantage for bioethanol production, resulting in improved extraction efficiency and diminished enzymatic pretreatment demands during processing, as highlighted by Krasileva et al. (2017). By mitigating lodging and enhancing overall plant vitality, solid-stemmed wheat holds the potential to increase global food production and promote sustainable agricultural practices (Nilsen et al., 2020). Incorporating solid pith into papermaking aligns with sustainability goals and offers potential advantages (Lavania, 2021). This integration enhances fiber properties like bulk and stiffness, potentially reducing the need for additives. The cellulose and hemicellulose-rich composition of solid pith further supports sustainability objectives. Addressing challenges related to extraction methods and supply consistency through continued research is essential (Lavania, 2021; Tribune News Service, 2021).

In conclusion, the solid-stemmed trait in wheat showcases versatile benefits, from resistance against sawflies and lodging issues to enhanced disease resistance and adaptability to environmental challenges. Its integration into wheat breeding offers promise for sustainable agriculture, addressing multiple challenges and contributing to increased food production and resilience in changing climates.

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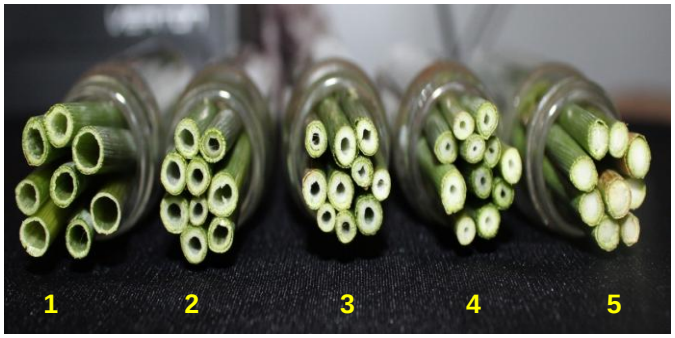
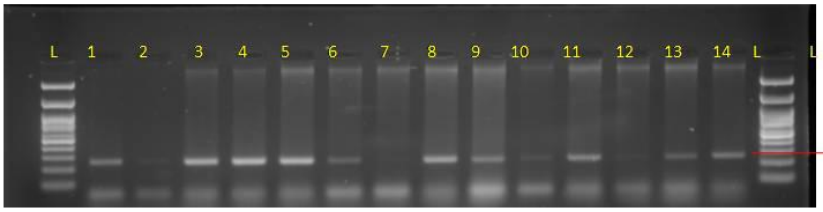


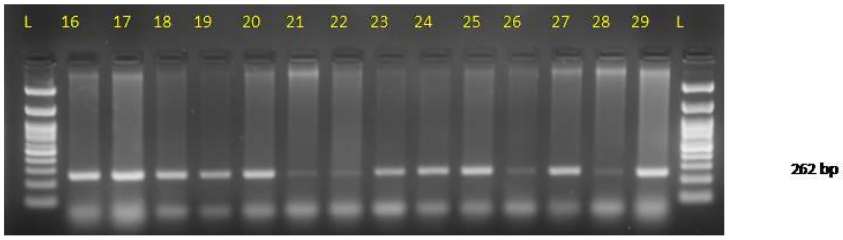

**Figure 1**: **The assessment of the stem-solidness according to the methodology developed by DePauw and Read (1982): 1—hollow pith (0% filled), 2—25% filled, 3—50% filled, 4—75% filled, 5—solid stem (100% filled)**

**Figure 2: 1- Solid stem with Stem rust resistance**
**2- Hollow Stem with Stem rust susceptible**


**Figure 2:**
**1- Solid stem with Stem rust resistance**
**2- Hollow Stem with Stem rust susceptible**

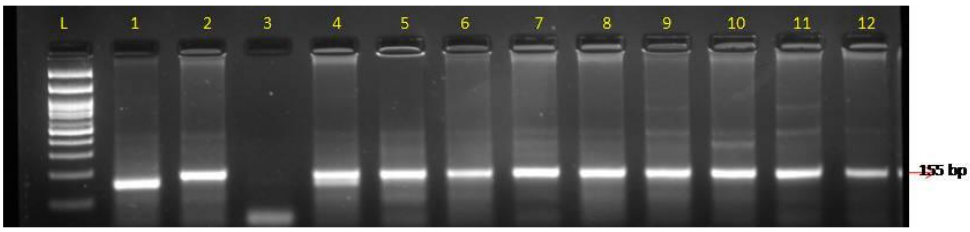
**Figure 3 :Molecular Confirmation of Lr37 gene using marker the VENTRIUP/LN2**




L:100bp ladder, 1- RI 6081( Positive control), 2: Lok-1Negative control), 3:WTN613,4-WTN 614, 5-WTN 615, 6-WTN 616, 7-WTN 617, 8-WTN 618, 9-WTN 619, 10- WTN 620, 11- WTN 621 , 12- WTN 622, 13- WTN 623, 14- WTN 624, 15- WTN 625, 16- WTN 626, 17- WTN 627, 18-WTN 628, 19- WTN 629, 20- WTN 630, 21- WTN 631, 22- WTN 632, 23- WTN 633, 24- WTN 634, 25- WTN 635, 26 –WTN 636, 27 –WTN 637, 28-WTN -638, 29- WTN -639

**Figure 4 : Molecular Confirmation of Sr36 gene using marker the STM 773**



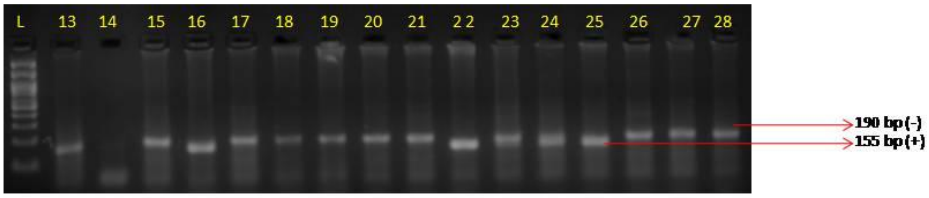
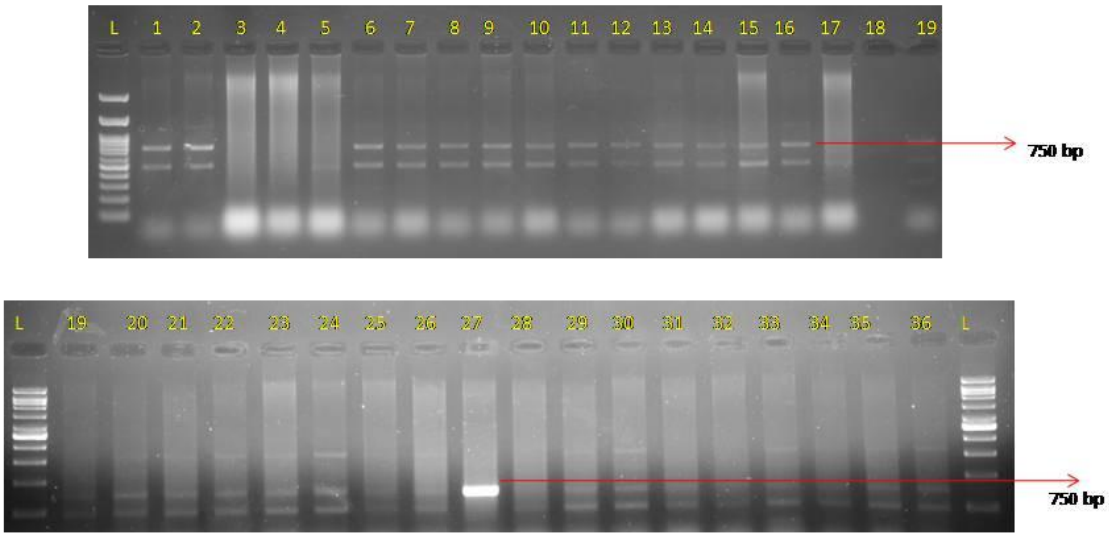
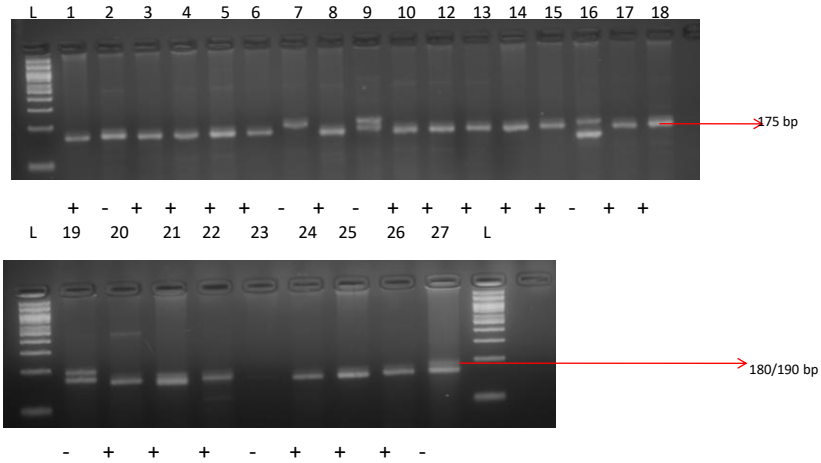



**Figure: 5 Molecular Confirmation of Yr10 gene using marker the E1**




**L: 50bp ladder, 1- HW2436-1 (Positive control), 2: HW2436-2 (Positive control), 3:WTN613,4-WTN 614, 5-WTN 615, 6-WTN 616, 7-WTN 617, 8-WTN 618, 9-WTN 619, 10- WTN 620, 11-WTN 621 , 12- WTN 622, 13- WTN 623, 14- WTN 624, 15- WTN 625, 16- WTN 626, 17- WTN 627, 18-WTN 628, 19- WTN 629, 20- WTN 630, 21- WTN 631, 22- WTN 632, 23- WTN 633, 24- WTN 634, 25- WTN 635, 26 –WTN 636, 27 –WTN 637, 28-WTN -638, 29- WTN -639,30- WTN 640, 31-WTN 641, 32- WTN 642, 33-WTN 643, 34- WTN 644, 35- WTN 645, 36- WTN 646**

**Figure 6: Molecular characterization of solid stem trait in the advance lines (GWM 247)**




L:100bp ladder, 1- COW(W)1 (Positive control), 2: C306(Negative control), 3:WTN613,4-WTN 614, 5-WTN 615, 6-WTN 616, 7-WTN 617, 8-WTN 618, 9-WTN 619, 10- WTN 620, 11- WTN 621 , 12- WTN 622, 13- WTN 623, 14- WTN 624, 15- WTN 625, 16- WTN 626, 17- WTN 627, 18-WTN 628, 19- : C306( (Negative control), 20- COW(W)1 (Positive control), 21- WTN 629, 22- WTN 630, 23- WTN 631, 24- WTN 632, 25- WTN 633, 26- WTN 634, 27- WTN 635

## AWARDS AND RECOGNITIONS RECEIVED

### i. ICAR/National Awards

| S. No. | Name of the Scientist | Name of the Award | Awarding agency | Nature of award (Medal/ Certificate/amount of Cash price) | Achievement for which the award was given (Life-time achievement/ any specific discover / technology etc for which the ward was given) |
|---|---|---|---|---|---|
| 1. | Dr. M. Sivasamy<br>Dr. Vikas V.K. | BGRI-Gene Stewardship Award | Borlaug Global Rust Initiative (BGRI), USA | Certificate | Deployment of rust resistance genes in Indian wheat cultivars |
| 2. | Dr. M. Sivasamy,<br>Dr. P. Jayaprakash<br>Dr. Vikas V.K. | Nanaji Deshmukh ICAR Award for Outstanding Interdisciplinary Team Research in Agricultural and Allied Sciences | Indian Council of Agricultural Research | Certificate | For the development and release of high yielding rust resistance wheat varieties |
| 3. | Dr. Vikas V.K. | Education Ambassador for Australia | Australian Government Dept. of Education and Training | Certificate | To pursue post-doctoral research in Australia |
| **4** | Dr. Vikas V.K. | Endeavour Research Fellowship | Australian Government Dept. of Education and Training | Certificate & Fellowship | To pursue post-doctoral research in Australia |

### ii. Fellowship/Associateship of National academies

| S. No. | Name of the Scientist | Fellowship/ Associateship | Name of the Academy |
|---|---|---|---|
| 1. | Dr. M. Sivasamy | NAAS Fellow(2020) | National Academy of Agricultural Sciences (NAAS), New Delhi |
| 2. | Dr. Vikas V.K. | NAAS Associate | National Academy of Agricultural Sciences (NAAS), New Delhi |

## VISIT OF DELEGATIONS

**Visit of Borlaug Global Rust Initiative (BGRI) team Dr Ronniee Coffman, Maricelis Acevedo, Associate Director of science for the Delivering Genetic Gain in Wheat (DGGW) project & Dr Vijaya Ragavan, Cornell University to I.A.R.I. R.S., Wellington**

**Visit of Dr. Kuldeep Singh, Director, NBPGR to I.A.R.I. R.S., Wellington**

## Postings:

1**. Dr P. Nallathambi**, Principal Scientist (Plant Pathology) joined and served as Head(Acting) from 1st Jan, 2023 to 25, April 2023 forenoon as per council's guide lines O.M. No. 8(1)/2027-Per.IV, dt. 16.08.2022

**2. Dr.M.Sivasamy**, Principal Scientist, Joined as 'Regular Head' from 25th, April 2023 afternoon after selection through ASRB and the same was endorsed by council's order vide F.N. 10(17)/2020-Per-III dated 04th May 2023.