Journal of Cereal Research

Volume 14 (Spl - 1): 96-104

Review Article

Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Molecular Markers Associated with Heat Tolerance in Wheat

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Article history: Received: 14 Apr., 2022 Revised: 08 June, 2022 Accepted: 24 July, 2022

Citation:

Vedi A, A Tripathi and GC Pandey. 2022. Molecular Markers Associated with Heat Tolerance in Wheat. *Journal of Cereal Research* 14 (Spl-1): 96-104. <u>http://doi.org/10.25174/2582-</u> 2675/2022/126886

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Abstract

High temperatures have emerged as a major significant barrier for wheat production, which is generally adapted to the high temperature and sub-tropical environment. The sensitivity of wheat to high temperature a very high mainly during the grain filling stages due to the vulnerability of abiotic stresses. In the tropical and sub-tropical regions, each increase in ambient temperature can lower grain yield by 3-20%. Tolerance to high temperature is a complicated phenomenon and a quantitative trait that influences a variety of physiological and agronomic traits. No single trait thoroughly explains why certain wheat varieties can produce higher yield even when exposed to heat stress. Genes and molecular markers associated with stress tolerance mechanisms are crucial for improving crop productivity under high temperature environment. Different genotypes respond differently to heat stress, and this is mediated by genes or quantitative trait loci (QTL). Genotypes that are either high temperature stress resistant or mature early without yield loss, allowing them to avoid stress are needed to be produced. Plant breeders should concentrate on current yield patterns and environmental stress, as well as traits related to yield stability and sustainability. To increase the possibility of success of new methods like DNA marker technology will be needed despite the persistent yield enhancement from conventional breeding.

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Keywords: Quantitative trait loci (QTL), heat tolerance, molecular markers, chromosomes, wheat.

1. Introduction

Wheat (*Triticum aestivum* L.) is the second staple crop after rice in the world. Wheat production is essential for our national food security programme because the parliament of India passed the bill "Food for All" (Anonymous, 2013). In India, wheat production has scaled gradually to higher grounds with expanding in area, production and yield. With an estimated area of more than 218 million hectares, wheat is the largest cultivated crop globally (Giraldo *et al.*, 2019). In India wheat was cultivated in 29.55 mha during 2018-19.A landmark was achieved by India in wheat production during 2019-20 by producing 107.18 million tonnes (Anonymous, 2020). Due to the rise in temperature and erratic rains, the vulnerability in abiotic stresses is obvious from a considerable dip

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observed in wheat manufacture. By 2050 the global and India's wheat demand will be 1090 and 140 million tonnes respectively. This target can be achieved by maintaining annual growth production of 1.6% (Anonymous, 2016). Wheat is vulnerable to climate changebut then 1.2 billion poor masses rely on it. Wheat yields by 2050 in South Asia are predicted to reduce 20-30%. Increased demand for food is expected to anywhere between 59-98% by 2050. Wheat production in India is being constantly challenged by many threats as reported worldwide. It includes climatic changes which lead to extreme temperature, altered weather, erratic precipitation and changes in pest dynamics (Mamrutha *et al.*, 2014). Development of climate resilient wheat varieties is going to be the way forward



if the food demand for ever increasing population is to be met (Kumar *et al.*, 2019). In coming years' changes in the micro-environments are expected to be more severe, coupled with challenges due to a decrease in the arable land caused by increasing urbanization, land degradation and limited water for irrigation (Anonymous, 2011). The abiotic stresses are the leading factors that affect the crop in different ways like plant growth, height, and strength, etc., ultimately led to the variation in yield by interfering with normal metabolic activities of plants.

Abiotic stress alone causes a yield reduction of more than 50% worldwide insignificant crops (Acquaah, 2007). India is a country that possesses different climatic and agronomic zones affected by different abiotic stresses. To date, various abiotic stresses, drought, salinity, heat, waterlogging, cold, aluminium toxicity, micronutrient deficiency etc., are reported to hamper wheat yield. Among the abiotic stresses, extreme temperature, drought, and salinity are the utmost ones and affects the maximum area of wheat in India (Lobell *et al.*, 2008; Battisti and Naylor, 2009; Butler and Huybers, 2013; Makhloufi *et al.*, 2014).

As wheat is a winter season crop high temperature pressure can be a serious element in the reduction of yield. Wheat when late planted suffers a drastic loss in yield which may overreach to 40-50%. It is an urgent need to evolve genotypes which are either high temperature stress tolerant or that without losing yield mature early and thus escape stress. The major focus of plant breeders should be present yield trends, community growth and stress on the environment, yield stability and sustainability associated traits. Despite the persistent yield enhancement from conventional breeding, the genetic basis of tolerance to high temperature is essential for improving wheat productivity in stress-prone regions, and new methods such as DNA marker technology will be required to increase the possibility of success (Kamboj *et al.*, 2020).

Allelic variation in the genes underlying these traits can be detected by DNA markers which can be assisting in plant breeding to increase efficiency and precision. Various genotypes show different response to high temperature stress and this is controlled by genes or quantitative trait loci (QTL); the genomic regions containing genes which are related with a particular quantitative trait. It is found that different QTLs had been linked to high temperature tolerance in plants. QTL mapping is a well-grounded way for the genomic assisted enhancement of abiotic stress toleration of crops and using traits which indicates heat tolerance, QTLs were detected.DNA polymorphism occurs naturally which is the basis of molecular markers utilization. A marker must occur in different forms or types and called polymorphic so it can distinguish between chromosomes consisting of mutant gene from chromosomes with a normal gene. The occurrence of a trait in two variants or genotypes of the same population is genetic polymorphism.

2. Molecular markers related with: Morphological traits

2.1. Days to anthesis (DA):

When anthers are extruded from the plants after the complete emergence of headings in wheat. Two QTLs were detected, linked to DA *gwm251* and *wmc160* on chromosome 4B and 5B respectively were reported by Mason *et al.*

2.2. Days to physiological maturity (DM):

When the peduncle of plants converts into yellow colour. Paliwal*et al.*, reported markers*Xgwm3062-Xgwm4335* on chromosome 7D significant for this trait.

2.3. Grain filling duration/rate (GFD/GFR):

Grain yield differences are expressed for genotypes' sensitivity in heat stress environment. GFD is associated with grain development and it is anessential parameter to estimate the efficiency of wheat genotypes (Yang and Zhang, 2006). 144 RILs were generated from the cross of two cultivars Kauz and MTRWA116 to map and characterize QTL controlling high temperature resistance and one significant QTL identified for GFD was Xgwm484 on chromosome 2D (Mohammadi *et al.*, 2008a).

Three markers Xgwm132, Xgwm577 and Xgwm617 located on 6A, 6B and 7B chromosomeswas identified respectively in F_1 and F_2 generation of two cultivars Debra and Yecora Rojo RIL population (Barakat *et al.*, 2011). *Xbarc04* and *Xgwm314* located on chromosome 3D and 5B were detected in 111 RIL population of two crossed cultivars Raj4014 and WH730 (Pandey *et al.*, 2013a). In RIL population generated from a cross of Halberd & Cutter three markers were detected associated to GFD are *barc137*, *wmc407* and *cfd43* on chromosome 1B, 2A and 2D respectively (Mason *et al.*, 2010). NW1014 and HUW468 crossed F_5 , F_6 and F_7 population was evaluated



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to determine heat tolerance using GFD trait. QTL flanked by markers *Xgwm935-Xgwm1273* on chromosome 2B were associated with this trait (Paliwal *et al.*, 2012). Another three QTLs were detected associated with GFD in two crossed cultivars Berkut and Krichauff. Two QTLs flanked by markers *gwm349-wPt9797* and *cfd233-cfd044* on chromosome 2D and one on chromosome 7A flanked by *wmc065-wmc13 9* (Tiwari *et al.*, 2013).

2.4. Flag leaf length/width (FLL/FLW):

Grain yield and photosynthetic ability are associated with these two leaf traits. Two QTLs were detected associated with FLL, *barc147* and *wmc160* on chromosome 3B and 5B respectively and three QTLs associated with FLW flanked by *wmc336*, *barc200* and *gwm60* on chromosome 1D, 2B and 7A respectively.

2.5. Stress susceptibility index (SSI):

144 RILs were generated from the cross of Kauz and MTRWA116 to map and characterize QTLs controlling heat tolerance. Composite interval mapping method was used and three QTLs related with heat resistance were mapped on 1B, 5B and 7B chromosomes which were measured by SSI. Markers *gwm190*, *gwm133* and *gwm63* were located on chromosome 1B, 5B and 7B respectively.

2.6. Visual leaf wax (VLW):

Mason *et al.*, (2010) reported one QTL significant for this trait on chromosome 5A flanked by *wmc150*.

Table 1. Markers significant to morphological traits

Chromosome	Markers	\mathbb{R}^2	Traits	References
1B	barc137	12.3	GFD	Mason et al., 2010
1B	gwm190	44.3	SSI	Mohammadi et al., 2008b
1D	wmc336	7.8	FLW	Mason et al., 2010
2A	wmc407	29.6	GFD	Mason et al., 2010
2 B	barc200	25.9	FLW	Mason et al., 2010
2 B	gwm935-gwm1273*	20.15	GFD	Paliwal et al., 2012
2D	gwm484	22.5	GFD	Mohammadi <i>et al.</i> , 2008a
2D	cfd43	14.6	GFD	Mason et al., 2010
2D	gwm349-wPt9797*	21.01	GFD	Tiwari et al., 2013
Chromosome	Markers	\mathbb{R}^2	Traits	References
2D	cfd233-cfd044*	20.60	GFD	Tiwari <i>et al.</i> , 2013
3 B	barc147	20.7	FLL	Mason et al., 2010
3D	gwm314	6.0	GFR	Pandey <i>et al.</i> , 2013a
5A	wmc150	16.4	VLW	Mason et al., 2010
5B	gwm133	27.3	SSI	Mohammadi et al., 2008b
5B	barc04	10.0	GFR	Pandey et al., 2013a
5B	wmc160	13.0	FLL, DA	Mason et al., 2010
6A	gwm617	3.0	GFR	Barakat <i>et al.</i> , 2011
6B	gwm132	7.0	GFR	Barakat <i>et al.</i> , 2011
7A	gwm60	19.0	FLW	Mason et al., 2010
7A	wmc065-wmc139*	12.27	GFD	Tiwari <i>et al.</i> , 2013
7B	gwm63	34.0	SSI	Mohammadi et al., 2008b
7B	gwm577	25.0	GFR	Barakat et al., 2011
7D	gwm3062-gwm4335*	7.42	DM	Paliwal <i>et al.</i> , 2012

*- Marker interval

SSI-Stress Susceptibility Index, GFD/GFR-Grain Filling Duration/Rate, FLL-Flag Leaf Length, FLW-Flag Leaf Width, VLW-Visual Leaf Wax, DA-Days to Anthesis, DM-Days to Physiological Maturity.



3. Agronomic traits:

3.1. Grain weight (GW):

It has a major effect on the formation of yields. It is a direct reflection of the effective use of nutrients and their translocation into the plant's generative parts. Two QTLs were detected on chromosome 3B linked to GW trait were flanked by *wmc527* and *wmc326*.

3.2. Grain number (GN):

Mason *et al.*, reported three QTLs significant to Grain Number. One QTL on chromosome 2B was *gwm111* and two QTLs on chromosome 3B *gwm389* and *barc147*.

3.3. Thousand grain weight (TGW):

It is one of the traits that contribute to yield. Yields can be approximate and measured easily (Baril, 1992). Three QTLs major to TGW reported by Paliwal *et al.*, (2012) located on chromosome 2B, 7B and 7D were flanked by markers *Xgwm935-Xgwm1273*, *Xgwm1025-Xgwm745* and *Xgwm3062-Xgwm4335* respectively. Two QTLs linked to this trait were identified on chromosome 1D and 6B flanked by markers *wPt9664-cfd083* and *gwm626wPt4924* respectively (Tiwari *et al.*, 2013). Another four QTLs *Xpsp3094*&*Xgwm282* mapped on chromosome 7A, *Xbarc114* and *Xbarc51* on 1A and 2B respectively (Pandey *et al.*, 2013b).

3.4. Yield (YLD):

Paliwal *et al.*, (2012) and Tiwari *et al.*,(2013) identified *Xgwm1025-Xgwm745*at chromosome 7B and *wmc216-cfd19* on chromosome 1D respectively associated with YLD trait.

Table 2. Markers significant to agronomic traits

3.5. Tiller:

Two cultivars Huapei3 and Yumai57 were crossed to generate 168 sets of double haploid (DH) lines and it is randomly permutated intermating immortalized F_2 (IF₂) population to investigate the QTLs for tillering: Effective Tillering in Harvest (ETH), Maximum Tillering in Spring (MTS) and Maximum Tillering of pre-Winter (MTW). Six QTLs (two in DH lines and four in IF₂ lines) significant to MTW, nine QTLs (three in DH and six in IF₂ lines) for MTS and ten QTLs (four in DH and six in IF₂ lines) for ETH (Li *et al.*, 2010). Advanced wheat lines were used to assign correlation between number of tillers and SSR marker *Xgwm136* associated with tin gene (Kumar *et al.*, 2015).

QTLs significant to MTW were flanked by markers *Xgwm459-Xgwm334*, *wmc215-barc345*, *cwem32-wmc59*, *cfd19-wmc93*, *wmc334-wmc331* and *barc320-wmc215* mapped on chromosome6A, 5D, 5A, 1D, 4D and 5D respectively.QTLs significant for MTS were flanked by markers *swes679-cfa2129*, *cfe188-Xbarc224*, *wmc215-barc345*, *wmc445-gwm111*, *cfe026-cwem32*, *barc320-wmc215*, *wmc553-gwm732*, *barc054-gwm55* and *cfa2134-wmc527* located on chromosome 6D, 4D, and 5D, 2B, 5A, 5D, 6A, 6D and 3A respectively.QTLs significant for ETH were flanked by markers *barc232-wmc235*, *wmc764-barc200*, *cbarc1177-barc276*, *gwm1055-wmc553*, *gwm194-cfa2173*, *gwm133-swes861*, *swes679-cfa2129*, *barc477-wmc175*, *gwm296-wmc112* and *gwm1055-wmc553* mapped on 5B, 2B, 3A, 6A, 4D, 6D, 6D, 2B, 2D and 6A chromosome respectively.

Chromosome	Markers	\mathbb{R}^2	Traits	References
1A	barc114	9.0	TGW	Pandey et al., 2013b
1D	cfd19-wmc93*	9.45	Tiller (MTW)	Li et al., 2010
1D	wmc216-cfd19*	10.24	YLD	Tiwari <i>et al.</i> , 2013
2A	gwm356	21.0	SGW	Mason et al., 2010
2A	gwm294	17.8	SGW	Mason et al., 2010
2 B	barc51	7.0	TGW	Pandey et al., 2013b
2 B	gwm111	12.7	GN	Mason et al., 2010
2 B	gwm935-gwm1273*	17.82	TGW	Paliwal <i>et al.</i> , 2012
2 B	wmc764-barc200*	4.49	Tiller (ETH)	Li et al., 2010
2 B	wmc445-gwm111*	6.43	Tiller (MTS)	Li et al., 2010

2B	barc477-wmc175*	9.12	Tiller (ETH)	Li et al., 2010
2D	gwm296-wmc112*	4.52	Tiller (ETH)	Li et al., 2010
3A	barc1177-barc276*	4.62	Tiller (ETH)	Li et al., 2010
3A	cfa2134-wmc527*	6.73	Tiller (MTS)	Li et al., 2010
3B	gwm389	11.3	GN	Mason et al., 2010
3B	barc147	20.7	GN, FLL	Mason et al., 2010
3B	wmc527	19.0	GW	Mason et al., 2010
3 B	wmc326	21.2	GW	Mason et al., 2010
4D	cfe188-barc224*	5.53	Tiller (MTS)	Li et al., 2010
4D	wmc334-wmc331*	7.94	Tiller (MTW)	Li et al., 2010
4D	gwm194-cfa2173*	21.32	Tiller (ETH)	Li et al., 2010
5A	cfe026-cwem32	15.91	Tiller (MTS)	Li et al., 2010
5A	cwem32-wmc59	8.21	Tiller (MTW)	Li et al., 2010
5B	barc232-wmc235*	10.91	Tiller (ETH)	Li et al., 2010
5D	wmc215-barc345*	23.19	Tiller (MTS, MTW)	Li et al., 2010
6A	gwm459-gwm334*	8.06	Tiller (MTW)	Li et al., 2010
6A	wmc553-gwm732*	9.51	Tiller (MTS)	Li et al., 2010
6A	gwm1055-wmc553*	8.60	Tiller (ETH)	Li et al., 2010
6B	gwm626-wPt4924	13.97	TGW	Tiwari <i>et al.</i> , 2013
6D	swes679-cfa2129	16.28	Tiller (MTS, ETH)	Li et al., 2010
6D	barc054-gwm55	11.28	Tiller (MTS)	Li et al., 2010
6D	gwm133-sews861	22.85	Tiller (ETH)	Li et al., 2010
7A	Xpsp3094	14.0	TGW	Pandey et al., 2013b
7A	gwm282	11.0	TGW	Pandey et al., 2013b
7B	$gwm1025$ - $gwm745^{*}$	20.34, 13.21	TGW, YLD	Paliwal et al., 2012
7D	gwm3062-gwm4335*	9.78	TGW	Paliwal <i>et al.</i> , 2012

*- Marker interval

TGW-Thousand grain weight, YLD-Yield, GN-Grain no., MTW-Maximum Tillering of Pre-Winter, MTS-Maximum Tillering in spring, ETH-Effective Tillering in Harvest, GW-Grain weight.

4. Physiological traits:

4.1. Thylakoid membrane damage (TMD), Cell membrane thermostability (CMT) and Chlorophyll content (CC):

Plasma membrane damage is also called as cell membrane thermostability, it's related to cellular thermo-tolerance and it is utilized as a measure of high temperature stress resistance in plants since it gives an indication of reduced membrane stability when there is loss of electrolytes. It is reported to be correlated with grain yield (Reynolds *et al.*, 1994 and Fokar *et al.*, 1998). Chlorophyll fluorescence is correlated to heat tolerance and it indicates photosystem II activity and thylakoid membrane damage (Moffatt *et al.*, 1990). Chlorophyll content provides a relative indication of chlorophyll in the plants.

Significant QTLs for these traits were located on five genomic regions which are 1B, 1D, 2B, 6A and 7A chromosomes in 101 RIL population generated by the cross of two cultivars Ventnor and Karl92.

QTL flanked by markers *Xbarc113* and *AGCTCG-347* associated with CC and TMD on chromosome 6A. On chromosome QTLs flanked by *Xbarc121* and *Xbarc49*markers are associated with all three traits. QTLs on 1B chromosome were flanked by *gwm18* and *Bin1130*, was associated with CC and QTLs on 2B chromosomewere flanked by *Bin178* and *Bin81*, was



associated with CMT. QTL flanked by markers *Bin747* and *Bin1596* identified on chromosome 1D was significant for all three traits. Markers *CGA.GAC-347* and *GTG.AGCT-85* on chromosome 7B were associated with CC in Ventor and Karl92 RIL population.

4.2. Senescence (SEN):

Senescence is a process which is genetically engineered and environmentally driven mechanism in which chlorophyll is lost and nutrients are transferred to younger or reproductive sections of plants.

RIL population generated by the cross of Ventnor and Karl92 identified QTL for senescence trait under high temperature. Green leaf area was calculated to determine the percentage of greenness retained over the reproductive period. The progression of senescence traits was calculated as 75 percent green (75 percent G), 25 percent green (25 percent G), and 50 percent green (50 percent G). This duration was converted into percent green leaf area and progression of senescence traits were estimated as: 75% green (75% G), 25% green (25% G) and 50% green (50% G).On chromosome 4B and 5D markers *Xgwm368* and *Xgwm292* respectively were linked to 50% G and PGMS (percent greenness at maximum senescence).

Similarly, marker *Xgwm111* on chromosome 7D was linked to 25%G and TMRS (time to maximum rate of

Table 3.Markers significant to physiological traits

senescence). On 5A chromosome marker *XCGA.CGCT*-485 was linked to 75% G. Markers *Xgwm356-XCGT.TGCG*-349 on 2A chromosome are linked to QTL for 25% G, 50% G, 75% G, MRS (maximum rate of senescence) traits and on 3B chromosome TMRS.*XCGT.CTCG-146* was linked to 75% G and *XCGT.CTCG-406* on chromosome 6B linked to TMRS. *Xgwm5* linked to PGMS on chromosome 3A and *XCGT.GTG-343* linked to 50% G, TMRS and PGMS (Vijayalakshmi *et al.*, 2010).

4.3. Chlorophyll fluorescence (Fv/Fm):

PhotosystemII activity is assessed by chlorophyll fluorescence, which is a non-invasive process. The yield of chlorophyll fluorescence emission provides us with essential knowledge about photosynthesis quantum efficiency and heat dissipation. Vijayalakshmi *et al.*, (2010) reported two markers *CGA.CGCT-272* and *Xbarc121* for this trait on chromosome 7A.

4.4. Canopy temperature (CT):

It is measured after anthesis by infra-red temperature sensors and it provides water status, water use and plant's functioning metabolism. One QTL linked to CT trait was mappedon chromosome 7B flanked by markers*Xgwm1025-Xgwm745*. Another QTL linked to CT was reported by Tiwari *et al.*, (2013) mapped on chromosome 1D flanked by *wPt9664-cfd083*.

Chromosome	Markers	\mathbb{R}^2	Traits	References
1B	gwm18	12.63	CC	Talukder et al.,2014
1B	Bin1130	12.63	CC	Talukder et al.,2014
1D	wPt9664-cfd083*	11.76	СТ	Tiwari <i>et al.</i> , 2013
1D	Bin747	11.59, 14.12, 16.64	CMT, TMD, CC	Talukder et al.,2014
1D	Bin1596	11.59, 14.12, 16.64	CMT, TMD, CC	Talukder et al.,2014
2A	gwm356	17.0	SEN	Vijayalakshmi <i>et al.</i> , 2010
2A	CGT.TGCG-349	26.0	SEN	Vijayalakshmi <i>et al.</i> , 2010
2 B	Bin178	17.22	CMT	Talukder et al.,2014
2 B	Bin81	17.22	CMT	Talukder et al.,2014
3A	gwm5	8.0	SEN	Vijayalakshmi <i>et al.</i> , 2010
3 B	CGT.CTCG-146	10.0	SEN	Vijayalakshmi <i>et al.</i> , 2010
4B	gwm368	17.0, 10.0	SEN	Vijayalakshmi <i>et al.,</i> 2010
5A	CGA.CGCT-485	30.0	SEN	Vijayalakshmi <i>et al.,</i> 2010
5D	gwm292	9.0, 10.0	SEN	Vijayalakshmi <i>et al.</i> , 2010

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6A	barc113	15.38, 14.87	CC, TMD	Talukder et al.,2014
6A	AGCTCG-347	15.38, 14.87	CC,TMD	Talukder et al.,2014
7A	barc121	32.03, 30.62, 26.59	CMT, TMD, CC	Talukder et al.,2014
7A	barc49	32.03, 30.62, 26.59	CMT, TMD, CC	Talukder et al.,2014
7A	CGA.CGCT-272	11.0	Fv/Fm	Vijayalakshmi <i>et al</i> ., 2010
7A	barc121	-	Fv/Fm	Vijayalakshmi <i>et al</i> ., 2010
7B	CGA.GAC-347	9.0	CC	Vijayalakshmi <i>et al</i> ., 2010
7B	GTG.AGCT-85	-	CC	Vijayalakshmi <i>et al</i> ., 2010
7B	gwm1025-gwm745*	19.81	СТ	Paliwal <i>et al.</i> , 2012
7D	gwm111	12.0, 10.0	SEN	Vijayalakshmi <i>et al.</i> , 2010

*- Marker interval

5. Conclusion

High temperature pressure is a serious element in the reduction of yield. In tolerance mechanism, distinct stress responsive genes are activated to withstand stress conditions and molecular markers can be utilized to identify and understand the inheritance of responsive genes for specific traits from wheat genome sequence. Marker-assisted breeding by combining and assembling different QTLs can lead to high temperature tolerant varieties with adequate economic yields under stress conditions.

Compliance with ethical standards

NA

Conflict of interest

No

Author contributions

AV and GCP: Conceptualization, Data curation, AV and AT: Writing & updating the manuscript for publication, GCP: Supervision, and Validation. All the listed authors read and approved the manuscript.

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