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TilhanTec-SUNH-1 (IIOSH-15-20): A new high seed and oil yielding and downy mildew resistant sunflower hybrid suitable for rainfed condition of India

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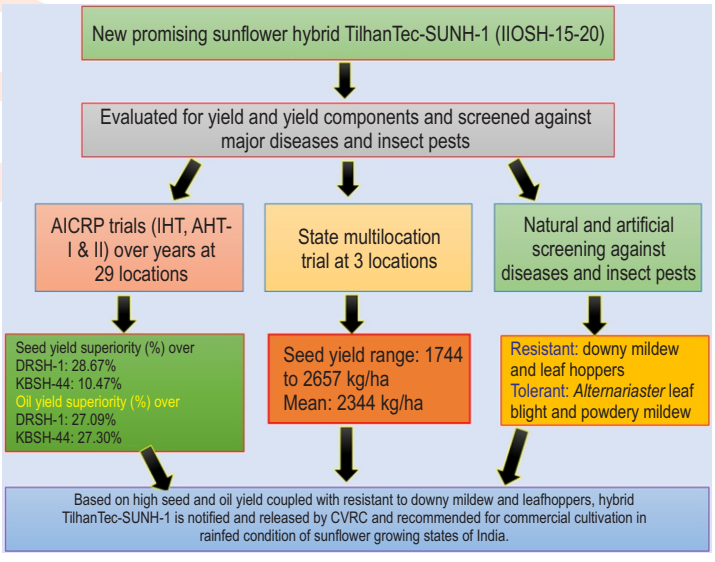
Abstract

Aim: To develop more heterotic hybrids for seed and oil yield coupled with resistant or tolerant to major disease (downy mildew) and insect pests (leafhoppers) for commercial cultivation in rainfed conditions and DNA profiling of the newly developed hybrid (TilhanTec-SUNH-1) along with 2 female lines, 4 male lines and 3 hybrids using sunflower specific simple sequence repeat (SSR) markers.

Methodology: Two parentages (ARM-243A and RGP-100) were utilized for the development of a new promising sunflower hybrid, TilhanTec-SUNH-1 (IIOSH-15-20). Performance of entry was assessed at 29 and 14 locations, respectively, during the *kharif* and *rabi* seasons over three years through All India Coordinated Trials (IHT, AHT-I & II) and a state multilocation trial at three locations. A total of nine quantitative observations were recorded. A total of 80 SSR primers were used on the 4 hybrids for DNA fingerprinting.



Results: Considerable seed yield superiority (28.7% and 10.5%) was observed in AICRP trials over DRSH-1 and KBSH-44, respectively. Oil yield superiority was 27.1% and 27.3% compared to national checks DRSH-1 and KBSH-44. TilhanTec-SUNH-1 was found resistant (0%) to downy mildew and leafhoppers (1.1 grade) while tolerant to *Alternariaster* leaf spot. Four SSR markers, *viz.*, ORS-57, ORS-203, ORS-1028 and ORS-598 can be confidently used for the identification of TilhanTec-SUNH-1 as well as for genetic purity tests.

Interpretation: The results of this investigation revealed that TilhanTec-SUNH-1 is superior in terms of seed and oil yield and resistance to downy mildew and moderately resistant to leafhoppers and tolerant to *Alternariaster* leaf spot. It is notified and released by CVRC for commercial cultivation in rainfed conditions of the sunflower growing states of India.

Key words: Downy mildew, Heterosis breeding, Molecular markers, Oil yield, Seed yield

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Introduction

Sunflower (*Helianthus annuus* L.; 2n = 2x = 34) is a major oilseed crop, primarily used for extracting cooking oil. It is an important source of unsaturated fatty acids like oleic and linoleic acids, which are desirable in human nutrition. Sunflower oil is the largest selling oil in the branded oil segment. Sunflower cultivation in India was initiated in the late 1960s with four promising introductions, *viz.,* VNMIK-8931, Peredovick, Armavirskii, and Armaverts from USSR. Its wider adaptability to different climatic conditions, high seed yield potential, thermo and photo insensitive nature, shorter duration, responses to high input agriculture and more profitability have played a significant role for becoming popular crop across various agro-climatic zones in the country. In India, sunflower cultivation is concentrated in the southern parts, mostly in five states, *viz.,* Karnataka, Maharashtra, Andhra Pradesh, Telangana and Tamil Nadu, which account for 80 per cent of the total acreage and 68 per cent of the production. In all these five major sunflower growing states, the productivity ranges from 436 (Maharashtra) to 2231 kg ha^{-1} (Telangana). However, there is a great yield gap between the national average yield of 913 kg ha^{-1} and the world average yield of 2048 kg ha^{-1} (FAOSTAT, 2021).

This production limitation may be due to low yielding ability of dominant varieties grown by farmers, the influence of several biotic and abiotic stresses and poor adoption of sunflower production package technologies. Highly cross-pollinated nature of the crop, is ideal for heterosis exploitation. In India, based on seed yield, oil content, yield stability and synchronization of flowering in male and female parents, the first CMS based hybrid, BSH-1 (CMS-234A x RHA-274), was released for commercial cultivation in 1980 (Seetharam *et al.,* 1980). Since then, more than 19 open-pollinated varieties and 30 hybrids have been released by various agricultural universities and/or public sector research institutes (Sujatha *et al.,* 2019) for commercial cultivation to suit different agro-production situations, including the kharif (central and southern parts), *rabi* (central, southern and north-eastern parts) and spring (northern parts) seasons of the country. In a rainfed situation, farmers are realising poor yields owing to many abiotic factors, of which terminal stress during the crop period is the most prominent (Neelima *et al.,* 2018). Long duration hybrids are prone to terminal stress, resulting in poor seed filling and drastically reduced seed and oil yields (Meena *et al.,* 2017).

Under this situation, the cultivation of hybrids with a short (85-90 days) to medium (90-100 days) duration would be one of the strategies to mitigate terminal stress and realise higher seed yield. During the past decade, two hybrids (KBSH-44 and DRSH-1) from the public sector were predominant among sunflower hybrids for seed yield and oil content, respectively, and were used as national checks in rainfed and irrigated conditions. Unfortunately, both the hybrids were highly susceptible to downy mildew disease and were more than 15 years old. Hence, there is a need to develop high seed and oil yielding hybrids coupled with resistance to downy mildew and tolerance to *Alternariaster* leaf spot to sustain the competitive ability of sunflower (Meena *et al.,* 2013). In view of the above, present study was carried out to breed a high seed and oil yielding, medium duration sunflower hybrid with desirable traits that could increase the production and profitability of sunflower farmers and develop the signature molecular profiles of the identified hybrids and their parental lines.

Materials and Methods

Breeding materials: Two parentages were utilised for the development of a novel promising hybrid, IIOSH-15-20. The female parent (ARM-243A) was developed from the Russian high oil content variety Armavirskii through selfing. In the S_6 generation, all the newly developed inbreds were crossed with a cytoplasmic genetic male sterile line (CMS-234A) to test maintainer or restorer reactions. Based on high seed yield, oil content, and maintainer reaction coupled with tolerance to powdery mildew, inbred ARM-243 was selected for conversion into CMS-234A background through repeated conventional backcrossing. After BC_6, a new CMS line, ARM-243A, was developed for utilization in heterosis breeding programme. Using ARM-243A as a female parent, a high oil content hybrid (DRSH-1) was released by ICAR-Indian Institute of Oilseeds Research, Hyderabad (formerly Directorate of Oilseeds Research) for commercial cultivation in 2005 (Sujatha *et al.,* 2019). The male parent, RGP-100, of the IIOSH-15-20 hybrid was developed through population improvement. Based on high seed yield, high oil content, good combining ability, resistance to downy mildew and promising root traits, a total of six inbreds were selected for developing base material and allowed for random mating in isolation up to 3rd cycle. Individual plants were selected based on morphological traits like head diameter, days to flowering, plant height, stem thickness, etc., and advanced through selfing up to S_6 generation. Stabilized inbreds were evaluated for yield and yield contributing traits. Based on good plant type, high seed yield, oil content, good combining ability and resistant reaction to downy mildew, RGP-100 was selected for utilization in the heterosis breeding programme.

Hybridization: New entry IIOSH-15-20 (TilhanTec-SUNH-1) was developed through hybridization between the stable cytoplasmic genetic male sterile line ARM-243A (female) and the newly developed downy mildew resistant restorer line RGP-100 (male). The female parent was characterized by medium maturity, strong stem, medium leaf size, medium head diameter, dark black seed colour, ovoid elongated seed shape, medium oil content (36-37%) and tolerance to powdery mildew. The male parent is shorter, full-branched with small leaves, light petiole pigmentation, and a black seed colour with an elongated shape and resistance to downy mildew. Hybridization between these two parents was attempted during 2016-17 at ICAR-IIOR, Hyderabad with the objective of developing a high seed and oil yielding sunflower hybrid coupled with resistance to downy mildew for rainfed and irrigated conditions. Preliminary and advanced stages testing of hybrid was done during *kharif*-2017

and *rabi*-2017-18, respectively. Based on seed and oil yield superiority coupled with resistance to downy mildew, IIOSH-15-20 was nominated to coordinated trial during *kharif*-2018.

Evaluation trials: The crossed product, IIOSH-15-20 (TilhanTec-SUNH-1) was tested in a hierarchy of preliminary hybrid yield trials (PHYT) and advanced hybrid yield trials (AHYT) at ICAR-IIOR, Hyderabad during *kharif*-2017 and *rabi*-2017-18, respectively, initial hybrid trial (IHT) and advanced hybrid trials I & II (AHT-I & II) across 29 locations over three years (2018 to 2020) during *kharif* and 14 locations during *rabi* 2019-20 through All India Coordinated Research Project (AICRP) trials and the Karnataka state multilocation trial during *Kharif* 2020. In the All India Coordinated Sunflower Improvement Program (AICSIP), cultivars are evaluated in an MLT before release. The test locations of an MLT represent diverse climatic and geographical diversity and are distributed across varied macro-climatic conditions. In the coordinated trial, it was evaluated along with two national checks, *viz.,* DRSH-1 (for oil content) and KBSH-44 (for seed yield) and a private hybrid check (GK-2002) of Kaveri Seeds, while in the state multilocation trial, it was compared with a national check (KBSH-44), a popular private check (PAC-303) of Advanta Company and local checks (RSFH-1887, KBSH-53 and KBSH-78) in a randomized block design (RBD) with four replications.

The plot size in IHT was 5 rows of 4.5 m and 7 rows of 4.5 m in AHT-I and II, with plant spacing of 60 cm between rows and 30 cm between plants within a row. A total of nine quantitative observations, *viz.,* days to 50% flowering, days to maturity, plant height (cm), head diameter (cm), seed yield (kg ha^{-1}), 100 seed weight (g), volume weight (g 100 ml^{-1}), oil content (%) and oil yield (kg/ha) were recorded. To avoid the edge effect, seed yield was recorded on plants from the middle rows (net plot). Seed yield was expressed in kg ha^{-1} and was adjusted to 11% moisture. Reactions to major diseases (downy and powdery mildew, Alternariaster leaf spot and sunflower necrosis disease) and insect pests (leafhoppers, white fly, stem, head borer and thrips) under natural and artificial conditions were recorded at the hot spot as per the standard procedures. The downy mildew screening was done at the Oilseeds Research Station, Latur, Maharashtra, under epiphytotic conditions (sick plot) over three years.

Molecular analysis: Plant DNA was isolated from seedlings of four hybrids, two female lines and four male lines (Table 1) using the CTAB (Doyle and Doyle, 1990). A total of 80 SSR primers were used for PCR amplification. PCR was performed in a reaction mixture volume of 20 µl containing 30 ng of template DNA, 1 x PCR buffer with 1.5 mM of $MgCl_2$, 0.2 mM of each dNTPs, 10 µmol of each primer and 1U of Taq DNA polymerase. PCR was carried out in a Thermal Cycler programmed for 35 cycles of 95 ^{0}C (5 min), 94 ^{0}C (1 min), 56 ^{0}C (30 sec.), 72 ^{0}C (1 min), followed by a final extension at 72 ^{0}C for 10 min. PCR products (10 µl) were used for electrophoresis and the amplicons were resolved on a 2.5% agarose gel stained with ethidium bromide at 1 µg ml^{-1} and visualised under UV in a gel documentation system.

Statistical analysis: The data on seed yield per hectare, oil yield per hectare and pest and disease were recorded from different trials and analysed as per the standard procedures.

Results and Discussion

Investigation of developed promising varieties/hybrids in different environments over the years is one of the most challenging tasks for plant breeders. Testing of genotypes, populations or hybrids in multi-environment trials is essential because of the presence of genotype × environment interaction, since genotypes respond in different ways to different environmental conditions (Arief *et al.,* 2019). There is a need to have high seed and oil yielding hybrids that can perform better across environments and locations to sustain the crop and make it remunerative to farmers. In this context, the new hybrid IIOSH-15-20 was tested at 29 locations for three consecutive years (2018 to 2020) during *kharif* and 14 locations during *rabi* season (2019-20), respectively, in All India coordinated trials to check the performance and select the most stable genotype. It was revealed that IIOSH-15-20 resulted in the highest seed yield (2015 kg ha^{-1}), which was 28.7% and 10.5% higher than national checks, DRSH-1 (1566 kg ha^{-1}) and KBSH-44 (1824 kg ha^{-1}), respectively (Table 3).

It is well known that oil yield per unit area (ha) is the ultimate goal of sunflower breeding. In this context, hybrid IIOSH-15-20 recorded an oil yield of 746 kg ha^{-1}, which was 27.3% and 27.1% higher than DRSH-1 (586 kg ha^{-1}) and KBSH-44 (587 kg ha^{-1}), respectively (Table 2). Also, the performance of IIOSH-15-20 in the All India coordinated trials taken up during *rabi* 2019-20 at 14 locations in different zones of India revealed that IIOSH-15-20 out yielded compared to KBSH-44 by 12.1% and DRSH-1 by 5.5%. It was at par for days to 50% flowering, days to maturity, plant height, head diameter, 100 seed weight, volume content and oil content with national check hybrids (Table 3). Škorić *et al.* (2007) suggested that a special focus in sunflower breeding

Table 1: List of sunflower hybrids and their parental lines used for DNA fingerprinting

Hybrid	Seed parent (A line)	Maintainer line (B line)	Pollen parent (R line)
IIOSH-15-20	ARM-243A	ARM-243B	RGP-100
DRSH-1	ARM-243A	ARM-243B	RHA-6D-1
KBSH-44	CMS-17A	CMS-17B	RHA-95C-1
LSFH-171	CMS-17A	CMS-17B	RHA-1-1

Table 2: Mean value of growth parameters of IIOSH-15-20 along with check hybrids in coordinated trials

Character	Mean value of three years			Mean value of three years	
	New hybrid	National check		CD at 5%	CV (%)
	IIOSH-15-20	Check 1 (KBSH-44)	Check 2 (DRSH-1)		
Days to 50% flowering	63.0	58.3	60.7	1.55	2.01
Days to maturity	93.7	88.7	90.7	2.07	1.61
Plant height (cm)	191.0	175.3	180.0	11.34	4.82
Head diameter (cm)	15.8	15.4	15.0	1.40	6.64
100 seed weight (g)	5.4	5.6	4.9	0.71	8.10
Volume weight (g/100 ml)	42.3	43.1	41.3	2.45	4.02
Oil content (%)	37.5	32.5	37.9	1.12	4.73

Table 3: Performance of IIOSH-15-20 in all India coordinated trials over the years

Entry	Seed yield (kg ha^{-1})				% increase over	Oil content (%)				% increase over	Oil yield (kg ha^{-1})				% increase over
	2018	2019	2020	Mean		2018	2019	2020	Mean		2018	2019	2020	Mean	
IIOSH-15-20	2411	1987	1648	2015	-	37.2	36.2	39.0	37.5	-	902	720	647	756	-
DRSH-1 (C)	1932	1586	1179	1566	+28.7	38.5	37.8	37.3	37.9	-1.1	736	608	443	596	+26.8
KBSH-44 (C)	2186	1880	1407	1824	+10.5	33.4	32.9	31.2	32.5	+15.4	728	621	440	596	+26.8
No. of location	09	10	10			08	10	10			08	10	10		
Name of trial	IHT	AHT-I	AHT-II			IHT	AHT-I	AHT-II			IHT	AHT-I	AHT-II		
CD at 5%	97.0	84.7	67.9	83.2		-	-	-			-	1.1	0.5		
CV (%)	10.8	10.9	11.5	11.1		-	-	-			-	7.1	3.0		

IHT=Initial hybrid trial AHT-I=Advanced hybrid trial-I AHT-II=Advanced hybrid trial-II

programmes should be placed on the development of high-yielding hybrids with a high genetic potential for oil yield, resistance to the dominant diseases, and wide environmental adaptability to increase the area, production and productivity. Basford and Cooper (1998) and Comstock and Moll (1963) also suggested multi environment trials for selecting the best performing and most stable wheat genotypes. Arief *et al.* (2015) suggested that multiyear data analysis would provide better estimates of genotype performance. In the process of releasing new genotypes for cultivation, multi-location testing (MLT) is a prerequisite for all crops including sunflower (Shah *et al*., 2023). In the state multilocation trial, conducted at three locations, *viz.,* Bengaluru, Hiriyur, Gunjevu in Karnataka state during *kharif* 2020, entry IIOSH-15-20 registered a mean seed yield of 2344 kg ha^{-1} and had a yield advantage of 25.6% and 43.7% over the popular hybrids KBSH-53 and KBSH-78 (Table 4). Whereas, it exhibited 11.2% higher seed yield over private popular hybrid GK-2002.

Apart from seed yield, IIOSH-15-20 recorded a 677 kg ha^{-1} oil yield, which was 65.9%, 40.8%, 30.2% and 9.5% higher than KBSH-78, KBSH-53, KBSH-44 and RSFH-1887, respectively (Table 4). This hybrid can replace the existing popular public and private sector hybrids and will be beneficial for farmers and growers. Rakshit *et al.* (2017) suggested that in multi-location evaluation, the agronomic superiority of test genotypes over existing cultivars receives more emphasis whereas less importance is given to genotype × environment interactions. The productivity of sunflowers in India is much lower than the world average productivity, mainly because the crop is vulnerable to several diseases like downy mildew, *Alternariaster* leaf spot, powdery mildew and necrosis (Saharan *et al.,* 2005).

About 251 insect pests are reported to infest the sunflower and among these major pests in the Indian sub-continents, leafhoppers, thrips, whiteflies, defoliators and head borers are key pests of the crop (Ghante *et al*., 2022). Incidences

Table 4: Performance of IIOSH-15-20 in Karnataka state multilocation trial (*Kharif* 2020)

Entry	Days to 50% flowering at Bengaluru	Plant height (cm) at Bengaluru	Head diameter (cm) at Bengaluru	100 seed weight (g) at Bengaluru	Volume weight (g 100^{-1} ml) at Bengaluru	Oil content (%) at Bengaluru	Oil yield (kg ha^{-1}) at Bengaluru	Seed yield (kg ha^{-1}) at			
								Bengaluru	Hiriyur	Gunjevu	Mean
IIOSH-15-20	58	214	14.1	4.7	42.8	38.7	677	1744	2630	2657	2344
GK-2002 (PC)	60	207	13.9	4.3	42.5	38.6	661	1714	2418	2192	2108
RSFH-1887 (C)	61	236	14.3	5.1	48.1	37.8	618	1633	2268	2724	2208
KBSH-53 (C)	66	217	13.9	3.9	40.8	40.9	458	1118	2107	2372	1866
KBSH-78 (C)	55	163	14.1	4.1	38.9	34.4	408	1186	1571	2135	1630
KBSH-44 (C)	52	217	13.5	4.4	43.2	30.9	520	1722	2523	2655	2300
Expt. Mean								1586	2351	2464	2134
CD at 5%								295.4	344.5	289.3	309.7
CV (%)								10.9	8.6	6.9	8.8

PC=Private check

Table 5: Reaction of IIOSH-15-20 to major diseases at multlocations over the years

Entry	Downy mildew incidence (%)				Disease reaction	Alternariaster leaf spot (%)				Disease reaction	Powdery mildew (%)				Disease reaction	Sunflower necrosis (%)				Disease reaction
	2018	2019	2020	Mean		2018	2019	2020	Mean		2018	2019	2020	Mean		2018	2019	2020	Mean	
IIOSH-15-20	0	0	0	0	R	23.3	25.3	32.5	27.0	S	47.6	34.6	21.9	34.7	S	9.0	5.9	0.7	5.2	MR
DRSH-1 (C)	90.0	85.0	75.0	83.3	S	30.5	22.8	37.0	30.1	S	52.2	22.6	22.1	32.3	S	15.3	4.0	1.2	6.8	MR
KBSH-44 (C)	0	60.0	70.0	43.3	S	31.8	39.4	38.1	36.4	S	51.2	32.7	48.9	44.3	S	27.8	6.5	1.9	12.1	S
No. of location	1	1	1	-		6	4	4	-		3	4	3	-		4	4	4	-	
Name of trial	IHT	AHT-I	AHT-II			IHT	AHT-I	AHT-II			IHT	AHT-I	AHT-II							

IHT=Initial hybrid trial AHT-I=Advanced hybrid trial-I AHT-II=Advanced hybrid trial-II R=Resistant MR=Moderately resistant S=Susceptible

Table 6: Reaction of IIOSH-15-20 to major insect pests at multilocations over two years

Entry	Leafhopper injury grade			White flies per plant			Stem borer (%)			Head borer/plant			Thrips/plant		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
IIOSH-15-20	1.42	0.87	1.15	1.75	1.20	1.47	5.0	0.0	2.50	0.57	0.65	0.61	5.86	4.65	5.25
DRSH-1 (C)	1.65	2.07	1.86	1.55	1.30	1.42	12.0	5.0	8.50	0.82	0.70	0.76	6.10	3.62	4.86
KBSH-44(C)	1.90	2.87	2.38	1.80	1.10	1.45	19.0	6.7	12.85	0.93	1.22	1.07	6.53	5.45	5.99
No. of location	4	4		2	2		1	1		4	4		3	4	
Name of trial	AHT-I	AHT-II		AHT-I	AHT-II		AHT-I	AHT-II		AHT-I	AHT-II				

IHT=Initial hybrid trial AHT-I=Advanced hybrid trial-I AHT-II=Advanced hybrid trial-II

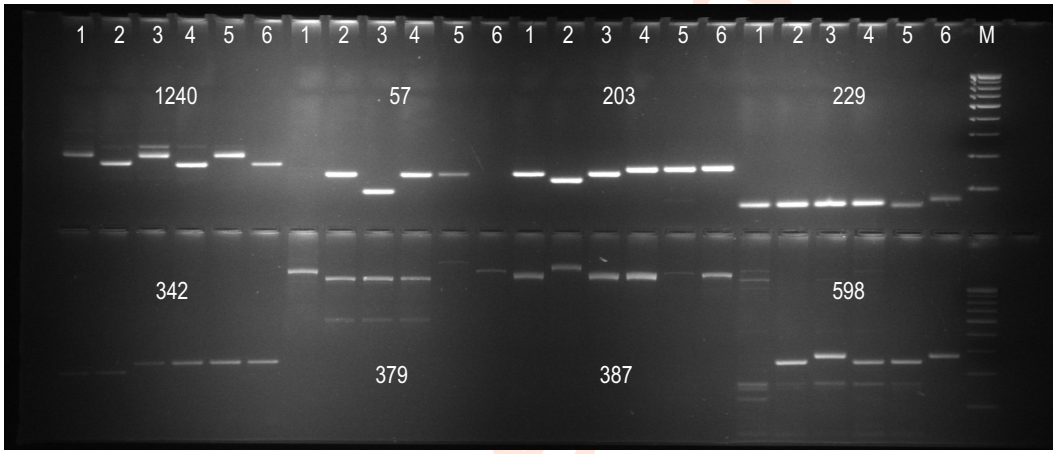

Fig. 1: Polymorphism between the test parents along with other A and R lines; 1: CMS-17A, 2: ARM-243A, 3: RGP-100, 4: RHA-6D-1, 5: RHA-1-1, 6: RHA-95C-1, M: 100 bp DNA ladder.

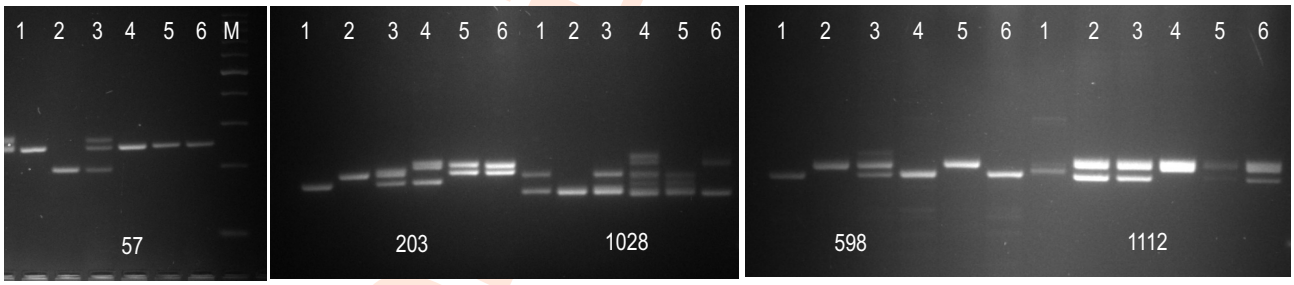

Fig. 2: Validation of polymorphic primers in the test hybrid IIOSH-15-20 along with the reference hybrids; 1: ARM 243A, 2: RGP-100, 3: IIOSH-15-20, 4: DRSH-1, 5: KBSH-44, 6: LSFH-171, M: 100 bp DNA ladder.

of diseases and insect pests are the major constraints for sustaining the higher productivity of sunflowers in India (Shanwad *et al.,* 2016). However, chemical management control measures are available for these biotic stresses, but they are not always economically or physically feasible. Thus, host plant resistance is the most reliable, eco-friendly, and economical way to minimize yield losses in sunflowers. To sustain sunflower in India, the development of high seed and oil yielding hybrids coupled with resistance to biotic stresses assumes priority. In this context, the hybrid IIOSH-15-20 was screened for downy mildew under the epiphytotic condition at the Latur centre which revealed that it was free from downy mildew for three years, while both the national check hybrids DRSH-1 (83.3%) and KBSH-44 (43.3%) exhibited highly susceptible reactions. At the same time, it showed the

lowest incidence of *Alternariaster* leaf spot (47.6%), powdery mildew (34.7%), and sunflower necrosis diseases (5.2%) (Table 5). The seed yield loss due to defoliators in a rainfed sunflower crop was recorded at up to 268 kg ha^{-1} by Naresh Kumar *et al.* (2017). Among insect pests, leafhopper appears in serious form and causes yield loss of up to 46 per cent (Srinivas *et al.,* 2023). The national released hybrid KBSH-44 is susceptible to leafhoppers. Hence, a new resistant hybrid is essential to minimize the yield loss due to these sucking pests. The entry IIOSH-15-20 showed the lowest leafhopper injury grade (1.15; moderately resistant) compared to DRSH-1 (1.86; moderately resistant) and KBSH-44 (2.38; susceptible). The new hybrid IIOSH-15-20 was at par for whiteflies, head borer and thrips per plant, while it showed the lowest (0%) stem borer compared to DRSH-1 (5.0%) and KBSH-44 (6.7%), respectively (Table 6). Six germplasm lines, *viz.,* GMU-25, GMU-339, GMU-504, GMU-922, GMU-570 and GP9-472-4-13, recorded the least hopper population and injury grade and hence, can be used for further genetic improvement programmes (Ghante *et al.,* 2019). Conventionally, morphological characters (DUS descriptors) are routinely used for establishing the identity of varieties or hybrids across the crops and the same for sunflowers also. But these descriptors suffer from many drawbacks, such as the influence of the environment on trait expression, epistatic interactions, pleiotropic effects, etc. Identification of varieties or hybrids and parental lines using molecular markers overcome these problems due to the discrimination power and reliability of the test. The sunflower hybrid IIOSH-15-20 was subjected to molecular analysis using sunflower specific simple sequence repeat (SSR) markers as per the standard protocols.

The molecular profiles of IIOSH-15-20 parentages were compared with those of check hybrids, DRSH-1, KBSH-44, and LSFH-171. Of the 80 SSR primers tested, 76 produced amplification products and 30 disclosed polymorphisms between the A and R lines (Fig. 1). Among these, 19 primers (ORS-953, ORS-955, ORS-988, ORS-998, ORS-1008, ORS-1013, ORS-1028, ORS-1088, ORS-1108, ORS-1112, ORS-1144, ORS-1159, ORS-1197, ORS-1240, ORS-57, ORS-203, ORS-342, ORS-387 and ORS-598) disclosed polymorphism between the test parents. However, some of these primers were common in the other R lines. Out of the 19 polymorphic primers, two primers (ORS-57 and ORS-203) were specific to RGP-100 and five primers (ORS-1028, ORS-1112, ORS-1240, ORS-203 and ORS-387) were specific to ARM-243A. The primers ORS-1197 and ORS-379 distinguished both the CMS lines (CMS-17A and ARM-243A). From these, 12 markers (ORS-998, ORS-1008, ORS-1028, ORS-1088, ORS-1112, ORS-1144, ORS-1159, ORS-57, ORS-203, ORS-342, ORS-387 and ORS-598) of the hybrid IIOSH-15-20 were validated on the test hybrid along with the national check hybrids (Fig. 2).

Only four primers (ORS-57, ORS-203, ORS-1028 and ORS-598) differentiated the test hybrid IIOSH-15-20 from the reference hybrids (KBSH-44, DRSH-1 and LSFH-171). DNA fingerprinting of 7 hybrids, 8 parental lines, and 6 varieties of sunflower was done through RAPD markers (Nandini and Chikkadevaiah, 2005). A set of SSR markers have been identified by Solodenko *et al.* (2003) and Antonova *et al.* (2006) to distinguish sunflower inbreds. Meena *et al.* (2020) used SSR markers for hybridity confirmation between cultivated sunflower and *H. argophyllus*. SSR markers were used for the hybrid purity of five hybrids, four female and two male lines of sunflower by Pallavi *et al.* (2011). At the National Research Centre on DNA fingerprinting, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, over 2000 varieties, parental lines and elite germplasm of 32 important crops, including cereals, pulses, oilseeds, vegetables and horticultural crops, have been fingerprinted using molecular markers and their suitability for DUS applications is being evaluated (Ibrar *et al.*, 2022).

In conclusion, the released and notified (S.O. 8(E) 24-12-2021) new hybrid, TilhanTec-SUNH-1, is found to be promising for seed and oil yield and highly resistant to downy mildew disease. At the same time, it showed a moderately resistant reaction to leafhoppers and was moderately resistant to *Alternariaster* leaf spot. Four SSR markers, *viz.,* ORS-57, ORS-203, ORS-1028 and ORS-598, can be reliably used for the identification of TilhanTec-SUNH-1. It is suggested that the new hybrid will not only increase the area, production and productivity of the sunflower crop in rainfed condition, but will also help to improve the livelihood of farmers.

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