Romanian Biotechnological Letters *Vol. 23, No. 4, 2018*

ORIGINAL PAPER

Physiological Characterization of Heat Tolerant Hybrids in Rice (*Oryza sativa* L.)

Received for publication, May 6, 2014
Accepted, April 7, 2015

DODDABHIMAPPA R. GANGAPUR[1, 2]*, K. THIYAGARAJAN[1], SANTOSHKUMAR MAGADUM[1, 3], C. VIJAYLAKSHMI[1]
[1]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore - 641003, Tamil Nadu, India; [2]CSIR- Central Salt and Marine Chemicals Research Institute, Bhavnagar- 364002, Gujarat, India; [3]ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India.
***Address for correspondance to:** db4334@gmail.com, Tel: +91-8139998260*

Abstract

A total of 83 genotypes consisting of 43 hybrids, four lines, 29 testers and seven checks were evaluated to identify heat tolerant hybrids, through physiological parameters such as chlorophyll fluorescence ratio, SPAD value, and membrane thermal stability. Regarding chlorophyll fluorescence (F_v/F_m) ratio at booting stage, the maximum values were observed in parental line CB00-13-55 (0.67) followed by HTL 31 (0.67) and COMS 24B (0.66). The hybrids which recorded followed by maximum (F_v/F_m) ratio were COMS 24A x CB00-13-79 (0.66) and COMS 24A x HTL 35 (0.66) followed by TNAU CMS 2A x CB00-14-56 (0.65) and COMS 24A x CB00-14-82 (0.65). At flowering stage, the chlorophyll fluorescence (F_v/F_m) ratio was maximum in the parental genotypes CB00-13-126 (0.66) and HTL 35 (0.66) followed by HTL 25 (0.66) and COMS 24B (0.65). The hybrids which recorded maximum (F_v/F_m) ratio were TNAU CMS 2A x CB00-14-56 (0.70) followed by TNAU CMS 2A x CB00-14-82 (0.67) and TNAU CMS 2A x IR 36 (0.66). Regarding thechlorophyll content, maximum SPAD value was observed in the parent CB00-13-79 (39.73) followed by CB00-13-97 (39.10) and HTL 39 (38.53) at booting stage. The hybrid which recorded maximum SPAD values were IR 68897A x CB00-13-52 (44.57) followed by COMS 24A x HTL 34 (43.83) and IR 68897A x CB00-13-104 (42.93). At flowering stage, the maximum SPAD values were recorded in the parental genotypes CB00-13-52 (44.67) followed by TNAU CMS 2B (41.80) and CB00-13-79 (39.63). The hybrid which recorded maximum SPAD values were IR 68897A x CB00-13-55(42.90) followed by TNAU CMS 2A x CB00-14-37 (41.77) and IR 68897A x CB00-13-52 (41.50). The results indicated that parents CB00-13-52, TNAU CMS 2B and CB00-13-79 and the hybrids IR 68897A x CB00-13-55, TNAU CMS 2A x CB00-14-37 and IR 68897A x CB00-13-52 have relative tolerance to high temperature. These hybrids may be recommended for cultivation under high temperature conditions.

Keywords: Chlorophyll fluorescence ratio, heat tolerance, membrane thermal stability, rice hybrids, SPAD value.

1. Introduction

Rice (*Oryza sativa* L.) is an important food crop, and nearly half of population in the world is dependent on rice for food. The climate is one of the most dominant factors that affect crop cultivation and productivity (O.H. FRANKEL [1]). Originating from tropical and sub-tropical areas, and adapting to ecological environments with high temperature and short day length, rice can survive certain high temperatures; however, its average growth is impaired once the temperature becomes higher than the threshold of its growth-optimal temperature (R.E. DAVID & al, [2]; R.W. JARROD & al, [3]). High temperature, *i.e.* heat stress is a major disastrous factor affecting rice productivity in many areas (A. GROVER & al, [4]; A. GROVER

& al, [5]), and it occurs more frequently and severely because of the global greenhouse effect (R.E. DAVID & al, [2]). It was reported that the annual mean maximum and minimum temperatures increased by 0.35 and 1.13°C from 1979 to 2003, and rice grain yield would decline by 10% for each 1°C increase of minimum temperature during the growing-season (S. PENG & al, [6]). According to IPCC (Intergovernmental Panel on Climate Change), the global mean surface temperature would increase by 2.0-4.5°C in 2100 (IPCC [7]) in recent decades. Therefore, high temperature will become a more severe abiotic stress in crop production. Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperature (A. WAHID & al, [8]). As plants cannot move, the only option they have to defend themselves from various stress is to make metabolic and structural adjustments (U. YAMANOUCHI & al, [9]). At later stages, high temperature may adversely affect photosynthesis, respiration, water relations and membrane stability and also modulate levels of hormones and primary and secondary metabolites. Heat stress causes a series of physiological damages to crop plants, including degrading of thechloroplast, reducing of chlorophyll content, declining of photosynthesis, and membrane thermal stability (H. SONG & al, [10]). High temperature disrupts water, ion and organic solute movement across plant membranes, which interferes with photosynthesis and respiration (M.N. CHRISTIANSEN [11]). Damage to membranes may be assayed by the membrane thermal stability (MTS), which measures electrolytic leakage from leaves subjected to elevated temperatures (C.Y. SULLIVAN [12]).

2. Materials and Methods

The present study was conducted at Paddy Breeding Station,Tamil Nadu Agricultural University, Coimbatore, India during summer 2011 to identify heat tolerant hybrids. A total of 83 genotypes comprising of 43 hybrids,four lines, 29 testers and seven checks *viz*., CORH 3, CORH4,IR86977-104-1,IR86970-126-3, IR86977-92-3, IR86930-145-1, IR86977-95-1 were raised in a randomized block design with two replications. For each genotype, single seedling per hill was planted at 20 x 20 cm, and the distance between rows was 1.2 m. Recommended management practices were adopted. The physiological parameters *viz*., chlorophyll fluorescence (F_v/F_m) ratio, SPAD value, and membrane thermal stability were recorded and analyzed in order to identify heat tolerant hybrids.

2.1. Chlorophyll fluorescence (F_v/F_m) ratio

Chlorophyll fluorescence ratio was measured in light-adapted leaves with the help of a portable chlorophyll fluorometer PAM-210 WALZ (K. MAXWELL & al, [13]) at booting and flowering stages.

2.2. SPAD Value

The relative value of Chlorophyll (SPAD Value) was measured by using a portable chlorophyll meter (Minolta SPAD 502) at booting and flowering stages. The Minolta SPAD-502 measures chlorophyll content as the ratio of transmittance of light at 650 nm and 940 nm. The SPAD value was measured on each side of the leaf at the point of three fourths of the way from base to leaf tip. Five readings were taken from each replication, and the average values computed using method described by C. MINOLTA [14] and O.A. MONJE & al, [15].

2.3. Measurement of membrane thermal stability

Fully expanded leaves of hybrids and parents were taken. The cut leaves were immediately placed into a plastic bag lined with moistened filter paper and transported to the laboratory in a cold box. Thereafter, midribs were removed, and leaves were thoroughly washed with de-ionized water and completed hydrated by soaking in de-ionized water for 2 h in a refrigerator. Then, each leaf was cut into 1 cm circular sections and six sections put into each vial containing 30 ml of de-ionized water. Half of the vials were used as controls (kept at room temperature, 25°C) and the other vials were subjected to heat treatment (45°C for 12 h) in a hot water bath. After thetreatment period, both control and heat-treated samples were kept refrigerated at 5°C for 12 h. Thereafter, the samples were brought to room temperature, and conductivity readings of the aqueous phase were taken at 25°C using an electrical conductivity meter. The samples were then autoclaved for 15 min (120°C and 0.10 MPa). After the samples cooled to the room temperature a second conductivity reading of the aqueous phase was taken at 25°C.

Leaf cell membrane thermal stability was estimated using the following equation (A. BLUM & al, [16]).

$\text{MTS (\%)} = [1- (T_1/T_2)]/ [1- (C_1/C_2)]\ 100$

Where, T_1- Before boiling; T_2 - After autoclaving; C_1- Before boiling; C_2- After autoclaving

Membrane thermal stability was expressed as relative injury (RI) using the following equation RI (%) = 100 - MTS

Where, RI- Relative injury; MTS - Membrane thermal stability

3. Results and Discussion

3.1. Chlorophyll fluorescence (F_v/F_m) ratio

Alterations in various photosynthetic attributes under heat stress are good indicators of thermo tolerance of the plants as they show correlations with growth. Any constraint in photosynthesis can limit plant growth at high temperature. Photo chemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast have been suggested as the primary sites of injury at high temperature (R.R. WISE & al, [17]). Chlorophyll fluorescence, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m), and the base fluorescence (F_o) are physiological parameters that have been shown to correlate with heat tolerance (M. YAMADA & al, [18]). Regarding chlorophyll fluorescence (F_v/F_m) ratio at booting stage, the values ranged from 0.58 (IR68897A x CB00-13-55) to 0.67 (CB00-13-55). The maximum values were observed in CB00-13-55 (0.67), HTL 31 (0.67) followed by COMS 24B (0.66), HTL 39 (0.66) and IR68897B (0.65) in case of parents. The hybrids which recorded maximum (F_v/F_m) ratio were COMS 24A x CB00-13-79 (0.66), COMS 24A x HTL 35 (0.66), followed by TNAU CMS 2A x CB00-14-56 (0.65) and COMS 24A x CB00-14-82 (0.65) (Table 1).

Table 1. Impact of high temperature stress on chlorophyll fluorescence (F_v/F_m) ratio, chlorophyll content (SPAD value) and membrane thermal stability (MTS) (%) in promising rice hybrids and their parents

Sl. No.	Genotypes	Chlorophyll fluorescence		SPAD Readings		Membrane thermal stability
		Booting stage	Flowering stage	Booting stage	Flowering stage	Flowering stage
1	TNAUCMS 2B	0.63	0.63	37.93	41.80	51.77
2	IR68897B	0.65	0.63	35.63	37.40	52.02
3	COMS 24B	0.66	0.63	35.20	34.27	44.82
4	COMS 23B	0.63	0.65	35.00	30.77	53.66
5	CB00-14-56	0.64	0.65	31.30	35.43	44.82
6	CB00-14-82	0.64	0.62	33.77	34.40	59.06
7	CB00-14-47	0.64	0.63	35.60	37.47	58.48
8	CB00-13-61	0.60	0.63	33.60	36.57	53.15
9	IR36	0.62	0.62	34.40	38.53	44.83
10	CB00-14-37	0.64	0.63	31.77	26.90	61.96
11	IR8	0.64	0.62	30.43	34.80	52.24
12	CB00-13-97	0.64	0.63	39.10	36.23	48.32
13	CB00-13-55	0.67	0.62	35.77	34.97	47.03
14	HTL2	0.64	0.63	31.07	35.23	47.18
15	CB00-13-164	0.65	0.63	31.30	37.47	53.79
16	CB00-13-104	0.62	0.65	30.80	36.43	48.51
17	CB00-13-126	0.62	0.66	30.63	32.83	77.20
18	CB00-13-73	0.65	0.63	33.93	38.77	53.84
19	CB00-13-171	0.60	0.62	36.17	39.50	79.43

20	CB00-13-52	0.61	0.65	35.73	44.67	49.77
21	CB00-14-65	0.62	0.61	26.60	34.07	75.40
22	CB00-13-117	0.61	0.65	35.57	35.90	51.83
23	CB00-13-79	0.63	0.62	39.73	39.63	55.80
24	CB00-14-12	0.63	0.64	34.10	34.70	64.29
25	HTL 35	0.63	0.66	32.97	38.97	64.10
26	HTL 33	0.62	0.61	28.37	32.37	44.23
27	HTL 31	0.67	0.64	34.53	32.83	61.21
28	BALILLA	0.62	0.61	31.00	32.73	59.92
29	HTL 34	0.62	0.62	34.10	35.10	46.31
30	CB00-13-88	0.64	0.61	34.60	37.13	41.92
31	HTL 40	0.64	0.62	33.03	27.27	74.75
32	HTL 25	0.63	0.66	32.13	28.83	75.13
33	HTL 39	0.66	0.65	38.53	31.00	68.65
34	CORH 3	0.64	0.62	35.83	36.80	67.38
35	CORH 4	0.64	0.63	36.10	33.33	45.02
36	IR86977-104-1	0.60	0.63	35.53	29.23	43.44
37	IR86970-126-3	0.61	0.69	37.80	31.93	71.54
38	IR86977-92-3	0.60	0.65	33.60	33.03	43.79
39	IR86930-145-1	0.60	0.63	36.50	32.67	42.79
40	IR86977-95-1	0.62	0.63	33.73	32.70	63.20
41	TNAUCMS2A X CB00-14-56	0.65	0.70	40.33	39.10	63.53
42	TNAUCMS2A X CB00-14-82	0.63	0.67	39.53	41.20	42.68
43	TNAUCMS2A X CB00-14-47	0.63	0.61	36.87	38.83	53.80
44	TNAUCMS2A X CB00-13-61	0.64	0.64	35.30	41.37	55.27
45	TNAUCMS2A X IR36	0.61	0.66	36.23	35.33	41.70
46	TNAUCMS2A X CB00-14-37	0.61	0.66	39.13	41.77	37.64
47	TNAUCMS2A X IR8	0.61	0.64	38.17	37.80	67.11
48	TNAUCMS2A X CB00-13-97	0.61	0.63	37.97	38.73	38.25
49	TNAUCMS2A X CB00-13-55	0.62	0.62	37.27	36.87	38.13
50	TNAUCMS2A X HTL 2	0.60	0.63	36.50	39.07	67.24
51	IR68897A X CB00-13-55	0.58	0.62	38.97	42.90	67.53
52	IR68897A X CB00-13-164	0.63	0.62	32.90	38.60	69.58
53	IR68897A X CB00-13-104	0.62	0.65	42.93	39.23	46.41
54	IR68897A X CB00-13-126	0.63	0.65	35.77	39.80	45.62
55	IR68897A X CB00-13-73	0.61	0.62	37.07	39.73	69.60
56	IR68897A X CB00-13-171	0.61	0.61	37.13	39.93	44.65
57	IR68897A X CB00-13-52	0.61	0.66	44.57	41.50	79.81
58	IR68897A X CB00-13-97	0.61	0.66	40.80	39.33	65.57
59	IR68897A X CB00-14-82	0.61	0.66	41.70	42.20	54.75
60	IR68897A X CB00-14-65	0.59	0.64	39.60	37.17	65.40
61	IR68897A X CB00-14-56	0.63	0.64	42.50	40.83	67.48
62	COMS 24A X CB00-14-56	0.58	0.62	41.10	38.73	52.62
63	COMS 24A X CB00-14-82	0.65	0.61	39.70	33.97	63.66
64	COMS 24A X CB00-13-97	0.63	0.61	37.33	33.47	68.21
65	COMS 24A X CB00-13-117	0.63	0.62	41.10	35.20	58.01
66	COMS 24A X CB00-13-55	0.61	0.63	37.57	30.00	57.37
67	COMS 24A X CB00-13-79	0.66	0.63	39.80	31.83	58.85
68	COMS 24A X CB00-14-12	0.61	0.65	39.83	38.93	58.02
69	COMS 24A X HTL 35	0.66	0.63	35.83	35.73	57.79
70	COMS 24A X HTL 33	0.60	0.65	38.53	34.90	69.03
71	COMS 24A X HTL 31	0.62	0.62	38.97	35.30	57.57
72	COMS 24A X BALILLA	0.60	0.61	34.23	32.43	65.57
73	COMS 24A X HTL 34	0.61	0.66	43.83	33.60	47.94
74	COMS 24A X IR8	0.62	0.64	35.20	32.03	50.97
75	COMS 24A X CB00-13-126	0.61	0.62	39.03	35.23	43.73
76	COMS 24A X CB00-13-88	0.61	0.60	37.20	37.00	41.41
77	COMS23A X CB00-13-55	0.61	0.62	37.53	35.97	61.14
78	COMS23A X CB00-14-37	0.63	0.60	37.57	38.33	54.20
79	COMS23A X HTL 40	0.60	0.64	40.30	33.47	50.95
80	COMS23A X HTL 25	0.62	0.61	36.53	35.60	60.66
81	COMS23A X CB00-14-82	0.60	0.60	40.47	36.67	42.80
82	COMS23A X HTL 39	0.61	0.65	37.93	34.67	40.60
83	COMS23A X IR8	0.62	0.61	36.53	35.60	40.70
	Grand Mean	**0.62**	**0.63**	**36.40**	**36.08**	**55.74**
	SED	**0.02**	**0.02**	**2.77**	**2.49**	**0.56**
	CD (5%)	**0.03****	**0.04****	**5.48****	**4.91****	**1.10****
	CD (1%)	**0.04****	**0.06****	**7.23****	**6.48****	**1.45****

* Significant at 5% level, ** Significant at 1% level

At flowering stage, the chlorophyll fluorescence (F_v/F_m) ratio ranged from 0.60 (COMS 23A x HTL31) to 0.70 (TNAUCMS 2A x CB00-14-56) and was maximum in the parental genotypes CB00-13-126 (0.66), HTL 35 (0.66), HTL 25 (0.66) and COMS 24B (0.65). The hybrids which recorded maximum (F_v/F_m) ratio were TNAU CMS 2A x CB00-14-56 (0.70) TNAU CMS 2A x CB00-14-82 (0.67) and TNAU CMS 2A x IR 36 (0.66) (Fig.1).

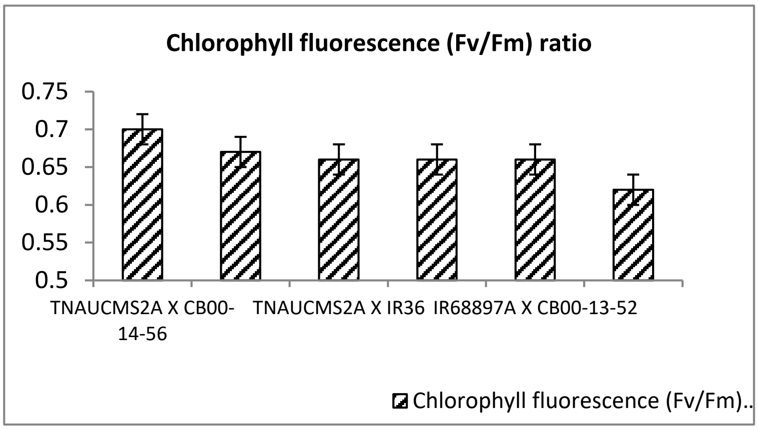

Figure 1. Best hybrids based on chlorophyll fluorescence (F_v/F_m) ratio over check hybrid, CORH 3

The results indicated that the above parents and hybrids have relative tolerance to high temperature. S. SHEIKH & al, [19] reported that the genotypes PBW 435 and hybrid PBW x WH542 had high temperature tolerance in wheat by using chlorophyll fluorescence (F_v/F_m) ratio.

3.2. Chlorophyll Meter Reading (SPAD value)

Leaf chlorophyll content is tightly associated with photosynthetic potential and biomass production (J. IMANISHI & al, [20]), and is easily affected by plant nutrition status, especially nitrogen level (J.W. LI & al, [21]) and abiotic stress (G.A. CARTER [22]; G.A. CARTER & al, [23]; B. QIU & al, [24]). E. KUMAGAI & al, [25] proved that the SPAD value could reflect chlorophyll content and photosynthetic capacity of leaves. M.P. REYNOLDS & al, [26] found that leaf chlorophyll content was highly correlated with wheat yield under heat stress. F. HAN & al, [27] demonstrated that the maximal quantum yield of PS II photochemistry (F_v/F_m) in rice leaves was significantly affected by high temperature (40°C). U.R. ROSYARA & al, [28] observed that the chlorophyll contents of 11 wheat genotypes were significantly reduced when exposed to heat stress and confirmed that high temperature reduced chlorophyll content and thermo-sensitive genotypes had more reduction than tolerant ones.

With regard to chlorophyll content, the values ranged from 26.60 (CB00-14-65) to 44.57 (IR68897A x CB00-13-52). Maximum SPAD values were observed in the genotypes CB00-13-79 (39.73) CB00-13-97 (39.10) and HTL 39 (38.53) at booting stage. The hybrids which recorded maximum SPAD values were IR 68897A x CB00-13-52 (44.57) COMS 24A x HTL 34 (43.83) and IR 68897A x CB00-13-104 (42.93) (Table 1).

At flowering stageit ranged from 26.90 (HTL 30) to 44.67 (CB00-13-52) and the maximum SPAD values were recorded in the parental genotypes CB00-13-52 (44.67) TNAU CMS 2B (41.80) and CB00-13-79 (39.63). The hybrid which recorded maximum SPAD values were IR68897A x CB00-13-55(42.90) TNAU CMS 2A x CB00-14-37 (41.77) and IR68897A x CB00-13-52 (41.50) (Fig. 2).

The results indicated that parents CB00-13-52, TNAU CMS 2B and CB00-13-79 and the hybrids IR 68897A x CB00-13-55, TNAU CMS 2A x CB00-14-37 and IR 68897A x CB00-13-52 have relative tolerance to high temperature. H.R. BALOUCHI [29] reported that heat stress decreased significantly the total chlorophyll content in wheat. T. REZA [30] observed that the genotypes with high yield had high chlorophyll content. W. ZHOU & al, [31] reported that when rice seedlings were exposed to heat stress chlorophyll content (SPAD values) were dramatically reduced.

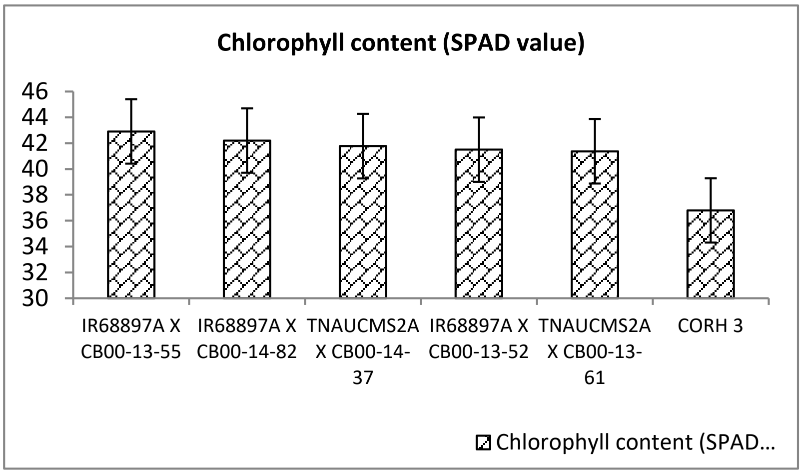

Figure 2. Best hybrids based on SPAD value over check hybrid CORH 3

3.3. Membrane Thermal Stability (MTS)

The sustained function of cellular membranes under stress is pivotal for processes such as photosynthesis and respiration (A. BLUM [32]). Heat stress accelerates the kinetic energy and movement of molecules across membranes thereby loosening chemical bonds within molecules of biological membranes. This makes the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated fatty acids (G.E. SAVCHENKO & al, [33]). The integrity and functions of biological membranes are sensitive to high temperature, as heat stress alters the tertiary and quaternary structures of membrane proteins. Such alterations enhance the permeability of membranes, as evident from increased loss of electrolytes. The increased solute leakage, as an indication of decreased cell membrane thermo-stability (CMT), has long been used as an indirect measure of heat stress tolerance in diverse plant species.

The values for membrane thermal stability ranged from 37.64 percent (TNAUCMS 2A x HTL31) to 79.82 (IR68897A x CB00-13-52) (Table 1). The parents CB00-13-171, CB00-13-126, CB00-14-65, HTL 25 and HTL 40 and the hybrids IR 68897A x CB00-13-52, IR 68897A x CB00-13-73, IR 68897A x CB00-13-164, COMS 24A x HTL 33 and COMS 24A x CB00-13-97 had high membrane thermal stability values at flowering stage and were inferred to be tolerant to high temperature stress (Fig.3). The thermo tolerance can be attributed due to the ability to maintain their cellular membrane integrity under high temperature conditions. Similar findings have also been reported by J.F. SHANAHAN & al, [34]; M. FOKAR & al, [35]; A.M.H. IBRAHIM & al, [36]; N.B. SINGH & al, [37].

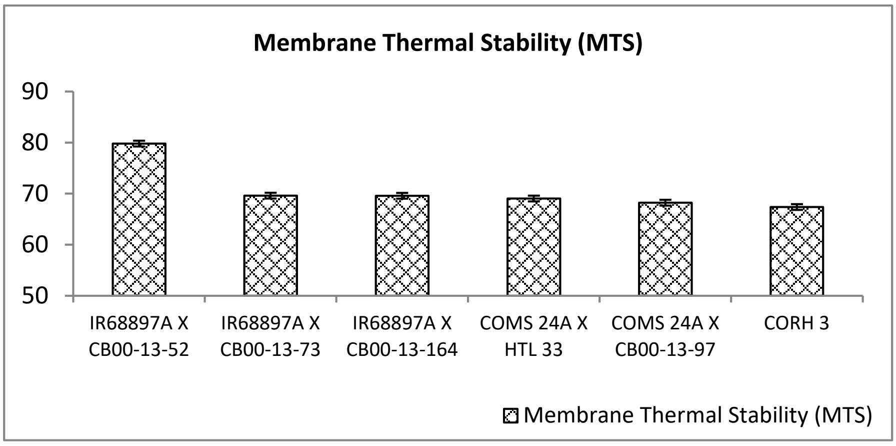

Figure 3. Best hybrids based on membrane thermal stability over check hybrid CORH 3

4. Conclusion

The studied rice genotypes (83) were characterized for chlorophyll fluorescence (F_v/F_m) ratio, SPAD value, and membrane thermal stability. Based on these physiological parameters, the hybrids TNAUCMS 2A x IR 36, TNAU CMS 2A x CB00-14-37, IR68897A x CB00-13-52 and COMS 24A x HTL 33 were identified to possess heat tolerance paving theway to recommend these hybrids for cultivation under high temperature conditions.

5. Competing interest

The authors declare no conflict of interest.

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