

## Original Research Article

### Screening of Rice Hybrids for Cold Tolerance at Seedling and Vegetative Stage Under Low Temperatures

#### Abstract:

Low temperature is **the one** of the most important abiotic constrain in rice **crop**. Rice plant growth and development are affected by cold stress from germination to reproductive stages. Development of 48 hybrids involving twelve restorer lines and four CMS lines by using line x tester mating design. Evaluation of **sixty nine** rice genotypes including checks based on leaf score (SES, IRRI, 2013), root length, shoot length, fresh weight, dry weight **and** SPAD analysis at seedling stage as well as leaf score, number of tillers seedling growth and establishment at vegetative stage. Results indicated that the hybrids IR72081 A x JGL 11118, IR72081 A x JGL 21071, IR80559A x JGL 29662, IR80559A x KNM 110, IR80559A x SN 470, IR58025A x JGL 29662 identified as cold tolerant genotypes. Screening for **cold tolerance** during early growth stages potentially be an effective way **for** assessing cold tolerance in breeding **programmes**.

**Comment [M1]:** Check Grammarly (highlight yellow color)

**Key words:** Cold tolerance, leaf score, root length, shoot length, SPAD analysis

#### 1. Introduction:

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. For more than half of the world's population, it is a staple food crop. Rice is a member of the Gramineae (grass) family. It belongs to the Oryza genus, which contains more than 20 species, only two of which, *Oryza sativa* and *Oryza glaberrima*, are cultivated globally. Rice is an angiosperm that is monocotyledonous. It is a diploid with 24 chromosomes that may be recognised using cytogenetic methods.

**Comment [M2]:** writing species according to the binomial system

**In Northern** and central Telangana area, rice is the most important crop to be grown in both **the seasons**. Rice sowings are confined to a few months per year, despite the availability of adequate irrigation systems, because the main reason for the restriction is cold stress. When sowings are delayed in the **kharif** crop, which often happens due to late canal water

release or a delay in monsoon rains, the reproductive phase of the crop coincides with low temperatures in November/December, resulting in poor seed set and yields. In addition, throughout the winter, minimum temperatures frequently fall below 10°C, resulting in poor seedling growth in the rabi crop. Low temperature stress can appear at various growth stages, including germination, seedling, vegetative, reproductive, and grain maturity, in addition to the two key stages (Andaya and Mackill, 2003).

Comment [M3]: Year...??

Cold sensitivity and damage symptoms differ depending on the rice plant's growth stage (Yoshida 1981). The most typical indications of cold temperature damage are delayed and a reduced percentage of germination occurs during the germination stage (da Cruz and Milach 2000). Chilling damage manifests itself in the vegetative stage as yellowing of the leaves, a reduction in height, and a reduction in rice plant tillering. When the rice plant's reproductive period coincides with the cold, the most common indicator of harm is sterility of the spikelets, but incomplete panicle exertion and spikelet abortion are also possible (Satake and Hayase 1970). Pollen abortion owing to cold during microsporogenesis, while pollen grains are being produced, at the booting stage, can cause spikelet sterility.

Comment [M4]: update this reference

## 2. Materials and method:

### 2.1 Study site:

The experiment was conducted in Regional Agricultural Research Station farm, Jagtial during 2021-2022. Three staggered sowings with 7 – 10 days intervals. A total of 69 rice genotypes (48 Hybrids, 12 testers and 4 CMS lines) and along with resistant and susceptible checks used as experimental material (Table.1).

### 2.2 Method of collection

In Rabi 2021-2021, evaluation of hybrids, parents and along with checks against cold stress through SPAD analysis, root length, shoot length, fresh weight, dry weight and leaf score (1-9) (SES IRRI,2013) at seedling and vegetative stage (Table .2&.3). The mean values and score of the genotypes given below (Table.4)

Comment [M5]: Check this sentence again

Comment [M6]: Explain this abbreviation

Deviation for SPAD analysis=

$$\frac{\text{Normal conditions (30DAS)} - \text{cold stress conditions (15DAS)}}{\text{Normal conditions (30DAS)}} \times 100$$

From the past five years temperatures are in optimum level but now, the weather conditions are very low during October – January (fig.1). Due to minimum temperatures

often results in poor germination, seedling mortality, leaf chlorosis, stunted growth, reduced tillering at seedling and vegetative stage. After transplanting of seedlings there will be transplanting shock may occur and take some time for recovery. But, due to cold stress there will be chances of getting more time for recovery of the seedlings.

### 3. Results and Discussion:

Cold tolerance was linked to physiological indicators such as root length, shoot length, and their fresh weight, dry weight as well as SES scoring and SPAD reading in a study on per se performance. These characteristics were chosen because they were the most effective in distinguishing cold tolerance from cold resistance genotypes at the seedling stage (Cruz and Milach, 2004). Because the rice crop grown during the rabi/boro season is affected by cold stress during the nursery stage is a better knowledge of the mechanism of cold tolerance at the seedling stage is critical. Results revealed that, at seedling stage the genotypes IR72081A × JGL11118, IR72081A × KNM 110, IR80559A × JGL 29662, IR80559A × KNM 110, IR80559A × NSR 64, IR80559A × WGL 14, IR80559A × SN 470, IR58025A × JGL 11118, IR58025A × JGL 29662, IR58025A × KNM 110, IR58025A × NSR 64, IR58025A × WGL 23985, JGL18264-1A × WGL 23985 and JGL 24423 having score 1(Highly tolerant) and IR72081A × IR 72, JGL 11470 and JGL 1798 having score 7 (Sensitive). (Fig. 2) (Table.2)

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Based on chlorophyll content through SPAD analysis, the hybrids IR72081A × JGL 11118, IR72081A × JGL 21071, IR80559 A × JGL 29662, IR58025A × JGL 29662, IR72081 A × JGL 29662, IR72081 A × KNM 110, IR80559 A × SN 470 shows less deviation which indicate tolerant genotypes. while, more deviation seen in IR72081 A × IR 72 and JGL18264-1A × SN S33 which are sensitive to cold stress. (Table.4)

For root length, the crosses IR72081A × JGL 11118, IR72081A × JGL 21071, IR80559A × KNM 110, IR80559A × WGL 23985, IR58025A × JGL 11118, IR58025A × KNM 110 and check JGL 24423 (fig.3) (Table.2) and for shoot development, the crosses IR80559A × JGL 29662, IR80559A × IRTON 270, IR80559A × SN 470, IR58025A × JGL 11118, JGL 18264-1A × SN 470 exhibit tolerance. (Fig.4) (Table.4)

At vegetative stage, due to cold stress the colour of leaf and seedling establishment is quite different. Among the genotypes, 15 were tolerant, 52 were moderately tolerant and 5 were sensitive (Fig.4) (Table.4)

Cold tolerance at the germination stage was linked to those at the seedling, booting, and flowering stages, according to Ye et al. (2008), while cold tolerance at the seedling stage was linked to those at the booting stage. Cruz and Milach (2004), Xia et al. (2006), Fallah and Elyasi (2010), Donoso Nanculao et al. (2013), Sahu et al. (2014), Kumari and Jaiswal (2017), lone et al. (2018) and Sumayya et al. (2020) all published similar studies.

**Comment [M8]:** Discussion: very minimal because it is only one paragraph, it is recommended to add an updated and appropriate reference citation to increase the scientific value of this article

#### 4. Conclusion:

The genotypes that come under scale 1-3 are considered as tolerant. Among the genotypes, the crosses IR72081A x JGL 11118, IR72081A x JGL 21071, IR80559A x JGL 29662, IR80559A x KNM 110, IR80559A x KNM 110, IR80559A x SN 470 exhibit highly cold tolerance, therefore these genotypes were good for rabi crop and IR72081A x IR 72, JGL18264-1A x SN S33 exhibit susceptible to cold by comparing with parents and checks. The maximum shoot length was observed in the genotype IR80559A x JGL 29662 as well as fresh weight and considered as cold tolerant genotype. The genotype IR72081A x IR 72 identified as susceptible for all the parameters, therefore it is not recommended for rabi paddy.

#### 5. Further Research:

Developing and identifying the cold tolerant genotypes. In Northern Telangana temperature falls below 10°C but the optimum temperature of the rice is 12°C. So, the farmers are facing this problem. In this way it is useful to develop cold tolerant genotypes.

#### 6. Reference:

Andaya, V.C., Mackill, D.J., 2003. Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. *Journal of Experimental Botany* 54, 2579-2585.

Da Cruz, R. P. and S. C. K. Milach., 2000. Breeding for cold tolerance in irrigated rice. *Ciencia Rural* 30, 909-917

Cruz. RP and Milach SCK., 2004. Cold to tolerance at the germination stage of rice: Methods of evaluation and characterization of genotypes. *Scientia Agricola* 61, 1-8

Fallah, A. and Elyasi, H., 2010. Quantity evaluation of rice plant in under cold stress at different growth stages. *AGRIS*.

**Comment [M9]:** ancient reference updates with updated references of the last 5 - 10 years.

Gholizadeh, A., Amin, M.S.M., Anuar, A.R and Aimrun, W., 2009. Evaluation of SPAD chlorophyll meter in two different rice growth stages and its temporal variability. *European Journal of Scientific Research* 37 (4), 591-598.

Kumari, P. and Jaiswal, H.K., 2017. Effect of cold stress on boro rice seedlings. *Journal of Applied and Natural Science* 9(2), 1036-1041.

Lone, J.A., Khan, M.N., Bhat, M.A., Shikari, A.B., Wani, S.H., Sofi, N.R., Khan, M.I. and Lone, R.A., 2018. Cold tolerance at germination and seedling stages of rice: methods of evaluation and characterization of thirty rice genotypes under stress conditions. *International Journal of Current Microbiology and Applied Sciences* 7, 1103-1109.

Ñanculao, D. G., Cárcamo, P. M., de los Santos, A. O. and Velásquez, B. V. (2013). Cold tolerance evaluation in Chilean rice genotypes at the germination stage. *Chilean journal of agricultural research*, 73(1), 3-8.

Rahul, N.S., Bhadru, D., Sreedhar, M and Vanisri, S., 2017. Screening of cold tolerant Rice genotypes for seedling traits under low temperature regimes. *International Journal of Current Microbiology and Applied Sciences* 6(12), 4074-4081.

Sahu, A., Verma, R.K., Sarawgi, A.K., Verulkar, S.B., Guhey, A. and Saxena, R.R., 2014. Impact of low temperature tolerance at seedling stage on rice genotypes. *Journal of Rice Research* 7(1), 2.

Satake, T. and H. Hayase., 1970. Male sterility caused by cooling treatment at the young microspore stage in rice plants: V. Estimation of pollen developmental stage and the most sensitive stage to coolness. *Japanese journal of crop science* 39(4), 468-473.

Comment [M10]: update this reference

Satya, P., Saha, A. and Singh, N.K., 2010. Screening for low-temperature stress tolerance in boro rice. *International Rice Research Notes* 35, 1-6.

Standard evaluation system for rice blast and cold stress screening. 2013. 5<sup>th</sup> edition, June, IRRI-SES. P.No 18 and 35.

Sumayya, K., Rajanna, M., Deepak, C., Shivakumar, K. and Denesh, G., 2020. Evaluation of rice (*Oryza sativa* L.) genotypes for cold tolerance at seedling stage. *ORYZA-An International Journal of Rice* 57(1), 43-48.

Xia L., Chuan Chao D., Yu. C., Ting, C and De Mao J., 2006. Identification for cold tolerance at different growth stages in rice (*Oryza sativa* L.) and physiological mechanism of differential cold tolerance. *Acta Agronomica Sinica* 32 (1), 76.83.

Xiong Z.M., Min S.K., Wang G.L., Cheng S.H and Cao L.Y., 1990). Genetic analysis of cold tolerance at the seedling stage of early rice (*O. sativa* L. subsp. Indica). *Chinese Journal of Rice Science* 4 (2), 75-78.

Ye C., Fukai S., Reinke R. Godwin I., Snell P and Basnayake J., 2008. Screening of rice genetic resources for cold tolerance at different growth stages. In: *Proceedings of the 14th Australian Society of Agronomy Conference, Adelaide, South Australia, 21-25 September 2008.*

Yoshida, S. 1981. 1–63 in *Fundamentals of rice crops science*. International Rice Research Institute. Los Banos.

**Comment [M11]:** ancient references update and this reference has not been included in the manuscript

**Comment [M12]:** update this reference

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**Table 1. Genotypes used as experimental material in this study****Comment [M13]:** Table writing is adjusted to the table writing guidelines

S.No	Genotype	S.No	Genotype
1.	IR72081 A × JGL 11118	36	IR58025A × SN 470
2.	IR72081 A × JGL 21071	37	JGL 18264-1A × JGL 11118
3.	IR72081 A × JGL 29662	38	JGL 18264-1A × JGL 21071
4.	IR72081 A × KNM 110	39	JGL 18264-1 A × JGL 29662
5.	IR72081 A × NSR 64	40	JGL 18264-1A × KNM 110
6.	IR72081 A × NSR103	41	JGL 18264-1A × NSR 64
7.	IR72081 A × IRTON 270	42	JGL 18264-1A × NSR 103
8.	IR72081 A × IR 72	43	JGL 18264-1A × IRTON 270
9.	IR72081 A × WGL 14	44	JGL 18264-1A × IR 72
10.	IR72081 A × WGL 23985	45	JGL 18264-1A × WGL 14
11.	IR72081 A × SN 233	46	JGL 18264-1A × WGL 23985
12.	IR72081 A × SN 470	47	JGL 18264-1A × SN S33
13.	IR80559 A × JGL 11118	48	JGL 18264-1A × SN 470
14.	IR80559 A × JGL 21071	49	IR72081B
15.	IR80559 A × JGL 29662	50	IR80559B
16.	IR80559 A × KNM 110	51	IR58025B
17.	IR80559 A × NSR 64	52	JGL 18264-1B
18.	IR80559 A × NSR 103	53	JGL 11118
19.	IR80559 A × IRTON 270	54	JGL 21071
20.	IR80559 A × IR 72	55	JGL 29662
21.	IR80559 A × WGL 14	56	KNM 110
22.	IR80559 A × WGL 23985	57	NSR 64
23.	IR80559 A × SN 233	58	NSR 103
24.	IR80559 A × SN 470	59	IRTON 270
25.	IR58025 A × JGL 11118	60	IR 72
26.	IR58025 A × JGL 21071	61	WGL 14
27.	IR58025 A × JGL 29662	62	WGL 23985
28.	IR58025 A × KNM 110	63	SN 233
29.	IR58025A × NSR 64	64	SN 470
30.	IR58025A × NSR 103	65	JGL 24423 (Tolerant check)

31.	IR58025A × IRTON 270	66	Tellahamsa (Tolerant check)
32.	IR58025A × IR 72	67	US – 312
33.	IR58025A × WGL 14	68	JGL 11470 (Susceptible check)
34.	IR58025A × WGL 23985	69	JGL 1798 (Susceptible check)
35.	IR58025A × SN 233		

**Table 2. Leaf scoring (1-9) (SES IRRI, 2013) at seedling stage.**

Score	Observations	Tolerance
1	Seedlings dark green	Highly tolerant
3	Seedlings light green	Tolerant
5	Seedlings yellow	Moderately tolerant
7	Seedlings brown	Sensitive
9	Seedling dead	Highly sensitive

**Table 3. Leaf scoring (1-9) (SES IRRI, 2013) at vegetative stage.**

score	observations	tolerance
1-3	All leaves normal color	Tolerant
4-6	Pale green leaves	Moderately tolerant
7-9	Yellowing of leaves and stunted growth	Sensitive

**Table. 4. The mean values and scoring of rice genotypes at seedling /vegetative stage under low temperature**

S.no.	Genotype	Before transplanting								After transplanting
		score (seedling stage) 20das	spad reading (mm) 15das	spad reading (mm) 30das	deviation (%)	root length (cm)	shoot length (cm)	fresh weight (g)	dry weight (g)	score (vegetatives tage) 15dap
	<b>Hybrids</b>									
1.	IR72081 A × JGL 11118	1	26.14	27.12	3.61	12.8	20.75	0.74	0.23	3
2.	IR72081 A × JGL 21071	3	25.76	26.74	3.66	13	20.25	1.17	0.30	5
3.	IR72081 A × JGL 29662	3	23.18	24.78	6.45	9.25	18.55	0.86	0.25	4
4.	IR72081 A × KNM 110	1	21.48	22.88	6.11	7.2	17.15	0.76	0.17	3
5.	IR72081 A × NSR 64	3	18.16	24.28	25.20	8.35	18.2	0.79	0.19	5
6.	IR72081 A × NSR103	3	18.64	25.1	25.73	8.55	17	0.88	0.20	6

7.	IR72081 A × IRTON 270	3	22.26	25.36	12.22	8.35	14.25	0.99	0.28	4
8.	IR72081 A × IR 72	7	12.84	23	44.17	8.55	15.4	0.61	0.14	7
9.	IR72081 A × WGL 14	5	18.06	24.44	26.10	9.35	17.95	0.88	0.21	3
10.	IR72081 A × WGL 23985	3	15.72	23.3	32.53	8.35	16.75	0.71	0.20	5
11.	IR72081 A × SN 233	3	15.78	26.98	41.51	6.65	18.45	1.04	0.34	5
12.	IR72081 A × SN 470	3	20.36	25.02	18.62	11.95	18.25	1.09	0.43	3
13.	IR80559 A × JGL 11118	3	24.44	28.94	15.54	10.5	19.2	1.01	0.42	3
14.	IR80559 A × JGL 21071	3	23	26.06	11.74	8.05	21.05	0.76	0.27	3
15.	IR80559 A × JGL 29662	1	24.1	25.22	4.04	8	24.05	1.36	0.42	3
16.	IR80559 A × KNM 110	1	22.56	3.67	12.21	13.55	21	0.67	0.35	3
17.	IR80559 A × NSR 64	1	21.32	23.38	8.81	9.7	20.05	0.63	0.27	5

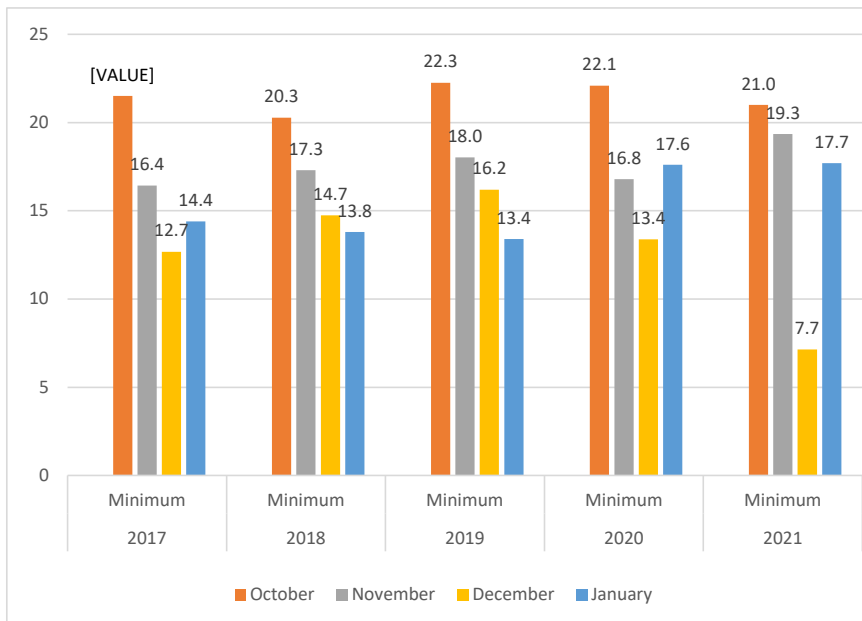
18.	IR80559 A × NSR 103	3	22.04	27.26	19.14	8.75	19.1	0.63	0.27	5
19.	IR80559 A × IRTON 270	3	22.42	25.02	10.39	5.55	23.05	1.42	0.39	5
20.	IR80559 A × IR 72	3	19.76	25.12	21.33	11.1	20.85	1.25	0.42	3
21.	IR80559 A × WGL 14	1	19.54	24.68	20.8	6.1	21.95	1.17	0.30	5
22.	IR80559 A × WGL 23985	3	19.82	24.88	20.33	13.15	19.95	1.47	0.41	4
23.	IR80559 A × SN 233	5	17.98	24.48	26.55	5.35	18.7	0.86	0.36	5
24.	IR80559 A × SN 470	1	23.82	25.16	5.32	9.8	23.3	1.47	0.54	3
25.	IR58025A × JGL 11118	1	19.86	27.62	28.09	12.55	21.85	1.25	0.36	5
26.	IR58025A × JGL 21071	3	18.14	26.56	31.70	7.95	20.45	1.33	0.39	5
27.	IR58025A × JGL 29662	1	23.88	24.9	4.09	11.7	20.65	1.55	0.34	5
28.	IR58025A × KNM 110	1	17.2	25.7	33.07	12.55	20.9	1.29	0.40	5

29.	IR58025A × NSR 64	1	17.42	25.8	31.63	8.95	14.55	1.17	0.21	3
30.	IR58025A × NSR 103	3	15.92	19.32	17.59	11.9	20.15	1.24	0.32	5
31.	IR58025A × IRTON 270	3	15.12	24.26	37.67	8.25	21.9	1.56	0.42	5
32.	IR58025A × IR 72	3	16.24	18.2	10.76	8.15	16.6	0.82	0.36	5
33.	IR58025A × WGL 14	3	16.04	24.16	33.60	7.3	18.4	0.88	0.22	4
34.	IR58025A × WGL 23985	1	17.16	22.84	24.86	8.75	16.9	0.73	0.20	4
35.	IR58025A × SN 233	3	20.66	25.8	19.92	9.35	17.9	0.80	0.19	3
36.	IR58025A × SN 470	3	18.08	23.64	23.51	9.3	18	0.64	0.25	5
37.	JGL18264-1A× JGL 11118	3	18.04	22.88	21.15	7.3	18.95	0.63	0.18	5
38.	JGL18264-1A × JGL 21071	5	17.4	20.4	14.70	5.85	16.8	0.43	0.15	5
39.	JGL18264-1A × JGL 29662	3	17.86	19.72	9.43	8.35	19.05	0.82	0.32	5

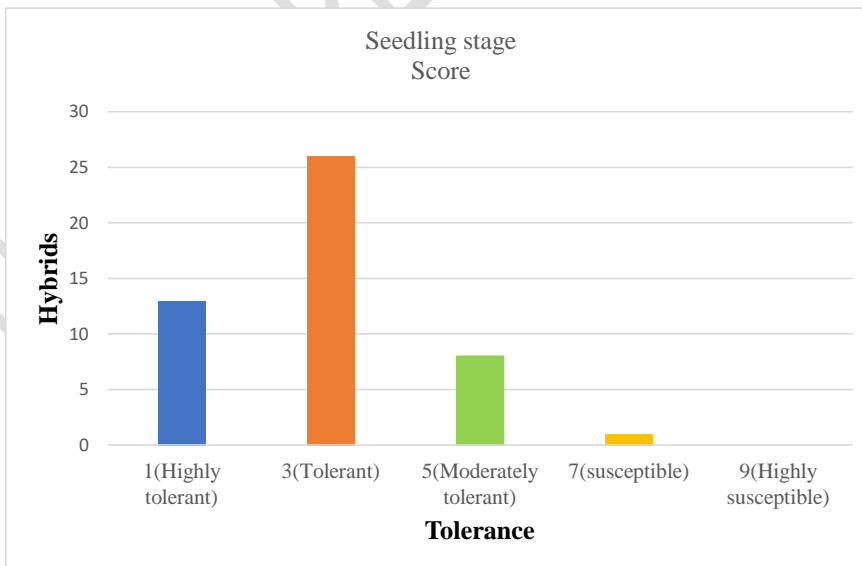
40.	JGL18264-1A × KNM 110	5	12.3	18.46	33.36	6.5	15.05	0.97	0.30	4
41.	JGL18264-1A × NSR 64	5	14.9	22.14	32.70	8.9	17.65	0.78	0.20	5
42.	JGL18264-1A × NSR 103	3	11.23	25.74	56.37	7.25	14.5	0.15	0.07	5
43.	JGL18264-1A × IRTON 270	5	17.96	22.82	21.29	9	18.7	1.08	0.42	5
44.	JGL18264-1A × IR 72	3	15.2	25.04	39.29	6.9	18.7	0.65	0.26	5
45.	JGL18264-1A × WGL 14	3	15.86	22.32	28.94	9.5	21.25	1.23	0.33	5
46.	JGL18264-1A × WGL 23985	1	17.18	23.06	25.49	8.3	19.3	0.67	0.43	5
47.	JGL18264-1A × SN S33	5	12.7	22.64	43.90	9.4	20.1	0.81	0.38	7
48.	JGL18264-1A × SN 470	5	15.02	23.08	34.92	9.55	22.35	1.48	0.42	5

	<b>Lines</b>									
49.	IR72081B	5	16.22	23.78	31.79	6.15	13.8	0.33	0.11	5
50.	IR80559B	5	12.96	19	31.78	5.65	12.8	0.25	0.07	5
51.	IR58025B	5	13.12	20.96	37.40	4.7	13.35	0.26	0.15	5
52.	JGL 18264-1B	5	14.9	19.8	24.74	2.7	10.95	0.07	0.20	7
	<b>Testers</b>									
53.	JGL 11118	3	19.4	19.54	0.07	5.3	14.75	0.42	0.19	6
54.	JGL 21071	3	17.62	22.5	21.6	3.75	14.55	0.60	0.25	5
55.	JGL 29662	3	16.32	22.54	27.59	5.8	15.25	0.45	0.16	5
56.	KNM 110	3	17.38	17.56	1.02	6.5	12.6	0.34	0.09	6
57.	NSR 64	3	18.3	21.08	13.18	2.15	15.5	0.28	0.18	5
58.	NSR 103	5	16.48	19.08	13.62	2.3	11.75	0.61	0.25	5
59.	IRTON 270	5	15.62	20.5	23.80	11.3	14.85	0.77	0.25	5
60.	IR 72	5	16.86	19.04	11.44	2.05	12.35	0.61	0.23	5

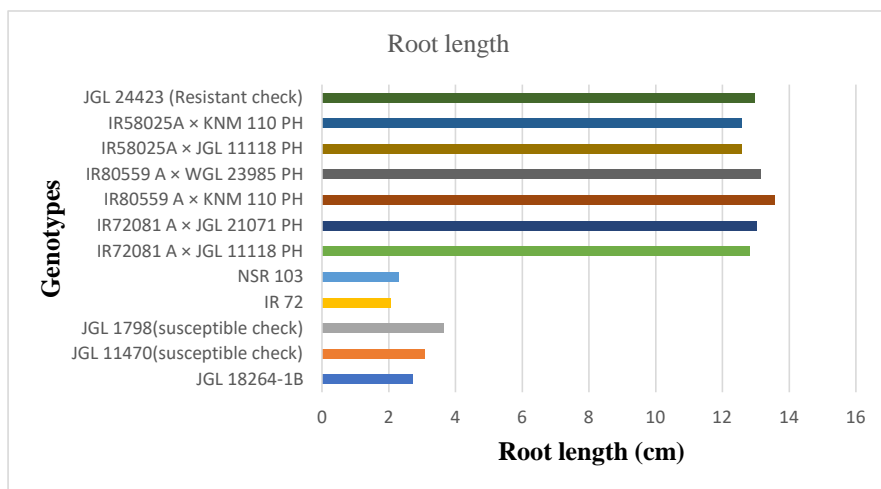
61.	WGL 14	5	17.1	17.56	2.61	5.95	16	0.75	0.20	6
62.	WGL 23985	5	17.52	20.78	15.68	4.35	14.55	0.55	0.24	5
63.	SN 233	5	17.42	17.56	0.79	6.1	10.95	0.52	0.23	5
64.	SN 470	3	18.42	18.08	1.88	6.05	14.45	0.44	0.19	5
	<b>Checks</b>									
65.	JGL 24423(Tolerant check)	1	17.24	22.68	23.98	12.95	20.11	0.66	0.19	3
66.	Tellahmsa(Tolerant check)	3	21.48	23.7	9.36	5.15	17.75	0.40	0.15	3
67.	US – 312	3	17.2	20.42	15.76	5.55	17.5	0.40	0.07	5
68.	JGL 11470(Susceptible check)	7	13.58	17.08	20.49	3.05	11.45	0.13	0.07	7
69.	JGL 1798(Susceptible check)	7	13.02	17.7	26.44	3.65	11.35	0.13	0.05	7



**Fig.1. Average weather status from 2017 -2021**

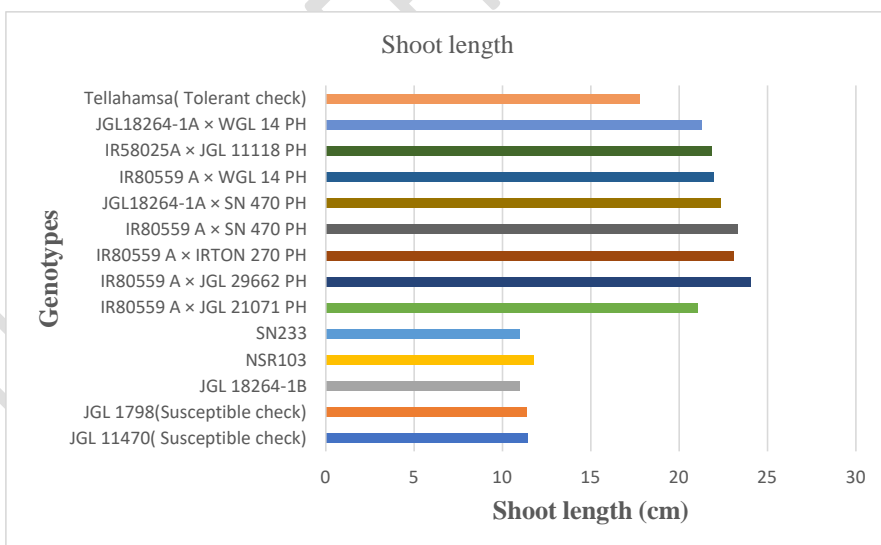


**Fig. 2. Frequency distribution of Hybrids and their tolerance at seedling stage**



PH: Promising hybrids

**Fig.3. Frequency distribution of genotypes and root length**



PH: Promising hybrid

**Fig.4. Frequency distribution of genotypes and shoot length**



**Fig.5. Frequency distribution of Hybrids and their tolerance at vegetative stage**

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