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ORIGINAL RESEARCH

Identification of Heat Stress Tolerant Wheat Genotype Using Stress Tolerance Indices

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ABSTRACT: This experiment was conducted to identify heat stress tolerant wheat genotypes using stress tolerance indices. A total of twenty wheat genotypes, provided by the National Wheat Research Program (NWRP) in Bhairahawa, were evaluated in both irrigated and heat stress environments. These genotypes comprised three Bhairahawa Lines (BL), fifteen Nepal Lines (NL), and two commercial checks—Bhrikuti and Gautam. The research was conducted at the Institute of Agriculture and Animal Science (IAAS) in Paklihawa, using alpha lattice design. Results showed that the mean grain yield of wheat was reduced by 24.82% under heat stress conditions as compared to irrigated conditions. Notably, mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), and yield index (YI) exhibited strong and highly significant positive correlations with yield under both irrigated and heat stress conditions. In contrast, tolerance index (TOL) and stress susceptibility index (SSI) displayed negative correlations under heat stress conditions. Genotype NL 1384 exhibited the highest MP, GMP, and STI, closely followed by NL 1417, establishing them as the most stable and productive genotypes. These findings suggest that these genotypes have the potential to be selected for high yields under both irrigated and heat stress conditions. The biplot analysis showed a positive correlation of MP, STI, GMP, YI, and yield stability index (YSI) with yield in the irrigated environment (Ys) and yield in the heat stress environment (Yp), and a negative correlation of stress susceptibility index (SSI), TOL, and reduction (Red). Hence, these indices could potentially be used for the evaluation of wheat genotypes under both irrigated and heat stress conditions.

KEYWORDS: *Triticum aestivum* L., abiotic stress, heat stress, tolerant, yield, stability

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1. Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop that supplies substantial quantities of protein and calories worldwide (Chand et al., 2022; Fu et al., 2023). It contributes to around 30% of world grain production and 20% of grain production in Nepal (Akter & Rafiqul Islam, 2017; Timsina et al., 2018). Wheat ranks first in world grain production, cultivated across approximately 217 million hectares with a productivity of 3460 kg ha-1 as of 2018 (Erenstein et al., 2022). In Nepal, wheat is the third most important crop, grown on around 711,067 hectares with a productivity of 2990 kg ha^{-1} (MoALD, 2021). Its contribution to Nepal's GDP and AGDP is

2.30 percent and 6.98 percent (Gairhe et al., 2017). In the year 2020-2021, wheat production in Nepal reached 2.13 million metric tons, showing a growth of only 15.22% from 2011 to 2020 (MoALD, 2021).

Temperature range for the cultivation of wheat is relatively narrower whose suitable range during sowing is 10°C-15°C and during ripening period is 21°C-26°C (Poudel et al., 2020). Increase in temperature above the specified range for a significant period damages the plant growth and development (Iqbal et al., 2017). Most of the wheat growing areas of South Asia are affected by heat stress. Anthesis in wheat occurs in mid-march and at this time, western hot wind blows with sudden increase in temperature in Terai area of Nepal (Poudel et al., 2021). Constant high temperature or transition of temperature cause change in morphology, physiology and biochemistry of a plant. These effect plant growth and cause heavy reduction in economic yield (Hossain et al., 2012). The early flowering or anthesis stage is regarded to be most sensitive to heat stress (Riaz et al., 2021). High temperature during development of wheat pollen inhibits translocation of nutrient and decrease pollen viability (Kumar & Nagora, 2023). Complete sterility may occur when temperature is greater than 30°C during floret formation. Grain yield is reduced when ambient temperature exceeds 22°C during the period between anthesis to grain maturity (Kamrani et al., 2018). It is estimated that for each degree rise in temperature, 3-17% yield loss occurs (Pokhrel et al., 2019).

There is an urgent need to enhance crop yield to fulfill demand due to the rapid increase in population (Poudel et al., 2020). Additional 198 million tons of wheat would be required for world by 2050 as per FAO (Singh et al., 2021). Breeders are trying hard to develop heat and water tolerant wheat varieties (Poudel et al., 2020). Increasing heat stress tolerance in wheat has been felt as great challenge by wheat breeders. The step required to increase heat stress tolerance in wheat is to screen wheat genotypes by breeders to recognize germplasm having better heat tolerance (Kamrani et al., 2018).

Effect of heat stress can be evaluated by the use of various indices and some of these are Tolerance index (TOL), Stress Susceptibility Index (SSI), Yield Stability Index (YSI), Mean Productivity (MP), Geometric Mean Productivity (GMP), Yield Index (YI), and Stress Tolerance Index (STI) (Fernandez et al., 1992). Stress Tolerance (TOL) is defined as the difference between yield in stress environment (Ys) and yield in non-stress environment (Yp) and mean productivity as average of Ys and Yp (Rosielle & Hamblin, 1981). (Fischer & Maurer, 1978) proposed Stress Susceptibility Index (SSI). (Fernandez et al., 1992) defined STI to identify high yielding genotypes under stress condition. This experiment was conducted to evaluate heat stress tolerance in wheat genotypes using various stress tolerance indices and disclose heat tolerant genotype in Terai region of Nepal among the tested twenty wheat genotypes.

2. Materials and methods

2.1 Experimental site

The field experiment was carried out at Institute of Agriculture and Animal Science (IAAS), Paklihawa, Nepal. Research site is located at 27°30′N, 83°27′E and 79 meter above sea level. The experimental materials consisted of twenty wheat genotypes provided by National Wheat Research Program (NWRP), Bhairahawa. There were three Bhairahawa Lines (BL), fifteen Nepal Lines (NL), and two commercial check Viz., Bhrikuti and Gautam. The list of all the genotypes used in the experiment are presented in Table 1.

2.2. Experimental design

The field experiment was conducted using alpha lattice design consisting of two replications and five blocks. The size of the experimental unit was 4 m^2 (2m × 2m) and each genotype was planted at a row to row spacing of twenty-five cm. The inter-plot space was kept fifty cm and inter block spacing was kept one m. Spacing between two replications was one m. Experiment was conducted in two environments: irrigated as normal season and heat stress as late season.

Land preparation was done by tractor and final levelling was done manually. Wheat genotypes were sown in their respective plot by line sowing method. Eight rows were made in each plot leaving 12.5 cm border and inter-row spacing 25 cm. Sowing was done on November 25, 2022 in irrigated environment and on December 25, 2022 in heat stress environment.

The seed rate was maintained at 100 kg per hectare. The recommended dose of 120:50:50 kg NPK per hectare was applied in both conditions (MoALD, 2021). Full dose of DAP and MOP and half dose of nitrogen was applied as basal dose at the time of sowing. The remaining dose of nitrogen was applied in two splits: one at 30 DAS another at 70 DAS. Pre-sowing irrigation was done and remaining irrigation was done at crown initiation stage, booting stage, flowering stage, heading stage, milking stage and soft dough stage One weeding was done at 45 DAS.

2.3. Measurement and analysis

The grain yield was taken by harvesting wheat from 2 m^2 area using sickle except from the border lines and threshing was done manually. Grain were weighed and converted to kg per hectare (kg ha^{-1}). Mean daily maximum and minimum along with the precipitation during wheat growing season at the experimental site is shown in Figure 1.

Eight stress tolerance indices were used in the evaluation of the genotypes. These were calculated by using following relationships:

1. Tolerance Index (TOL) = $Ys - Yp$ (Hossain et al., 1990)
2. Stress Susceptibility Index (SSI) =$(1 - (Ys/ Yp)) / SI$ (Fischer & Maurer, 1978)

   Where, Stress Intensity (SI)= $1 - (\frac{\bar{Ys}}{\bar{Yp}})$(Fischer & Maurer, 1978)
3. Mean Productivity (MP) = $(Ys + Yp)/2$ (Hossain et al., 1990)
4. Geometric Mean Productivity (GMP) = $\sqrt{(Yp \times Ys)}$(Fernandez et al., 1992)
5. Stress Tolerance Index (STI) = $(Ys \times Yp)/\bar{Yp}^2$ (Fernandez et al., 1992)
6. Yield Index (YI) = $Ys/\bar{Ys}$ (Khan & Kabir, 2015)
7. Yield Stability Index (YSI) = Ys/Yp (Bouslama & Schapaugh Jr., 1984)
8. Reduction (Red) = $[(Yp - Ys)/100] * 100$ (Bennani et al., 2017)

Table 1: List of all genotypes used in experiment.

S. N	Genotypes	Origin
1	Bhrikuti	CIMMYT, Mexico
2	BL 4407	Nepal
3	BL 4669	Nepal
4	BL 4949	Nepal
5	Gautam	Nepal
6	NL 1179	CIMMYT, Mexico
7	NL 1346	CIMMYT, Mexico
8	NL 1350	CIMMYT, Mexico
9	NL 1368	CIMMYT, Mexico
10	NL 1369	CIMMYT, Mexico
11	NL 1376	CIMMYT, Mexico
12	NL 1381	CIMMYT, Mexico
13	NL 1384	CIMMYT, Mexico
14	NL 1386	CIMMYT, Mexico
15	NL 1387	CIMMYT, Mexico
16	NL 1404	CIMMYT, Mexico
17	NL 1412	CIMMYT, Mexico
18	NL 1413	CIMMYT, Mexico
19	NL 1417	CIMMYT, Mexico
20	NL 1420	CIMMYT, Mexico

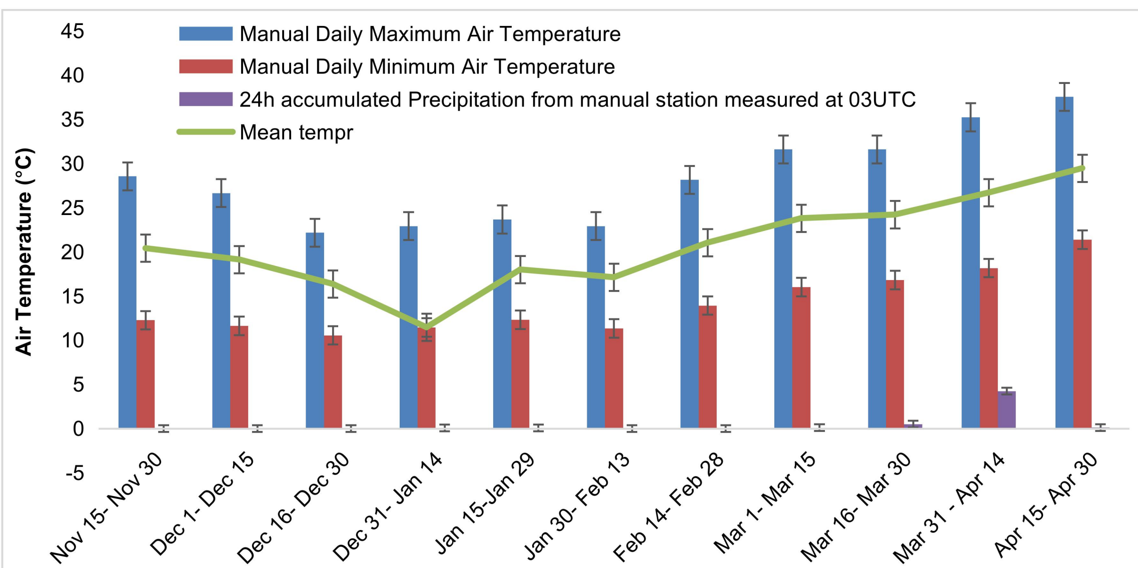

Figure 1. Manual daily maximum and minimum temperature along with precipitation from November 15 to April 30.

Whereas Ys indicates yield under heat stress condition, YP indicates yield under irrigated condition, $\bar{Ys}$ indicates mean of grain yield under heat stress and $\bar{Yp}$ indicates mean of grain yield under irrigated condition.

2.4. Statistical Analysis

Data entry and processing was done on Microsoft Excel office 2019. Stress tolerance indices were also calculated for evaluation of genotypes using Microsoft Excel office 2019. Analysis of variance for mean comparison, correlation among stress tolerance indices, principal component analysis and biplot analysis were performed using IBM SPSS statistics V. 25.

3. Results and Discussions

3.1. Yield performance

Under heat stress environment, days to booting (DTB), plant height (Ph), number of spikes per meter square (NSPMS), and thousand kernel weight (TKW) has shown significant difference among different genotypes as shown in Table 2 while DTB, days to heading (DTH), days to anthesis (DTA) and NSPMS has shown significant difference among wheat genotypes under irrigated environment as shown in Table 3. NL 1384 (3755 kg ha^{-1}), NL 1413 (3210 kg ha^{-1}), NL 1417 (3185 kg ha^{-1}) and NL 1420 (3010 kg ha^{-1}) yielded highest under irrigated condition. While NL 1384 (2473.33 kg ha^{-1}), NL 1412 (2320 kg ha^{-1}), Gautam (2293.33 kg ha^{-1}) and BL 4919 (2286.67 kg ha^{-1}) had shown highest yield under heat stress condition. Whereas, NL 1369 (1795 kg ha^{-1}) and NL 1368 (2015 kg ha^{-1}) had yielded lowest under irrigated condition. NL 1387 (1386.67 kg ha^{-1}) and NL 1369 (1406.67 kg ha^{-1}) had yielded lowest under heat stress condition as shown in Table 4. Similar result was reported by (Poudel et al., 2021), where maximum grain yield was observed in NL 1179 and Bhrikuti under normal and heat stress condition. Mean grain yield was found to be reduced by 24.82% under heat stress as shown in Table 4. Heat stress decreases growth cycle, number of tillers, photosynthetic area, chlorophyll content and increases photorespiration (Aberkane et al., 2021). This forces premature ripening which shortens number of grains/spikes and finally results in low grain yield (Jatoi et al., 2021). Yield reduction of wheat varies according to the severity of heat stress. Puri et al., 2015 reported 27.45 % reduction on yield. Higher temperature being linked with limitation of water is observed which causes rapid shrinkage of grain volume (Jatoi et al., 2021).

3.3. Stress tolerance indices

The highest TOL was recorded in NL 1413 followed by NL 1384 and NL 1386. TOL has negative correlation with yield under stress condition as shown in Table 5. So, these genotypes had high grain yield under non-stress condition while low yield under stress condition. So, they could be considered stress susceptible genotypes. Lowest TOL was recorded in NL 1179 and NL 1404. Both of these genotyp es yielded low in both irrigated and heat stress condition. Low TOL was due to low difference among yield in two conditions. NL 1413 had highest SSI while NL 1179 had lowest SSI which means NL 1413 is most susceptible genotype and NL 1179 is least susceptible genotype to heat stress. SSI value higher than one indicates above-average susceptibility, while SSI less than one indicates below-average susceptibility.

Table 2. Yield attributing characteristics of different wheat genotypes under heat stress environment.

Genotypes	DTB	DTH	DTA	Ph	SL	NSPMS	NSPS	NGPS	TSW	TKW	GY
1	65	68	71	81.2	9.7	205.0	14.8	33.1	22.3	41.4	1760.0
2	63	68	70	77.1	9.6	175.5	15.0	39.8	24.3	39.4	2240.0
3	66	69	71	75.7	8.6	199.5	17.2	34.1	18.8	36.2	1720.0
4	63	68	71	84.4	9.1	163.5	16.6	35.5	21.9	36.9	2286.7
5	67	68	70	82.9	8.7	258.5	16.4	40.7	20.2	32.7	2293.3
6	66	68	69	72.4	8.3	216.5	15.2	31.4	20.0	36.9	2260.0
7	64	68	69	75.9	9.2	223.0	14.5	33.0	20.2	38.4	2180.0
8	62	66	68	84.8	9.9	183.0	12.1	30.9	23.2	47.1	1633.3
9	65	68	70	73.3	8.6	220.5	15.0	33.3	18.8	36.7	1753.3
10	63	67	69	66.3	8.5	200.0	13.7	25.0	18.7	43.2	1406.7
11	66	68	71	79.4	9.2	127.5	16.7	31.9	21.5	38.4	1446.7
12	64	68	70	77.1	8.3	171.0	18.6	31.9	17.8	33.9	1673.3
13	68	71	73	82.1	9.7	273.5	17.1	41.6	21.5	32.9	2473.3
14	68	71	74	71.0	8.8	140.0	16.3	34.7	23.6	39.4	1480.0
15	63	67	72	65.6	8.1	186.5	13.4	27.4	17.6	39.0	1386.7
16	64	67	71	72.6	8.4	208.0	14.0	30.3	19.5	39.3	1946.7
17	66	68	69	84.4	8.9	232.0	16.0	32.4	20.0	39.5	2320.0
18	64	68	69	75.1	9.2	231.5	15.5	34.4	21.9	34.4	1766.7
19	66	69	71	80.1	9.5	215.5	17.8	38.4	21.4	32.9	2186.7
20	65	68	69	83.2	9.7	225.0	17.0	38.6	21.8	33.2	2206.7
Mean	65	68	70	77.2	9.0	202.8	15.6	33.9	20.8	37.6	1921.0
F value	**	ns	ns	**	ns	**	ns	ns	ns	**	ns

Note: DTB: Days to booting, DTH: Days to heading, DTA: Days to anthesis, Ph: Plant height, SL: Spike length, NSPMS: Number of spikes per meter square, NSPS: Number of spikelets per spike, NGPS: Number of grains per spike, TSW: Ten spike weight, TKW: Thousand kernel weight, GY: Grain Yield. For Genotypes see table 1.

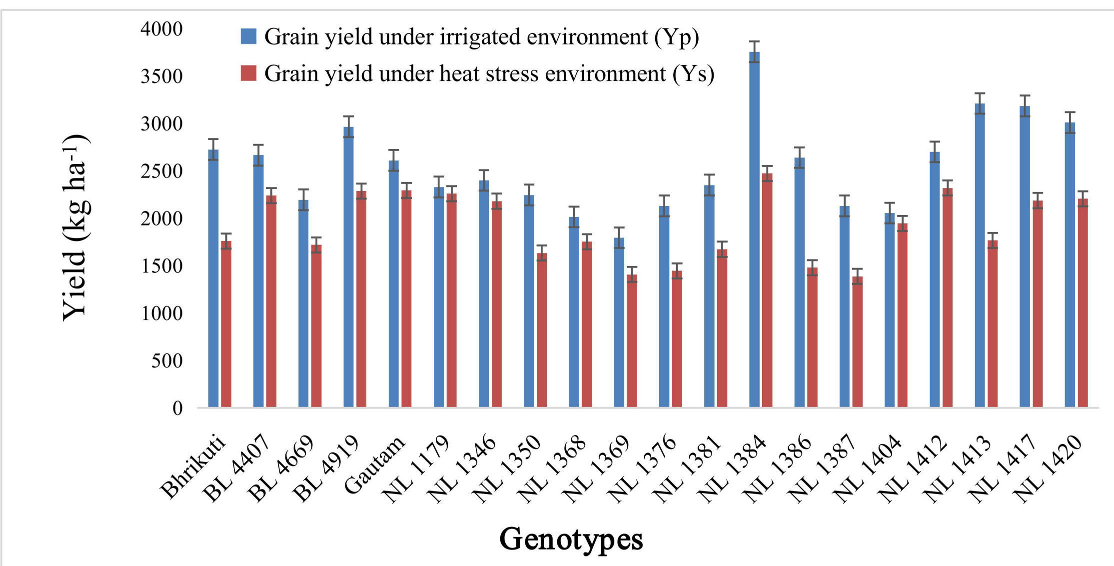

Figure 3. Yield of twenty wheat genotypes under irrigated (Yp) and heat stress condition (Ys).

Low TOL and SSI does not mean the genotype is high yielding. Grain yield must also be taken under consideration while selecting stress tolerant genotype. (Thapa et al., 2022) also stated to take grain yield into consideration. Highest MP, GMP and STI was obtained in genotype NL 1384 followed by NL 1417. Hence, they were concluded as most stable and most productive genotypes among the cultivated 20 genotypes. (Kamrani et al., 2018) also concluded genotypes having highest MP, GMP and STI as highest producing genotypes. According to YSI, NL 1179, NL 1404 and Gautam were identified as more stable and heat tolerant genotypes under heat stress condition.

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Table 3: Yield attributing characteristics of different wheat genotypes under irrigated environment

Genotypes	DTB	DTH	DTA	Ph	SL	NSPMS	NSPS	NGPS	TSW	TKW	GY
1	75	80	82	92.4	9.8	272.0	17.3	695.0	38.2	45.8	2725.0
2	71	74	79	86.2	9.6	256.5	15.1	601.5	36.7	43.4	2665.0
3	73	77	80	85.9	9.6	247.5	17.4	569.0	37.4	42.6	2195.0
4	68	73	78	88.4	9.5	239.5	15.9	595.0	39.4	46.9	2965.0
5	77	82	84	90.5	9.4	314.5	17.5	585.0	37.7	38.1	2610.0
6	75	80	82	82.8	9.3	264.0	18.4	586.5	36.3	38.6	2330.0
7	69	75	78	74.6	9.5	201.0	16.8	590.0	32.5	38.2	2400.0
8	68	74	78	89.6	10.3	209.5	14.3	518.0	37.0	46.7	2245.0
9	70	75	80	78.3	9.9	259.5	18.0	629.0	36.0	37.2	2015.0
10	70	75	80	76.6	9.3	179.5	16.2	486.0	34.3	43.0	1795.0
11	72	78	81	79.4	8.9	203.5	15.0	536.5	31.0	39.2	2130.0
12	72	79	81	84.3	9.9	266.0	18.1	814.5	40.5	34.8	2350.0
13	77	82	84	93.8	10.6	379.0	19.3	674.0	36.3	35.8	3755.0
14	76	81	85	87.2	10.8	209.5	18.9	701.0	44.6	44.2	2640.0
15	72	80	81	80.0	10.1	225.5	17.1	501.5	37.8	42.2	2130.0
16	70	75	79	76.8	9.2	282.0	15.7	538.0	32.8	38.6	2055.0
17	69	79	81	91.6	9.0	350.5	15.5	386.0	28.7	46.8	2700.0
18	73	79	82	91.5	9.9	268.5	18.0	720.5	43.4	40.2	3210.0
19	75	80	81	94.2	10.2	273.5	18.2	645.5	40.2	44.1	3185.0
20	76	81	82	89.2	10.5	339.0	18.4	687.5	41.7	33.9	3010.0
Mean	72	78	81	85.7	9.8	262.0	17.1	603.0	37.1	41.0	2555.5
F value	**	**	**	ns	ns	**	ns	ns	ns	ns	ns

Note: DTB: Days to booting, DTH: Days to heading, DTA: Days to anthesis, Ph: Plant height, SL: Spike length, NSPMS: Number of spikes per meter square, NSPS: Number of spikelets per spike, NGPS: Number of grains per spike, TSW: Ten spike weight, TKW: Thousand kernel weight, GY: Grain Yield.

Table 4. Yield under irrigated and heat stress environment (kg ha^{-1}) with stress tolerance indices.

S. N	Genotype	Yp	Ys	TOL	SSI	MP	GMP	STI	YSI	YI	Red (%)
1	Bhrikuti	2725	1760.00	965.00	1.43	2242.50	2189.98	0.73	0.65	0.92	35.4
2	BL 4407	2665	2240.00	425.00	0.64	2452.50	2443.28	0.91	0.84	1.17	15.9
3	BL 4669	2195	1720.00	475.00	0.87	1957.50	1943.04	0.58	0.78	0.90	21.6
4	BL 4919	2965	2286.67	678.33	0.92	2625.83	2603.84	1.04	0.77	1.19	22.8
5	Gautam	2610	2293.33	316.67	0.49	2451.67	2446.55	0.92	0.88	1.19	12.1
6	NL 1179	2330	2260.00	70.00	0.12	2295.00	2294.73	0.81	0.97	1.18	3.0
7	NL 1346	2400	2180.00	220.00	0.37	2290.00	2287.36	0.80	0.91	1.13	9.1
8	NL 1350	2245	1633.33	611.67	1.10	1939.17	1914.90	0.56	0.73	0.85	27.2
9	NL 1368	2015	1753.33	261.67	0.52	1884.17	1879.62	0.54	0.87	0.91	12.9
10	NL 1369	1795	1406.67	388.33	0.87	1600.83	1589.01	0.39	0.78	0.73	21.6
11	NL 1376	2130	1446.67	683.33	1.29	1788.33	1755.39	0.47	0.68	0.75	32.0
12	NL 1381	2350	1673.33	676.67	1.16	2011.67	1983.01	0.60	0.71	0.87	28.7
13	NL 1384	3755	2473.33	1281.67	1.37	3114.17	3047.52	1.42	0.66	1.29	34.1
14	NL 1386	2640	1480.00	1160.00	1.77	2060.00	1976.66	0.60	0.56	0.77	43.9
15	NL 1387	2130	1386.67	743.33	1.41	1758.33	1718.60	0.45	0.65	0.72	34.9
16	NL 1404	2055	1946.67	108.33	0.21	2000.83	2000.10	0.61	0.95	1.01	5.2
17	NL 1412	2700	2320.00	380.00	0.57	2510.00	2502.80	0.96	0.86	1.21	14.0
18	NL 1413	3210	1766.67	1443.33	1.81	2488.33	2381.39	0.87	0.55	0.92	44.9
19	NL 1417	3185	2186.67	998.33	1.26	2685.83	2639.04	1.07	0.69	1.14	31.3
20	NL 1420	3010	2206.67	803.33	1.07	2608.33	2577.22	1.02	0.73	1.15	26.6
Mean		2555.5	1921.0	634.5	0.9	2238.2	2208.7	0.7	0.7	1.0	23.9

Note: YP; yield under irrigated condition, Ys-yield in the irrigated environment, TOL; tolerance index , SSI; stress susceptibility index ; GMP: geometric mean productivity, STI: stress tolerance index, YSI: yield stability index , YI: Yield index, Red: Yield Index.

Table 5. Correlation among yield under irrigated and heat stress condition and stress tolerance indices.

	Yp	Ys	TOL	SSI	MP	GMP	STI	YSI	YI	Red
Yp	1									
Ys	0.621**	1								
TOL	0.692**	-0.136	1							
SSI	0.416	-0.446*	0.936**	1						
MP	0.931**	0.865**	0.381	0.058	1					
GMP	0.901**	0.899**	0.312	-0.012	0.997**	1				
STI	0.909**	0.882**	0.337	0.014	0.994**	0.996**	1			
YSI	-0.416	0.446*	-0.936**	-1.000**	-0.058	0.012	-0.014	1		
YI	0.621**	1.000**	-0.136	-0.446*	0.865**	0.899**	0.882**	0.446*	1	
Red	0.416	-0.446*	0.936**	1.000**	0.058	-0.012	0.014	-1.000**	-0.446*	1

Note: * and ** denotes level of significance at 5 and 1%, respectively. Check table 4 for other abbreviation.

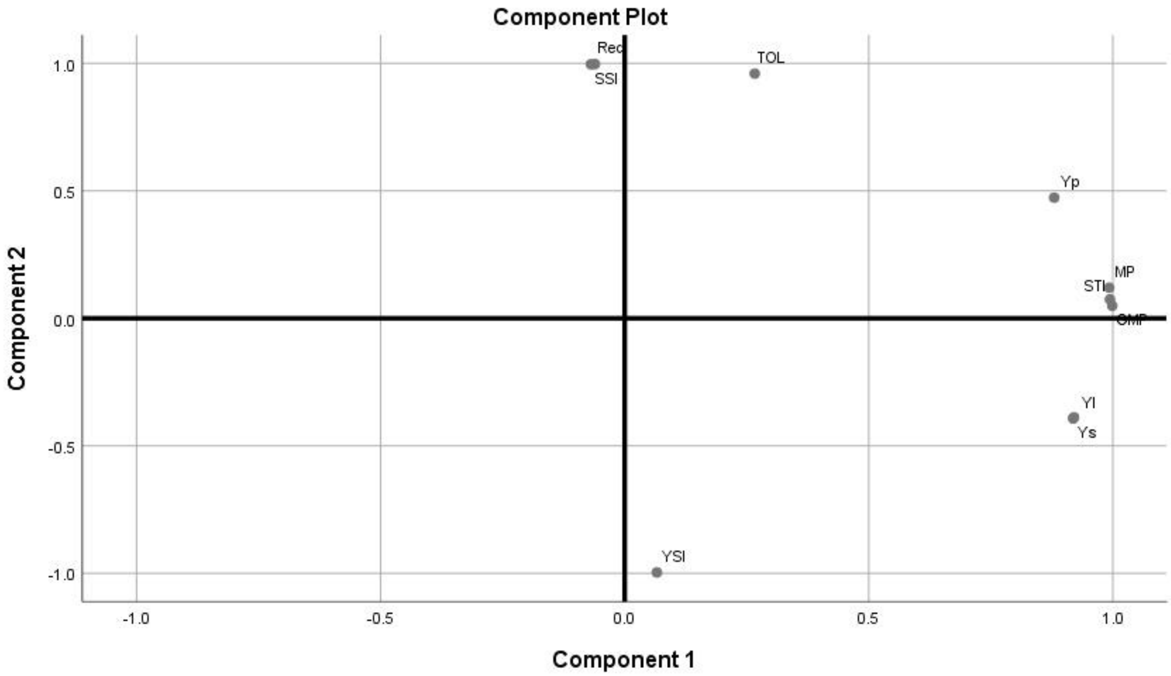

Figure 5. Biplot based on PC1 and PC2 using result of principal component analysis.

3.4. Correlation

To determine the most suitable heat stress tolerance selection criterion, we calculated the correlation between Yp, Ys, and other stress tolerance indices (Table 5). The analysis revealed a positive and significant relationship between grain yield under irrigated and heat stress conditions. This indicates that genotypes with high grain yield in normal irrigated conditions are likely to exhibit higher grain yields under heat stress conditions. A similar result was reported by Thapa et al. (2022), who found a positive and significant relationship between grain yield in irrigated and heat stress conditions.

On the other hand, SSI displayed a negative and significant correlation with yield under heat stress conditions, suggesting that an increase in SSI will lead to a significant decrease in yield. YSI exhibited a negative but non-significant correlation with yield under irrigated conditions, while it displayed a positive and significant correlation with yield under heat stress conditions. Therefore, selecting genotypes based on higher YSI and lower SSI values will help identify heat stress-tolerant genotypes. Poudel et al. (2021) also identified genotypes with higher YSI and lower SSI as heat-tolerant.

Additionally, MP, GMP, STI, and YI demonstrated positive and highly significant correlations with yield under both irrigated and heat stress conditions. As a result, these parameters MP, GMP, STI, and YI should be taken into consideration when selecting high-yielding genotypes under both conditions. These findings align with the results reported by Chand et al. (2022), Poudel et al. (2021), and Thapa et al. (2022).

4. Conclusion

Under heat stress condition, tested genotypes showed significant reduction in yield. So, it can be considered as one of the major causes of low wheat production. Grain yield had shown positive and highly significant correlation with MP, GMP, STI and YI in both environments. As a result, these are considered appropriate indices for the selection of high yielding genotypes under both irrigated and heat stress environment. NL 1384, NL 1412, Gautam and BL 4919 had shown higher production under heat stress condition with grain yield of 2473.33 kg ha^{-1}, 2320 kg ha^{-1}, 2293.33 kg ha^{-1} and 2286.67 kg ha^{-1} respectively. Hence, these genotypes can further be used for breeding program to cultivate in heat prone areas.

Conflicts of Interest: The authors declare no conflict of interest.

Availability of Data and Materials: Data will be available on formal request from the corresponding authors.

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