LR-4888
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RESEARCH ARTICLE Legume Research- An International Journal, Volume Issue : ()

Application of Stress Indices to Identify Terminal Heat Tolerance Genotype in Field Pea (*Pisum sativum* var. *arvense*)

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10.18805/LR-4888

ABSTRACT

Background: The exposure of field pea crop to high-temperature stress during the anthesis and pod development stage led to poor pollination, development of shriveled pods and poor performance that results in reduced seed yield. The study aims to screen and identify heat-tolerant genotypes based on stress tolerance indices. One hundred forty-three field pea genotypes, including three checks evaluated in augmented block design for seed yield per plant under normal and late sown conditions to identify terminal heat-tolerant genotypes of field pea.

Methods: Stress indices were computed based on seed yield per plant under normal (Yp) and late (Ys) sown conditions. The stress indices for seed yield are calculated as per the suggested method *i.e.* tolerance index (Hossain *et al.*, 1990), heat susceptibility index (Fischer and Maurer, 1978), yield stability index (Bouslama and Schapaugh, 1984), mean productivity (Hossain *et al.*, 1990), geometric mean productivity (Fernandez, 1992), stress tolerance index (Fernandez, 1992). Correlation coefficient and principal component analysis were done by using R (version 4.1.1) statistical software.

Result: Correlation coefficient analysis reveals that seed yield under stress conditions was positively correlated with the indices *viz*., MP, GMP, STI and YSI whereas, it was negatively correlated with HSI, signifying that higher estimates of MP, GMP, STI and YSI and lower of HSI correspond to heat tolerance. The seed yield under both environments had a positive and significant association with MP, GMP and STI. Genotypes *viz*., EC-341743, P-1679, P-1384-3, P-179 and P-781 were found with high indices scores and showing their suitability for the breeding program for heat tolerance. The first two principal components (PCs) accounted for 97.46% of the total variation present in the genotype.

Key words: Correlation, Field pea, Heat stress, PCA, Stress indices.

INTRODUCTION

Field pea (*Pisum sativum* var. *arvense* L.) is an annual, self-pollinated grain legume crop grown in high altitudes in tropical climates with temperatures between 7-30°C. Pea seeds are a good source of protein (21-25%), with high levels of lysine and tryptophan amino acids reflecting high nutritional value (Bhat *et al.*, 2013; Gregory *et al.*, 2016). In India, field pea occupies about 8.2 lakh hectares of area and production of 9.87 lakh tonnes with a productivity of 1204 kg/ha during 2017-18 (Anonymous, 2019). In India, the top field pea producers states are Uttar Pradesh and Madhya Pradesh. Uttar Pradesh alone occupies a 2.94 lakh-hectare area and produces 4.32 lakh tonnes of field pea seeds with productivity of 1469.3 kg/ha (Anonymous, 2019).

Field pea is a thermo-sensitive cool-season crop. In some cases, it is exposed to high-temperature stress during the anthesis and pod development stage which led to poor pollination, flower abortion and development of poor or shriveled pods. Terminal heat stress is one of the major abiotic stress that causes poor growth and performance of field pea, especially in the warmer region. High-temperature stress in field pea had resulted in a reduction of 60-80% loss of seed yield (Makasheva, 1984). Temperatures above 28°C reduce seed yield and the duration of flowering to maturity of field pea under dry soil conditions in western Canada (Bueckert *et al.*, 2015). High temperatures reduced the seeds number in field pea (Guilioni *et al.,* 2003). Jiang *et al.* (2019) studied the effects of thermal stress on phenology and yield of field pea and they found that heat stress reduced seed yield by speeding up the plant life cycle and reducing pod number and seed size. As a consequence, breeding for heat tolerance has now become an essential component of the pea improvement program. Considering the foregoing facts into account, the current study was carried out to identify heat-tolerant genotypes by employing

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How to cite this article: Seepal, Y.S., Sharma, V., Singh, C.M., Shukla, G., Gangwar, V., Kamaluddin and Singh, S.K. (2022). Application of Stress Indices to Identify Terminal Heat Tolerance Genotype in Field Pea (*Pisum sativum* var. *arvense*). Legume Research. DOI: 10.18805/LR-4888.

Submitted: 01-02-2022 **Accepted:** 02-07-2022 **Online:** 23-07-2022

some selection indices based on mathematical equations between stress and non-stress conditions.

MATERIALS AND METHODS

Planting material and experimental layout

The present investigation was done to evaluate 140 field pea germplasm lines with three check varieties *viz.*, IPF 4-9, Adarsh and IPFD 10-12 comprising experimental material, collected from ICAR-IIPR, Kanpur; CSAUAT, Kanpur and CCSHAU, Hisar. The experimental material was evaluated in Augmented Block Design in two environments during *rabi* 2020-21 at the Research Block of BUAT, Banda. The environments were created by two dates of sowing *viz.*, normal sown (November 10, 2020) and late sown (December 10, 2020). The experimental field was divided into 7 blocks and each block occupied 23 entries. Twenty test genotypes along with three checks were accommodated in each block. Each row was 4 m in length with a row's distance of 30 cm and plant to plant distance was 15 cm. Recommended cultural practices were followed to raise a healthy crop.

Environmental characterization

During the crop season, the maximum temperature was ranged from 31°C to 42°C and the minimum temperature was varied from 14°C to 21°C at the experimental site. In comparison to normal sown, the late sown crop faces a higher temperature than the optimum at the reproductive stage *i.e.* flowering to pod formation stage. This indicated that field pea was exposed to terminal heat stress in late sown condition (Fig 1).

Observations recorded

The observations were noted on five randomly sampled competitive plants from each genotype for seed yield per plant under normal (Yp) and late (Ys) sown conditions. The averages of the data from the sampled plant from each genotype were used for statistical analysis.

Statistical analysis

The correlation coefficient and principal component analysis (PCA) were calculated by using R (version 4.1.1) statistical software. The stress indices for seed yield per plant were calculated by using the following formulas:

(1) Tolerance index (TOL) = Yp - Ys

(Hossain *et al.*, 1990)

(2) Heat susceptibility index (HSI) =

$$\frac{\left(1 - \frac{Y_s}{Y_p}\right)}{\left(1 - \frac{\bar{Y}_s}{\bar{Y}_p}\right)}$$

(Fischer and Maurer, 1978)

(3) Yield stability index (YSI) = $\frac{Y_s}{Y_p}$

(Bouslama and Schapaugh, 1984)

(4) Mean productivity (MP) =

$$\frac{Yp + Ys}{2}$$

(Hossain *et al.*, 1990)

(5) Geometric mean productivity (GMP) =

$$\sqrt{Yp \times Ys}$$

(Fernandez, 1992)

(6) Stress tolerance index (STI) =

$$\frac{Yp \times Ys}{\bar{Y}p^2}$$

(Fernandez, 1992)

Where,

Yp and Ys- Seed yield of genotypes under normal sown and late sown conditions, respectively.

$\bar{Y}p$ and $\bar{Y}s$- Mean seed yield of all genotypes under normal sown and late sown conditions, respectively.

RESULTS AND DISCUSSION

Yield performance

The descriptive statistics including the lowest and highest values of genotypes and overall mean together obtained based on average data of one hundred forty-three germplasm lines (including three checks) of field pea for seed yield per plant in normal sown (Yp), seed yield per plant in late sown (Ys) and stress indices are summarized in Table 1.

The highest seed yield per plant in non-stress and heat stress conditions was 31.06 g (P-1545-1) and 11.80 g (EC 341743), respectively, while the lowest seed yield per plant in non-stress and heat stress conditions was 1.81 g and 0.27 g were found in KPMR 874 and EC 564812, respectively. The general mean for seed yield per plant in non-stress and heat stress conditions was 13.36 g and 4.15 g, respectively. Under heat stress conditions, the average seed yield is reduced by 68.94%. Makasheva (1984) reported a 60-80% loss of seed yield in field pea under hot and dry conditions.

Stress indices

In the present study, the seed yield of field pea genotypes under non-stress and heat stress conditions was used to calculate stress tolerance indices. Many researchers (Kumar *et al.*, 2020 in Mungbean, Shehrawat *et al.*, 2020 in wheat and Poudel *et al.*, 2021 in wheat) have used stress tolerance indices of seed yield to identify stress-tolerant genotypes.

The heat susceptibility index (HSI) measures the reduced performance of test genotypes under stress (heat stress) conditions and is used to identify heat-tolerant genotypes. To determine relative tolerance, the heat susceptibility index values were estimated for seed yield per plant of genotypes.

The minimum value of HSI was associated with genotypes P-1531 (0.30), P-1384-1 (0.33), P-1384-3 (0.49), P-864 (0.52), KPMR 874 (0.53), P-1679 (0.55), P-179 (0.56), KPMR 400 (0.58), P-781 (0.60), P-1448-2 (0.62) and the maximum value of HSI was related to genotypes KPMR 839 (1.28), 02/1088 (1.28), P-DMR-11 (1.30), 02/1118 (1.31),

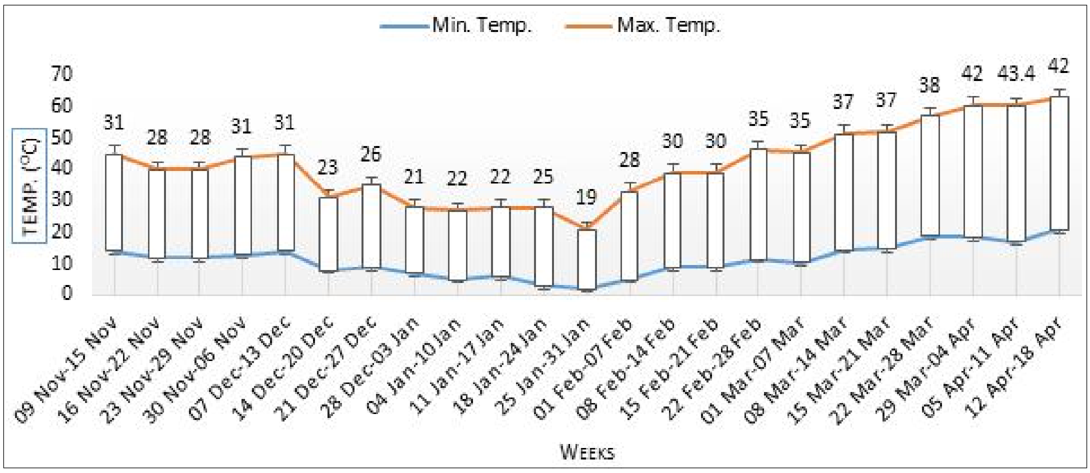

Fig 1: Weekly temperature data recorded during cropping period.

Table 1: Range and mean of seed yield per plant under the normal and late sown condition and stress indices for seed yield in field pea.

S.N.	Range		Mean
	Min.	Max.	
Yp	1.81	31.06	13.54
Ys	0.27	11.80	4.01
HSI	0.30	1.39	0.97
TOL	0.67	27.93	9.53
YSI	0.02	0.79	0.32
MP	1.47	17.37	8.77
GMP	1.39	16.45	7.08
STI	0.01	1.48	0.33

EC 564816 (1.31), P-1541-19 (1.31), EC 564805 (1.34), P-1697 (1.34), EC 564812 (1.37), 02/1119 (1.39). According to Kamrani *et al.* (2017), HSI-based selection aids in the identification of high-yielding genotypes under both conditions. Various genotypes were ranked as heat-tolerant (HSI < 0.75), moderately heat tolerant (HSI: 0.76-1.00) and heat susceptible (HSI > 1.00) based on the value and direction of desirability.

Based on the heat susceptibility index of seed yield, twenty-nine genotypes *viz.,* P-1384-3, P-1531, P-1384-1, P-864, KPMR 874, P-1679, P-179, KPMR 400, P-781, P-1448-2, P-1440-10, EC-341792, P-107, KPMR 522, P-1300, EC-341743, P-600, P-91-3, P-1535-2, P-122-19, IPF 15-8, P-1297-97, VL 202, P-133-2-1, NDP 2014-4, KPMR 913, HF 13-14, KPMR 928 and P-1597-11 showed least value of HSI (less than 0.75), indicating that these genotypes were slightly affected under late sowing conditions. In addition, forty genotypes showed HSI values (0.76 to 1.00), indicating moderately heat-tolerant responses under stress conditions.

The highest value of TOL exhibited by genotypes *viz.*, P-1545-1 (27.93), P-1697 (22.13), 02/1129 (21.21), P-1451 (20.97), P-108-2 (19.93), P-1440-21 (19.53), JM-6 (19.37), HFP 916 (19.21), P-1378 (18.93), 02/1119 (18.71). Under heat stress conditions, these genotypes had a low seed yield per plant. As a result, these genotypes were classified as heat susceptible. The lowest value of TOL belongs to genotypes *viz*., KPMR 874 (0.67), KPMR 400 (1.47), KPMR 225 (1.67), P-1535-2 (1.67), P-1531 (1.87), IPF 15-8 (2.01), P-1384-1 (2.47), P-864 (2.87), LFP 431 (3.01), NDP 2014-4 (3.01). Shehrawat *et al.* (2020) suggested that a lower TOL value is advantageous for the selection of high-yielding genotypes under stress conditions.

The greatest YSI value observed for genotypes P-1531 (0.79), P-1384-1 (0.77), P-1384-3 (0.66), KPMR 874 (0.63), P-864 (0.63), P-1679 (0.61), P-179 (0.61), KPMR 400 (0.59), P-781 (0.58), P-1448-2 (0.57) and lowest value of YSI associated with 02/1119 (0.02), EC 564812 (0.04), EC 564805 (0.06), P-1697 (0.06), 02/1118 (0.08), EC 564816 (0.08), P-1541-19 (0.08), P-DMR-11 (0.09), 02/1088 (0.1), VRP-3 (0.1). The genotypes with a high YSI value are considered stable under both heat stress and non-stress conditions.

The highest value of MP recorded for genotypes EC-341743 (17.37), P-1545-1 (17.1), Shikha (17.02), P-1532 (16.47), P-1451 (15.62), P-108-2 (15.1), JM-6 (15.02), P-1679 (14.92), EC-329761 (14.67), P-1438-1 (14.4). Under both heat stress and non-stress conditions, genotypes with a high MP value are more desirable for seed yield.

The highest value of GMP exhibited by genotypes EC-341743 (16.45), Shikha (15.65), P-1679 (14.48), P-1532 (14.36), P-1438-1 (12.62), EC-324131 (12.27), P-179 (12.24), EC-329761 (12.12), P-1384-3 (11.76), P-107 (11.63). The genotypes with a high value of GMP are also more desirable for seed yield in both environments.

The maximum value of STI associated with genotypes EC-341743 (1.48), Shikha (1.34), P-1679 (1.14), P-1532 (1.12), P-1438-1 (0.87), EC-324131 (0.82), P-179 (0.82), EC-329761 (0.80), P-1384-3 (0.75), P-107 (0.74). Higher value of STI revealed higher level of tolerance of genotypes to heat stress.

The genotypes with the highest value of MP, GMP and STI indicate that these genotypes were also productive under heat stress conditions when compared to other genotypes under study. Similar results were also reported by Kumar *et al.* (2020) in Mungbean and Poudel *et al.* (2021) suggested that choosing genotypes based on MP, GMP and STI would identify high yielding genotypes with heat tolerant. Identifying suitable stress indices would facilitate the crop improvement process for high-temperature tolerance.

Correlation among Yp, Ys and stress tolerance indices

The knowledge about the correlation coefficient between seed yield (Yp and Ys) and stress indices facilitate the development of an effective selection strategy aimed at improving seed yield for terminal heat tolerance.

The simple correlation coefficient among seed yield per plant under non-stress and stress conditions and stress indices of field pea were estimated between all possible pairs and the results are presented in Table 2. The heat map of the correlation between seed yield per plant (Yp and Ys) and stress indices are represented in Fig 2. The color assigned to the point in the heat map grid indicates the strength of a particular correlation between two characters. The level of correlation is indicated by red for positive correlations and blue for negative correlations, as represented in the color key.

In the present study, correlation analysis showed that the seed yield per plant under a stress environment had a positive and significant association with seed yield under a non-stress environment. Therefore, the selection of genotypes for terminal heat stress based on their performance in both environments (non-stress and heat stress) is fruitful.

Stress indices that had a significant correlation with seed yield under both heat stress and non-stress environments had been selected as the best ones, because of their ability to separate and identify the genotypes with high seed yield in both environments.

It was observed that seed yield under stress conditions was found to be positively correlated with the indices *viz.*, MP, GMP, STI and YSI whereas, it was negatively correlated with HSI, signifying that higher estimates of MP, GMP, STI and YSI and lower of HSI correspond to heat tolerance. The stress indices STI and GMP exhibited a maximum positive correlation with seed yield under stress conditions; therefore, they can be regarded as the best selection criteria for heat stress tolerance. Under a non-stress environment, seed yield recorded a significant positive correlation with all the stress indices except YSI. The seed yield under both environments had a positive and significant association with MP, GMP and STI. These results conform with those of Puri *et al.* (2015) in wheat. HSI and TOL had a significant positive association with seed yield (Yp) under a non-stress environment, while in a stressed environment, HSI was negatively and significantly correlated with seed yield (Ys), YSI, GMP and STI. TOL showed a non-significant and positive association with seed yield (Ys); a positive and significant correlation with MP, GMP and STI while, negative and significant association with YSI. GMP and STI showed a positive and significant relationship with all other stress indices and with each other, whereas, HSI had a negative and significant association with GMP and STI. YSI had a positive significant correlation with GMP and STI, while, negative and significant correlated with HSI and TOL. MP reflects a positive and significant correlation with TOL, GMP and STI. YSI had a significant and negative relationship with Yp, but a positive

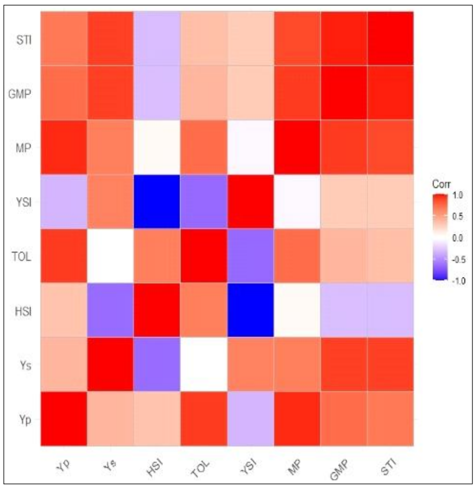

Fig 2: Heat map of simple correlation between seed yield (Yp and Ys) and stress indices.

Table 2: Correlation coefficients between different stress indices in field pea germplasm.

Stress indices	Yp	Ys	HSI	TOL	YSI	MP	GMP	STI
Yp	1.00	0.40**	0.34**	0.91**	-0.34**	0.95**	0.74**	0.69**
Ys		1.00	-0.65**	0.00^{NS}	0.65**	0.66**	0.90**	0.90**
HSI			1.00	0.66**	-1.00**	0.06	-0.30**	-0.31**
TOL				1.00	-0.66**	0.74**	0.40**	0.36**
YSI					1.00	-0.06^{NS}	0.30**	0.30**
MP						1.00	0.91**	0.87**
GMP							1.00	0.97**
STI								1.00

** Significant at 1% probability level, NS- Non-significant.

Yp: Seed yield of genotypes under the normal sown condition; Ys: Seed yield of genotypes under the late sown condition; YSI: Yield stability index; MP: Mean productivity; HSI: Heat susceptibility index; GMP: Geometric mean productivity; TOL: Tolerance index; STI: Stress tolerance index.

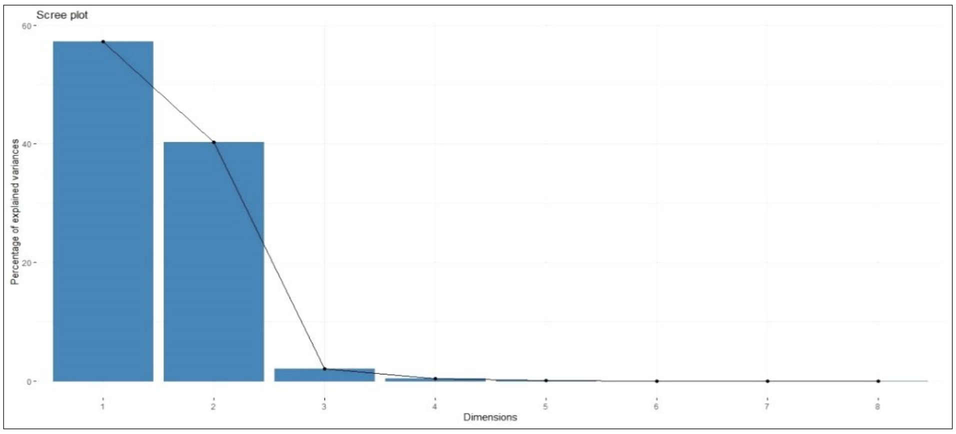

Fig 3: Scree plot showing variation for different PC values.

Table 3: Eigenvalue and variability (%) for seed yield (Yp and Ys) and stress indices in field pea.

Variables	PC1	PC2	PC3
Yp	-0.400	-0.280	-0.310
Ys	-0.382	0.312	0.263
HSI	0.071	-0.542	0.421
TOL	-0.266	-0.446	-0.457
YSI	-0.070	0.542	-0.426
MP	-0.454	-0.125	-0.166
GMP	-0.457	0.096	0.164
STI	-0.445	0.109	0.460
Eigenvalue	4.58	3.22	0.16
Variability (%)	57.21	40.26	2.02
Cumulative%	5 7.21	97.46	99.48

and significant correlation with Ys. As a result, picking genotypes with lower HSI values and higher YSI values aids in the identification of heat-tolerant genotypes under stress conditions.

Principal component analysis (PCA)

The result of PCA revealed that the first two principal components (PCs) displayed more than 1.00 eigenvalue and explained maximum variability of around 97.46% for seed yield (Table 3 and Fig 3). The eigenvalues are often used to determine how many factors to retain. The first principal component (PC1) showed the highest variability (57.21%) followed by the second principal component (40.26%). For PC1, HSI was the major positive contributor, while, GMP, MP and STI had negative weights. The PC2 was described only by YSI, STI, GMP and Ys with positive factor loadings, meanwhile, the remaining variables in that PC obtained negative loadings. The positive and negative loading indicates the existence of positive and negative correlation patterns between the components and the variables. Therefore, the variables in the PCs that loaded strongly positively or negatively contributed most to the diversity and they were the ones that most differentiated the clusters. Puri *et al.* (2015) in wheat crop reported that the first two components explained 97% of the variation.

CONCLUSION

Under the late sown condition, there was a significant reduction in seed yield indicating that terminal heat stress is one of the major constraints for field pea production. For estimating the effectiveness of selection indices for heat stress tolerance, 143 genotypes were evaluated in normal sown and late sown conditions. Seed yield had a positive and significant relationship with MP, GMP and STI in both environments. As a consequence, these indices are found to be appropriate for the selection of high-yielding genotypes in both non-stress and heat stress environments. Genotypes *viz.*, P-1384-3, EC-341743, P-1679, P-179 and P-781 had high yield potential under both conditions and showed their suitability for the breeding program for heat tolerance. The first two principal components (PCs) accounted for 97.46% of the total variation in genotypes. The variables that fall under these two PCs may be given due weight in terms of heat tolerance in field pea.

Conflict of interest: None.

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