Method for selection of superior lines with combined stay-green and stem reserve mobilisation traits in wheat under multi-environment stress conditions

Abstract

To meet the ever-increasing demand for food, there is a huge concern about the selection of superior genotypes with enhanced resilience to abiotic stress. However, choice/selection of suitable genotype/line is complex due to significant interaction of genotypes with environmental factors. Aiming few traits for selection may simplify the statistical analysis, however selection of genotypes using multiple traits is quite difficult. Furthermore, use of any single index may bias the selection of genotypes.Here,we proposed one method for selection of superior genotypes using combined approach of **four selection indexes** such as, multi-trait genotype-ideotype distance index (MGIDI), factor analysis and genotype-ideotype distance (FAI-BLUP), Smith-Hazel index (SHI) and multi-trait stability index (MTSI) under multi-environment stress **conditions by** using R programming. From our study,twenty random recombinant inbred wheat lines among the 220 lines developed by crossing HD3086 and HI1500 has been taken for analysis. The selection intensity (SI) was 15% for all the indexes. The lines were tested for stay-green and stem reserve mobilisation traits under control, drought, heat and combined stress conditions. Result found that RIL-10 was common to these **four indexes by using venn diagram** and was considered to be superior among the lines, which can perform better under multi-environment stress conditions. Thus, we recommend that combined approach of several indexes can be used a robust method for selection of ideal genotypes in wheat and other crop as well.

Keywords- FAI-BLUP, MGIDI, MTSI, SH index, Selection, Wheat

Introduction

To ensure global food and nutritional security by 2050, there must be a major improvement in the rate of genetic gain in crop yield, quality, input use efficiency, and adaptation to biotic and abiotic challenges (Kumar *et al*., 2016). In recent past, many physiological traits have been targeted for yield improvement. Furthermore, statistical analysts, geneticists, and plant breeders, all **havean** enduring fascination with exploring and incorporating Genotype (G) and Genotype× Environment (E) to choose better genotypes for use in breeding trials (Yan *et al.*, 2007). Several selection indexes have been used commonly for selection of genotypes namely, Smith-Hazel index (SHI), factor analysis and genotype-ideotype distance (FAI-BLUP). However, on account of multicollinearity issues amongst various traits, it is crucial to select superior genotypes or parents by appropriate use of selection indexes for use in future breeding programmes (Olivoto &Nardino, 2021). Several innovative data analysis techniques like principal component analysis (PCA), clustering has been used to remove data analysis bottleneck (Arya *et al.*, 2024).Currently, multi-trait stability index (MTSI) and the multi-trait genotype–ideotype distance index (MGIDI) are used for genotype selection in multi-environment trials based on desired idiotypes free from weighting coefficients and multicollinearity issues (Olivoto *et al.*, 2019; Olivoto & Nardino, 2021). However, use of single selection index may bias the selection of superior genotypes.

Considering above facts in mind, we hypothesized that combined use of various indexeswill be a robust method for selection of superior high-performance genotypes. Here, we proposed combined use of multi-trait genotype-ideotype distance index (MGIDI), factor analysis and genotype-ideotype distance (FAI-BLUP), Smith-Hazel index (SHI), multi-trait stability index (MTSI) for selection of superior genotypes using R programming.

Materials and Methods

Twenty recombinant inbred lines (RILs) out of 220 RILs have been taken for selection of superior genotypes. The recombinant inbred lines had been developed by crossing two contrasting wheat genotypes namely, HD3086 and HI1500. The lines were tested for its stay-green (SG) and stem reserve mobilisation (SRM) traits during rabi season, 2021-22 (Taria *et al.*, 2023) under control, drought, heat and combined stress conditions.**The normal sowing of wheat RILs was accomplished on 26th November 2021, while late sowing was done on 24th December 2021 to impose heat stress and combined stress.**

Under normal sown condition, drought stress was imposed during anthesis stage by withholding irrigation after booting stage. Heat stress was imposed by one-month late sowing as compared to normal sown condition to expose terminal heat stress. Combined heat and drought stress was imposed by one-month delayed sowing to normal sown condition along with withholding of irrigation after booting stage to impose drought stress. In all the stress conditions, stress was imposed from anthesis stage to physiological maturity. The populations were evaluated using an alpha lattice design with two replications. Each line (3 rows of 1 m each) was maintained with 22.5 cm distance between rows and 10 cm distance between plants. Under field conditions, proper agronomic measures were followed for uniform plant establishment. For stay-green traits, we recorded leaf senescence rate (LSR), soil plant analysis development (SPAD) value at different developmental stages, 1000 grain weight (TGW). For stem reserve mobilisation, we recorded SRM efficiency, stem-specific weight difference (SSWD) along with ear weight difference (EWD) as a proportion of mobilisation of stored carbon to grain under stress conditions.The minimum and maximum temperatures (°C), as well as precipitation (mm) during the wheat crop growing season (2021-22) are depicted in Fig.1. The average temperature at anthesis during normal sown condition was 15.5°C, whereas average temperature of 24.3°C during anthesis was recorded during late-sown condition.

For soil moisture content (SMC%), soil samples from each experimental units were collected using augers and stored in aluminium boxes with secure lids at anthesis, 10 days after anthesis (A+10), and 20 days after anthesis (A+20) in timely sown plots, and at anthesis, 5 days after anthesis (A+5), and 10 days after anthesis (A+10) in late-sown plots. The samples were immediately weighed and oven-dried for 72 hours at 105 °C. The SMC was calculated according to the formula given by Faulkner *et al.* (1989).The mean SMC in the root zone during the anthesis stage was 25.14% for control, 17.53% for drought stress, 23.49% for heat stress, and 14.34% for the combined stress conditions. The SMC at different developmental stages under control (a), drought (b), heat (c) and combined stress (d) conditions is depicted in Fig.2(a-d).

Selection of superior RILs across all the environments were accomplished by computing multi-trait genotype-ideotype distance index (MGIDI), factor analysis and genotype-ideotype distance (FAI-BLUP), Smith Hazel index (SHI) and multi-trait stability index (MTSI). Computation of multi-trait genotype-ideotype distance index (MGIDI) was based on ideotypes input (Olivoto & Nardino, 2021). MGIDI was calculated as follows-

$$\text{MGIDI} = \sqrt{\sum_{j=1}^{f} \left(F_{ij} - F_j\right)^2}$$

Where MGIDI is the multi-trait genotype-ideotype distance index, F_{ij} is the score of the **i^{th}genotype** in the j^{th} factor (i = 1, 2, ..., g; j = 1, 2, ..., f), being g and f are the number of genotypes and factors, respectively, and F_j is the j^{th} score of the ideotype. For the ideotype plan, all the traits except LSR&SSI were given higher for the selection of genotypes/lines.

For FAI-BLUP index, the desired ideotypes were defined by setting vector for minimum and maximum values of traits (Rocha *et al.*, 2018). Then, distance of each genotype from ideotype was calculated and converted into spatial probabilities as follows

$$P_{ij} = \frac{\frac{1}{d_{ij}}}{\sum_{i=1;j=1}^{i=n;j=m} \frac{1}{d_{ij}}}$$

Where P_{ij} is the probabilities of the i^{th} genotype (i =1, 2…. n) to be similar to j^{th} ideotypes (j = 1,2…m); d_{ij} is the genotype-ideotype distance from the i^{th} genotype to the j^{th} ideotype based on standardized mean Euclidean distance.

Smith Hazel index (SHI) (Smith, 1936; Hazel, 1943) was calculated according to the formulae given below

$$b = \mathrm{P}^{-1}\mathrm{G}\mathrm{w}$$

Where P and G represent phenotypic and genetic variance-covariance matrices, respectively. b and w represent vectors of index coefficients and economic weightings, respectively.

Multi-trait stability index (MTSI) based on stabilitywas calculated by the method described by Olivoto *et al*., 2019. For selection using all the selection indexes, *Metan* package in R programming has been used. Selection intensity (SI) of 15% was used for selection of superior lines using MGIDI, FAI-BLUP, SHI and MTSI.

The selection differential (SD) is calculated as difference between means of selected parents and means of the population (Azam *et al*., 2023). SD was calculated using *Metan* package in R programming version 4.3.2.

Results

Using multi-trait genotype-ideotype distance index, it was found that, RIL-12, RIL-10 and RIL-9 were found to be superior among the twenty lines (Fig.3). In-addition, same lines (RIL-12, RIL-10 and RIL-9) were found to be superior using FAI-BLUP (Fig.4). It is worth noting that both the indexes (MGIDI & FAI-BLUP) selected genotypes were in the same position, indicating reliability of these indexes in the selection of genotypes/lines. However, lines such as RIL-12, RIL-20 and RIL-10 were found to be superior among the 20 lines using Smith-Hazel index (Fig.5). Based on multi-trait stability index (MTSI), RIL-17, RIL-8 and RIL-10 were found to be superior (Fig.6). This indicates that use of single index in the choice of high-performance line may biased the selection of genotypes.Using Venn diagram, it was found RIL-10 was common to these indexes (Fig.7).

It was noted that selection differential for SRM traits (SRM, SRE and SSWD) were positive using selection indexes except MTSI (Table 1). In addition, selection differential for

SPAD value was higher at 20 days after anthesis, indicating selection at later stage of crop life cycle *i.e.* retention of greenness, will help in better selection of superior high-performance lines under multi-environment stress conditions. However, selection differential for TGW and EWD were higher using MGIDI, FAI-BLUP and MTSI except SHI. From our study it was found that, RIL-10 was superior and can perform better under adverse climatic conditions without any yield penalty.

Discussion

Selecting superior genotypes is complex due to the quantitative nature of important agronomic traits (Nogueira *et al.*, 2012). Focussing directly on a few factors/traits statistically simplifies the problem (Van Oijen&Höglind, 2016); howeverimportant information might be missed in data analysis.Several studies have shown that using multiple selection measures is effective for making selection process easy in plant breeding (Zuffo et al., 2020; Farhad et al., 2022; Padmaja et al., 2022). For this, breeders use selection indexes to select high performance and superior genotypes (Harikrishna *et al.*, 2016; Céron-Rojas & Crossa, 2018). MGIDI (Taria *et al.*, 2023; Debnath *et al.*, 2024; Malakondaiah*et al.*, 2025), FAI-BLUP (Santana *et al.*, 2023; Das *et al.*, 2024) and SH index (Hannachi & Fellahi, 2023) has been used for genetic diversity study and selection of genotypes. In addition, multi-trait stability index (MTSI) has been used by various workers based on multiple traits (Lima *et al.*, 2022; Padmaja *et al.*, 2022). Venn diagram can be used to select the common genotypes that has been selected by the various indices, presenting good performance in different environment (Ambrósio*et al.*, 2024). In our study, lower expressive gain of SRM and EWD can be explained by the fact that the genetic gain per trait is reduced when many traits are selected at once (Ambrósio*et al.*, 2024). However, for the traits when genetic gain is low, the genetic gain in the traits set can balance this reduction (Zetouni *et al.*, 2017; Almeida *et al.*, 2021).

The index's performance depends on number of traits, number of genotypes and degree of correlation between the traits. Overall, MGIDI & FAI-BLUP provided satisfactory genetic gain (Table 1). Moreover, superiority of MGIDI over all the indexes has been evident in datasets having low correlation between the traits (Ambrósio*et al.*, 2024).In choosing traits with intended gains, MGIDI typically has a success rate of 71.7% (Olivoto & Nardino, 2021).When trying to minimise the variable in the ideotype design, negative selection differentials become intriguing. By using selection indexes, it is possible to select genotypes by reducing LSR and SSI.Here, we may use positive selection differentials for traits we wish to improve and negative selection differentials for traits we want to reduce to apply the principle of selection of superior genotypes.Breeders and agronomists can get benefit from this, since it offers a novel, easily interpretable selection technique that takes the correlation structure between traits into account and allowing them to simultaneously choose for average performance while considering many traits (Olivoto *et al.*, 2019).

From our study, RIL-10 can be used as a donor for both SG and SRM traits in wheat. Henceforth, combined use of selection indexes must be adopted for selection of high-performance lines/genotypes in wheat and other crops as well.

Conclusion

Under the scenarios of global climate change, there is urgent need to develop crop varieties with capability to withstand multiple abiotic stress conditions. For development of superior genotypes, breeders need to transfer traits of interest from donor parents to elite cultivars. However, G×E interaction impose severe limitation on selection of high-performance lines.We have used several selection indexes under multi-environment condition to select superior lines. The multivariate indexes proficiently selected superior wheat RIL-10, showing desirable selection gain in the most of the traits. From our study, it was concluded that, combined approach of MGIDI, FAI-BLUP, SHI and MTSI mustbe used for robust selection of superior high-performance lines under multi-environment conditions as well as in multi-location breeding trials.

Authors Contribution

AA (A. Arora): conceptualization; ST: methodology; ST: writing original draft; ST: statistical analysis; SK (Sudhir Kumar), HK, SK (Sushil Kumar), BA,AA (A. Arunachalam): Editing whole manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest-The authors declare that they have no conflict of interest.

Disclaimer (Artificial Intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

Almeida, G. Q. D., Rocha, J. R. A. S. D. C., Pessoa, H. P., Alves, F. M., & Chaves, L. J. (2021). Selection of Hancornia speciosa germplasm accessions based on the FAI-BLUP index. *Pesquisa Agropecuária Tropical*, 51, e67226.https://doi.org/10.1590/1983-40632021v5167226.

Ambrósio, M., Daher, R. F., Santos, R. M., Santana, J. G. S., Vidal, A. K. F., Nascimento, M. R. et al. (2024). Multi-trait index: selection and recommendation of superior black bean genotypes as new improved varieties. *BMC Plant Biology*, *24*(1), 525.

Arya, S., Sahoo, R. N., Sehgal, V. K., Bandyopadhyay, K., Rejith, R. G., Chinnusamy, V. et al. (2024). High-throughput chlorophyll fluorescence image-based phenotyping for water deficit stress tolerance in wheat. *Plant Physiology Reports*, 29 (4), 278-293. https://doi.org/10.1007/s40502-024-00783-7

Azam, M. G., Hossain, M. A., Sarker, U., Alam, A. M., Nair, R. M., Roychowdhury, R. et al. (2023). Genetic analyses of mungbean [Vigna radiata (L.) Wilczek] breeding traits for selecting superior genotype (s) using multivariate and multi-traits indexing approaches. *Plants*, *12*(10), 1984.

Céron-Rojas, J.J. & Crossa, J. (2018). Linear selection indices in modern plant breeding. Springer Nature, Cham, Switzerland. https://doi.org/10.1007/978-3-319-91223-3

Das, A., Pal, M., Taria, S., Ellur, R. K., Kumar, S., Sathee, L., et al. (2024). Multivariate analysis and genome wide association mapping for chlorophyll fluorescence and grain number per panicle under high temperature stress in rice. *Plant Physiology Reports*. https://doi.org/10.1007/s40502-024-00808-1

Debnath, P., Chakma, K., Bhuiyan, M.S.U., Thapa, R., Pan, R. & Akhter, D. (2024). A Novel Multi Trait Genotype Ideotype Distance Index (MGIDI) for Genotype Selection in Plant Breeding: Application, Prospects, and Limitations. *Crop Design*, 100074. https://doi.org/10.1016/j.cropd.2024.100074

Farhad, M., Tripathi, S. B., Singh, R. P., Joshi, A. K., Bhati, P. K., Vishwakarma, M. K. et al. (2022). Multi□trait selection of bread wheat ideotypes for adaptation to early sown condition. *Crop Science*, *62*(1), 67-82.

Faulkner, S. P., Patrick Jr, W. H. & Gambrell, R. P. (1989). Field techniques for measuring wetland soil parameters. Soil Science Society of America Journal, 53(3), 883-890.

Hannachi, A. & Fellahi, Z.E.A. (2023). Efficiency of index-based selection for potential yield in durum wheat [Triticum turgidum (L.) ssp. turgidum convar. durum (Desf.) Mackey] lines. *Italian Journal of Agronomy*, 18, 2182.

Harikrishna, Singh, G. P., Jain, N., Singh, P. K., Sai Prasad, S. V., Ambati, D. et al. (2016). Physiological characterization and grain yield stability analysis of RILs under different moisture stress conditions in wheat (*Triticum aestivum* L.). *Indian Journal of Plant Physiology*, 21, 576-582. https://doi.org/10.1007/s40502-016-0257-9

Hazel, L.N. (1943). The genetic basis for constructing selection indexes. *Genetics*, 28(6), 476-490.

Kumar, S., Raju, D., Sahoo, R. N. & Chinnusamy, V. (2016). Phenomics: unlocking the hidden genetic variation for breaking the barriers in yield and stress tolerance.

Indian Journal of Plant Physiology, 21, 409-419. https://doi.org/10.1007/s40502-016-0261-0

Lima, G. W., Silva, C. M., Mezzomo, H. C., Casagrande, C. R., Olivoto, T., Borem, A. et al. (2022). Genetic diversity in tropical wheat germplasm and selection *via* multitrait index. *Agron. J.*, 114, 887–899. doi: 10.1002/AGJ2.20991

Malakondaiah, A. C., Arora, A., Krishna, H., Taria, S., Kumar, S., Devate, N. B. et al. (2025) Genome-wide association mapping for stay-green and stem reserve mobilization traits in wheat (*Triticum aestivum* L.) under combined heat and drought stress. *Protoplasma*.https://doi.org/10.1007/s00709-025-02031-7

Nogueira, A.P.O., Sediyama, T., de Sousa, L.B., Hamawaki, O.T., Cruz, C.D., Pereira, D.G. et al. (2012). Análise de trilha e correlações entre caracteresem soja cultivadaem duas épocas de semeadura. *Bioscience Journal*, 28(6), 877-888.

Olivoto, T., A.D.C. L\'ucio, J.A.G. da silva, B.G. Sari, & M.I. Diel. (2019). Mean performance andstability in multi-environment trials II: Selection based on multiple traits. *Agron. J.*, 111, 2961-2969.doi: 10.2134/agronj2019.03.0220

Olivoto, T., and Nardino, M. (2021). MGIDI: Toward an effective multivariate selection in biological experiments. *Bioinformatics*, 37(10), 1383-1389.

Padmaja, P. G., Kalaisekar, A., Tonapi, V. A.& Madhusudhana, R. (2022). A multi-season analysis of barnyard millet (Echinochloa frumentacea) germplasm lines for shoot fly resistance and multi-trait stability. *Plant Breed.*, 141, 399–407. doi: 10.1111/pbr.13011

Rocha, J.R.D.A.S.D.C., Machado, J.C & Carneiro, P.C.S. (2018). Multitrait index based on factor analysis and ideotype□design: Proposal and application on elephant grass breeding for bioenergy. *Gcb Bioenergy*, 10(1), 52-60.

Santana, A.S., de Souza Marçal, T., Vicentino Salvador, F., de Souza, M.H., da Silva, L.M.B., da Silva, M.B.P. et al. (2023). Analysis of advanced generation multistage field trials data in autogamous plant breeding: An evaluation in common Bean. *Crop Science*, 63(3), 1221-1233.

Smith, H.F. (1936) A discriminant function for plant selection. *Annals of Eugenics*, 7(3), 240-250.

Taria, S., Arora, A., Krishna, H., Manjunath, K.K., Meena, S., Kumar, S. et al. (2023). Multivariate analysis and genetic dissection of staygreen and stem reserve mobilisation under combined drought and heat stress in wheat (*Triticum aestivum* L.). *Frontiers in Genetics*, 14, 1242048. doi: 10.3389/fgene.2023.1242048

Van Oijen, M., &Höglind, M. (2016). Toward a Bayesian procedure for using process-based models in plant breeding, with application to ideotype design. *Euphytica*, *207*(3), 627-643.

Yan, W., Kang, M. S., Ma, B., Woods, S., & Cornelius, P. L. (2007). GGE biplot vs. AMMI analysis of genotype□by□environment data. *Crop science*, *47*(2), 643-653.

Zetouni, L., Henryon, M., Kargo, M., & Lassen, J. (2017). Direct multitrait selection realizes the highest genetic response for ratio traits. *Journal of Animal Science*, *95*(5), 1921-1925.https://doi.org/10.2527/jas.2016.1324.

Zuffo, A. M., Steiner, F., Aguilera, J. G., Teodoro, P. E., Teodoro, L. P. R., & Busch, A. (2020). Multi□trait stability index: A tool for simultaneous selection of soya bean genotypes in drought and saline stress. *Journal of Agronomy and Crop Science*, *206*(6), 815-822.

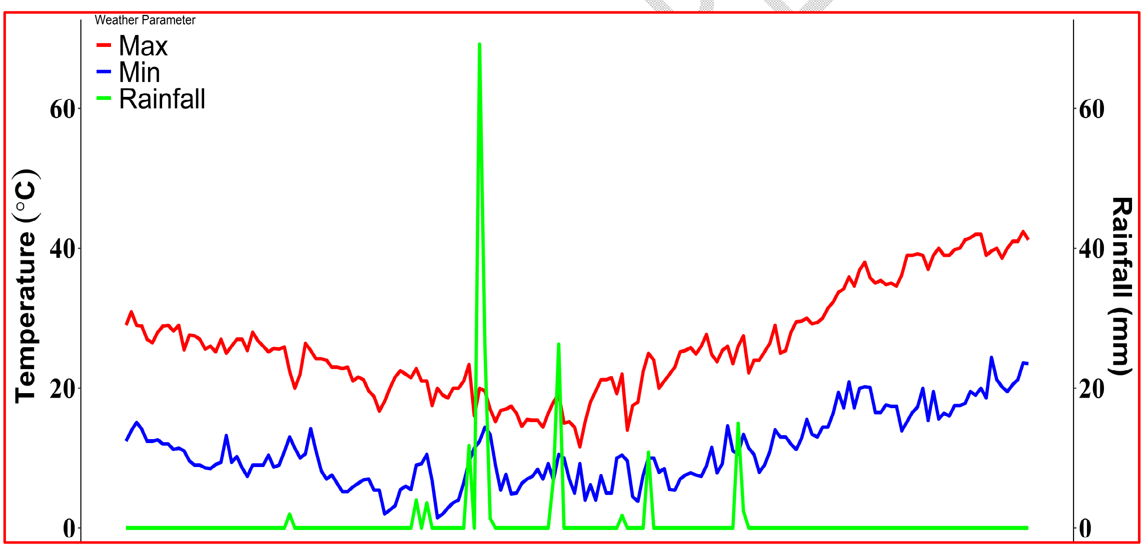

Fig.1 Weather parameters depicting maximum temperature (Blue lines), minimum temperature (red line), and rainfall (Green line) during 2021-22

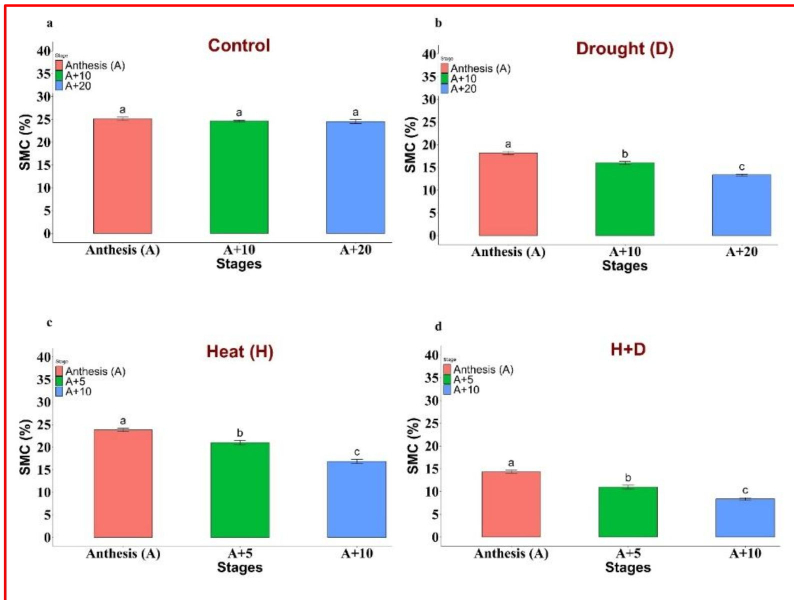

Fig.2 Soil moisture content (SMC %) recorded under control, drought (D), heat (H) and combined stress (H+D) conditions at different developmental stages during cropping season 2021-22

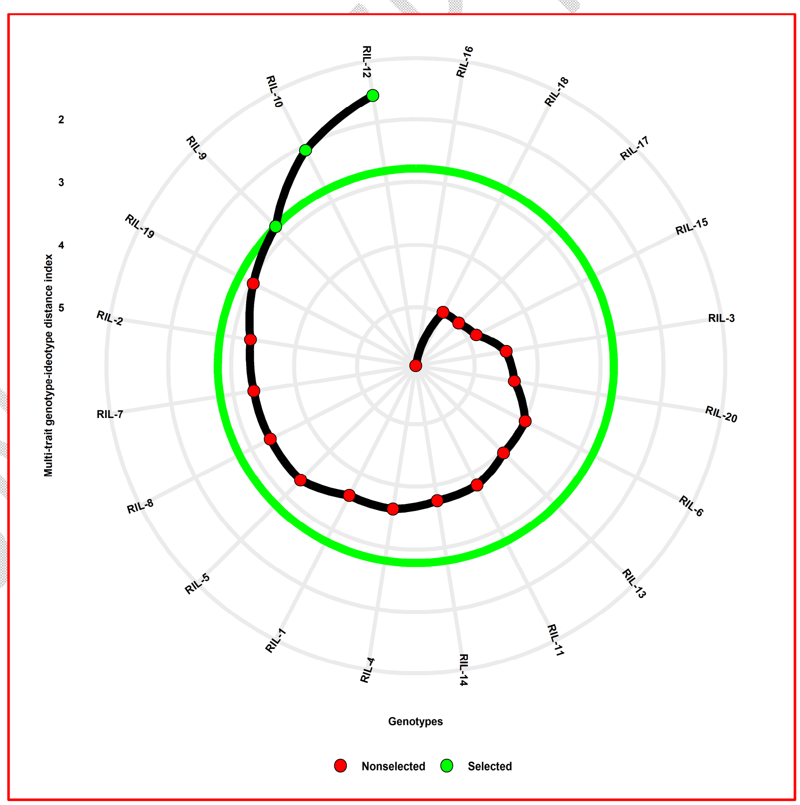

Fig.3 Ranking of RILs based on multi-trait genotype-ideotype distance index (MGIDI). Red and green dots represent non-selected and selected lines respectively.

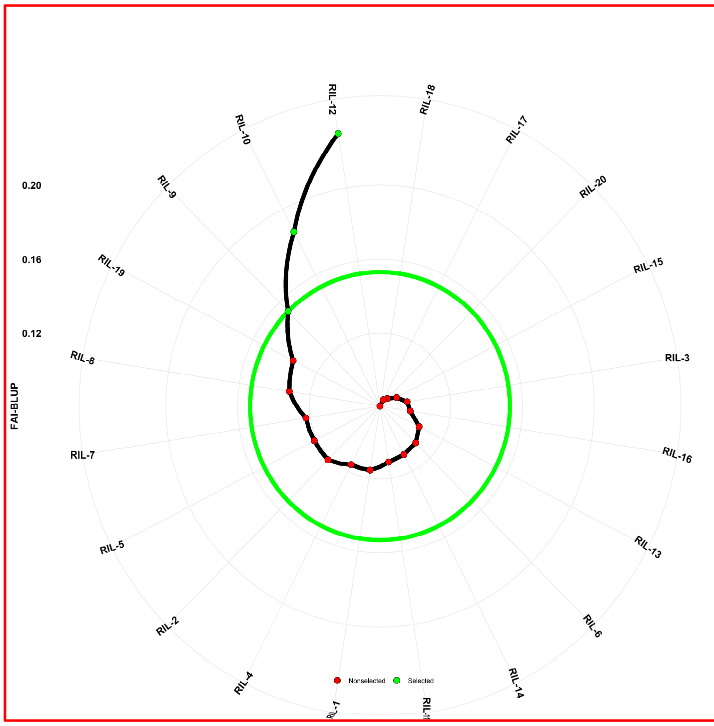

Fig. 4 Ranking of RILs based on factor analysis and genotype-ideotype distance (FAI-BLUP). Red and green dots represent non-selected and selected lines respectively.

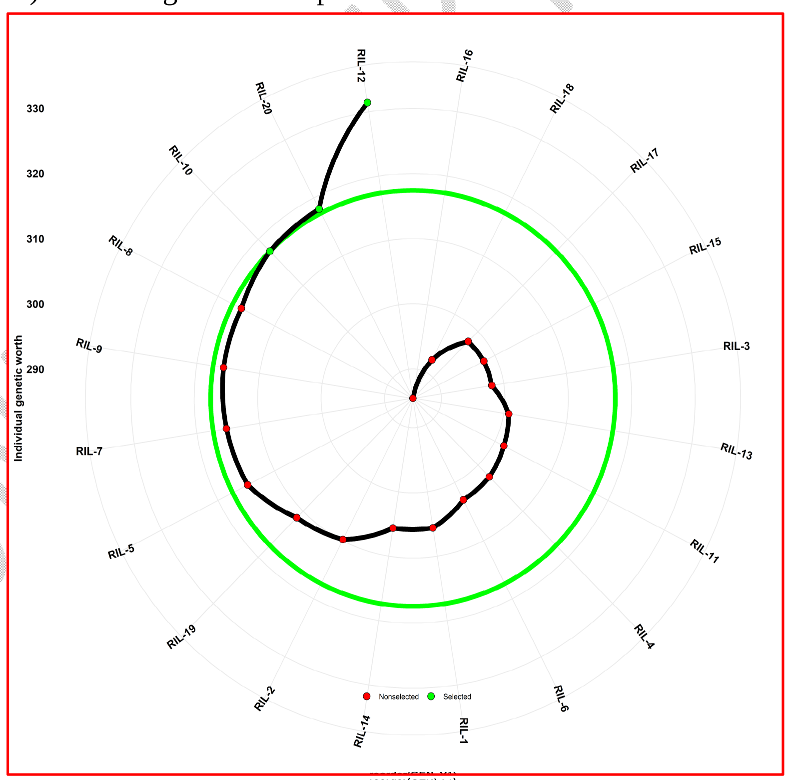

Fig. 5 Ranking of RILs based on Smith and Hazel index (SHI). Red and green dots represent non-selected and selected lines respectively.

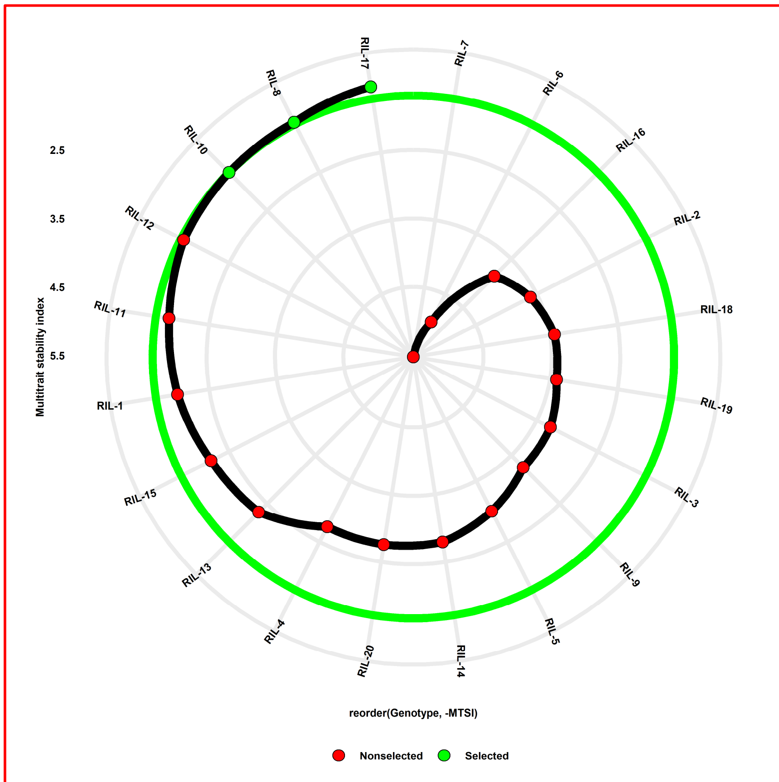

Fig.6 Ranking of RILs based on multi-trait stability index (MTSI). Red and green dots represent non-selected and selected lines respectively.

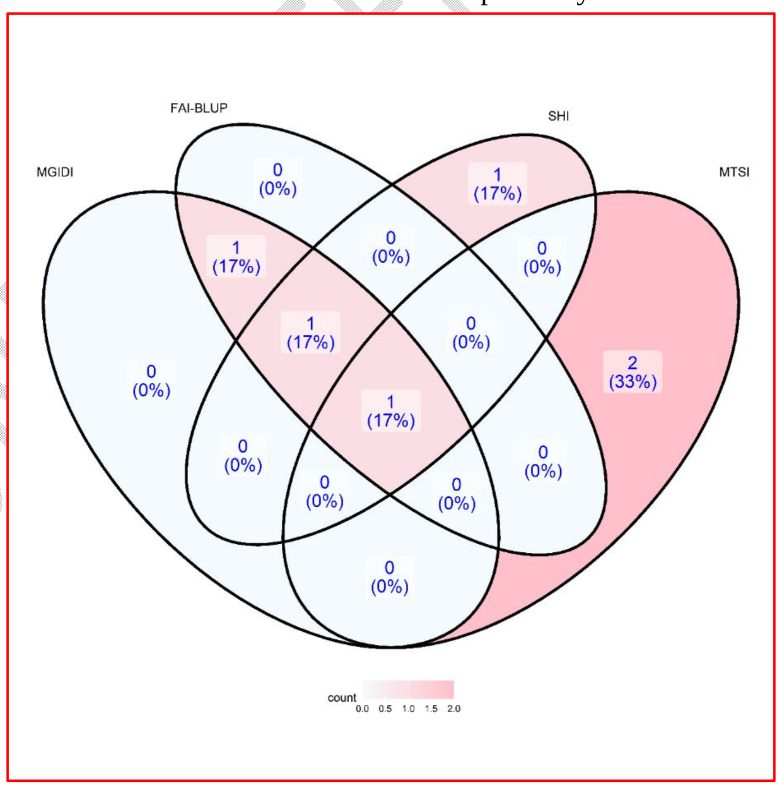

Fig.7Venn diagram depicting one best performing line (RIL-10) by employing MGIDI, FAI-BLUP, SHI and MTSI under multi-environment stress conditions.

Table 1Genetic selection differential based on indirect selection through MGIDI, FAI-BLUP, SHI and MTSI, considering selection intensity of 15%

Traits	Selection differential			
	MGIDI	FAI-BLUP	SHI	MTSI
LSR	-0.013	-0.013	0.000	-0.001
SRM	0.208	0.208	0.010	-0.020
SRE	5.161	5.161	0.350	-1.077
SSWD	6.567	6.567	0.780	-1.044
SPAD_A	1.157	1.157	1.091	-0.731
SPAD_A10	2.620	2.620	2.723	0.712
SPAD_A20	9.099	9.099	8.895	1.269
SSI	-0.282	-0.282	0.000	-0.144
TGW	3.354	3.354	0.973	2.342
EWD	0.116	0.116	0.055	0.252

(LSR- leaf senescence rate, SRM-Stem reserve mobilisation, SRE- SRM efficiency, SSWD- stem-specific weight difference, SPAD_A- Soil plant analysis development (SPAD) value at anthesis (A) stage, SPAD_A10- SPAD value at A+10 stage,SPAD_A20- SPAD value at A+20 stage, SSI- Stress susceptibility index, TGW- 1000 grain weight, EWD- Ear weight difference)