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Identification of Terminal Moisture Stress Tolerant Dolichos Bean (*Lab lab purpureus* L. Sweet) Recombinant Inbred Lines (RILs)

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ABSTRACT

Dolichos bean being rainfed crop frequently experiences of terminal moisture stress (TMS) with grain yield loss upto 50 per cent. Sustainable production of dolichos bean requires development and deployment of TMS tolerant cultivars. An effective strategy to breed dolichos bean for TMS tolerance is to screen the breeding lines for grain yield under both stress and non-stress environments, with selection based on an index (es) combining grain yield under TMS (Y_{TMS}) and MSF (Y_{MSF}) environments. Reported indices that integrate Y_{TMS} and Y_{MSF} are being used for selection of TMS tolerant genotypes in different crops but seldom in dolichos bean. The objectives of the present study were to (i) identify the most desirable index (es) to discriminate selected RILs for responses to TMS, (ii) identify index (es) that exhibit high correlation with YTMS and YMSF and (iii) identify most desirable TMS tolerant RILs. To address these objectives, we selected 33 RILs with 45-50 days to 50 per cent flowering from among 144 $F_{2:5}$ population. These RILs were evaluated under two moisture regimes (MSF and TMS) using alpha lattice design with two replications in four locations. Reported four drought tolerance indices, namely arithmetic mean productivity (AMP), geometric mean productivity (GMP), harmonic mean productivity (HMP) and stress tolerance index (STI) were used to detect and quantify variability among RILs and identify those tolerant to TMS environment. Grain yield of RILs reduced up to 64 per cent due to TMS. Based on the criteria of (1) better ability to discriminate RILs for response to TMS environment and (2) high correlation of indices with YTMS and YMSF, STI and HMP were found desirable. Based on STI and HMP, RIL 61, RIL 68 & RIL 120 were identified as TMS tolerant ones with high grain potential under both MSF and TMS environments.

Keywords : Terminal moisture stress, Moisture stress free, Recombinant inbred lines, Drought tolerance indices

DOLICHOS bean is one of the ancient traditional grain legume crops extensively grown in Southern India, especially in southern districts of Karnataka and Andhra Pradesh and northern districts of Tamil Nadu. It is grown both as a food grain (Ramesh and Byregowda, 2016) and fodder legume (Ramesh *et al.,* 2018). It is grown for fresh beans for use as vegetable, and for whole grain and split dhal for use in various culinary products. When grown for culinary purposes, matured dry pods are the harvestable products, while grains and split dhal are marketable and consumable products (Basanagouda *et al.,* 2022). When grown for fresh beans, fresh pods are harvestable and marketable economic products, while fresh beans are consumable products (Shivakumar *et al.,* 2016). Fresh beans, grains and split dhal are one of the good sources of protein for millions of people who depend on vegetarian diet as the major source of energy. Dolichos bean grain productivity in farmer's field (1.2 t ha^{-1}) is rather low compared to its potential productivity (2.0 t ha^{-1}) under well-managed production practices in research stations (Sushmita and Ramesh, 2020). This is because, dolichos bean frequently experience soil moisture stress (SMS) at pod filling and grain maturity stages as it is predominantly grown as a rainfed crop (Ramesh *et al*., 2018). SMS at pod filling and grain maturity stages is referred as terminal moisture stress (TMS). TMS occurs when soil moisture recedes mostly during pod filling stage (Pushpavalli *et al.*, 2014) and it affects growth and development in grain legumes (Baroowa and Gogoi, 2013). Vivek *et al.*, (2016) reported significant reduction in photosynthetic rate, number of seeds pod^{-1}, grain yield $plant^{-1}$, 100-grain weight and total biomass in dolichos bean genotypes subjected to managed moisture stress at 90 days after sowing which coincides with grain filling stage. In dolichos bean, grain yield losses attributable to TMS are estimated at 40 to 50 per cent (Susmitha and Ramesh, 2020).

Sustainable production of dolichos bean requires development and deployment of TMS tolerant cultivars. The adoption of drought tolerant cultivars is not only rapid, but also does not involve additional costs. Breeding dolichos bean for TMS environment is still in infancy (Susmitha and Ramesh 2020). Three approaches have been used to breed crop plants for tolerance to drought stress (Mitra, 2001). In the first approach, direct selection for grain yield under moisture stress free (MSF) environment is practiced. This approach is based on the hypothesis that genotypes that perform well under MSF environment do so under TMS environment as well (Blum, 2011). Most often, this hypothesis is not necessarily true. In the second approach, direct selection for high yield under TMS environment is practiced. Due to significant genotype × TMS interaction coupled with low heritability, direct selection for grain yield potential under TMS environment has been less effective. As a result, progress of breeding for tolerance to TMS is rather slow (Mitra, 2001). The arguments favouring ineffectiveness of the second approach are as follows. Differential performance of selected genotypes across diverse moisture regimes (MR) is caused either by differential responses of same set of genes to changes in MR or by expression of different sets of genes in different MR (Falconer, 1990; Holmes & Robertson, 1959; Atlin and Frey, 1989) and Falconer (1990) further opined that grain yield in TMS and MSF environments, therefore not necessarily could be maximized. Theoretical investigation has shown that selection under TMS environments is most likely to result in reduced grain yield under MSF environments (Rosielle & Hamblin, 1981; Hohls, 2001 and Mardeh *et al.,* 2006). In third approach, the ability of high yielding geno types to tolerate TMS is enhanced by transferring genes controlling morphological, physiological and biochemical traits contributing to tolerance to TMS. Even this third approach proved less effective due to inadequate understanding of genetic basis of these traits contributing to TMS tolerance (Richards, 1996 and Mitra, 2001). Considering the demerits of these three approaches, an alternative one which is the combination of the first two is suggested.

In the alternative breeding approach, direct selection for high grain yield under MSF environment and stability of yield (with minimal reduction in yield) under TMS environment is practiced (Mitra, 2001 and Bennani *et al.,* 2017). Rosielle and Hamblin (1981), Atlin & Frey (1989), Ceccarelli *et al.* (1998) and Hohls (2001) have theoretically demonstrated the effectiveness of alternative approach. They opined that to improve grain yield across a range of environments which include both TMS and MSF ones, selection should be based on mean productivity. Hohls (2001) further showed that correlation between tolerance and grain yield in TMS environments is always positive and selection for high mean productivity is always positively correlated with mean grain yield in TMS environments. The alternative approach has been proved empirically effective in tropical maize and wheat (Banziger and Cooper, 2001) and rice (Venuprasad *et al.,* 2007).

Researchers such as Calhoun *et al.* (1994), Ceccarelli *et al.* 1998) and Venuprasad *et al.* (2007) based on yet another empirical data opined that an effective strategy to breed crops for drought tolerance is to screen breeding lines for grain yield under both stress and non-stress conditions, with selection based on an index yield that combines grain under TMS and MSF environments. The alternative approach however, is not empirically verified adequately in dolichos bean. Suitable indices that are based on the combination of grain yield under MSF and TMS environments are necessary to select TMS tolerant genotypes. Several indices are being frequently used by breeders to identify drought tolerant genotypes in different crops. These indices are based on reduction in economic product yield under TMS environment (Y_{TMS}) in comparison to that under MSF environment (Y_{MSF}). In dolichos bean, four indices namely, arithmetic mean productivity (AMP), geometric mean productivity (GMP), harmonic mean productivity (HMP) and stress tolerance index (STI) have been suggested as most desirable ones for quantification of TMS tolerance and selection of TMS tolerant genotypes (Sushmita and Ramesh, 2020). For effective use of these indices, it is necessary to identify those that discriminate well the test genotypes and have high correlation with Y_{TMS} and Y_{MSF}. Under these premises, the objectives of the present study were to (i) identify the most desirable index (es) to discriminate selected RILs for responses to TMS, (ii) identify index (es) that exhibit high correlation with Y_{TMS} and Y_{MSF} and (iii) identify most desirable TMS tolerant RILs.

Material and Methods

Experimental Material

The experimental genetic material consisted of 33 RILs derived from HA 4 × HA 5 biparental cross. Both HA 4 and HA 5 (Ramesh *et al.* 2018) are high yielding released pureline varieties. While HA 4 is photoperiod insensitive (PIS) pureline variety with determinate growth habit, HA 5 is PIS pureline variety with indeterminate growth habit. HA 4 and HA 5 are contrasting for number of racemes plant^{-1}, number of fresh pods plant^{-1} and fresh pod yield plant^{-1} (Ramesh *et al.* 2018). These RILs were carefully chosen such that their 50 per cent flowering ranged between 45 to 50 days. This narrow window of 50 per cent days to flowering help avoid possible confounding effects of differential timing of imposition of TMS environment.

Evaluation of Experimental Material

The RILs were evaluated in two moisture regimes (MR), namely, TMS and MSF environments during 2023 summer season following alpha lattice design with two replications at four locations. The four locations are (i) experimental plot of Department of Genetics and Plant Breeding (GPB), College of Agriculture (CoA), University of Agricultural Sciences (UAS), Bangalore, (ii) experimental plot, CoA, Chintamani, UAS, Bangalore, India, (iii) experimental plot, CoA, Hassan, UAS, Bangalore, India (iv) farmer's field, Belagavi, Karnataka, India. In both the MR, the seeds of each 33 RILs were sown in a single row of 3m length. 10 days after planting, seedlings were thinned to maintain recommended spacing of 0.3m between the plants within a row. In all the locations, RILs were maintained by providing need-based irrigation till grain maturity to create MSF environment. To create TMS environment, the RILs were maintained by with holding irrigation during pod filling stage till the grain maturity. All the other recommended crop production and protection practices were followed to maintain the crop free from other abiotic stresses and biotic stresses at both the locations. Eight to 10 plants survived to maturity in each MR.

Sampling and Data Collection

Dry pods were harvested from five randomly selected plants (avoiding border ones) from each RIL evaluated in both MSF and TMS environments. The pods were hand-threshed, sundried and weighed and the data was recorded as grain yield plant^{-1} using standard protocol (Byregowda *et al.*, 2015). Replication-wise means of grain yield plant^{-1} were used for statistical analysis.

TABLE 1

The formulae to estimate the indices which were used to quantify the responses of RILs to managed TMS relative to MSF experiments

Index	Formula	Reference
Harmonic mean productivity (HMP)	$HMP = 2 \times \frac{(Y_P \times Ys)}{(Y_P + Ys)}$	Fischer and Maurer(1978)
Stress tolerance index (STI)	$STI = \frac{Y_P \times Ys}{Y_P}$	Fischer and Maurer (1978)
Arithmetic mean productivity (AMP)	$MP= (Y_P+ Ys)\ 2$	Rosielle and Hamblin (1981)
Geometric mean productivity (GMP)	$GMP= (Y_P\times Ys)^{0.5}$	Fernandez (1992)

Statistical Analysis

Location-wise ANOVA was performed to examine the significance/otherwise of differences among RILs for grain yield plant^{-1} at all the locations under MSF and TMS environments. The analysis was implemented in R version 4.2.1 (R core team, 2021). Pooled ANOVA was performed to detect interaction of RILs with two MR for grain yield plant^{-1} using three factor analysis in OPSTAT (Sheoran *et al.* 1998). The mean grain yield of RILs in both the MR was estimated as two different statistics namely, Best Linear Unbiased Predictors (BLUPs) and yield relative to environment maximum (YREM). While, BLUP was estimated using meta-R software (Alvarado *et al.,* 2015), YREM was estimated using MS Excel software. YREM of RIL/genotype, Yij = Xij /MAX ij (Yan, 1999), where Yij and Xij are the YREM and trait value, respectively of 'ith' RIL in moisture stress 'j'. MAX j is the maximum grain yield (of any genotype) observed in environment 'j'. The efficiency of correlated (indirect) response (CR) in TMS environment to selection in MSF environment relative to response to direct selection in TMS environment was predicted as CR/DR = rg. hy/hx, where, rg = genetic correlation coefficient between Y_{TMS} and Y_{MSF} and hy and hx are square roots of heritabilities in MSF and TMS environments. For further statistical analysis, BLUPs estimated across the four location was used.

Identification of RILs with Tolerance to Managed TMS Environment

Quantification of Responses of Genotypes to TMS

Previously developed and reported four indices (Table 1) were estimated to quantify the responses of RILs to TMS environment for grain yield plant^{-1}. The four indices were estimated based on the extent of reduction in grain yield plant^{-1} of the RILs evaluated under MSF environment relative to those evaluated under TMS environment.

Criteria to Identify Desirable Indices for Effective Discrimination of the RILs for Responses to Managed TMS

First-degree statistics such as standardized range (SR) and second-degree statistics such as phenotypic coefficient of variation (PCV) for grain yield plant^{-1} were estimated as SR = ([(Highest grain yield Lowest grain yield)])/ (mean grain yield) and PCV = (grain yield standard deviation)/ (mean grain yield) × 100. Discriminating ability of the RILs was assessed based on the magnitudes of SR and PCV. Higher the estimate of SR and PCV better is the discriminating ability of the indices.

Relationship of Indices with Grain Yield Plant^{-1} of RILs Evaluated Under MSF and TMS Environments and Among the Indices

Correlation coefficients of the indices with grain yield plant^{-1} of RILs evaluated under MSF and TMS environments and among the indices were estimated.

Criteria to Identify TMS Tolerant RILs

TMS tolerant RILs were identified as those with (i) higher magnitudes of STI, AMP, GMP and HMP / their combination along with better ability to discriminate the RILs and (ii) high correlation of such indices with grain yield under MSF and TMS environments. As TMS tolerant genotypes varied with the indices, rank sum (RS) method (Farshadfar *et al.,* 2012) which combine all the indices into one integrated index was used to select TMS tolerant RILs. Lower the rank-sum, better is the tolerance of RILs.

Selection of TMS Tolerant RILs based on the Combination of Indices and Grain Yield Under MSF and TMS Environments

Based on the (i) combination of indices identified as desirable to select TMS tolerant RILs and (ii) high grain yield plant^{-1} under MSF and TMS environments, the genotypes were grouped into four classes (A, B, C and D) of responses to TMS (Fernandez, 1992). Class 'A' response RILs are those which expressed superior performance in both MSF and TMS environments. Class 'B' response RILs are those which performed better only in MSF environments. Class 'C' response RILs are those which performed better only in TMS environment. Class 'D' response RILs are those which performed poorly in both MSF and TMS environments. Three dimensional graphs were drawn by plotting grain yield plant^{-1} under MSF and TMS environments on X-axis and Y-axis, respectively and indices on Z-axis (Fig.1) to group the RILs into A, B, C and D response classes. These graphs were plotted using 'NCSS' software (NCSS software, 2023).

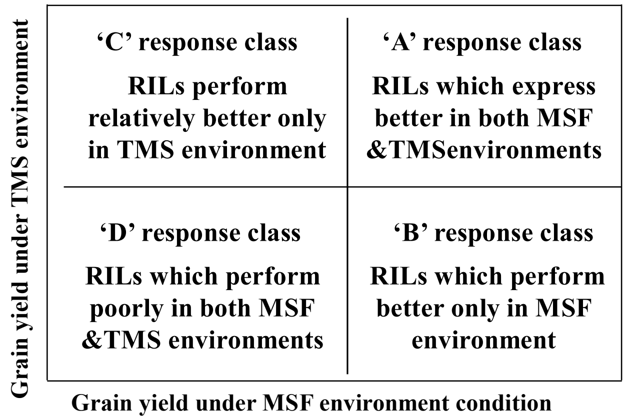

Fig. 1: Schematic illustration to demonstrate the protocol to classify the RILs into 'A', 'B', 'C' and 'D' response classes

Results and Discussion

Analysis of Variance

RILs differed significantly for grain yield plant^{-1} as revealed from significant mean squares attributable to RILs (Table 2). These results justified the selection of the RILs for the study. Further, the RILs performed differentially across the two MR for grain yield plant^{-1} as indicated from significant mean

Table 2

Pooled analysis of variance of RILs evaluated for grain yield plant^{-1} under TMS and MSF environments

Source	Degrees of freedom	Mean sum of squares	'F' statistics	Probability value
RILs	32	225.84	9.45	1×10^{-10}
Replication	01	7.213	0.30	00.86
Location	03	843.02	35.28	1×10^{-5}
Moisture Regimes (MR)	01	5,973.83	249.99	1×10^{-3}
RILs × Location	96	8.35	0.35	01.00
RILs × MR	32	203.46	8.51	1×10^{-7}
Genotypes × Location × MR	96	11.02	0.46	00.99
Residuals	263	23.89		

squares attributable to RIL × MR interaction. Significant RILs × MR interaction results from either significant difference among RILs / significant variability attributable to the study environments (Gauch, 2013) such as contrasting MR in the present study, although the latter dominated (Table 2). However, non-significant mean squares attributable to locations and RILs × location interaction suggested that location environments appeared to exert comparable responses of RILs in both MSF and TMS environments. Non-receipt of rains during intended crop growth stage, *i.e.,* during pod filling and grain maturity stages in all the locations suggested successful imposition of TMS, which amply reflected by significant mean squares attributable to RILs × MR interaction in pooled ANOVA (Table 2) and substantial reduction in mean grain yield plant^{-1} of RILs in the two MR (Fig. 2).

Effect of TMS on Grain Yield of RILs

TMS environment considerably affected grain yield plant^{-1} of RILs. The RILs varied widely for grain yield plant^{-1} under TMS environment relative to that under MSF environments. The mean *per cent* reduction in grain yield plant^{-1} varied between 1.02 to 63.43 per cent (Fig. 3). A wide range of reduction in grain yield plant^{-1} suggest that the imposed level of TMS was sufficient enough to discriminate the RILs for their responses and hence their degree of tolerance to TMS environment.

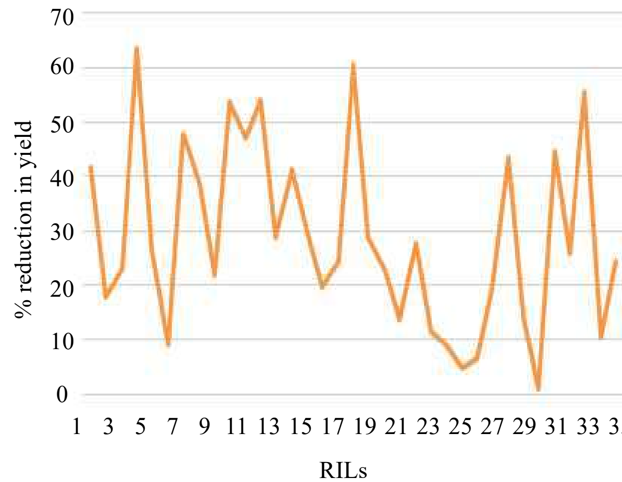

Fig. 3 : Per cent reduction in grain yield plant^{-1} of RILs evaluated under TMS and MSF environments

Relationship Between Grain Yield of RILs Under MSF and TMS Environments

A rather low phenotypic correlation coefficient (0.45) between grain yield under MSF and TMS environments (Fig. 4) could be attributed to change in performance ranks and significant RILs × MR interaction (Table 2). These results also suggest that RILs' performance for grain yield under MSF is not a good indication of their performance under TMS environments and *vice-versa.* Low correlation could be attributed to possible involvement of differential physiological processes in partitioning of photosynthates into grains (Falconer, 1990) under MSF and TMS environments. Theoretical

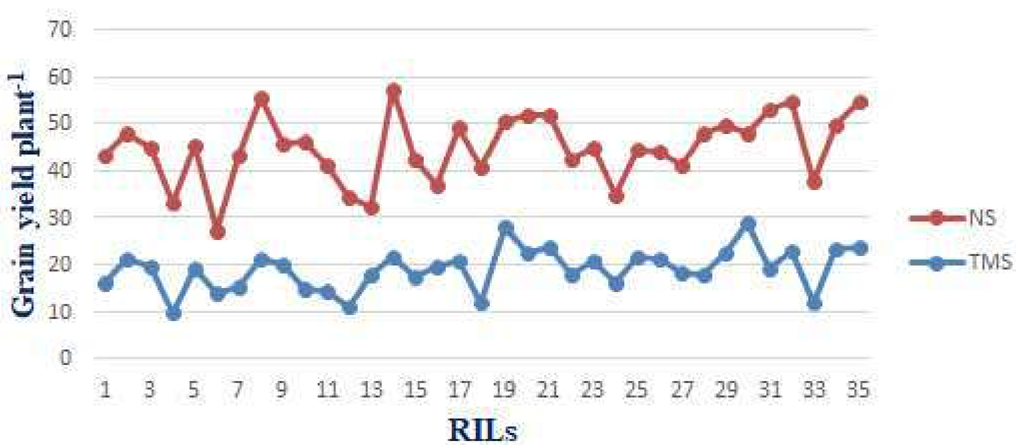

Fig. 2 : Grain yield plant^{-1} of RILs evaluated under TMS and MSF environments

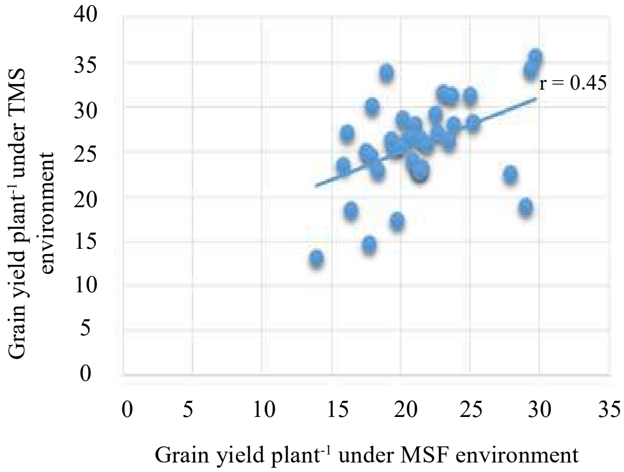

Fig. 4 : Relationship for grain yield plant^{-1} of RILs evaluated under MSF and TMS environments

results reported by Rosielle and Hamblin (1981) implicitly indicate that at each of the several loci controlling the target trait (grain yield in the present study), alleles controlling grain yield under MSF environment are different from those controlling grain yield under TMS environment. It is therefore argued that grain yield measured under MSF and TMS environments must be treated as two different traits and are likely to be influenced to certain extent by different genes, though partly also by same genes (Falconer, 1990). The two traits are genetically correlated and the magnitude of correlation reflects the extent to which the same genes are involved. Hence, grain yield under MSF and TMS environments cannot be maximized when correlation is low and a little compromise in grain yield is therefore necessary.

Choice of Selection Environment

The choice of selection environments is one of the most frequently debated researchable issue especially while breeding crops for abiotic stress tolerance (Ceccarelli *et al.*, 1998). Conventionally selection is implemented in experimental research stations under MSF environments with an assumption that magnitudes of genetic differences and heritability (h^2) in MSF environments are greater than those in TMS environments. This assumption is based on the several empirical studies which have indicated that estimates of h^2 are most often higher for target traits measured under MSF environments than those measured under stress environments (Blum, 2005 and Blum, 2011). However, this assumption is not necessarily true as evident from our and other empirical studies. In our study, the estimates of h^2 of grain yield plant^{-1} were higher under TMS environment than those under MSF environments (Table 3). Other researchers such as Ceccarelli *et al.* (1998) have also shown that it is not always true that h^2 is higher in MSF than in stress environments (Falconer, 1990 and Simmonds, 1991).

TABLE 3

Estimates of relative magnitudes of variability due to genetic and non-genetic sources, heritability and predicted correlated response (CR) to selection in dolichos bean

	TMS environment	MSF environment
Genotypic variance	21.30	24.65
Residual variance	20.89	24.23
Heritability	0.48	0.42
Correlated response in TMS environment to selection in MSF environment	0.93	

The efficiency of indirect response in TMS environment to selection under MSF environment relative to direct selection for grain yield under TMS environment can be predicted using the estimates of h^2 under TMS and MSF environments and genetic correlation (r_g) between YTMS and YMSF. Higher h^2 in TMS environment with low r_g (0.45) between YTMS and YMSF suggest that selection should be implemented under TMS environment to identify RILs with improved tolerance to TMS. This inference is amply supported by less than unit (0.93) ratio of indirect response of RILs in TMS environment to selection in MSF environment to the direct response of RILs to selection in TMS environment (Table 3). Indirect response of RILs in TMS environment to selection in MSF environment can never be more effective than direct response even when estimates of h^2 in TMS and MSF environments are comparable given that r_g between YTMS and YMSF cannot exceed 1.00. This further means that h^2 in MSF environment should be twice as large as h^2 in TMS environment and r_g between YTMS and YMSF should be more than 0.50 for indirect response of RILs in TMS environment to selection in MSF environment to be more effective than direct response. Such results in real situation are a remote possibility. Thus, though our results indicate effectiveness of direct selection in TMS environment, Rosielle & Hamblin, (1981) theoretically showed that selection in TMS environment results in reduced grain yield in MSF and reduced average grain yield in both TMS and MSF environments. This is further supported by lower genetic variance in TMS environment compared to that in MSF environment (Table 3) However, farmers prefer cultivars that produce optimum grain yield under MSF environment with least reduction in grain yield under TMS environment. Several researches such as Clarke *et al.* (1992), Ud-Din *et al.* (1992) in wheat and Byrne *et al.* (1995) in maize have concluded that selection of genotypes for moisture stress tolerance will be most effective when they are evaluated under both MSF and TMS environments. Trethowen *et al.* (2002) showed that selection in alternating MSF and TMS environments at international center for Maize and Wheat Improvement (CIMMYT), Mexico has resulted in significant progress in development of wheat germplasm adapted to dry areas. It is in this context, the indices which provide objective measures of tolerance based on the reduction in grain yield under TMS environment relative to MSF environments have been proposed and are being used for identifying TMS tolerant genotypes in different crops (Mitra, 2001). There indices have proved handy to discriminate the test genotypes for responses to TMS environments and to select the ones with better tolerance to TMS environment.

Previously reported four indices namely AMP, GMP, HMP and STI in dolichos bean (Sushmitha and Ramesh, 2020) are used in the present study to quantify the responses of RILs to TMS environment and to select TMS tolerant RILs. We used two criteria to identify desirable indices. These are (i) indices with good discriminating ability and (ii) indices with high magnitude of correlation with YTMS and YMSF.

Indices with Good Discriminating Ability and High Correlation with YTMS and YMSF

Good discrimination ability of indices helps effective identification of most desirable one's for selection of RILs tolerant to TMS environment. In the present study, STI most discriminated the RILs for their responses to TMS environment as indicated from high magnitude of estimates of both SR and PCV (Table 4). This means that, STI can discriminate the RILs which have high grain yield in both MSF and TMS environments from those which have relatively low grain yield under MSF and TMS environments. It is therefore desirable to preferentially use STI for screening the RILs for responses to TMS environment in dolichos bean. Safavi *et al.* (2015) in sunflower, Uday *et al.* (2016) in chickpea, Bennani *et al.* (2016) and Bennani *et al.* (2017) in bread wheat and Sushmitha and Ramesh (2020) in dolichos bean, have also suggested the use of STI for discriminating the test genotypes for their responses to TMS environment.

TABLE 4

Estimates of descriptive statistics of drought tolerance indices based on grain yield $plant^{-1}$ of RILs evaluated under MSF and TMS environments in dolichos bean

	Standardized Range	PCV (%)
Harmonic mean productiviy	0.54	17.23
Arithmetic mean productiviy	0.67	15.69
Geometric mean productiviy	0.53	16.35
Stress tolerance index	0.99	22.78

Based on only correlation criterion, all the four indices used in the study namely AMP, GMP, HMP and STI with significant positive and high magnitude of correlation with grain yield $plant^{-1}$ under both MSF and TMS environments (Fig. 5) were considered desirable ones. These results suggest any one or any combination of these four indices could be used to

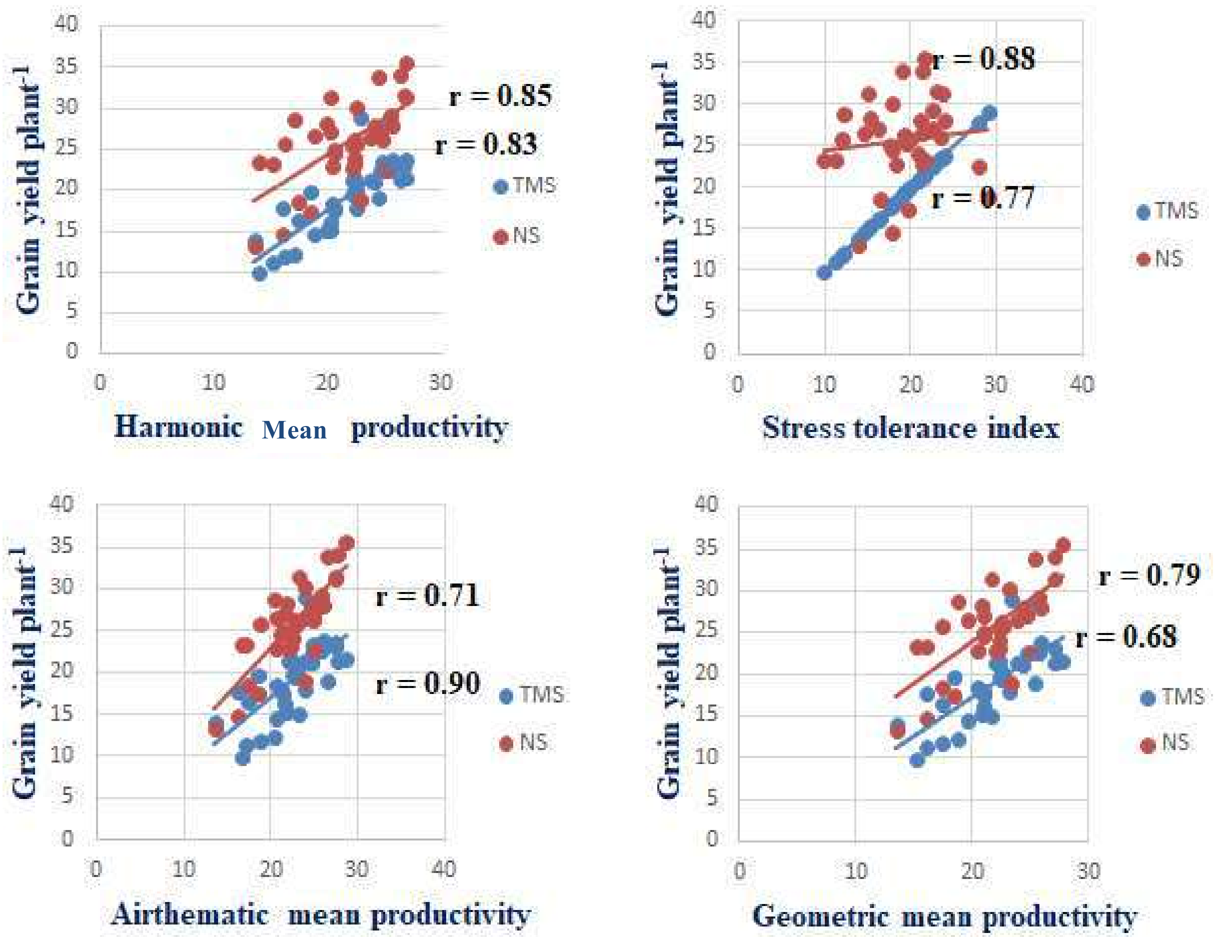

Fig. 5 : Relationship between arithmetic mean productivity (AMP), geometric mean productivity (GMP), harmonic mean productivity (HMP) and stress tolerance index (STI) with grain yield plant^{-1} of RILs evaluated under MSF and TMS environments

select TMS tolerant genotypes. Several researches have used this criterion and identified STI, MP, HMP and GMP as most desirable indices for selection of drought tolerant genotypes in different crops. To illustrate a few, Moosavi *et al.* (2008) and Seyyed *et al.* (2014) in soybean, Bennani *et al.* (2016) and Bennani *et al.* (2017) in wheat have reported the utility of AMP, GMP, HMP and STI for selection of drought tolerant genotypes based on the correlation criterion.

Identification of TMS Tolerant RILs based on Discriminating Ability and Correlation Criteria or Indices

Selection of indices should not be just based on their discriminating ability, but also should be based on their high magnitude of correlation with Y_{TMS} and Y_{MSF}. This is because, mere drought tolerance of crop varieties doesn't guarantee their acceptance and adoption by farmers. Only those drought tolerant varieties with optimum economic product yield potential under MSF production environments with minimum reduction in grain yield under TMS environment receive immediate and wider acceptance by the farmers (Serrai *et al.*, 2011; Dixit *et al.*, 2014 and Sushmitha and Ramesh, 2020). Hence, indices which exhibit good discriminating ability as well as significant positive and high magnitude of correlation with Y_{TMS} and Y_{MSF} are considered desirable. When both correlation and discriminating ability criteria were considered, only two indices namely STI and HMP were found desirable to identify TMS tolerant RILs. Among these two, HMP showed high correlation (Fig. 6) with AMP and GMP. Thus, it is desirable to use composite index which combines all the four indices to select TMS tolerant RILs. Rank sum (RS) method which effectively combines all the four indices into one index was used to select TMS tolerant RILs. Based on RS method, RIL 16, RIL 40, RIL 61, RIL 66, RIL 68 and RIL 129 were found TMS tolerant (Table 5). RILs selected based on RS method are characterized by TMS tolerance with high yield under both MSF and TMS environments (Thiry *et al.,* 2016).

Identification of TMS Tolerant RILs Based on all the Four Indices and YTMS and YMSF

Considering the cues from other studies, we identified TMS tolerant RILs based on the combination of all the indices and YTMS and YMSF. Based on the combination of YTMS and YMSF and magnitude of four indices (AMP, GMP, HMP and STI), four RILs (with class 'A' response) namely RIL 61, RIL 68 and RIL 112 with high YTMS and YMSF (Fig. 7) were identified. Of these, two (RIL 61 and RIL 68) were identified

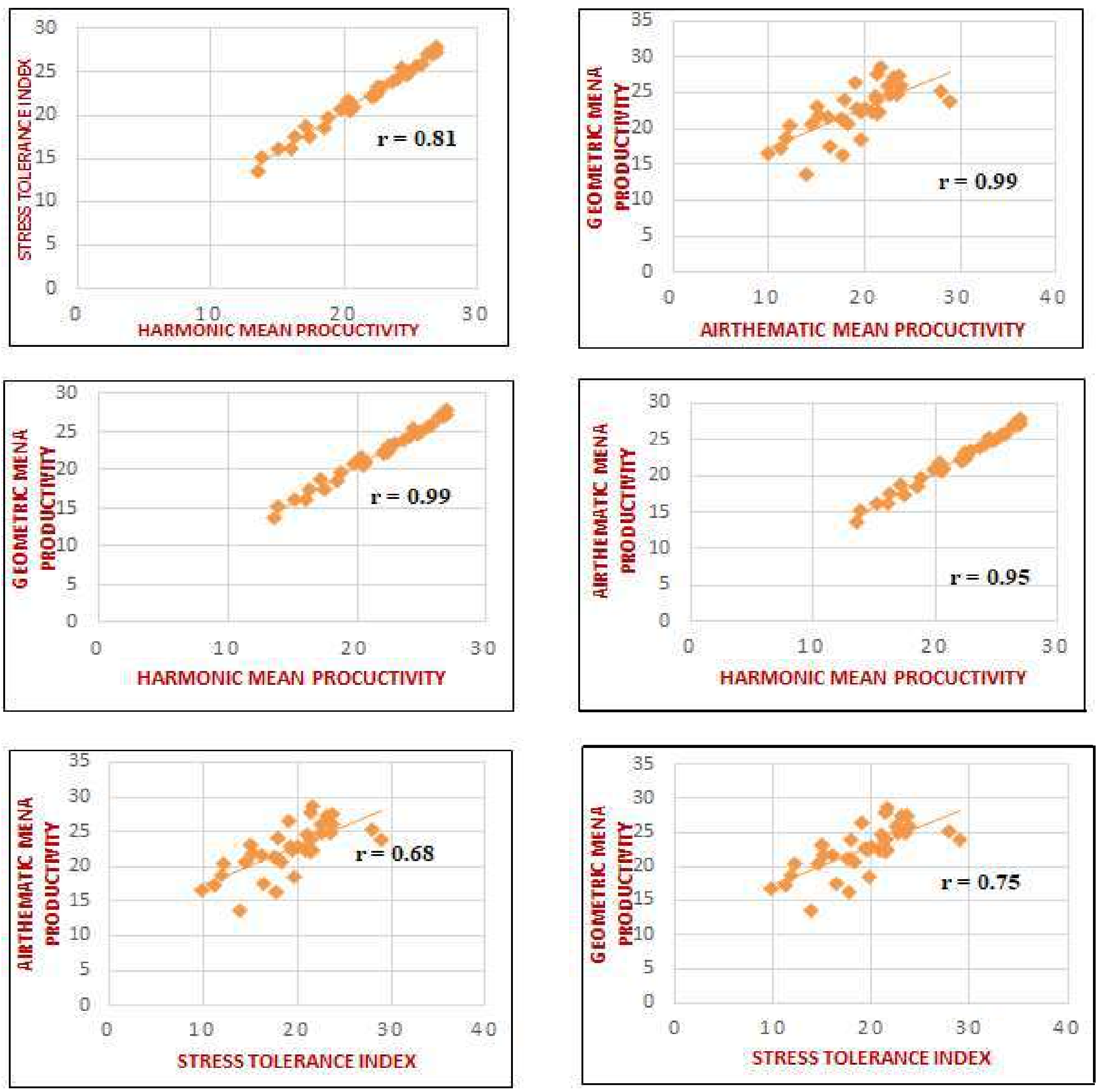

Fig. 6 : Relationship among drought tolerance indices based on grain yield plant^{-1} of RILs evaluated under MSF and TM S environments

TABLE 5

Ranks of RILs based on the combination of the four indices for grain yield plant^{-1} of RILs evaluated under MSF and TMS environments

Genotypes	RANK based on all four indices	HMP	Rank	AMP	Rank	STI	Rank	GMP	Rank
RIL - 40	2	26.94	2	28.63	1	21.66	9	27.77	1
RIL - 129	3	26.66	3	27.32	4	23.08	6	26.98	4
RIL - 68	4	25.75	5	25.92	6	23.80	3	25.84	5
RIL-112	5	25.54	6	25.90	7	28.98	1	25.72	6
RIL - 16	6	26.29	4	27.76	2	21.37	12	27.02	3
RIL - 61	7	24.90	7	25.19	8	27.89	2	25.05	8
HA 4	19	20.202	25	24.81	10	23.45	5	24.77	9
HA 5	1	26.94	1	27.47	3	23.64	4	27.20	2

HMP : Harmonic mean productivity GMP : Geometric mean productivity;
AMP : Arithmetic mean productivity STI : Stress tolerance index

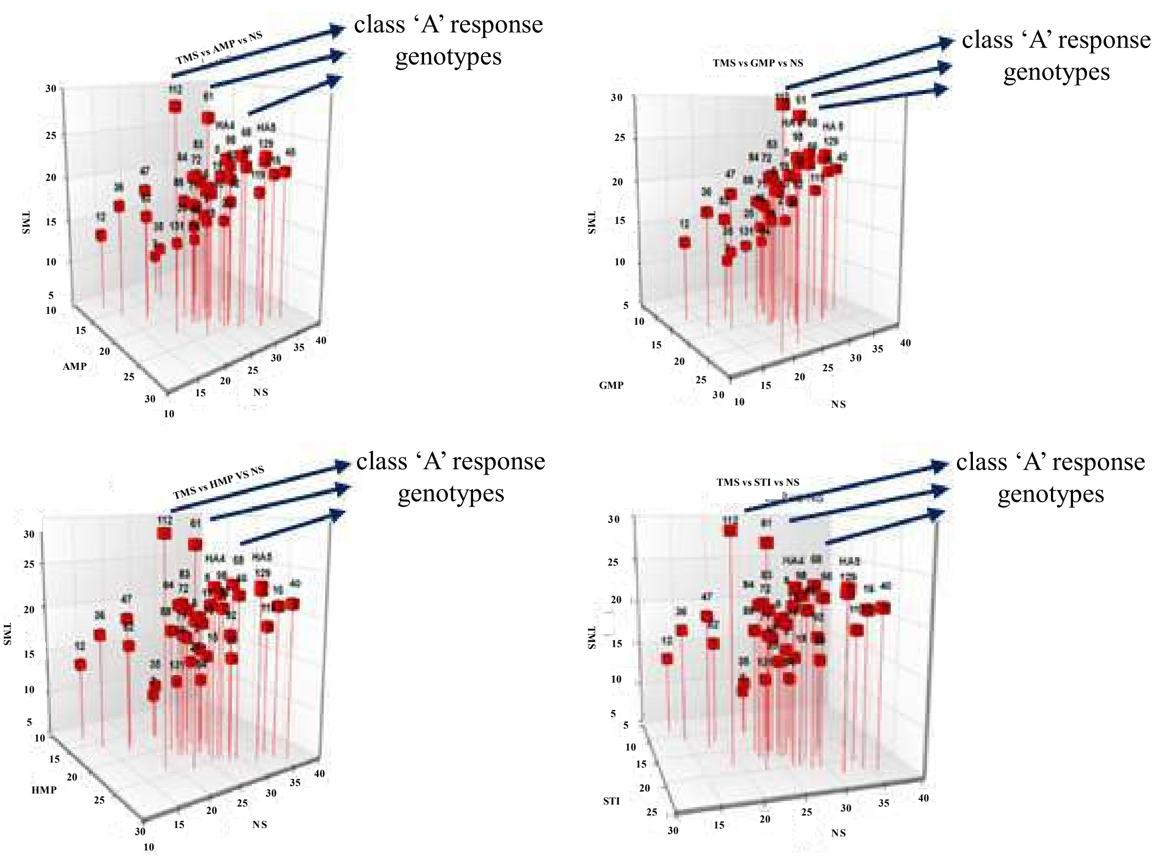

Fig. 7 : 3D graphs for grouping of the RILs into A, B, C & D classes of responses of RILs for tolerance to TMS environments for grain yield based on AMP, HMP, STI and GMP and Y_{TMS} and Y_{MSF}

based on the combination of only the indices. RIL 112 was the additional RIL identified based on the combination of both indices and Y_{TMS} and Y_{MSF}. Previous researchers such as Farshadfar and Javadinia (2011) in chickpea and Farshadfar and Elyasi (2012) and Farshadfar *et al.* (2012) in bread wheat have also identified class 'A' response genotypes. It is likely that a complex interplay of antioxidant enzymes such as peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and polyphenol oxidase (PPO) and non-enzymatic components such as ascorbate (ASC), glutathione (GSH), phenols, etc. (D'souza and Devaraj, 2011) could be playing a significant role in imparting TMS tolerance in these three RILs. Taking clues from report by Yao *et al.* (2013) on the key genes associated with drought tolerance using suppression subtraction hybridization (SSH) technique in dolichos bean, it is hypothesized that the genes encoding enzymes involved in the phenylalanine metabolism, flavonoid biosynthesis pathways and putative genes encoding protein located on cell membrane are likely to have over-expressed in RIL 61, RIL 68 and RIL 112. It is also possible that ß-amylase, a metabolic enzyme which plays a major role in cell survival (Kokila *et al.*, 2014) could be involved in imparting TMS tolerance in these three RILs.

YREM as a Measure of Predicted Loss in Grain Yield of TMS Tolerant RILs Relative to others

The estimates of YREM suggested that the RIL 61, RIL 68 and RIL 112 (Table 6) are expected to suffer a much lower loss in attainable grain yield plant^{-1} than the other RILs. This inference is based on the innate property of YREM. YREM is an intuitive, genotypes' attendance-independent dynamic statistics (Yan, 1999). The best genotype's performance is its potential grain yield attainable in a given environment. Hence, expected YREM of genotypes tested across diverse environments such as MSF and TMS environments in the present study must be unity. Any departure of genotype's YREM from unity is attributable to reduction in grain yield attributed to cross-over RILs × MR interaction. The extent of reduction in attainable grain yield of a genotype depends on the extent of departure of its YREM from unity (Yan, 1999). For example, if a genotype's average YREM across tested environments is 0.90, then 10 per cent of its attainable grain yield is lost due to cross-over RILs × MR interaction. Based on this argument, RIL 61, RIL 68 and RIL 112 with higher estimates of YREM (Table 6) are likely to suffer lower attainable grain yield losses attributable to significant cross-over RILs × MR interaction than other RILs with relatively lower estimate of YREM. of these three RILs, RIL 112 with highest YREM (Table 6) under both MSF and TMS environments is predicted to suffer least reduction in attainable grain yield if evaluated in any MR. Ashwini *et al.* (2021) and Kirankumar *et. al.* (2023) have used YREM to select horse gram genotypes that suffer lower reduction in grain yield attributable to cross-over genotype by environment interaction.

TABLE 6

Estimates of YREM of selected RILs under MSF and TMS environments for grain yield plant^{-1}

Genotypes	Terminal moisture stress environment	Moisture stress free environment
RIL - 129	0.80	0.89
RIL - 68	0.82	0.79
RIL - 40	0.75	1.00
RIL - 112	1.00	0.84
RIL - 61	0.96	0.63
RIL -16	0.74	0.96
HA 4	0.81	0.73
HA 5	0.82	0.88

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