How can we improve food crop genotypes to increase stress resilience and productivity in a future climate?

# A new crop screening method based on productivity and resistance to abiotic stress

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# Developing a new crop breeding tool to improve genotypes selection for sustainable production allowing understanding whether a high yield under stress is due to resilience or productivity or both.

# Abstract

The need to accelerate the selection of crop genotypes that are both resistant to and productive under abiotic stress is enhanced by global warming and the increase in demand for food by a growing world population. In this paper, we propose a new method for evaluation of wheat genotypes in terms of their resilience to stress and their production capacity. The method quantifies the components of a new index related with yield under abiotic stress (Ys), based on previously developed stress indices: Stress Susceptibility Index (SSI), Stress Tolerance (TOL), Mean Production index (MP), Geometric Mean Production index (GMP) and Stress Tolerance Index (STI), created originally to evaluate drought adaptation. The method, based on a scoring scale, offers a simple and easy visualisation and identification of resilient, productive and/or contrasting genotypes according to grain yield. This new selection method could help breeders and researchers by defining clear and strong criteria which identify genotypes with high resilience and high productivity and provide a clear visualisation of contrasts in terms of grain yield production under stress. It is also expected this methodology will reduce the time required for first selection and the number of first-selected genotypes for further evaluation and provide a basis for appropriate comparisons of genotypes that would help reveal the biology behind high stress productivity of food crops.

**Keywords:** abiotic stress indices, bread wheat, crop breeding, drought tolerance index, productivity, resilience.

# Abbreviations

# CIMCOG CIMMYT Core Germplasm

# GMP Geometric mean productivity index

# GMPs Score geometric mean productivity index

# masl Meters above sea level

# MP Mean productivity index

# MPs Score mean productivity index

# MSI Mean Score Index

# PC Production capacity

# PCI Production Capacity Index

# PT Physiological traits

# RC Resilience capacity

# RCI Resilience Capacity Index

# SSI Stress susceptibility index

# SSIs Score stress susceptibility index

# STI Stress tolerance index

# STIs Score stress tolerance index

# TOL Stress tolerance index

# TOLs Score stress tolerance index

# WAMI Wheat Association Mapping Initiative trial

# Yp Grain yield under yield potential conditions

# YPSI Yield Potential Score Index

# Ys Grain yield under abiotic stress environment

# YSSI Yield Stress Score Index

# Introduction

In agriculture, drought is by far the most important environmental stress that constrains crop yield (Blum, 2011). More than 40 percent of the world is classified as dry land, of which 8% is dry sub-humid area and 16% is semiarid area (Pretty *et al.*, 2005; UNDP, 2011). In addition, increasing temperature is an important component of climate change and its negative impact on yield is expected to increase in the future. Indeed, it has been demonstrated that growing wheat crop under heat stress (30/25 Cº) can lead to a 30-35% reduction in yield grain weight, when compared with control (18/13 Cº) (Wardlaw *et al.*1989), and the importance of incorporating a heat tolerance trait into wheat germplasm has been highlighted (Sareen *et al*. 2012). We need to develop genotypes with the capacity to yield significantly under heat stressed environments (Sareen *et al*. 2012). Therefore, understanding more about the mechanisms involved in plant tolerance/resistance to high temperature and drought stress becomes key for future improved crop production under stress as the climate in many food producing regions becomes hotter and drier (Blum, 2011; Macková *et al.*, 2013).

Many efforts have been made to improve crop productivity under water-limiting conditions. While breeding activity has directed selection towards increasing the economic yield of cultivated species, natural selection has favoured mechanisms of adaptation and survival (Cattivelli *et al.*, 2008). More than 80 years of breeding activities have focused on the increase of yield under drought environments for different crop plants. Meanwhile, significant gains in the understanding of the physiological and molecular responses of plants to water deficits have been provided by fundamental research (Cattivelli *et al.*, 2008).

However, in both conventional breeding and biotechnology the drought resistant ideotype is not always well defined and traits which might deliver high drought productivity are not always clear (Blum, 2005). Further, we have made little progress in identifying key mechanisms involved in delivering high productivity and stress resilience.

There is a need to define and characterize properly what it is meant by the term ‘stress tolerant genotype’. The concepts of drought tolerance as set out in the literature can differ significantly. Effectively, the ecological definition of drought resistance is the ability to stay alive during periods of low water supply (Levitt *et al.*, 1960 cited in Turner, 1979). Alternatively, for crop species, drought tolerance is defined as the ability of plants to grow and reproduce satisfactorily to produce harvestable yield with limited water supply or when under periodic water deficit (Turner, 1979; Fleury *et al.*, 2010). It has been suggested that yield stability is a better indicator of genotypic drought-resistance compared to grain yield under stress (Blum *et al.*, 1989). In terms of physiological mechanism, drought resistance is often considered as a compromise between ‘dehydration avoidance’ and ‘dehydration tolerance’ both of which can have variable impacts on yield (Fischer and Maurer, 1978; Turner, 1979, 1986; Levitt, 1980). Additionally, the concept of escape strategy mentioned by Turner (1979) and Levitt, (1980) includes phenological development speed as a criterion for selection, in order to avoid selecting early genotypes within a population which also contains genotypes with a longer phenological development under stress.

Plant breeding programmes mainly focus on selecting genotypes which have high yield firstly under yield potential conditions (non-stress) and secondly under stress conditions. To reach this aim, the classical postulate, widely accepted by breeder for selection, is that a genotype with high yield potential will perform well under most environments (Blum, 2005). However, this selection method does not include the concept of yield stability neither consider adaptation to a stress environment. Such shortcomings can be a cause of slow progress in breeding (Ceccarelli and Grando, 1991; Blum, 1996).

Several stress indices, described in supplementary appendix A, have been proposed to allow screening for drought stress adaptation. Fisher & Maurer (1978) developed a stress susceptibility index (SSI), Rosielle & Hamblin (1981) defined the stress tolerance index (TOL) and the mean productivity index (MP), and Fernandez (1982) analysed the latter and created two new indices, the geometric mean productivity index (GMP) and the stress tolerance index (STI) in an attempt to improve the MP index so that it would identify highly productive genotypes under both – stress and non-stress - environments. These various indices consider the relationships between traits, in non-stress (yield potential, irrigated conditions) and stress (drought mainly) environments. These indices were grouped into 2 classes, according to Rosielle and Hamblin (1981); Fernandez (1992) and Sareen *et al.*, (2012). The first class represents the susceptibility indices (SSI and TOL) which tend to distinguish between the stress-tolerant and the stress-susceptible genotypes, showing a negative relationship with yield. The second class represents the tolerance indices (MP, GMP and particularly STI) which tend to identify genotypes with stress-tolerance and high average yield, showing a positive relationship with yield. However, tolerance and susceptibility indices are not ideal to characterize genotypes with high yield performance and high stress tolerance under both environments. Genotype yield performance under stress and non-stress conditions has been categorized by Fernandez (1992) into four groups: A) genotypes express uniform superiority in both stress and no-stress condition, B) genotypes express good performance only in yield potential but not under stress conditions, C) genotype presents a relatively higher yield only under stress, and D) poor yield performance in both environments. Additionally, Fernandez (1992) evidenced some failures of the defined indices to distinguish between certain of these groups and suggesting that STI is generally able to distinguish better group A from group B and C (Table 1).

There is a clear need to develop an accurate tool able to identify the yield performance and resilience capacity of genotypes under stress conditions, since previous research has focused only on yield performance without taking resilience or stability into account. Currently, STI, GMP and MP are the most recommended indices to identify genotypes with high yield under both non-stress and stress environments (heat and drought) (Khodarahmpour *et al.*, 2011; Mohammadi *et al.*, 2011; Sareen *et al.*, 2012). In contrast, Khayatnezhad *et al.*(2010) stated that none of these indices could clearly identify cultivars with high yield under both environments (stress and non-stress).

Importantly, there is not yet an accurate screening index which can be recommended in breeding programmes to select genotypes for abiotic stress adaptation and high yield under both stress and non-stress environments. However, it has been suggested that a combination of stress indices (tolerance and suceptibility indices) might provide a more useful criterion for improving drought stress tolerance selection in common bean and heat stress tolerance selection in maize (Ramirez-Vallejo and Kelly, 1998; Khodarahmpour *et al.*, 2011). Nevertheless, it is not yet clear how to combine stress indices appropriately.

Therefore, the main objective of the present work was to develop a new simple tool based on the complementarities of two classes of indices (class 1: susceptibility indices, and class 2: tolerance indices, in Table 1) to express crop yield, in order to elucidate the characteristics of the best performing and adapted genotypes under stress. To achieve this goal, we develop a methodology to enable us to combine indices. We suggest how this tool can be used in a crop breeding programmes and show how the new indices can be used to provide a focus for mechanistic research aimed at understanding the basis of the sensitivity of crop yield to environmental stresses.

# Materials and Methods

#### Site of Experiments

Field trials were conducted at the Mexican Phenotyping Platform (MEXPLAT), located in the highly productive irrigated spring wheat growing environment in the Yaqui Valley, near Obregon City, NW Mexico (27.20º N, 109.54º W, 38 meter above sea level (masl). This site is a temperate high radiation environment, and with adequate irrigation, average yield of the best lines is approximately 8 t/ha (Sayre *et al.*, 1997).

#### Experimental Material and Stress Treatments

Ten lines selected from the CIMCOG trial (acronym of CIMMYT Core Germplasm) representing contrasting genotypes for partitioning and related traits were used in this study. These wheat lines were evaluated during two cropping seasons, 2012-13 and 2013-14, in three different environments: irrigated conditions (yield potential) for the two cropping seasons (from November to early May), and under drought and irrigated heat stress during the later cropping season, i.e., from December 2013 to late May 2014, and from February to June 2013, respectively. All trials were conducted with optimal crop management following a preventive biotic stress control strategy in order to control the others stresses and with conventional nutrients supplied.

For all the experiments, the testing area was surrounded by durum wheat (*Triticum durum*) that acted as a windbreak to reduce edge effects. The experimental design was a total randomized block design with three replications for the Yp and drought trials, and two replications for the heat trial. Irrigation was gravity-fed flood applied for all experiments. For the drought stress trial, the last irrigation was at 50% of seedling emergence, and in the case of Yp and irrigated heat trial, 4 additional irrigations have been applied, after 50 % of emergence, every three weeks until 15 days before maturity.

Additionally, data from a set of 294 elite genotypes - the WAMI trial (acronym of Wheat Association Mapping Initiative) - grown under yield potential conditions during the 2012-13 cycle (November to May), and under heat conditions from February to June 2013, have been used to test the robustness of the indices.

## Methods

## Concept of stress adapted genotypes selection within a population under field conditions

The selection under field conditions presents additional difficulties to screen genotypes due to the variability, intensity, timing and duration of stress, as well as having several stresses at the same time (e.g. pest invasion and nutrient stress).

Therefore, it is important to compare genotypes within the population response in order to identify genotypes more or less susceptible and/or tolerant to the stress in study. Screening for stress adapted genotypes under field conditions is made through the susceptibility to accumulative stress and the interaction between responses. Consequently, to study a specific mechanistic response to environmental stress by plants under field conditions it is highly important to control as much as possible the other collateral stresses that can appear during a growing season (biotic stress, nutrient stress, etc…) in order to reduce their effects on the crops (Chapin *et al.*, 1987; Herms and Mattson, 1992; Dolferus *et al.*, 2011). However, the control will never completely reduce the pressure of the other stresses, and we therefore assume that a population of genotypes grown during the same season would have suffered a similar pressure of cumulative stress (abiotic and biotic stress). Therefore, we suggest that each genotype should be compared within the population response each season to better understand the stress adaptation.

## Basis of the development of the new stress indices and their uses

As mentioned above, different approaches are used to identify tolerant genotypes between the indices from class 1 and 2 (Table 1), as class 1 tends to discriminate the tolerant from the susceptible, and class 2 tends to distinguish the tolerant with high mean yield. However, the failures (Table 1) have shown that a high yield under non-stress conditions does not automatically indicate a good performance under stress, and similarly, a high yield under stress does not automatically indicate high resilience. The outcome of a stress challenge will depend on the severity of the stress and obviously on the characteristics of the genotype (genetic effects).

Nevertheless, both classes (Table 1) explain a part of the behaviour of the genotypes under stress. Therefore, based on the previous concept developed by Fernandez (1992), Fisher & Maurer (1978) and Rosielle & Hamblin (1981), we propose here two new indices which are compiled through the combination of the score indices that show a high correlation with yield under stress and non-stress environments. The score indices have been classified within two new scales called resilience and production capacity, based on classes 1 and 2 (Table 1), respectively.

Then, the resilience and production capacity indices can be defined as follow:

**Resilience Capacity Index (RCI)** expresses the yield decrease of the genotypes under stress (Ys) within a population, compared to yield potential conditions (Yp).

**Production Capacity Index (PCI)** expresses the mean production of the genotypes under both stressed (Ys) and non-stressed (Yp) environments within a population.

These indices RCI and PCI constitute an attempt to improve the use of the five previous indices (SSI, TOL, MP, GMP, STI), as both new indices (RCI and PCI) are required if it is wanted to understand the basis of any yield limitations under stress. Indeed, it is generally accepted (by ecologists) that an ecosystem shows a complex relationship among the resilience, productivity and stability (Xu and Li, 2002) and therefore are key issues to increase future crop production.

**Why to combine the indices?**

It is important to analyse the different groups of yield responses (from A to D). Groups A and D represent the extremes – in terms of grain yield - as the best and worst genotypes. However, extreme responses are rare and genotypes in these two groups would tend to group with B or C like AB or AC and DB or DC. Nevertheless, this could explain why both classes of indices have a relatively good relationship with both yields (non-stress and stress), as shown by Fernandez (1992), as they both fail to correctly identify the middle index values of the linear regression with yield (non-stress and stress). In turn, the middle values can have two tendencies, a medium-high or a medium-low value, for both environments. For example, group A with a value close to the boundary line value, which discriminates group A from C under non–stress and from group B under stress conditions (Fig. 1). Indeed, to distinguish these values which are more A than C under non-stress and vice versa, we will use the terms medium-high and medium-low, respectively. Considering this, medium values in the linear regression obtained with the indices, have to be readjusted in order to express better the yield trait under non-stress and stress environments. This can be achieved by combining the indices.

**How can the indices be combined, as their values are totally different?**

In order to classify the trait (e.g. tolerance) from the highest to the lowest, the indices (SSI; TOL; MP; GMP; STI) are given their own numerical value each, as individual index values can only be interpreted inside each index itself, because the scale or reference of the different indices is not the same . Additionally, indices of class 1 have a reverse scale to that of class 2 where low values mean high tolerance. Therefore, to enable comparison of the different indices, a scale has been created on an equal reference for all indices by scoring the results from 1 to 10. Afterwards, the five indices show a value for each genotype which is comparable between the different indices. The idea of scoring is to have an easy visualization of the information given by the indices for the population under study, and to be able to compare one index with the others. A simple number, on a 1 to 10 scale, provides an easier interpretation than decimal values allocated to the original equations. Additionally, it opens new insights by permitting arithmetic operations between the indices in a simple way.

**How to create the scoring scale?**

The scoring scale for each index is calculated on the global response within the overall population under study. Thus, the scale is adjusted with the minimum and maximum value obtained with the original equation of the index. The difference of these two values gives the range of the scale for each index. This range is divided into 10 parts and each part has a score from 1 to 10. Therefore, each part represents the 10%, 20%,.., or 100% of the range value.

Additionally, we have inverted the value of TOL and SSI, so a high value obtained with the original equation will receive a lower score. It allows the two classes of indices to have the same scale, where a high score will always mean a good genotype. For example, score value 2 is obtained in the different indices for all the values within the 10% and 20% of the range for MP, GMP, STI and 80% and 90% of the range for TOL and SSI. A tool developed on MS Excel has been created to assign a score to each value.

Once the scores have been obtained, we can easily combine and test them against yield under stress and non-stress conditions, and figure out if they are better adapted to express yield under stress and/or non-stress by identifying or distinguishing better groups B and C. Finally, the score index based on SSI or TOL equations is now called Resilient Capacity Index (RCI) and the score index based on MP, GMP, STI is termed Production Capacity Index (PCI). These are terms which indicate much more specifically what the indices are showing.

# Results and discussion

### Testing the methodology and the score indices

Firstly, the score indices have been tested against their original value from each index. Table 2 shows the Pearson correlation coefficient between the Score stress susceptibility index and the Score Tolerance Index values (SSIs and TOLs, respectively) and their original index values (SSI and TOL), calculated on yield data from the WAMI trial (294 genotypes). This correlation is highly negative (ranging from -0.78 to -0.98), as the score scale has been inverted in order to create a scale showing resilience instead of susceptibility. On the other hand, the Pearson correlation coefficient between the original value (MP, GMP and STI), and the score indices (MPs, GMPs, STIs), is highly significant. These high Pearson correlation coefficient values demonstrates that the score indices can be used as a surrogate of their original index value.

**How to combine the score indices? What is the best combination?**

Fig. 2 shows the linear regression and the coefficient of determination of the different score indices versus yield under non-stressed and stressed environments, calculated on 294 genotypes from the WAMI trial.

Fig. 2 shows that no index, used individually, could clearly identify the high yielding genotypes, independently of the environment. This result confirms the conclusion of Khayatnezhad *et al.*(2010), who concluded the same in a study with 22 genotypes of durum wheat. Into each class of indices (susceptibility and tolerance), SSI and STI show the highest relationship with yield under heat stress. In contrast, TOL and MP show a high relationship with yield potential. These responses would suggest that the combination of the score indices from each class would improve the relationship between the indices *per se* and grain yield.

**New indices**

The new indices are based on the combination of score indices. In order to show and illustrate easily the score indices value and the contrast within the whole population, data from a smaller trial of ten genotypes from the CIMCOG-ROOT trial has been used to make the visualization easier, compared with a table with 294 genotypes. Nevertheless, the method to interpret and use the score is the same for ten, 294 or even more genotypes.

Table 3 illustrates an example of a score index table using grain yield data of ten genotypes from the CIMCOG-ROOT trial. The Yp data used in this table are a mean of the grain yield under Yp conditions for each genotype from two cropping seasons (2012-13 and 2013-14), in order to provide more consistent information on the yield potential of the genotype, considering that the Yp represents the maximum grain yield that a genotype is able to produce. Indeed, the ten score indices provide an illustration of small differences between SSI and TOL. On the other hand, GMP and STI were very similar, but both were slightly different from MP. Table 2 indicates also the slight differences between the 2 class of indices into the 294 genotypes trial. It is important to observe that in both cases, the values are generally of the same magnitude within the two classes. Thus, these score values (Table 3) and the Pearson correlation coefficient (Table2) confirm that SSI and TOL, as well as MP, GMP and STI, can be associated to class 1 and 2, respectively (Table 1), demonstrating that these classes address on two different characteristics, resilience capacity (RC) and production capacity (PC), respectively.

At this point, it is asked which combination of these score indices could be considered as the best indicator of yield under stress and non-stress conditions. Several combinations have been studied in order to generate a new index with the two components (RC and PC). The method and the different combinations and formula are exemplified in supplementary data (Table S1). The combinations taken into account, for each case, were achieved by pairs or groups of four score indices (combinations of two by two score indices from class 1 and 2), adding or subtracting components. Each combination was correlated with grain yield values under stress (Ys) and non-stress conditions (Yp) by calculating the Pearson correlation coefficient (Table S2 to S4). Some combinations show a better correlation with Ys and Yp than others, and two of them are outstanding. Indeed, Equation 1 presents the highest Pearson correlation coefficient with Ys, and Equation 2 with Yp, for the three trials (CIMCOG-root under drought and irrigated heat, and WAMI). In both equations, the first and second components correspond to PCI and RCI, respectively.

The relationship between Ys and Yp and Equations 1 and 2 is illustrated in Fig. 3 and Fig. 4, respectively. At this point, Equation 1 will be called Yield Stress Score Index (YSSI) and Equation 2 will be called Yield Potential Score Index (YPSI). These results have demonstrated that yield, either under stress and non-stress, can be expressed by two components, resilience (RCI) and production (PCI). Moreover, the combination of score indices has improved the use of the original indices and their relationship with yield. Additionally, to demonstrate the robustness of this index, it has been calculated using different multiyear populations (WAMII, Seri/Babax, CIMCOG-ROOT) previously studied in CIMMYT under different abiotic stresses (Pinto *et al.*, 2010; Lopes *et al.*, 2015; Sukumaran *et al.*, 2015). Table 4 shows the Pearson correlation coefficient and coefficient of determination (R2) of yield under stress versus YSSI. The consistency of the correlations demonstrates the efficiency of the index.

Equation 1

Equation 2

In supplementary appendix B, it is explained why PCI and RCI are complementary and why these combinations work better than the previous indices (SSI, TOL, MP, GMP, STI).

A further combination of the five score indices, named Mean Score Index (MSI) (Equation 3), shows a slightly better correlation with Ys (Fig. S4). Nevertheless, this formula contains a disproportion between its productivity and resilience components, giving more weigh to the first one with 3 indices, while containing only 2 indices for resilience, with the consequent greater impact of the productivity on the output. As the aim of this paper is to identify an easy method to distinguish resilience and productivity, the MSI has not been addressed.

Equation 3

Therefore, YSSI and its components PCI and RCI, which are the Scored STI and the Scored SSI indices, respectively, are the focus indices of this work in order to improve the selection and identify contrasting genotypes in terms of PC and RC, both under stress conditions.

**How these indices can be used to identify resilient and productive genotypes?**

The score indices provide two things. Firstly, the interpretation of the data is much easier as everything is on a similar scale, allowing the visualization of the score, being 1 to 10 to detect the lowest, medium or highest response. Secondly, the score indices enable to understand better the genotype behaviour under stress, indicating if a high yield under stress is due to tolerance (resilience) or due to the high production capacity (mean yield performance), or both. This can be achieved by analysing the components of YSSI, where high resilient/tolerant and high productive genotypes should have a high value in both indices (RCI and PCI).

Table 5 presents an extended summary of the different indices and their combined value as a function of the values of Yp and Ys. The groups are delimitated by a boundary line (Fig. 1) which represent the minimum or maximum for each groups. The boundary line could be represented by the average grain yield within the population under the corresponding environments or by using the yield of a local check (both could be used depending on the aim of the research). Consequently, depending on the range of values of Yp and Ys inside the groups, a range of values for RCI and PCI is expected to correspond to the variation of Yp and Ys.

As shown in Table 5, a unique combination of RCI and PCI values identifies and differentiates perfectly the four groups defined by Fernandez (1992), the only case where the combination is not unique is for the low yield under stress which could be obtained from group B or D, both with low PCI and RCI value. These responses could be differentiated using the Yp value, which is higher for genotypes B than genotypes D. In general, the unique combination and distinction of the different groups is illustrated by an example in Fig. 5, where the resultant value, in this case YSSI, can be similar for genotypes included into groups A and C, however RCI and PCI will be different between these groups. In this particular case, genotypes C show a better resilience (RCI) than genotypes A, and genotype A shows a better yield performance under non-stress.

These differentiations can be very useful for a crop breeding program focussed on discovering high resilient and productive genotypes or only highly resilient ones for crossing with highly productive genotypes. For mechanistic research, contrasting genotypes in terms of resilience or productivity could provide an understanding of the impact of specific traits expression such as stomatal conductance, waxiness, hormone production, etc... For example, high yield production under stress can be derived from a genotype which is tolerant or has a good yield performance under non-stress, or both. Indeed, some genotypes from groups B and C can have a similar yield value under stress conditions, but genotypes from group C will present a lower yield under a non-stress condition (compared with genotypes from group B) but they will not reduce much their yield under stress and consequently, will have a better resilience to the stress which can be identified by a higher RCI value. Therefore, the score indices offer the possibility of easily visualizing the plasticity of genotypes in response to a particular stress by looking at the RCI and PCI values. Table 6 shows a simple example for a small trial of ten genotypes under heat stress during the 2012-13 cropping season. One example of a contrasting genotype selection for fundamental research can be taken from these data: Genotypes 6 and 1 have a similar YPSI (Yp) but the YSSI (Ys) values are totally opposed, being the highest and the lowest, respectively. Additionally, genotype 6 has the highest PCI and RCI, and genotype 1 shows the lowest index values within whole population.

At this point, an important question has to be raised. Does phenology influence the index selection method? Actually, it is widely known that the ability of plant to recover from abiotic stress (drought or heat) principally depends on the developmental stage at which the plant suffers it (Jäger *et al.*, 2008). In wheat, meiosis is a very sensitive stage to abiotic stress and results in reduce pollen fertility and consequently the final number of grain (Saini *et al.*, 1984; Acevedo *et al.*, 2002; Jäger *et al.*, 2008). Additionally, Tewolde *et al.*, 2006 state that early-heading genotypes under heat stress had a longer grain ﬁlling period and completed a greater fraction of the grain ﬁlling earlier in the season when air temperatures were lower and generally more favourable compared to the later-heading cultivars. However, early-heading could be considered as an escape strategy instead of having a tolerance and/or resilient adaptation. Consequently, in order to improve genotype selection for mechanistic research to discover new traits for stress resilience and to avoid selecting genotypes which may have an escape strategy, phenology should be taken into account. Effectively, as the index compares genotypes within the whole population, if some genotypes have an escape strategy and present good resilience and productivity they would modify the general scale and could favour them more with the risk to discriminate genotypes with late phenology which actually could present better adaptive/tolerant traits to endure the stress. Considering this, in order to improve a contrasting selection, two attempts should be tested. First attempt, with a small trial with dozens of genotypes, to analyse the whole population and identify separately the early, mid and late genotypes, thus selecting genotypes into groups of similar phenology. For example, in a small trial like CIMCOG-ROOT used for fundamental research, it was observed that genotypes 2 and 4 show an early phenology, reaching booting stage 5 and 7 days before the late genotypes (9 and 10), respectively, when the mean population reaches this stage 3 days before genotypes 9 and 10. Additionally, when genotypes 9 and 10 started meiosis, genotypes 2 and 4 were at the middle in progress of that phase. Two observations can be made from the point of view of the early genotypes. Firstly, genotype 4 is the earliest genotype which starts meiosis 4 days before the mean population, currently behaving as resilient (RCI = 6). So, it could present an escape strategy, then turning susceptible, compared to genotypes 7 or 8 if it would receive the stress at the same phenological stages with the same intensity like genotypes 7 and 8. Secondly, genotypes 2 and 10 show similar susceptibility (RCI=4), although genotype 10 received a higher stress during the susceptible phenological stages. Consequently, genotype 10 could be considered more resilient to heat stress than genotype 2 if phenology is taken into consideration. Therefore, in this specific case of the CIMCOG-ROOT trial, genotype 6 seems to be the best to be selected, in terms of adaptation to stress and harvestable yield under stress. However, this genotype will be classified into the group C instead of the group A, because its Yp is lower than the mean yield of the population. So, selection for abiotic stress tolerance and suitable yield performance should consider genotypes from groups A and C.

A second attempt to integrate phenology into the use of the index for a huge panel, would be to analyse the whole population and/or analyse separately the early, mid and late genotypes. For a breeding program using the selection method based on the proposed index, the problem of different phenology into a panel is similar compared to the conventional selection on yield, with the only difference that the index method allows to create “new populations” like the early, mid and late genotypes, and compare each with the check. The more uniform the population is, in terms of phenology, the better the index will perform in identifying contrasting genotypes or with high yield performance, as one of the basis of the score index method is to compare the genotypes response within the whole population. For example, the WAMI trial has been compared between the conventional selection method - based on yield under both environments (stress and no stress) – and 1) the index selection method, and 2) the index selection by grouping the genotypes according to their phenology (in this case, heading). For all these cases the selection is done using a check line (Sokoll) as the reference. The yield method selects genotypes with higher yield under both environments, compared with Sokoll. The index selection refers to the score obtained by Sokoll and selects genotypes with similar score or higher.. The first observation is that the index selection reduces 33% in the number of selected genotypes, and the index and phenology method reduces a 48%, compared to the yield check method. The second observation is that the index method shows a 64% of matching genotypes with the yield method, being these genotypes classified into group A, while 36% are genotypes that would never have been selected with the conventional method, coming from the group C. The third observation comparing the index method and the index integrating the phenology, is that late genotypes are generally discarded using the index method. Thus, the use of the index will depend on the aim of the research.

As mentioned above, the selection methods used by breeders are mainly based on yield production, where genotypes are firstly selected for their ability to produce more yield under both environments, compared to the performance of a local check. Subsequently, there is a deeper study in a second phase of selection. However, Blum (1996) has noted that an apparently negative association between yield potential and drought resistance has been found in different researches, where genotypes with a superior adaptation to drought stress may have a lower yield under yield potential environments. However, he specifies that it is not always the case, and suggested that the identification of factors involved in the negative relationship will be important to enable designing a more efficient approach to be used in breeding for high yield and yield stability. We suggest that a wheat improvement program could beneficially use the score indices, RCI and PCI, simultaneously, in order to identify those rare genotypes which do not show these negative relationships. Such a course of action could reduce considerably the number of selected genotypes, focusing on resilience and productivity, allowing breeders to reduce costs and save time. Finally, the use of a uniform criterion in fundamental research like RCI and PCI, would help to ensure more valid and useful comparisons between research results obtained across a selection panel where there is only a hazy understanding of potential selection criteria.

# Conclusions

Score indices offer an easy-to-use new method to classify and visualize quickly which are the best or the worst genotypes within a population, in terms of resilience and production. Additionally, score indices allow arithmetic operations to create a new index, YSSI, which express yield under stress into a simple score scale value. This expression of yield has demonstrated that yield under stress can usefully be perceived as a function of two major crop characteristics, the resilient capacity (RC) and the production capacity (PC).

This analysis opens new insights for selection of genotypes in crop breeding programmes, helping breeders and researchers to understand better the genotypic responses under stress. However, it has been observed that high productive and high resilient genotypes (Group A) are rare in nature. Therefore, we suggest considering also those genotypes with high resilience (high RCI) and medium-high Yp (high PCI), included in Group C. These new indices also offer the opportunity to focus the analysis on resilience to the increasing stress environment in order to improve and assure yield sustainability.

Improvements of this new method could be done by grouping the genotypes by their phenology in order to avoid an incorrect interpretation, or by considering a similar resilience when the stress appears at different sensitive phenological stages, meaning therefore that the response of plants could be different.

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**Table 1:** Summary of the interpretation of tolerance according to the value given by the previously developed indices (Stress Susceptibility Index (SSI), Tolerance Index (TOL), Mean Productivity Index (MP), Geometric Mean Productivity Index (GMP), and Stress Tolerance Index (STI)) and their failures to distinguish the different groups (A, B, C and D) defined by Fernandez (1992). Class 1 and 2 correspond to susceptibility indices and tolerance indices, respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Index | Index value | Tolerance | Fails | Class |
| SSI | High | Low | Fails in distinguish A and C | 1 |
| TOL | High | Low | Fails in distinguish A and C | 1 |
| MP | High | High | Fails in distinguish A and B | 2 |
| GMP | High | High | Same fail as MP distinguishing better A compared to MP | 2 |
| STI | High | High | Same fail as MP distinguishing better A compared to MP and GMP | 2 |

**Table 2:** Pearson correlation coefficient (R2) between the score indices (SSIs, TOLs, MPs, GMPs, STIs) and their original index (SSI, TOL, MP, GMP, STI), *P*<0.05

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | SSI | TOL | MP | GMP | STI |
| Class 1 | SSIs | -0,98\* | -0,80\* | 0,07 | 0,38 | 0,38 |
| TOLs | -0,78\* | -0,97\* | -0,51 | -0,21 | -0,21 |
| Class 2 | MPs | -0,09 | 0,49 | 0,98\* | 0,93\* | 0,93\* |
| GMPs | -0,40 | 0,19 | 0,93\* | 0,98\* | 0,98\* |
| STIs | -0,39 | 0,19 | 0,93\* | 0,98\* | 0,99\* |

* P<0.05

**Table 3:** Example of a Score index table based on grain yield data from the CIMCOG-root trail (ten genotypes) under yield potential and heat stress environments, for the Stress Susceptibility Index (SSI), Tolerance Index (TOL), Mean Productivity Index (MP), Geometric Mean Productivity Index (GMP), and Stress Tolerance Index (STI) during the 2012-13 cropping season (Y12-13).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Entries | Score SSI | Score TOL | Score MP | Score GMP | Score STI |
| 1 | **1** | **3** | **3** | **1** | **1** |
| 2 | **4** | **6** | **4** | **3** | **3** |
| 3 | **2** | **3** | **6** | **4** | **4** |
| 4 | **6** | **10** | **1** | **1** | **1** |
| 5 | **4** | **2** | **10** | **9** | **9** |
| 6 | **10** | **10** | **9** | **10** | **10** |
| 7 | **5** | **7** | **6** | **5** | **5** |
| 8 | **5** | **5** | **8** | **7** | **7** |
| 9 | **2** | **1** | **10** | **7** | **7** |
| 10 | **4** | **3** | **9** | **7** | **7** |

**Table 4:** Summary table showing the Pearson correlation coefficient and coefficient of determination (R2) of Yield Score Stress Index (YSSI) versus yield under stress (Ys) from multiyear populations (WAMII, Seri/Babax, CIMCOG-ROOT) previously studied in CIMMYT under different abiotic stresses (Heat, drought, drought under drip; semi-drought: drought applied at booting stage). The consistency of correlations demonstrates the efficiency of the index.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trials | Entries | Environment | Year | Correlation | R2 |
| WAMI | 294 | Heat | 2009-2010 | 0,988 | 0,976 |
| WAMI | 294 | Heat | 2010-2011 | 0,983 | 0,967 |
| WAMI | 294 | Heat | 2011-2012 | 0,987 | 0,975 |
| WAMI | 294 | Drought | 2009-2010 | 0,987 | 0,973 |
| Seri/Babax | 169 | Heat | 2004-2005 | 0,993 | 0,986 |
| Seri/Babax | 169 | Heat | 2005-2006 | 0,992 | 0,984 |
| Seri/Babax | 169 | Heat | 2009-2010 | 0,955 | 0,912 |
| Seri/Babax | 169 | Drought | 2005-2006 | 0,994 | 0,988 |
| Seri/Babax | 169 | Drought (Drip) | 2007-2008 | 0,989 | 0,978 |
| Seri/Babax | 169 | Drought | 2008-2009 | 0,991 | 0,982 |
| Seri/Babax | 169 | Drought (Drip) | 2009-2010 | 0,974 | 0,948 |
| CIMCOG ROOT | 10 | Semi-drought | 2012-2013 | 0,996 | 0,992 |
| CIMCOG ROOT | 10 | Heat | 2012-2013 | 0,975 | 0,950 |
| CIMCOG ROOT | 10 | Drought | 2013-2014 | 0,981 | 0,962 |

**Table 5:** Summary of the value expected for the different index scales within a population and their addition or subtraction. In this table, a unique combination of the Resilience Capacity Index (RCI) and the Productivity Capacity Index (PCI) values differentiates the four groups (A, B, C and D) defined by Fernandez (1992), according to Yield Stress Score Index (YSSI) and Yield Potential Score Index (YPSI) values. Yp = grain yield under yield potential conditions, Ys = grain yield under stress conditions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Groups | Yp | Ys | RCI | PCI | Combination’s range of values | |
| YSSI | YPSI |
| A | Med-high to  High | Med-high to  High | Med-high | High | High to Med-high | High to Med-high |
| B | Med-high  To  Med | Med-low to  Low | Med | Med | Med | Med |
| Low | Low | Low | Med. |
| Med | Med-low | Med-high |
| High | Med-high | High |
| C | Med  To  Med-low | Med-high to  high | High | Med | High to Med-high | Med to Med-Low |
| D | Med-low to  Low | Med-low to  Low | High | Low | Med-high | Low |
| Med | Med-Low | Med-low |
| Low | Low | Med-Low |

**Table 6:** Values of the Resilience Capacity Index (RCI), the Productivity Capacity Index (PCI), and the resultant of their combination, the Yield Stress Score Index (YSSI) and the Yield Productivity Score Index (YPSI), calculated using the grain yield data from the CIMCOG-root trial (ten genotypes) under yield potential conditions and heat stress, for the 2012-13 cropping season (Y12-13).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Yield Y12-13 | | | | |
| Entries | RCI | PCI | YSSI | YPSI |
| 1 | **1** | **1** | **1,00** | **0,00** |
| 2 | **4** | **3** | **3,50** | **-1,50** |
| 3 | **2** | **4** | **3,00** | **2,50** |
| 4 | **6** | **1** | **3,50** | **-7,00** |
| 5 | **4** | **9** | **6,50** | **6,50** |
| 6 | **10** | **10** | **10,00** | **-0,50** |
| 7 | **5** | **5** | **5,00** | **-0,50** |
| 8 | **5** | **7** | **6,00** | **2,50** |
| 9 | **2** | **7** | **4,50** | **7,00** |
| 10 | **4** | **7** | **5,50** | **4,50** |

**Figure 1:** Representation of the different groups (A, B, C and D), defined by Fernandez 1992, according totheir yield performance under stress (Ys) and non-stress (Yp) environments. The bold line represents the limit between one group and the others, the value to trace these boundary lines could come from, 1) the average yield value of the population, or 2) the yield value from a check under each environment (Ys and Yp).

**Figure 2:** Linear regression and the coefficient of determination of the different score indices *versus* grain yield under non-stressed and stressed environments. Calculations use data from WAMI trial: (a) Yield potential *versus* Score SSI; (b) Yield potential *versus* Score TOL; (c) Yield potential *versus* Score MP; (d) Yield potential *versus* Score GMP; (e) Yield potential *versus* Score STI; (f) Yield under heat stress *versus* Score SSI; (g) Yield under heat stress *versus* Score TOL; (h) Yield under heat stress *versus* Score MP; (i) Yield under heat stress *versus* Score GMP; (j) Yield under heat stress *versus* Score STI.

**Figure 3:** Linear regression of grain yield under heat stress (Ys) and the Yield Stress Score Index (YSSI).

**Figure 4:** Linear regression of grain yield under a yield potential environment (Yp) and the Yield Potential Score Index (YPSI).

**Figure 5:** Schematic illustration of a particular case of two genotypes according to grain yield performance: A) Schematic illustration of two genotypes from groups A and C with a similar grain yield value under stress (Ys) and different grain yield value under yield potential conditions (Yp). B) Schematic representation of the distribution of values of the Productivity Capacity Index (PCI) and the Resilience Capacity Index (RCI) where genotypes A show a higher PCI compared with genotypes C, and *vice versa*, in terms of RCI.