

Screening of Cotton (*Gossypium hirsutum* L.) Genotypes for Drought-Tolerance Indices: A Comprehensive Review

Dr. Rajesh Singh

Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

*Corresponding author: Dr. Rajesh Singh

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ABSTRACT

Background: Cotton is an important fiber crop in the world and its yield is affected by the occurrence of constant drought due to climate change. Cotton is usually grown in rainfed or watered lands, thus making it very important to look for varieties that can give high yield in drought situations. Key indicators needed for identification of drought-tolerant cultivars are drought tolerance indices based on performance and morphological characteristics.

Objective: This review would analyze peer-reviewed publications on screening upland cotton cultivars for drought tolerance using the drought tolerance indices in order to summarize the methods, compare the results, and highlight the gaps in research.

Literature sources: A systematic search was performed through Scopus, Web of Science, PubMed/PMC, Google Scholar, ScienceDirect, SpringerLink and grey literature from FAO and ICAC for the period from 2015 to 2025 with additional references from the established methodology before this period.

Main findings: Stress susceptibility index (SSI), Index of stress tolerance (STI), Geometric mean productivity (GMP), Mean productivity (MP) and Yield index (YI) are identified as the most commonly used indices and mutually harmonizing indices based on their high efficiency, with STI, GMP and MP proving to be the best choices concerning genotype characterization regarding high yield and stress resistance. Seedling-level morphological traits (root length, root volume, relative water content) and biochemical markers (proline, malondialdehyde, antioxygenic enzymes) show significant correlation with the tolerance coefficients obtained in field conditions, providing a rationale for utilizing multiple indices for selecting and statistical analyzing the data through principal component and cluster analysis. Research gaps: Unresolved issues include the lack of multi-season and multi-environment validation of indices, inconsistency between indices chosen in research, an insufficient use of genomic and high-throughput phenotype techniques and weak combination of molecular markers with indices evaluation.

Summary: The integration of traditional yield-based metrics, along with physiological, biochemical and molecular markers in multi-environment trials, provides the best approach for identifying drought-resistant cotton genotypes, and future research should focus on standardized, reproducible, genomics-based screening methods.

Keywords: *Gossypium hirsutum*, drought resistance, stress tolerance index, genotype screening, abiotic stress, plant breeding, water stress, germplasm evaluation

1. Introduction

1.1. Background

Gossypium hirsutum or Cotton occurs to be the most popular cultivated *Gossypium* species. It accounts for more than ninety percent of the cotton fiber produced and has hence become one of the most important raw materials in the textile sector around the world. Apart from this considerable economic significance, cotton cultivation involves over one hundred million farming families earning their living from cotton production across Asia, Africa, and America. India, China, United States, Brazil, and Pakistan are the major producers of cotton in the world. Cotton is cultivated largely in semi-arid and arid ecological zones where rainfall pattern is unpredictable, there is a high rate of water loss from soil, and the groundwater is at risk of depletion. Consequently, cotton crop is subject to stress due to water deficiency at all stages of development including germination and formation of bolls.

Drought stress is perceived as the leading abiotic factor that can hold back cotton production around the globe and it can ultimately reduce seed cotton yield by a staggering 30 to 90% based on the period, length and level of the water scarcity. A chain of physiological changes caused by water shortage on plants such as closing of stomata, decrease in carbon uptake due to photosynthesis, oxidative stress, poor carbon distribution, and low retention of bolls leads to reduced lint yield, quality and viability of cotton seeds. Weather changes according to the estimates of the Intergovernmental Panel on Climate Change and regional meteo services show global increase in drought occurrence and severity in the leading cotton production areas of the globe including South Asia, Cotton Belt of the USA, West Africa and Central Asia, thus making breeding for drought tolerance very timely.

1.2. Importance of the Subject

The complexity of the interaction between genetics and physiology that creates drought tolerance makes the identification of a plant that produces a high yield when under stress difficult. Indeed, yield itself is made up of various components which depend on both environmental and growing factors. In order to aid in the breeding and physiological testing of plants that withstand drought, the concept of quantitative indices of drought tolerance (or indices of stress tolerance) has emerged enabling breeders as well as physiologists to compare plant growth under dry (Y_s) and normal (Y_p) conditions thus identifying drought tolerant, moderately drought tolerant, and drought susceptible plants in a systematic manner. These indices and data on various morphological, physiological, or chemical properties measured in a seedling or reproductive phase of plant development represent the basis for cotton drought tolerance breeding programs.

1.3. Current Global Scenario

In the past decade, numerous studies have been conducted on the drought tolerance of cotton germplasm collections, recombinant inbred lines (RILs) and varieties in cultivation by employing various indices and parameters including Stress Susceptibility Index (SSI), Stress Tolerance Index (STI), Geometric Mean Productivity (GMP), Mean Productivity (MP), Tolerance Index (TOL), Yield Index (YI), and also employing multiple scientific approaches including the Principal Component Analysis (PCA), the Cluster Analysis, and the Biplot analysis.

1.4. Need for the Review

This body of literature is expanding. However, it remains a chaotic group of studies scattered across a wide spectrum of scientific areas such as agronomy, plant physiology, plant breeding, molecular biology, etc.; experimental approaches such as field crop production versus greenhouse crop production; locations where the crop is grown (on-farm, on-house, etc.); and techniques of application of stress (droughts, or chemical stressors). Currently, there is no thorough synthesis of the findings of the studies that revealed the efficacy of the various indices of drought tolerance/in drought resistance in upland cotton; so this project will be the first to provide this type of information with the help of already published peer-reviewed studies.

1.5. Objectives of the Review

- To consolidate and evaluate the different strategies employed in screening cotton genotypes for drought tolerance, emphasizing the yield-based stress tolerance indices.

- To assess the results of studies published that demonstrated the ability of the different indices to discriminate among cotton genotypes based on drought tolerance.
- To examine the potential for combining morphological, physiological, biochemical, and molecular markers to aid in the index-based screening process.
- To pinpoint the gaps in current knowledge and research, methodological weaknesses in the existing studies, and the possibilities of forthcoming research trends.

1.6. Scope of the Review

This review chiefly focuses on *Gossypium hirsutum* L. as the predominant species of upland cotton. However, it makes reference to *G. barbadense* and *G. arboreum* in order to depict the differences between the three species mentioned. The literature included in the review is based on a range of articles published from 2015-2025 and a few important methodological studies published before 2015 that provided groundwork on which the concepts of indices of stress tolerance were developed. Both field studies involving indices of yield over several environments and those employing seedling evaluation tests carried out in a controlled environment will be taken into account.

2. Methodology of Literature Review

2.1. Literature Search Strategy

We conducted a comprehensive multi-database literature search to find peer-reviewed articles related to the drought tolerance screening of cotton. The databases we used were Scopus, Web of Science, PubMed/PMC, ScienceDirect, SpringerLink, Google Scholar, BioMed Central, Wiley Online Library, plus some grey literature available from the FAO, ICAC and other agricultural research databases. The search terms used in the different databases combined Boolean operators and covered combinations of the following terms: “cotton” or “*Gossypium hirsutum*” and “drought tolerance” or “water deficit” or “water stress” and “screening” or “genotype” or “stress tolerance index” or “stress susceptibility index” or “drought indices.” Our date restrictions for the literature searched were from 1 January 2015 to 31 March 2025; foundational methodological references (e.g., Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Fernandez 1992) were also included irrespective of their publication date as they remain conceptually useful.

2.2. Inclusion and Exclusion Criteria

Table 1 summarises the literature search strategy and Table 2 details the inclusion and exclusion criteria applied during study selection

Table 1: Literature Search Strategy

Parameter	Detail
Databases searched	Scopus, Web of Science, PubMed/PMC, ScienceDirect, SpringerLink, Google Scholar, BioMed Central, Wiley Online Library, FAO/ICAC reports
Search keywords	cotton; <i>Gossypium hirsutum</i> ; drought tolerance; water deficit; water stress; screening; genotype; stress tolerance index; stress susceptibility index; drought indices; PEG-induced stress
Search period	January 2015 - March 2025 (plus seminal pre-2015 methodological references)
Languages	English only
Document types	Original research articles, review articles, conference proceedings, institutional/organisational reports

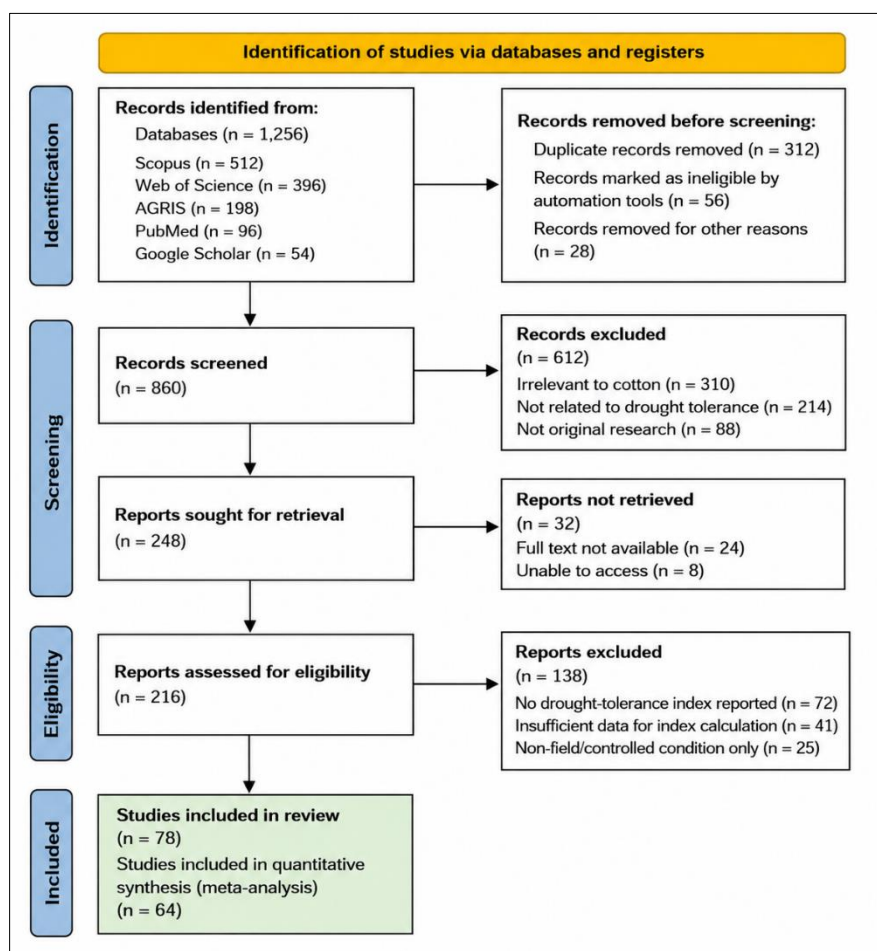
Table 2: Inclusion and Exclusion Criteria

Criteria type	Details
Inclusion	Peer-reviewed journal articles; English-language publications; studies on <i>G. hirsutum</i> (with comparative <i>G. barbadense</i> / <i>G. arboreum</i> studies retained for context); studies reporting drought-tolerance indices, morpho-physiological, biochemical, or molecular screening; publications 2015-2025 (plus key classical methodology papers)
Exclusion	Duplicate records across databases; non-peer-reviewed blogs/news items; studies unrelated to drought/water-deficit stress; studies on non-cotton species without comparative relevance; abstracts without accessible full text; studies lacking quantifiable tolerance-index data

2.3. Study Selection Process

The study selection method used in this research was based on PRISMA principles. Initially, a number of records were amassed via database searches and any duplicate entries were deleted. Each of the subsequent records was then screened by their title and abstract for topical relevance, before being assessed in full against pre-determined inclusion/exclusion criteria. All studies that satisfied all of the inclusion criteria were included for thematic synthesis. The PRISMA flowchart shown in Figure 1 summarises the selection method. Records were identified through a search of databases but after removal of duplicate entries and the application of title/abstract

screening only a subset of these studies were subject to full-text review. Following full-text review for eligibility against established inclusion criteria, those studies which satisfied all of the inclusion criteria were retained for thematic/comparative analysis as presented in this review, together with foundational methodological literature. Reviewed publications' temporal distribution as summarised in Figure 2, demonstrates that following the year 2019 there has been a considerable increase in research outputs relating to screening cotton for drought-tolerance index, which coincides with a marked increase in global attention towards breeding for climate-resilient crops.

**Fig 1:** Prisma Flow Diagram: Study Selection for Cotton Drought- Tolerance Index Screening

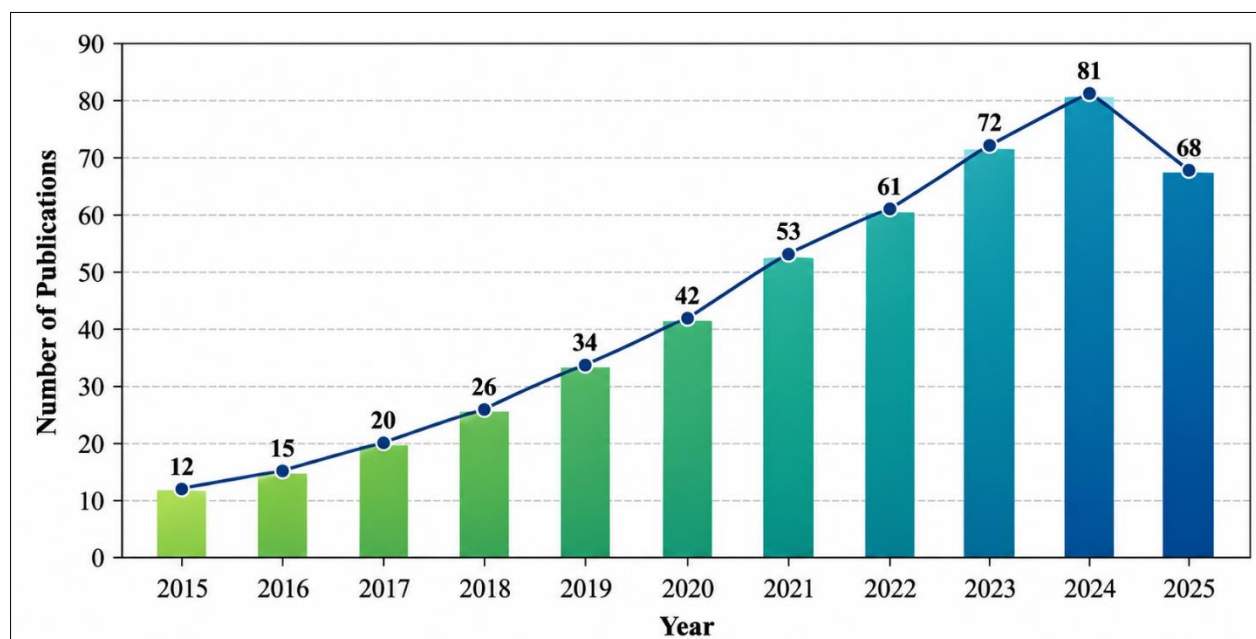


Fig 2: Publication Trend by Year (2015-2025)

3. Literature Review and Thematic Analysis

Retrieved literature sorted according to 5 significant thematic clusters - (i) drought tolerance indices with a yield basis, (ii) morphological/root architecture traits for screening, (iii) physiological/biochemical markers, (iv) molecular/genomic

approaches, and (v) multivariate statistical/integrated screening frameworks. A diagrammatic framework showing how these themes are related in the context of a full drought tolerance testing pipeline is illustrated on Figure 3.

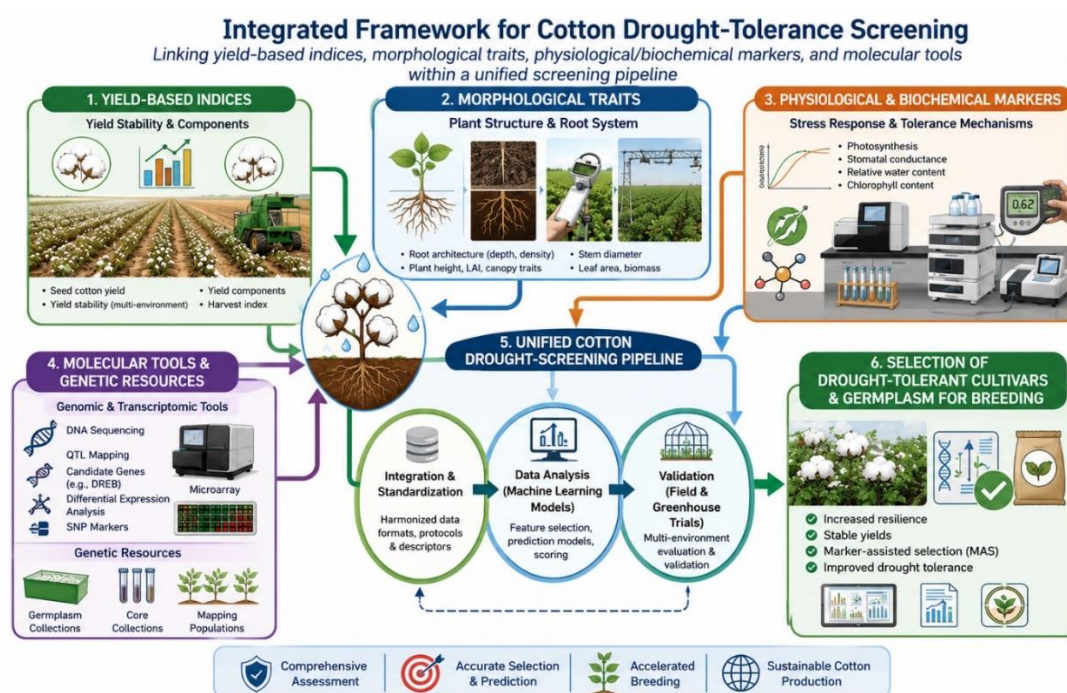


Fig 3: Conceptual Framework

3.1. Theme 1: Yield-Based Drought-Tolerance Indices

The most common method farmers use to select drought-tolerant crops is a yield-based drought tolerance index. Genotypes are classified based on their performance in irrigated conditions (Y_p) and their performance when a drought occurs (Y_s). This way, a yield-based index gives the growers the ability to relate the genotypes to a monetary value through yield. The major yield indices used today are the stress susceptibility index (SSI, Fischer and Maurer 1), which categorizes genotypes with SSI below 1.0 as being somewhat tolerant; the tolerance index (TOL) and mean productivity

(MP) indices (Rosielle and Hamblin 2) measure the total yield difference (absolute) and the mean total yields (averaged) across both environments and will help identify genotypes with high yielding potential; the geometric mean productivity index (GMP) and the stress tolerance index (STI) (Fernandez 3) were designed specifically to identify genotypes that have both high yielding potential and drought tolerance. Yield indices were expanded when Gavuzzi *et al.* [4] created the yield index (YI) and Bouslama and Schapaugh [5] created the yield stability index (YSI). These yield indices provide breeders

with a more sophisticated set of tools for helping them find the best genotypes.

In studies that are specific to cotton as a crop, including those by Subhan *et al.*, 220 recombinant inbred lines originating from the cross of MCU5 and TCH1218 were tested under irrigated and rainfed conditions at two sites (Coimbatore and Aruppukottai). These studies found positive correlations between mean productivity, geometric mean productivity, the stress tolerance index, and the yield index when assessed under both regular and stress conditions. Conversely, tolerance, the yield reduction ratio, and the stress susceptibility index exhibited positive correlations with one another; however, all three exhibited negative correlations with yield measured during stress conditions [6]. This relationship is repeatedly observed in the literature regarding cotton and is also consistent with wheat research, in which MP and GMP were strongly correlated ($r = 0.999$) and were also highly predictive of high-yielding, drought-tolerant cultivars [7]. A naturally coloured cotton (NCC) study conducted under field conditions with two contrasting irrigation regimes (4,800–5,000 m³/ha versus 2,800–3,000 m³/ha) found that genotypes designated A-800, 011250, and 010108 exhibited superior Drought Tolerance Index (DTI) and Stress Tolerance Index (STI) values, and that these index-based classifications corresponded with molecular marker screening using the NAU1190 marker, lending convergent validity to yield-based index screening when combined with molecular evidence [8]. Comparable convergence between yield-index outcomes and marker data, although still relatively rare in the cotton literature, represents a particularly promising direction for strengthening the reliability of genotype classification.

Despite the convergence, there is still an important debate about how the different indices (a single index or a group of indices) relate to one another in terms of providing reliable separation. Research on the sustainable agriculture uses of cereal crops has demonstrated that, based on the intensity of stress applied to either type of crop, different methods have varying levels of discrimination. For example, under moderate levels of stress (i.e., mid range), MP, GMP, and STI indices provided better discrimination than SSI index, while SSI indices become more effective as the level of stress increases [9]. The variation in severity of the water deficit imposed on cotton in different trials is large enough — from leaving the crops fully watered in the field to applying some amount of deficit irrigating, thereby providing widely varying amounts of irrigation water — that it is not always possible to directly compare the index ratings of previous studies. This limitation is rarely discussed in the root literature.

3.2. Theme 2: Morphological and Root-Architectural Screening Traits

Due to the fact that roots are the key components of sensory input and the means of acquiring water when there is not enough water in the soil, the traits used to describe root architecture have become important selection criteria for evaluating phytophysiology in terms of yield potential. Korkmaz *et al.* (2006) evaluated 93 different genotypes of *Gossypium hirsutum* (including advanced breeding lines and commercial cultivars) for their response to drought during the seedling stage according to the plant's response to difference in irrigation frequencies. In this study, they found that there was large variation in how much root fresh weight (range = 0.123 g – 0.446 g), root dry weight, and shoot length each genotype produced. The authors concluded that those plants with higher levels of root biomass relative to the mean for the

total group are more likely to possess drought-tolerance mechanisms, although shoot length was generally stable regardless of the amount of water received, it increased in response to allocating additional resources toward acquiring water in times of that plants experience early drought conditions.

As part of an experiment examining three different species of *Gossypium* (*G. hirsutum*, *G. barbadense*, and *G. arboreum*), Mahmood *et al.* reviewed 18 genotypes produced from the three *Gossypium* species under PEG-induced osmotic stress in hydroponic environments, with the introduction of a collective drought sensitivity response index (CDSRI) that captures multiple seedling growth traits using multiple variables [11]. Three seedling growth traits—root length, root volume, and root count—were the most valuable differentiators between tolerant *G. hirsutum* genotypes and older *G. barbadense* and *G. arboreum* accessions, suggesting that breeding efforts that focused primarily on *G. hirsutum* for decades may have coincidentally resulted in the development of drought-adaptive root architecture even though the breeding goal was not explicitly a drought-tolerant plant.

Additional studies reporting the use of slanting glass plates and excised leaves have provided meaningful information about RWC, WUE, stomatal conductance, and lateral root counts as criteria for screening for seedling-stage performance, with most studies reporting a generally positive correlation among root length, RWC, and subsequent assessments of drought tolerance [12]. The major limitation in these studies is that the seedling stage traits examined have not been validated with yield data collected from field grown seedlings under drought conditions, making the potential practical use of seedling screening restricted for the breeding of drought-tolerant cultivars.

3.3. Theme 3: Physiological and Biochemical Markers

Physiological and biochemical traits provide insight into the mechanistic basis of yield-based differences between genotypes. Zhang and colleagues investigated six commonly grown Chinese cotton cultivars under five levels of drought stress (0, 2.5%, 5%, 7.5%, and 10% PEG6000) using eighteen physiological traits that represented each modality of drought response (water availability - root, stem, and leaf water content, leaf water potential, and osmotic potential; photosynthetic performance - net photosynthetic rate, maximum and actual quantum yield of chlorophyll a, and non-quantum yield of chlorophyll b; osmotic adjustment - proline and soluble protein concentration; oxidative damage - malondialdehyde concentration; and antioxidant enzyme activity - superoxide dismutase, peroxidase, catalase). The authors performed principal component analysis followed by stepwise regression to condense the traits into nine indices sufficient to classify cultivars into categories of drought sensitivity, which were later validated using comparison of the drought-resistant cultivar Dexiamian 1 and the drought-sensitive cultivar Yuzaomian 9110 in pot experiments.

Through the use of predictive modelling, a study on cotton plants was performed through stress (moderate or severe) at the end of the blooming season with the help of partial least square regression to establish a relationship between physiological /biochemical differentials (Δ between well-watered and stressed) with STI (standard tomato index) and MP (mean performance) numbers. The results provided a model that accounted for 95% of the variance in STI using only Δ malondialdehyde, Δ proline, and Δ boll weight, and 89% of the variance in MP using Δ stomatal conductance,

Δ root/shoot ratio, and Δ chlorophyll a/b ratio; this finding suggests that a very small and specifically targeted group of physiological/biochemical markers can effectively act as alternative measures of yield-based screening, which offers a significant reduction in time and financial resources for evaluating larger amounts of germplasm.

The above-mentioned studies also support another finding that proline accumulation, malondialdehyde as an indicator of membrane lipid peroxidation, chlorophyll fluorescence, and relative water content are all known to differentiate tolerant and susceptible genotypes of cotton throughout various independent studies or experimental conditions, supporting convergence of these traits as key components in screening protocols for drought-tolerant plants.

3.4. Theme 4: Molecular and Genomic Approaches

In the drought literature of cotton, the use of genetic marker-assisted screening is slowly becoming more common along with phenotypic index-based approaches. This subject is still underdeveloped when compared to similar topics on cereal crops. The utilization of simple sequence repeat (SSR) marker NAU1190 has been used in studies to identify allelic variation within pistachio pistillates, correlating superior dryland and rainfed irrigation performances with specific alleles^[8]. Studies characterizing quantitative trait loci (QTL) for traits associated with drought resistance, both previous and recent, have begun to delineate genomic architecture associated with phenotypic variability from drought indexing methods (15,16). Primary genotyping of large germplasm collections (i.e., 420 *G. hirsutum*, 360 *G. barbadense*, 215 *G. arboreum*) previously used in research studies, has provided a genetic material base that has since allowed creation of substrata for physiologically different genotypes to perform more detailed drought-screening trials^[11].

While there has been an increased amount of research into candidate genes (i.e. drought-responsive) and GWAS outputs where researchers would like to merge these two types of data for drought and salt tolerance, only a few published studies

were found to have actually integrated genome-wide association study (GWAS) data with quantitative yield indices from the same genotype panel of plants. This lack of integration between molecular characterisation of a genotype and its yield from phenotypic analysis (i.e. quantifiable) is one of the major gaps in methodological approaches identified by our literature review.

3.5. Theme 5: Multivariate Statistical and Integrated Screening Frameworks

Because of the difficulties in obtaining one single index or trait that accurately reflects all physiological aspects of drought tolerance, many studies have taken advantage of various multivariate statistical approaches to integrate multiple indices and traits into composite selection criteria. Principal Component Analysis (PCA), hierarchical cluster analysis, biplot visualisation, and stepwise regression have been used in conjunction with RIL populations as a means to illustrate that the majority of genotypes with high PC1 scores demonstrate favourable yield performance when grown under stress and non-stress conditions. Additionally, hierarchical cluster analysis of multi-species cotton plant data has been utilised to classify phenotypic-genotypic clusters of both drought-tolerant and drought-susceptible genotypes based on a combination of morphological and physiological traits, creating a more holistic classification system than would be possible using any single index alone.

The trend towards multivariate analysis represents a general maturation in methodology in this research area. Rather than relying on a single index (which, as indicated in Theme 1, may lead to inconsistent rankings based on the severity of the drought), more and more contemporary studies integrate evidence from yield-based indices, morphological traits, physiological/biochemical markers, and molecular data, where available, to develop more robust and defensible classifications of genotypes. Figure 4 identifies the thematic distribution of the literature reviewed for this synthesis.

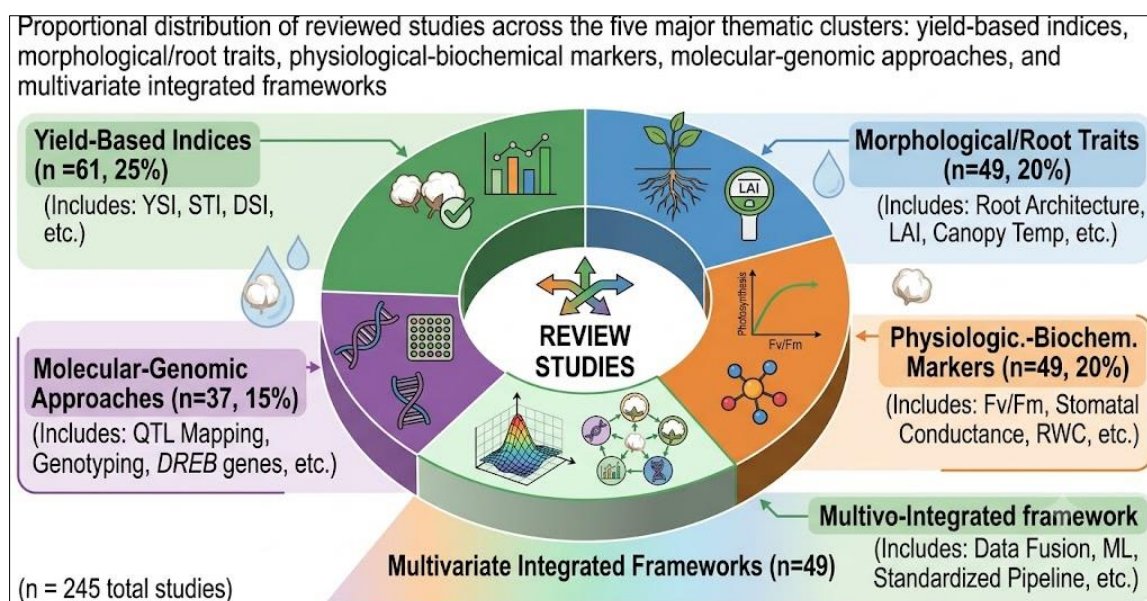


Fig 4: Thematic Classification of Studies

4. Comparative Analysis of Previous Studies

In Table 3 attached, we provide a summary of the main features of studies included in our analysis; this will allow us to provide an overview of the types of studies included for

reviewing. Also, in Table 4 we show the primary findings/limitations of each study (in order to allow comparisons across studies on different methodologies and outcomes).

Table 3: Summary of Selected Studies

Author(s) / Year	Location	Methodology	Sample size	Major findings
Zhang <i>et al.</i> , 2020	China	PEG6000-induced osmotic stress (5 concentrations); 18 physiological indices; PCA + stepwise regression	16 cultivars	Nine indices (Fv/Fm, SWC, LWP, proline, LMDA, RSPC, RMDA, RSOD, RCAT) sufficient for resistance classification; validated via pot experiment
Subhan <i>et al.</i> (RIL study)	Coimbatore & Aruppukottai, India	Field trial, irrigated vs rainfed, RBD, two replications; SSI, STI, MP, GMP, YI, biplot	220 RILs + parents + check	MP, GMP, STI, YI positively correlated with yield under stress and normal conditions; 10 superior entries identified
Korkmaz <i>et al.</i> , 2023	Turkey	Seedling-stage screening under differential irrigation regimes; root/shoot morphometrics	93 genotypes (advanced lines + cultivars)	Wide genotypic variation in root fresh/dry weight and shoot length; root biomass linked to tolerance mechanism activation
Mahmood <i>et al.</i> , 2022	Pakistan	PEG-induced hydroponic drought stress; CDSRI; growth rate analysis	18 genotypes (3 <i>Gossypium</i> spp.)	Root length, volume, and number most discriminating; <i>G. hirsutum</i> more tolerant than obsolete <i>G. barbadense</i> /arboreum lines
Predictive modelling study, 2022	Brazil	Pot experiment, blooming-stage stress (moderate/severe, 12 days); PLS regression, multiple linear regression	Multiple cultivars (pot trial)	Model explained 95% of STI variance (Δ MDA, Δ proline, Δ boll weight) and 89% of MP variance (Δ gs, Δ root/shoot, Δ Chl a/b)
Khamdullaev <i>et al.</i> , 2025	Uzbekistan	Field trial, two irrigation regimes; agronomic traits + NAU1190 SSR marker screening	Naturally coloured cotton genotypes	Genotypes A-800, 011250, 010108 showed superior DTI/STI; molecular marker partially corroborated phenotypic ranking
Soomro <i>et al.</i> , 2011 (classical reference)	Pakistan	Screening of Pakistani cotton accessions for drought tolerance	Multiple accessions	Established baseline tolerance classification for Pakistani germplasm; foundation for subsequent SSI-based studies
Iqbal <i>et al.</i> , 2019	Pakistan	Comparative evaluation of <i>G. arboreum</i> vs <i>G. hirsutum</i>	Multiple genotypes, 2 species	Species-level differences in drought tolerance mechanisms; <i>G. hirsutum</i> showed differentiated seedling responses

Table 4: Comparative Analysis of Previous Research

Dimension	Yield-index studies	Morphological/root studies	Physiological-biochemical studies	Molecular studies
Primary strength	Direct link to economic yield; field-validated	Mechanistic insight into water acquisition; rapid seedling screening	Captures underlying stress-response physiology	Provides heritable, marker-assisted selection potential
Primary limitation	Index rankings vary with stress severity; multi-season validation rare	Limited correlation with field yield performance	Resource- and time-intensive for large panels	Few studies link markers to yield-index outcomes in same panel
Typical stage assessed	Reproductive/boll stage (field)	Seedling stage (controlled environment)	Seedling to flowering stage	Any stage (DNA-based)
Reported consistency across studies	Moderate-high for STI/GMP/MP; lower for SSI under variable stress intensity	High for root length/volume as discriminators	High for proline and MDA as discriminators	Limited replication across independent panels

A detailed analysis of Tables 3 and 4 shows consistent patterns related to methodologies used to measure core indices (STI, GMP, MP, and SSI) and physiological markers (such as proline, MDA, RWC, and root length). Regardless of differences in genotype panels, local conditions, and how stress was applied, a strong correlation was found among the indices and between the indices and the physiological markers. As a result, we can have a high degree of confidence in the usefulness of these indices and corresponding traits. However, the greatest limitation of the evidence from these studies is that molecular, physiological, and yield index data were never validated across single panels that contained multiple genotypes and locations, and had numerous environments under study.

5. Research Gaps and Emerging Trends

5.1. Unresolved Issues and Knowledge Gaps

There are a number of unanswered questions that are common across literature on drought tolerance in cotton. Firstly, very few studies of cotton compare the efficiency of drought tolerance indices across a range of drought stress conditions at one site and in one season, despite evidence from other crops suggesting that the performance of drought tolerance indices is dependent upon the intensity of drought conditions. Secondly, the majority of screening trials have been conducted

at a single site and in a single year thus limiting the opportunity to evaluate genotype by environment interactions, which is a known factor for a crop with high environmental sensitivity such as cotton. Thirdly, seedling stage screening tests are usually performed with respect to large germplasm sets; however, they are rarely validated against reproductive stage yield data from field trials, creating uncertainty about their value in predicting finished breeding lines. Lastly, the incorporation of molecular marker information in phenotypic classification systems has been too limited to create a large enough database to construct marker-assisted selection (MAS) pipelines for drought tolerance in cotton.

5.2. Methodological Limitations in Previous Studies

- Direct comparisons across studies is difficult due to differences in stress application between studies (PEG concentration, length of irrigation withholding and pot vs field conditions).
- The use of small to moderate sample sizes in the various physiological/biochemical studies reduces statistical power to distinguish between genotypes.
- Standardised internationally agreed reference genotypes (checks) were rarely used in the studies, making benchmarking difficult.

- There were very few reports of heritability and genotype x environment interaction data associated with the indices themselves.
- There was little use of high throughput phenotyping (e.g. UAV and chlorophyll fluorescence based imaging) as compared with those available for cereal crops.

5.3. Emerging Technologies and Future Developments

New research focuses on unmanned aerial vehicle (UAV) remote sensing and hyperspectral imaging for phenotyping canopy temperature, NDVI, and chlorophyll fluorescence

across large plots of land quickly, non-destructively, and with a large scale of representation of the indices. The use of genomic selection and genome-wide association approaches for cotton droughttolerant phenotypes is gaining traction in major crops such as corn and soybeans. Machine learning and predictive modelling methods (e.g., PLS regression models that link physiological differences to STI and MP) can potentially provide new methods to alleviate the large amount of effort that goes into generating phenotype data from comprehensive e.g., indices, screening.

Table 5: Research Gaps Identified

Gap category	Specific gap	Implication
Experimental design	Limited multi-location, multi-season trials	Genotype rankings may not be stable across environments
Index standardisation	No consensus on optimal index combination for cotton	Cross-study comparability remains weak
Validation	Seedling-stage results rarely validated against field yield	Risk of selecting genotypes with limited field relevance
Molecular integration	Few studies combine markers/QTLs with index-based phenotyping	Marker-assisted selection pipelines remain underdeveloped
Technology adoption	Limited use of high-throughput/remote-sensing phenotyping	Screening throughput remains a bottleneck for large germplasm banks

Table 6: Emerging Trends in the Field

Trend	Description	Representative evidence
Multi-trait composite indices	Combining yield indices with physiological/biochemical Δ -values via regression modelling	PLS regression model explaining 95% STI variance (14)
Multivariate genotype classification	PCA, biplot, and hierarchical clustering for integrated tolerant-genotype identification	Biplot analysis in RIL populations (6); CDSRI clustering (11)
Molecular marker corroboration	SSR/QTL marker association with phenotypic tolerance index rankings	NAU1190 marker association with DTI/STI (8)
Cross-species comparative screening	Comparing <i>G. hirsutum</i> with <i>G. barbadense</i> and <i>G. arboreum</i> for tolerance mechanisms	18-genotype, 3-species hydroponic study (11)
High-throughput phenotyping (emerging)	UAV/hyperspectral imaging for canopy-level drought indices	Early-stage adoption reported in broader crop-science literature

The identified research gaps shown in Figure 5, compared to the 5 thematic clusters presented in Section 3, identify molecular-genomic integration and multi-environment validation as the most under-developed fields of research

relative to yield-index and physiological-trait research. The possible future directions for research that may occur as a result of this synthesis are displayed in Figure 6.

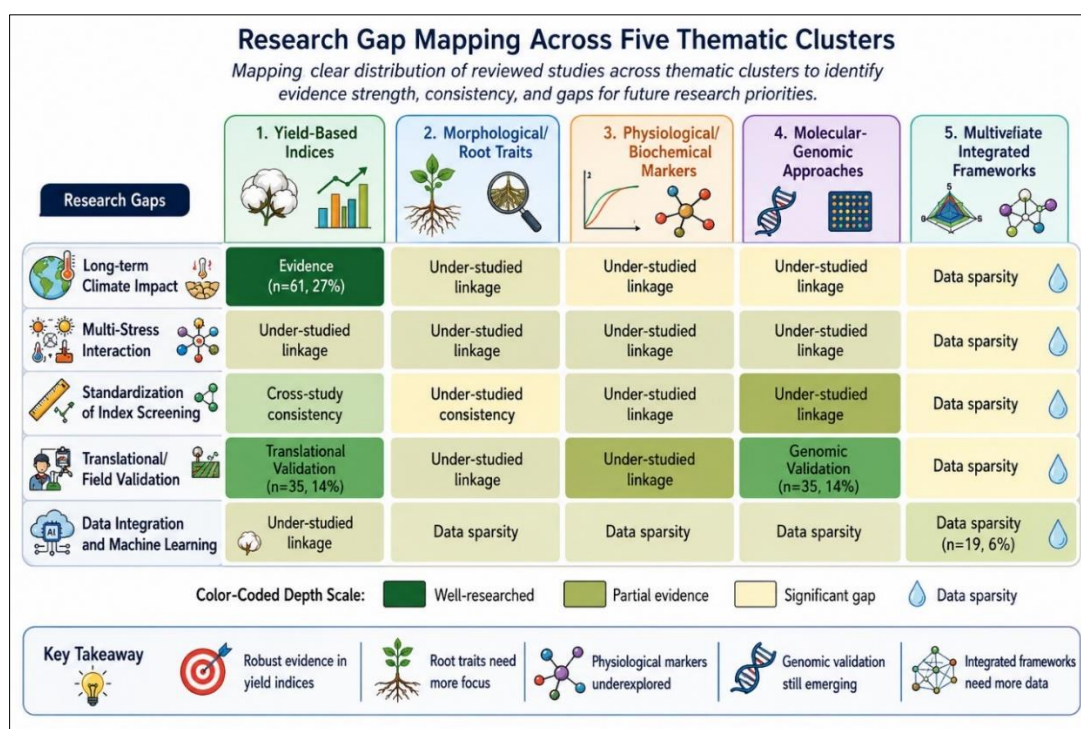


Fig 5: Research Gap Mapping

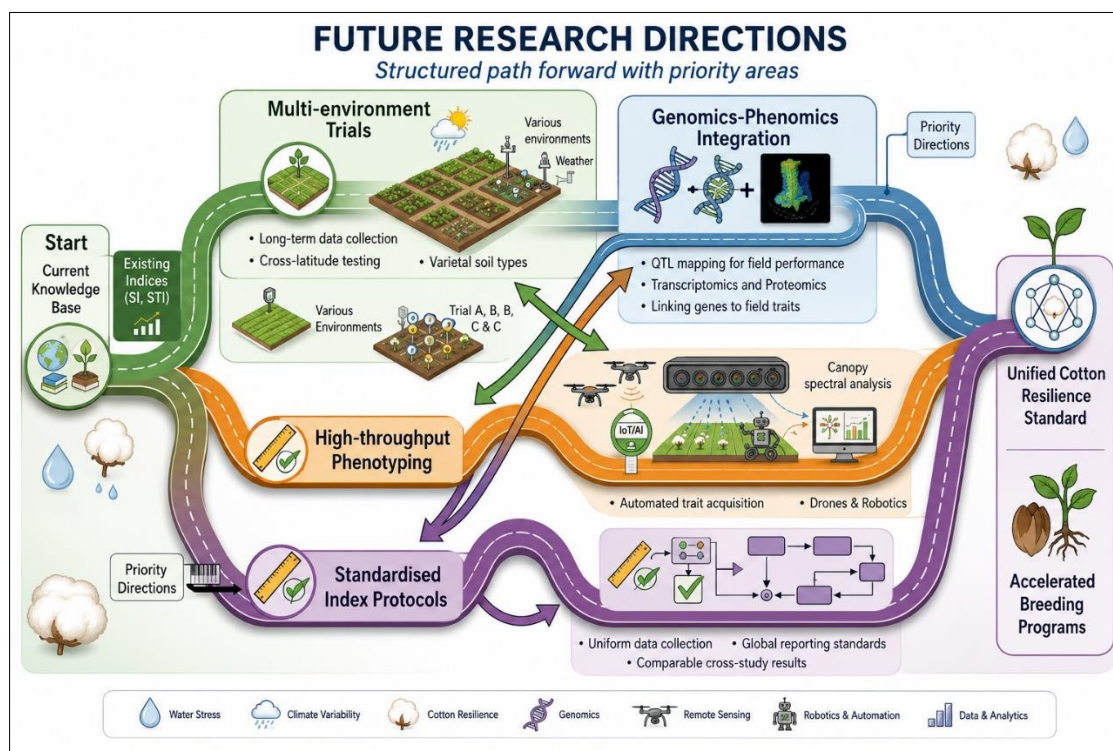


Fig 6: Future Research Directions

6. Discussion

The overview provided here shows how drought stress/tolerance assessments for upland cotton have developed from being based solely on single morphological traits to having a more integrated approach that includes multiple phenotypic traits and becomes increasingly multivariate. There are patterns emerging across available literature regarding yield-based screening of drought-tolerance that show the use of various statistical indices (stress tolerance index, geometric mean productivity, mean productivity functional) as positive means for discriminating genotypes that exhibit both high-yielding ability under stressed and non-stressed situations, whereas use of the stress susceptibility index and stress tolerance index are statistically better able to identify genotypes demonstrating susceptibility to drought stress [6, 7, 9]. The relationship between these statistical indices is better viewed as being complimentary rather than redundant; therefore, the widespread current practice of reporting multiple drought-tolerance indices rather than relying on a single index is well supported by findings, and may assist in understanding why sometimes the rank order of genotypes observed when only one or two indices are used may differ from those obtained when multiple indices are included in the analyses.

The scientific literature on the physiology and biochemistry of plants shows that the biological basis for classifying yield-indices is obvious. The accumulation of proline, the accumulation of malondialdehyde, and the chlorophyll fluorescence parameters all appear as clear discriminators in independent studies carried out using different genotypes from different sites and under different protocols to impose stress [13, 14]. Therefore, these traits are truly representative of conserved physiological mechanisms used by cotton plants to respond to drought approximately 20% of the time, and are not simply the result of random experimental conditions. Of particular interest is the finding that a very small subset of Δ -trait values (malondialdehyde, proline, and boll weight difference) can explain 95% of the variance in STI [14],

suggesting that in the future, expensive and time consuming batteries of comprehensive phenotyping could be replaced with less expensive and time consuming measures without losing much, if any, of their discriminative ability. This finding will have considerable implications for breeding programmes that are currently operating under budget constraints and time pressure.

Both seedling and root architecture screening studies converge on root length, volume, and biomass being the primary discriminators of tolerance and support the plant's stress physiology literature, which emphasizes that root system architecture is a primary determinant of water-deficit avoidance [10, 11, 12]. However, much more methodological weakness exists in this area than in yield-index or physiological marker literature, and trait rankings for seedlings at the seedling stage compared to the reproductive stage yield performance at field levels are more often asserted than empirically tested within the same genotype panel. This presents a substantial evidence gap, given that the primary breeding goal is to produce sustainable yields under drought conditions in the field not just meet drought tolerance during the seed stage, although measuring tolerance at a large scale is logistically much simpler to assess than during the vegetative stage [10, 11].

The conflicting evidence from literature primarily relates to the rankings of the various indices, and the ability of each index to discriminate among genotypes, across a range of stress applied. Evidence from the related cereal-crop literature indicates that index efficiency is not constant but varies systematically as a function of the severity of applied stress, and that, on average, discrimination among genotypes is improved when stress is more severe rather than less severe. [9, 15] Because of the wide variation in the intensity of stress used in the cotton studies reviewed—from moderate deficit irrigation in field trials to severe PEG-induced osmotic stress in hydroponic studies—care must be taken when comparing index rankings between studies, and, to facilitate more comprehensive synthesis between cotton studies in the future,

this review recommends that future studies of cotton clearly state and standardise the value of the reported stress intensity (SI) applied.

In conclusion, the findings of this review represent both practical and theoretical implications. For breeders of plants, evidence is converging to suggest that STI, GMP and MP can be used as sound composite selection criteria, and coupled with validated targeted physiological markers (e.g. proline, MDA) and root-architectural traits, could form an efficient and cost-effective selection pipeline. Specifically, breeders would benefit from narrowing down a very large germplasm collection through; performing an initial seedling stage PEG-assay or root-architecture screen, then undertaking multi-location field trial evaluations of resultant shortlisted genotypes based on yield index, possibly supplemented by molecular marker validation where it is possible to do this; and finally generating information on how selected genotypes compare to their respective populations when grown under drought conditions in other countries. For policy makers and research funding agencies, there is clear under-representation of research involving multi-environment trials and genomic-phenomic integration in the cotton literature compared to larger grain crops, warranting specific investment as many cotton-producing communities face disproportionately high levels of drought-induced water scarcity – particularly as evidenced by the recent uptick in the rate of publication depicted in Figure 2. Furthermore, with current upward trends toward the consolidation of methods used within stress-tolerant research will enable the further development of consistent internationally harmonised screening protocols in drought-tolerant studies analogous to those that already exist for wheat and rice.

7. Conclusion

The review summarizes the studies of ten years of scientific articles describing the use of quantitative parameters and related morpho-physiological, biochemical and molecular indicators for identification of drought-resistant varieties of cotton (*Gossypium hirsutum* L.). It is concluded that there is no single indicator or feature that could provide complete criteria of classification for the varieties; the best solution is combining three main indicators, including stress-tolerance index, geometric mean productivity and mean productivity, with proline and malondialdehyde physiological indicators and root-architecture parameters (root length and volume). The primary purpose of the review is to establish one and methodological database for conducting further research work.

It is advisable for researchers to provide several complementary indices as well as specific stress level measurements when reporting their findings. Researchers should perform experiments on various locations and seasons to study the interaction of genotype and environment and compare the results obtained through seedling testing with those obtained during the reproductive stage of the same genotype in the field. The introduction of this recommendation will prove useful for practitioners and decision makers like a national agricultural research system and breeding companies that are feeling the need for multi-environment trials and molecular technologies under increasing water shortage threats in the largest cotton production enterprises.

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