

# SCREENING OF PROMISING HIGH YIELDING MAIZE (ZEA MAYS L.) CULTIVARS FOR DROUGHT TOLERANCE USING CHLOROPHYLL STABILITY INDEX

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## ABSTRACT

Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. In the present investigation, an attempt was made for screening promising high yielding maize (*Zea mays* L.) cultivars for drought tolerance using chlorophyll stability index. Fourteen varieties of maize were evaluated in the present study under drought and rain fed and irrigated conditions. The following investigation was carried out to find out and determine chlorophyll stability index (CSI) in 14 genotype lines of maize as per the technique suggested by Kaloyereas (1958) Murthy and Majmudar (1962). Composite leaf samples used as a tool in screening germplasm for drought tolerance in breeding programmes in evaluate the efficiency of high yielding varieties resistant for drought tolerance. The results showed that maize grain yield was ranging between 48.75 to 64.50 Q/ha with a mean of 53.932 q/ha. Pusa Hybrid Makka 1 maize variety recorded the highest of 14 selected maize varieties and DHM – 103 recorded the lowest of all.

The results of Chlorophyll A & B and Total Chlorophyll contents in different maize varieties tested indicated that Chlorophyll A of these varieties is ranged between 1.675 and 2.450mg/g with a mean of 2.063 mg/g and Pusa Hybrid Makka 1 Maize variety has recorded highest Chlorophyll A content and NMH 1242 Maize variety has recorded lowest Chlorophyll A content. Chlorophyll B of these varieties is ranged between 0.782 and 1.144mg/g with a mean of 0.963mg/g. Pusa Hybrid Makka 1 Maize variety has recorded highest Chlorophyll B content and NMH 1242 Maize variety has recorded lowest Chlorophyll B content. Total Chlorophyll content was found to be highest of 3.594mg/g in Pusa Hybrid Makka 1 maize variety and lowest in NMH 1242 maize variety of 2.457mg/g. Total Chlorophyll content in different maize varieties tested were ranging from 2.457 mg/g to 3.594mg/g with a mean of 3.026mg/g. Chlorophyll Stability Index of these varieties ranged between 3.667 and 5.134 with an average of 4.361. In the first group of long duration variety

Pusa Hybrid Makka 1 maize variety performed well and recorded highest Chlorophyll stability Index. In the second group of medium duration variety DHM-101 and DHM - 107 maize varieties recorded highest CSI value and in third group of short duration varieties respectively. Maize variety Trisulatha recorded highest value of CSI. It is evident that while the maize varieties in kharif season with yield potential under stress have exhibited and recoded low CSI ranging from 4.030 to 4.431. The highest CSI observed in having low potential yield of the normal yield under stress condition.

**Key Words: Maize genotypes, Chlorophyll A, B and Total Chlorophyll, Chlorophyll Stability Index, Drought Tolerant.**

## INTRODUCTION

It is well known that water scarcity limits crop production and further expansion of agriculture. Maize cultivars and promising high yielding Maize genotypes that can endure and recover from drought are needed to minimize yield loss in dry land areas and to reduce the water needs of irrigated production. Several efforts have been taken to improve maize production under water limiting conditions through conventional breeding techniques. However, progress in traditional breeding approach has been slow due to limited knowledge on genetics of drought tolerance and involvement of several complex tolerance mechanisms. Water deficit or drought stress is a environmental factor and the major constraint on plant productivity with an evident on plant growth.

Maize (*Zea mays* L.) is a cereal grain called “queen of cereals” is the third most important cereal crop in India after rice and wheat. In India, is grown in an area of 8.69 m ha and production of 21.81 mt with the average productivity of 2509 kg ha<sup>-1</sup>.

In India, the production of maize witnessed a significant increase of more than 14 times from a mere 1.73 million tons in 1950-51 to 24.17 million tons in 2014-15. Presently it occupies 9.23 million hectare area with the mean yield of 2.56 tons/hectare. Maize grain is gaining popularity in our country due to huge demand, particularly for poultry feed industry. The most important maize growing states are Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, Rajasthan, Bihar, Uttar Pradesh, Gujarat and Madhya Pradesh, which account for more than 80% of the total maize area of the country and also account for similar share in production. Both area and production of maize have been steadily increasing. Apart from uses as food and feed, maize has great demand in the development of various products in

different industries viz., pharmacy, textile, paper, film, tyre, bio -fuel etc. Maize is utilized domestically for poultry and cattle feed, food, manufacturing of starch and other industrial purposes. Being a potential crop in India, maize occupies an important place as a source of human food (25%), animal feed (12%), poultry feed (49%), industrial products mainly as starch (12%) and 1% each in beverages and seed. In the last few years, good quantity of maize is also being exported from India to different countries. It is understood that with the increasing demand for value added foods and industrial requirements, from a growing economy and population, maize will hold its share as an important cereal crop.

Telangana, Andhra Pradesh and Karnataka is one of the major producers of maize with an area of 1.18 m ha and a production of 3.27 mt with the productivity of 2773 kg ha<sup>-1</sup> (Anon., 2016). Majority of the maize in Telangana, Andhra Pradesh and Karnataka is grown under rain fed conditions and the occurrence of drought limits the productivity during different growth stages. Drought stress during post-anthesis stages is responsible for kernel weight reduction (Oveysi *et al.*, 2010). It is reported that drought stress during kernel development is responsible for 20–30 % yield losses which are mainly due to undersized kernels (Heinigre, 2000). Another report mentioned that drought prevalence during kernel development can cause 2.5–5.8 % yield losses on a daily basis (Lauer,2003). Kumar, et al., 2003 investigated on two genotypes each of maize and rice were compared for their response to varying degrees of temperature stress (35/30, 40/35, 45/40°C) with controls growing at 30/25°C. At elevated temperatures of 40/35 and 45/40°C, the rice genotypes were inhibited to a significantly higher extent, especially for their shoot growth compared to maize genotypes. The stress injury measured as damage to membranes, loss of chlorophyll and reduction in leaf water status was significantly higher in rice plants, especially at 45/40°C. They have suggested that maize growth was inhibited to a lesser degree than rice genotypes under heat stress of 45/40°C. These differences might be attributed to lesser oxidative stress injury coupled with greater expression of enzymatic and non-enzymatic antioxidants, especially ascorbate and glutathione in maize that possibly conferred superior stability to membranes, chlorophyll and eventually growth. Since, maize and rice genotypes belong to C4 and C3 plant groups, respectively, these variations also imply that C4 plant types might be better equipped to deal with heat stress than C3 plants. ( (Kumar et.al., 2003).

Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots (Mirza et al., 2013; Srichaikul *et al.*, 2011). However the chlorophyll production is mainly depended on penetration of sun light and it is the main source of energy for plant (Srichaikul *et al.*, 2011). In the laboratory it is commonly determined by using pestle and mortar to extract the pigments using an organic solvent such as acetone or dimethyl formamide (Arnon 1949; Porra *et al.*, 1989; Ling *et al.*, 2011; Masood Kiani,2013). Chlorophyll a and Chlorophyll b are essential pigments of the plant photo systems (Richardson *et al.*, 2002). Moreover the chlorophyll A is the primary photosynthetic pigment in plants which helps to produce energy in plant (Srichaikul *et al.*, 2011). However the chlorophyll A concentration is 2-3 times higher than that of secondary chlorophyll b in plants (Srichaikul *et al.*, 2011). To obtain ratio of chlorophyll a and b, the readings should be taken at the wavelength of 650 nm, which was in between the absorption maximum of both (Arnon 1949; Porra *et al.*, 1989; Devmalkar et al., 2014).

The importance of chlorophyll stability index (CSI) in measuring the drought resistance capacity of the genotypes in crop plants has been reported by Kaloyereas (1958) in pines, Murthy and Majmudar(1962) in rice and Kilen and Andrew (1969) in maize, and Ramalingam et.al.,(1980) screened drought resistance varieties in in Maize genotypes and also they have standardized the method of chlorophyll stability index (CSI) in crop plants. Mathew and Ramadasan (1973) reported low CSI in high yielding hybrids of coconuts and suggested their possible association between drought resistance and chlorophyll stability index (CSI). Fanmous(1967) however, did not observe any association between chlorophyll stability index (CSI) and drought resistance capacity of the pearl millet material studied. Arunyanark, et.al., (2008) revealed that Chlorophyll stability during drought might be a promising criterion for selection for drought resistance in peanut. Inhibition of chlorophyll synthesis and enzymes of nitrogen assimilation by selenite in excised maize leaf segments during greening was reported by Jain and Gadre (1998).

In the present investigation, an attempt was made in Maize, Leaf samples of 14 different Maize Analysed for Chlorophyll A and B as per the standard chemical analytical procedures.. These 14 genotypes of Maize were estimated for Chlorophyll Stability Index as per the technique laid out by Kaloyereas (1958) and standard modified method established by Murthy and Majumdar (1962) and identified and differentiated among them which are most tolerant and resistant for rain fed and irrigated conditions by different heating temperatures and heating durations. These results of Chlorophyll A & Chlorophyll B and Chlorophyll Stability Index (CSI) of these 14 Maize varieties were compared and correlated with yield of these 14 varieties obtained from responsible research Institutes and conclusions were drawn how best Chlorophyll Stability Index (CSI) is useful for identifying and differentiating Maize genotypes for drought tolerance which may be helpful for future breeding research on Maize.

## **MATERIALS AND METHODS**

During the study, 14 Maize varieties leaf samples collected during October-November, 2019 for Kharif Maize varieties and during Jan- February, 2020 for Rabi Maize varieties. These composite leaf samples of each variety was processed and analysed for Chlorophyll A and Chlorophyll B and calculated Total Chlorophyll content of each variety. These chemical analyses were carried out at the Iota Laboratories, Hyderabad, India.

For Chlorophyll Stability Index (CSI), a composite consisting of 20 leaves (3<sup>rd</sup> fully open leaf from 4 week old seedlings of selected maize varieties were taken, washed thoroughly, air dried and after removing midrib, were cut in to pieces and weighed. Five grams of leaf samples were used for each kind for determination of Chlorophyll Stability Index (CSI) by subjecting them to different heating times and heating temperatures. Three heating temperatures viz., 50, 60 and 70 °C and two heating durations, 30 and 60 minutes studied. After heating to respective temperature and duration, the leaf sample extracts in 80% acetone were filtered using Whatman No.1 filter paper and measured their light intensity to determine Chlorophyll A and B at 645 and 663 wave lengths. Chlorophyll A, B as mg/g leaf fresh weight was calculated according to Dhopte and Manuel, (2002): The colour absorbance of the solution was estimated by a spectrophotometer using 645 and 663nm wavelength against the solvent. Acetone (80%) was used as a blank (APHA,1989). Leaf material

– crushed using mortar and pestle added Acetone and MgCO<sub>3</sub> –kept for 4 hours in freezer at 40C – centrifuge at 500 rpm for 5 minutes then measured absorbance on spectrophotometer.

Chlorophyll A in mg/g of leaf =  $11.75 \times A_{662.6} - 2.35 \times A_{645.6} \times V/1000 w$

Chlorophyll B in mg/g of leaf =  $18.61 \times A_{645.6} - 3.96 \times A_{662.6} \times V/1000 w$

Where, Chlorophyll A and Chlorophyll B are the chlorophylls A and B,

A = Absorbance recorded through spectrophotometer at 645.6 and 662.6 uM.

W = leaf fresh weight as gram

The chlorophyll content of leaves was measured by using Lichtenthaler and Wellbum (1983) method. A 5-g representative sample of healthy unblemished leaf samples are placed in a large tube one inch in diameter with 50 ml of distilled water and heated in a water bath at 56° + 1° C for exactly 30 minutes. At the end of this time the leaves were removed from the tubes and were ground in a Warring blender for five minutes with 100 ml of 80 % acetone solution in water. The chlorophyll extract was filtered and the filtrate examined immediately for light absorption with a Klett Summerson Photoelectric colorimeter using a no. 66 red filter or with spectrophotometer at 660 um wave length.. Pigment was extracted from a 5-g sample of unheated leaves and the light absorption is also measured. The difference between the two readings is defined as the "Chlorophyll Stability Index" (C.S.I.).

Chlorophyll stability index (CSI) was assessed according to the method suggested by Murthy and Majumdar (1962) and expressed as a percentage. CSI is calculated by using the following formula.

Chlorophyll Stability Index CSI (%) =  $\frac{\text{Total chlorophyll content (heated)} \times 100}{\text{Total chlorophyll content (control)}}$

Chlorophyll Stability Index= Difference between

(Colorimetric reading of heated sample – Colorimetric reading of unheated sample)

OR

(Spectrophotometer reading of heated sample–Spectrophotometer reading of unheated sample).

## RESULTS AND DISCUSSION

Maize Grain Yield, Chlopphyll A & B and Total Chlorophyll contents recorded in different varieties of Maize are presented in Table 1. Yield of selected maize varieties were obtained from the respective Research Institutes and farmers fields.

The results showed that maize grain yield was ranging between 48.75 to 64.50 Q/ha with a mean of 53.932 q/ha. Pusa Hybrid Makka 1 maize variety recorded the highest of 14 selected maize varieties and DHM – 103 recorded the lowest of all.

**Table 1. Maize Yield, Chlorophyll A , Chlorophyll B and Total Chlorophyll contents in Maize genotypes**

S. No.	Name of Maize Variety/Hybrid	Maize Grain Yield (Quintals /ha)	Chlorophyll A content (mg/g)	Chlorophyll B content(mg/g)	Total Cchlorophyll content(mg/g)
1.	DHM – 103	48.75	1.925	0.896	2.821
2.	DHM – 105	51.50	2.140	0.999	3.139
3.	DHM – 1	51.50	1.875	0.875	2.750
4.	Trisulatha	52.65	2.350	1.097	3.447
5.	DHM – 107	54.75	2.250	1.050	3.300
6.	DHM - 109	57.35	2.360	1.102	3.462
7.	NMH 713	50.25	2.380	1.111	3.491
8.	NMH 731	49.65	1.865	0.871	2.736
9.	NMH 1242	52.75	1.675	0.782	2.457
10.	Pusa Hybrid Makka 1	64.50	2.450	1.144	3.594
11.	Pusa Hybrid Makka 5	61.25	2.276	1.063	3.339
12.	DRONA (KMaharashtra-2589) Hybrid	49.65	1.750	0.781	2.531
13.	Pusa Jawahar Hybrid Maize-1	50.75	2.145	0.957	3.102
14.	DHM 121	45.75	1.750	0.817	2.567
	<b>MEAN</b>	<b>52.932</b>	<b>2.273</b>	<b>1.060</b>	<b>3.333</b>
	<b>S.Ed.</b>	<b>4.874</b>	<b>0.414</b>	<b>0.193</b>	<b>0.607</b>
	<b>CD (p&lt;0.05)</b>	<b>3.730</b>	<b>0.330</b>	<b>0.150</b>	<b>0.480</b>

The results indicated that Chlorophyll A of these varieties is ranged between 1.675mg/g and 2.450 mg/g with a mean of 2.063 mg/g and Pusa Hybrid Makka 1 Maize variety has recorded highest Chlorophyll A content and NMH 1242 Maize

variety has recorded lowest Chlorophyll A content. Chlorophyll B of these varieties is ranged between 0.782mg/g and 1.144mg/g with a mean of 0.963mg/g. Pusa Hybrid Makka 1 Maize variety has recorded highest Chlorophyll B content and NMH 1242 Maize variety has recorded lowest Chlorophyll B content. Total Chlorophyll content was found to be highest of 3.594mg/g in Pusa Hybrid Makka 1 maize variety and lowest in NMH 1242 maize variety of 2.457mg/g. Total Chlorophyll content in different maize varieties tested were ranging from 2.457 mg/g to 3.594mg/g with a mean of 3.026mg/g.

Chlorophyll A, Chlorophyll B and Total Chlorophyll contents in different Maize lines selected were in conformity with earlier researchers viz., Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots (Mirza et al., 2013; Srichaikul et al., 2011). However the chlorophyll production is mainly depended on penetration of sun light and it is the main source of energy for plant (Srichaikul et al., 2011). In the laboratory it is commonly determined by using pestle and mortar to extract the pigments using an organic solvent such as acetone or dimethyl formamide (Arnon 1949; Porra et al., 1989; Ling et al., 2011). Chlorophyll A and Chlorophyll B are essential pigments of the plant photo systems (Richardson et al., 2002). Moreover the chlorophyll A is the primary photosynthetic pigment in plants which helps to produce energy in plant (Srichaikul et al., 2011). However the chlorophyll A concentration is 2-3 times higher than that of secondary chlorophyll b in plants (Srichaikul et al., 2011). To obtain ratio of chlorophyll A and B, the readings should be taken at the wavelength of 650 nm, which was in between the absorption maxima of both (Arnon 1949; Porra et al., 1989; Devmalkar et al., 2014).

### **Chlorophyll Stability Index in Maize varieties:**

The results of Chlorophyll Stability Index of the selected 14 Maize varieties tested by standard procedures laid out by Kaloreas(1958) and Murthy and Majumdar (1962) are presented in Table 2 and depicted in Fig.1. Chlorophyll Stability Index of these varieties ranged between 3.667 and 5.134 with an average of 4.361. In the first



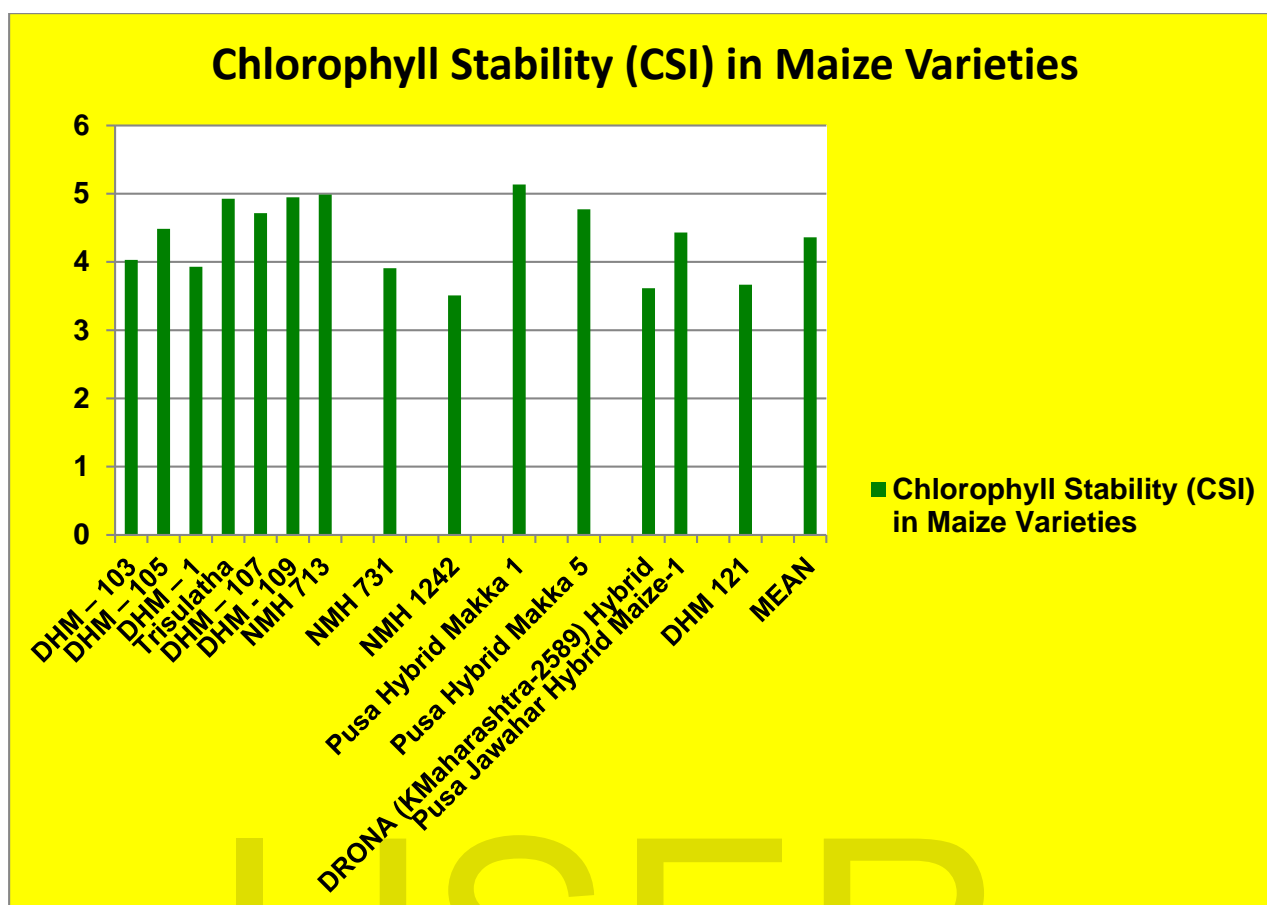
group of long duration variety Pusa Hybrid Makka 1 maize variety performed well and recorded highest Chlorophyll stability Index. In the second group of medium duration variety DHM-101 and DHM - 107 maize varieties recorded highest CSI value and in third group of short duration varieties respectively. Maize variety Trisulatha recorded highest value of CSI. It is evident that while the maize varieties in kharif season with yield potential under stress have exhibited and recoded low CSI ranging from 4.030 to 4.431. The highest CSI observed in having low potential yield of the normal yield under stress condition. Positive Correlations were observed between Maize yield and Chlorophyll A, B and Total Chlorophyll contents with correlation coefficients ( $R^2$ ) of 0.649, 0.658 and 0.653 respectively. High positive correlation coefficients ( $R^2$ ) were noticed among Chlorophyll A, B and Total Chlorophyll as 0.994; 0.997 and 0.999 respectively as per statistical analysis laid out by Snedecor, 1956.

**Table 2. Chlorophyll Stability (CSI) in Maize Varieties**

S. No.	Name of Maize Variety/Hybrid	Chlorophyll Stability (CSI) in Maize Varieties
1.	DHM – 103	4.030
2.	DHM – 105	4.485
3.	DHM – 1	3.930
4.	Trisulatha	4.925
5.	DHM – 107	4.714
6.	DHM - 109	4.946
7.	NMH 713	4.987
8.	NMH 731	3.909
9.	NMH 1242	3.510
10.	Pusa Hybrid Makka 1	5.134
11.	Pusa Hybrid Makka 5	4.770
12.	DRONA (KMaharashtra-2589) Hybrid	3.616
13.	Pusa Jawahar Hybrid Maize-1	4.431
14.	DHM 121	3.667

	<b>MEAN</b>	<b>4.361</b>
	<b>S.Ed.</b>	<b>0.549</b>
	<b>CD (p&lt;0.05)</b>	<b>0.620</b>

Based on this study following four lines of maize were outstanding. 1) Pusa Hybrid Makka-1 followed by 2) NMH 713; 3) DHM – 109 and 4) Trisulatha maize varieties. Among the 14 Maize varieties examined for Chlorophyll Stability Index drought tolerance was excellent in varieties viz., Pusa Hybrid Makka-1, NMH 713 closely followed by DHM – 101, Trisulatha and DHM – 109. Maize varieties namely Drona (KMaharashtra-2589) Hybrid, DHM 121 and NMH 1242 were recorded the lowest Chlorophyll Stability Index (CSI) and these may be considered as least drought resistance varieties which may not withstand drought and also moisture stress. It is evident that while the maize lines showing fairly high yield potential under stress (drought) have exhibited high CSI ranging from 4.770 to 5.134 in long duration maize varieties. But there were three lines which HAD EQUALLY low CSI ranging from 3.616 to 3.909 but have shown low yield potential of 45.75 and 49.65 q/ha of the normal yield under stress condition. Based on this study following four varieties of maize were outstanding. They are viz., Pusa Hybrid Mkka-1, Pusa Hybrid Mkka-5, DHM – 109 and DHM – 107.



**Fig. 1 Chlorophyll Stability Index (CSI) in different Maize varieties tested**

The results of Chlorophyll Stability Index of different Maize varieties tested in this study are in conformity with the earlier researchers like Roshni Vijayan(2017) evaluated Impact of drought stress on leaf chlorophyll content in Maize cultivars(*Zea Mays* L.). The chlorophyll stability index (CSI) is an indication of the stress tolerance capacity of plants. A high CSI value means that the stress did not have much effect on chlorophyll content of plants. A higher CSI helps plants to withstand stress through better availability of chlorophyll. This leads to increased photosynthetic rate, more dry matter production, and higher productivity. This indicates how well chlorophyll can perform under stress.

## CONCLUSIONS

CIMMYT, IITA, Monsanto, Syngenta and Pioneer are the leading maize research groups in the world. These groups have worked and working on numerous aspects of maize crop however, drought tolerance is also one of their key research objectives. Numerous research projects have been completed in which different

breeding methods were practiced for improvement of drought tolerance in maize. As demand of maize is increasing day by day and water scarcity is increasing so, there is dire need to further improve the level of drought resistance in maize. Different strategies are used for improvement of maize against drought stress e.g. managerial strategies and biological strategies. Managerial strategies involve the usage of water resources and adoption of water saving agronomic practices. On the other hand biological approaches deal with manipulation of genetic background of maize for improvement against drought stress. Biological strategies are preferred over managerial practices due to long term and economical effectiveness. Biological approach is practiced in different forms. Available germplasm has lot of genetic variability which can be exploited for higher drought tolerance through effective screening tools (Muhammad Aslam et.al., 2017). Screening Maize germplasm for drought resistance through estimation of Chlorophyll Stability Index (CSI) is found to be one of the best tools as per the study conducted in this research project.

Ananthi et.al.,(2013) investigated on drought-induced changes in Chlorophyll Stability Index, Relative Water Content and Yield of Cotton Genotypes and revealed that it paved way to utilize the most reliable parameter such as Chlorophyll Stability Index (CSI) and relative water content for screening drought tolerance. These parameters can be regarded as selection indices to pick up the most tolerant genotype from large number of population by the breeders. This investigation has opened new vistas for development of quantifiable drought Index for further exploitation. Based on the study, Maize varieties Makka 1 among long duration varieties, DHM 101 in medium duration and DHM 107 in short duration may be chosen for advanced research studies and it may be possible to release drought tolerance varieties for commercial exploitation after proper further evaluation.

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