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Response of physiological traits of maize to moisture stress induced at different developmental stages

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Abstract
Screening of plants germplasm may serve as important source of favorable alleles for enhancing the performance of new maize verities and hybrids under moisture stress conditions. For this, field study was carried by crossing of 15 inbreed lines with 3 testers in L x T and resultant 45 hybrids, inbreads with four checks are evaluated in 2015-16 Rabbi to avoid rainfall in order to determine the effect of irrigation and moisture stress imposed at different stages on physiological and yield contributing characters of corn. Tasseling and grain filling stages are targeted for giving moisture stress for 20 days at starting of both stages. The effect of moisture stress imposed at different developmental stages on anthesis-silking interval, grain yield per plant, chlorophyll content, relative water content, proline content and leaf area were evaluated. On the basis of evaluated trial, the study showed that all vegetative and yield parameters were significantly affected by water shortage in soil profile due to omitted irrigation during sensitive tasseling and grain filling stage. Water stress during tasseling stage increases the ASI and proline content while it lowers down the relative water content, chlorophyll content, grain yield per plant. Even 20 days of halting irrigation at anthesis and grain filling stage causes up to 42 and 14 per cent of grain yield loss respectively. Much greater loss of 70-80 per cent of grain yield could be expected as result of prolonged moisture stress during anthesis and grain filling stage.

Keywords: L x T, inbreds

Introduction
Water is vitally needed for every organism in specified amount and any deficiency in that particular imposes the stressful condition. Soil drought because of inadequate rainfall has become an acute problem that constrains plant growth and productivity in growing regions that already have tendency to moisture stress (Boyer, 1982; Ahuja *et al*., 2010; Dawson *et al*., 2011) [4, 1, 6]. Worldwide drought is one of the most important abiotic stress limiting crop yield including maize (Prasanna 2012; Mir *et al*., 2012) [13, 14], which cause up to 50 % or more (Wang *et al*., 2003) [17]. The interaction of crop to drought differs significantly at various organizational levels depending upon the intensity and duration of stress as well as the plant species and developmental stage (Doorenbos and Kassam, 1979, Chaves *et al*., 2003) [7, 5] in maize, during reproductive phase 8-9 mm water is needed per day in which each mm produce 15 kh of grain which is reason for being most sensitive stage for moisture stress. Doorenbos and Kassam 1979 [7] have reported corn is relatively tolerant to moisture deficit during vegetative stage and that the greatest decrease in grain yield is caused due to moisture stress during anthesis stage. For development of drought tolerant hybrids heritability of gene is important but the heritability of grain yield under drought condition is low and difficult to make genetic progress by selection for it per se. therefore the use of secondary traits has been suggested (Edmeades *et al*. 1997, Banziger *et al*. 2000) [8]. The secondary traits are plant characteristics other than grain yield that provide additional information about how the plant performs under a given environment (Lafitte *et al*. 2003) [11]. The present study was planned to identify tolerant genotype by investigating the effect of drought stress on physiological and yield contributing parameter when moisture stress was given at anthesis and grain filling stage.

Material and Methods
Experimental site and experimental procedure
The hybrid were attempted by adopting line x tester mating design among 15 inbreed lines and 3 testers in maize (*Zea mays* L.) during *rabi* 2014-15 at M.P.U.A.T.

Udaipur, Rajasthan (24^0 - 35' N and 73^0 - 42' E). The resultant hybrids along with their parents were evaluated in randomized block design with three replications in three environments during *rabi* 2015-16. The soil of experimental fields was clay loam, deep, well drained, alluvial in origin and fairly good moisture holding capacity.

In present investigation experiment were planted in three adjacent environments that receives different irrigation treatments. First environment receive irrigation throughout the life cycle of the crop whereas the second environment receive normal irrigation up to onset of anthesis stage and irrigation was halted for 20 days i.e. 125 DAS so that the water stress can coincide with time of flowering, third environment receive irrigation up to onset of grain filling stage and irrigation was halted for 20 days i.e. 150 DAS so that water stress can coincide with time of grain filling stage.

Table 1: Details of the environments

Environment	Moisture stress
Environment 1	Normal irrigation
Environment 2	Anthesis stage (Drought imposed at 125 DAS for 20 days)
Environment 3	Grain filling stage (Drought imposed at 150 DAS for 20 days)

The *in vivo* relative Chlorophyll concentration of leaves of five plants per hybrid were assessed using a portable chlorophyll miter (SPAD-502; Minolta; Tokyo, Japan). The ears of the ten randomly selected competitive tagged plants were shelled and grain from ear were weighed and divided by the total number of plants and mean grain yield per plant was worked out for each plot and recorded separately. Proline is estimated at 140 DAS in I and II environment while after 165 DAS in III environment for estimating stress imposed in I and III environment. It was determined as per method given by Bates *et al*. (1973) [3].

Results

Effect of water deficit on physiological parameters

Morpho-physiological traits were significantly affected by moistures tress and they differed significantly as compared to well-watered environment. Among these morphological traits and yield related traits, high variation was observed for grain yield, relative water content, chlorophyll content and anthesis-silking interval under moisture stress condition. On the basis of grain yield per plant L13 x T1, L5 x T3, L2 x T1, L12 x T2 and L1 x T1 exhibited good performance in controlled environment. These data of controlled environment was compared with the moisture stress given at anthesis and grain filling stage to get exact effect on the grain yield and other morpho-physiological characters. (Table 2)

Table 2: Interaction effect of moisture stress at anthesis and grain filing stage on best hybrids

	Situation	L_{13} x T_1	L_5 x T_3	L_2 x T_1	L_{12} x T_2	L_1 x T_1
Grain yield (g)	Control	197.20	187.77	185.20	171.90	172.15
	Drought at G	183.15	161.80	175.81	163.65	156.69
	Drought at A	113.83	126.63	111.70	127.37	131.40
Chlorophyll content (SPAD value)	Control	66.33	64.56	61.37	60.17	61.90
	Drought at G	62.96	53.50	56.54	49.25	55.07
	Drought at A	42.97	45.14	35.97	43.90	46.60
Leaf area (cm^2)	Control	435.27	448.66	576.63	495.98	459.12
	Drought at G	429.06	440.39	560.25	484.43	450.91
	Drought at A	422.20	423.00	450.68	422.62	405.58
Relative water content (%)	Control	80.75	79.08	77.33	80.10	82.46
	Drought at G	79.20	75.19	69.85	73.06	75.30
	Drought at A	53.45	63.59	45.54	54.10	60.59
ASI	Control	1	2	1.67	2	1
	Drought at G	1	2	1	2	1
	Drought at A	4.67	4	4.33	4.33	2.33
Proline content (µg)	Control	24.15	23.00	19.23	18.24	21.53
	Drought at G	197.20	158.23	133.33	96.05	112.87
	Drought at A	214.51	160.76	207.30	193.83	218.43

Table 3: Mean GYs and DTI of 10 highest yielding and 10 lowest yielding hybrids and their checks under drought at anthesis and grain filling stage.

Drought at anthesis			Drought at grain filling stage		
hybrids	**GY**	**DTI**	**hybrids**	**GY**	**DTI**
Top 10			**Top 10**		
L1 X T1	131.40	23.67	L13 X T1	183.15	7.12
L12 X T2	127.37	25.90	L2 X T1	175.81	5.07
L5 X T3	126.63	31.46	L12 X T2	163.65	4.79
L4 X T3	125.57	21.44	L15 X T1	161.91	6.13
L3 X T1	120.47	27.86	L5 X T3	161.80	12.43
L6 X T2	118.27	27.46	L7 X T1	159.90	1.96
L12 X T3	115.17	27.61	L10 X T2	158.60	4.72
L3 X T3	114.37	28.91	L4 X T2	157.00	6.32
L13 X T1	113.83	42.27	L1 X T1	156.69	8.98
L1 X T3	112.73	29.86	L6 X T2	152.75	6.31
Bottom 10			**Bottom 10**		
L14 X T1	43.03	41.25	L11 X T3	56.00	13.88
L11 X T3	43.53	33.06	L14 X T1	61.57	15.94

L14 X T3	44.90	54.15	L8 X T3	69.07	30.35
L9 X T3	60.30	37.51	L14 X T3	69.40	29.13
L7 X T3	64.17	37.49	L9 X T3	75.37	21.89
L11 X T1	64.87	36.65	L7 X T3	77.50	24.51
L15 X T2	65.33	35.88	L14 X T2	93.50	19.90
L14 X T2	67.60	42.08	L15 X T2	96.10	5.69
L15 X T1	76.07	55.90	L11 X T1	96.35	5.90
L11 X T2	78.93	51.62	L5 X T1	105.71	13.81
checks					
Prakash	98.67	35.13	Prakash	148.98	2.05
Bio-9681	107.83	29.67	Bio-9681	149.68	2.38
Pratap QPM-1	106	26.11	Pratap QPM-1	137.86	3.91
PHM-3	110.87	27.45	PHM-3	126.90	6.64

Anthesis-Silking interval

The result indicated a subsequent increase in the Anthesis-silking interval of all hybrids when drought was imposed before anthesis when compared with well-watered environment. Lower the values of ASI, higher will be the productivity and vice versa. Drought imposed at grain filling stage do not showed any increase in ASI as irrigation was supplied at anthesis stage. L13 x T1 showed 4.67 ASI which was maximum and also suffered from high level of decrease in grain yield (42.27 %) as compared with controlled environment. Similarly L5 x T3, L2 x T1, L12 x T2 and L1 x T1 also suffered from moisture stress when applied before anthesis stage as compared with controlled environment.

Chlorophyll content

Moisture stress in experiment caused the chlorophyll content of leaves. The strong moistures tress induce decrease of chlorophyll content, indicates that moistures stress cause to photosynthetic reaction centre (PSI and PSII). The result showed that high yielding hybrid L13 x T1 exhibited 35.21 per cent depilation in chlorophyll content when moisture stress was given before anthesis stage while 5 per cent loss of chlorophyll content when stress given before grain filling stage as compared with controlled environment. The susceptible inbreeds always showed large reduction in chlorophyll content under moisture stress. Hybrid L2 x T1 exhibited highest i.e. 41.38 per cent depilation in chlorophyll content when stress given before anthesis stage.

Leaf area

The leaf area varied significantly in developmental stages under different timing of moisture stress. The moisture deficit significantly reduces the leaf area across the growth stage. The maximum leaf area reduction was observed when stress was given before anthesis stage. Hybrid L2 x T1 had significantly reduced leaf area when moisture stress given at anthesis and grain filling stage of about 126 and 16.38 cm^2 when compared with controlled environment. The stress given at grain filling stage does not show significant reduction in the leaf area as the vegetative stage was almost completed at grain filling stage. The experiment revelled that hybrid L13 x T1 was tolerate to moisture stress as small amount of decrease in leaf area which can be positively correlated to the grain yield per plant and pro line content.

Relative water content

Leaf relative water content is a reliable indicator for leaf water deficit status. It is generally used to determine the response of a plant to progress of moisture stress. In response of the moisture stress RWC of leaf was decrease over time in both stages i.e. at stress at anthesis and grain filling stage. But the intensity was different for both stages. Hybrid L2 x T1 lost highest i.e. 41.10 and 9.67 percent of relative water content when imposed moisture stress at anthesis and grain filling stage respectively. Among the high yielding hybrids L5 x T3 exhibited lowest depletion of RWC i.e. 19.58 at anthesis and hybrid L13 x T1 showed 1.91 percent of depletion when stress at grain filling stage. Visual observation also indicated that L5 x T3 hybrid were most moisture stress tolerant and had stay green character.

Grain yield per plant

Data obtained from experiment showed that grain yield was significantly affected by soil moisture stress. The moisture stress at anthesis and grain filling stage result a grain yield reduction when compared with well-watered environment. Data obtained from above experiment showed that high yielding hybrid L13 x T1 depleted 42.27 percent grain yield loss as compared with well-watered environment at omitting of water at anthesis stage. It is observed that grain yield was not that much affected when moisture stress given before grain filling stage but it definitely affects the quality of seed in terms of the boldness and weight. Comparisons among the differ stages of crop clearly showed that flowering stage is most sensitive stage of the life cycle.

Proline content

Many of changes due to dehydration stress in plant include the accumulation of proline in addition to change in protein content. These changes are associated with somatic adjustment and the protection of membrane from damage as cell content desiccate. Results obtained that hybrid L1 x T1 (196.9 mg) stage showed highest accumulation of proline when stress was given at anthesis stage and yielded better in both environments.

Top 10 hybrids under moisture stress at anthesis and at grain filling stage given proper difference between the losses of grain yield. The drought tolerance index (DTI), which is indicator of hybrid yield loss due to moisture stress, ranged from 21.44 to 42.27 per cent at stress given at anthesis stage and 1.96 to 12.43 per cent when stress given at grain filling stage. Experiment conducted indicated that moisture stress sharply affect the grain yield per plant when given at stating of anthesis stage than grain filling stage. Three hybrids namely L1 x T1, L12 x T2 and L4 x T3 exhibited significant DTI of 23.67, 25.90 and 21.44 percent as compared with best check Bio-9681. Three hybrids L12 x T2 (25.90 and 4.79 %), L5 x T3 (31.46 and 12.43 %) and L6 x T2 (27.46 and 6.31%) were found best among the 45 hybrids as these hybrids exhibited drought tolerance under both stress condition. The bottom 10 hybrids at both stress stages are harshly affected in relation to the grain yield per plant and other traits associated with the plant physiology.

Discussion

Moisture stress, like other abiotic stresses affects many physiological & metabolic processes such as decreasing growth and development. Maize plant is susceptible to moisture stress with different impact at different stages. However, with respect to reproductive phase i.e. at anthesis stage, plant becomes more sensitive to moisture stress. Stress occurred at this stage can significantly reduces leaf area, plant height, chlorophyll content and overall growth (Heninger 2001) [10]. Identification and use of inbread line with superior drought tolerant traits is the way for success to develop drought tolerant hybrids. In present study, we examine the ability of 45 maize hybrids to withstand negative impact on the six important drought related characters.

Relative Water Content (RWC)

All the maize cultivar exposed to moistures stress had lowered biomass compared to control environment. The relative water content in leaves of maize hybrids in both stress environments is decreased significantly. These results were in agreement with the finding of (Efeoglu *et al*. 2009) [9]. At reproductive phase i.e. at anthesis phase moisture stress affects harshly as this is sensitive phase in relation with growth and development (Setter *et al*. 2001; Taiz and Zeiger 2006) [15, 16]. Result of experiment indicated that RWC was lowest when moisture stress given at antheis than grain filling stage.

Leaf area

Leaf area varied significantly under the moisture stress at different growth stages. Means, moisture stress significantly reduces leaf across the growth stages. The result suggested that leaf area is susceptible to water stress in early growth stages. As rule, leaf area index increased under favourable soil moisture condition until the anthesis stage and then decreased as older leaves died. Leaf area development is sensitive process among all the morphological traits which are directly relate and involve in moistures tress at above ground portion of plant (Chaves *et al*. 2003) [5]. Moisture stress induces reduction in both cell enlargement and cell division which is cause for decrease in leaf area (Marron *et al*. 2003). In present investigation, all the hybrids showed reduced leaf area under the moisture stress at both environments. This response is also attributed to an avoidance mechanism to minimize the water loss through stomatal closer.

Anthesis-Silking Interval

Release of pollen in large quantity and proper landing on the silk is necessary for pollination. In moisture stress before anthesis stage, process is disturbed which cause grain yield loss. In present study, moisture stress affect the anthesis-silking interval to increase i.e. pollen shedding is accelerated and silking is delayed by stress and this increase ASI followed by 40-50 % of yield loss (Nielsen 2005). The result indicated that moisture stress at anthesis stage is more sensitive than at grain filling stage when compared with well-watered environment.

Proline content

Proline is imino acid, which is osmo-protectant under different abiotic stress. It maintains water status by maintaining osmatic potential and maintains sub-cellular structures under osmatic stress (Ashraf and Foolad 2007). Under present investigation, the high yielding hybrids exhibited the 2-3 fold increase in the proline level when stress was given at both stages, the accumulation of proline content is directly proportional with drought tolerance which indicating that parents of hybrids have moisture tolerant gene which can be used for further purpose. Results were supported to Bruce *et al*. 2002 that proline is osmo-protectant and to be accumulated in maize under moisture stress and exhibited drought tolerance.

Grain yield

The moisture stress at grain filling stage cause the premature hanging of cobs and though water requirement is low during this stage but cause potential loss of yield and quality (Pannar 2012). The result indicated that, high yielding hybrids in controlled environment exhibited significant loss when moisture stress given at anthesis stage which is very sensitive stage and cause loss of 40 % of grain yield per plant. Result is also supported by Heinigre 2000. While the moisture stress at grain filling stage does not exhibited significant loss of grain yield but cause the grain development. It indicate that number of grain are determined during pre-anthesis stage where as grain weight is determined grain filling stage. The result is also supported by Oveysi *et al*. 2010.

Chlorophyll content

The result indicated that the moisture stress during anthesis and grain filling caused up to 45 and 20 per cent of loss respectively. Meena Kumari *et al*. (2004) also reported that chlorophyll content and stability index decreased under drought stress. They observed up to 60 and 50 per cent decrease in chlorophyll content when moisture stress was applied at anthesis and grain filling stage respectively. The susceptible inbreds showed large reduction in chlorophyll content as hybrid L2 x T1. Under moisture stress condition, the decrease in chlorophyll content was also reported by various workers (Gutierrez *et al*., 1998; Abdel-Rasoul *et al*., 1989 and Chaves *et al*., 2003) [5].

Conclusion

Moderate and severe moisture stress impaired the physiological traits of maize but the magnitude depended on the duration and severity of moisture stress at critical growth stages. Under experimental condition, well-watered environment showed better exposure to physiological traits and relatively higher grain yield irrespective of genotypes. Although the moisture stress was imposed for different periods at different growth stages under the experiments, the severe moisture stress had more damaging effect on the RWC, chlorophyll content and eventually grain yield per plant compared to the well-watered environment. The relative water content, chlorophyll content leaf area and grain yield per plant exhibited negative relationship with soil water profile while the grain yield exhibited a positive relationship with the relative water content, chlorophyll content, leaf area and negative relationship with ASI and proline content. As result of the evaluation trail it was concluded that reproductive i.e. anthesis phase of corn is strongly affected by moisture stress. It also showed that, all yield parameter which had presented in this research are significantly affected because of the moisture stress during sensitive stage. Higher grain yield will be obtained if the irrigation was applied at these sensitive stages.

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