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Quantification of Secondary Traits for Drought and Low Nitrogen Stress Tolerance in Inbreds and Hybrids of Maize (*Zea mays L.*)

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Abstract

A field experiment was conducted using twenty hybrids, twelve inbreds and four check varieties of Maize (*Zea mays L.*) under different level of moisture and nitrogen stress. Inbreds were crossed in line/tester mating design at National Maize Research Program, Rampur to produce hybrids. The inbreds and their hybrids were grown in the field to quantify secondary traits and study stress indices for selecting the best genotype for both drought and low nitrogen stress tolerance. The secondary traits do not affect the yield under stressed condition directly but assist in selecting the tolerant genotypes. The correlation of those traits with grain yield under stressed condition was studied. Canopy temperature depression (r=0.61**) and SPAD reading (r= 0.50**) showed positive correlation while leaf rolling score (r=0.49**), leaf senescence score (r=-0.57**) and anthesis silking interval (r=0.15) showed negative correlation. Cluster analysis showed six distinct clusters and cluster 4 represented tolerant genotypes. Hybrids were concentrated at a place while inbreds were scattered as shown by the Principal component analysis. The secondary traits along with stress tolerance indices (TOL and STI) were found useful for selecting stress tolerant genotypes. Based on quantification of secondary traits and stress indices, the hybrids were found to be more tolerant as compared with their inbred parents. The hybrids RML-4/RML-17, RML-32/RML-17, RML-8/RML-17, RML-32/RL-111 were found to be more tolerant compared with other hybrids based on secondary trait quantification and stress indices.

Keywords Secondary traits; Drought stress; Tolerance; Correlation; Stress indices

Introduction

Drought and low soil nitrogen are highlighted as potential major constraint to maize production in the tropics by maize breeders. Within the abiotic stress that the maize crop faces, drought is regarded as the most devastating. After drought, low soil nitrogen is the most important abiotic factor limiting maize yields in the tropics. Maize yields in Nepal are considerably lower than the world average as Nepalese maize cultivating fields are often prone to drought and low soil fertility in addition to biotic stresses [1]. There are also complex interactions among these stresses as drought also reduces soil nitrogen uptake [2]. Drought and low nitrogen stress together can reduce upto 80% of maize yield [3]. Drought events are expected to increase in the coming years due to climate change which will also limit N availability in soil. This will expose future maize crops under severe drought and low nitrogen stressed environment.

Drought, like many other abiotic stresses, has adverse effects on crop yield. It is an expanding and problematic threat in the world with the increase in cultivated area and increasing intensity every year [4]. It is a major limiting factor for maize production in all the growing areas, but it is a greater challenge for the rural poor farmers of developing countries particularly in the tropical region. Plants experience drought stress when the water supply to root system becomes difficult or when the transpiration rate becomes very high [5]. It is estimated that 20-25% of the global maize production area is affected by drought in any given year [6]. Drought occurring at anthesis and silking period leads to greater yield loss than when it occurs at other growth stages [7]. Drought stress causing a single day or more delay in silk emergence, results in increasing anthesis silking interval (ASI). This asynchrony between male and female flowering time is regarded to be highly associated with decrease in grain yield of maize. Water deficit lasting for only one or two may cause as much as 22% reduction in yield [8]. The major effect of drought is embryo abortion, which is related to the inhibition of photosynthesis and the subsequent reduction in assimilates available to the developing kernels [9]. Drought affects maize yield by restricting season length and through unpredictable stress that can occur at any time during the cropping cycle [10]. In addition, during flowering time the farmers can no longer adjust management practices, such as fertilizer application, weed control and replanting [11]. The genetic improvement of maize for higher drought stress tolerance is thus, highly desirable.

Low nitrogen stress is another major abiotic constraint to maize production. Mostly, the tropical maize are grown under sub optimal level of soil nitrogen. This causes a high drop in maize yield due to low nitrogen stress alone. N stress reduces crop photosynthesis by reducing leaf area development and leaf photosynthesis rate and by accelerating leaf senescence. More than 50% of all leaf N is directly involved in photosynthesis either as enzyme or as chlorophyll. Lower rate of nitrogen before flowering reduces leaf area development and photosynthesis rate during flowering and it ultimately results in kernel and ear abortion. Cultivars with improved tolerance to low N can be

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developed by simultaneously selecting for higher grain yields under stress condition and secondary traits are expected to add an advantage under such stress.

Secondary traits are the morpho-physiological traits that do not directly affect the yield but assist in the identification of the genotypes that can easily adapt with the stressed environment and indirectly affect the yield of the crop. Breeding progress for drought and low N stress tolerance has been slow since abiotic stress tolerance is a complex trait governed by polygenes. Tolerance to drought is a quantitative trait, with a complex phenotype, often determined by plant phenology. Therefore, breeders working towards improvement of abiotic stress tolerance like drought and low nitrogen in maize have been using secondary traits and selection indices for selecting the best performing genotypes under stressed condition. Secondary traits and selection indices are two major criteria in selecting drought and low nitrogen tolerant genotypes [12]. That's why; breeding for the development of drought and low nitrogen stress tolerance genotype seems to be one of the best breeding techniques to cope with drought and low nitrogen stress. Considering the above mentioned breeding strategy, this research was conducted to evaluate twelve inbred lines, their twenty line/tester hybrids along with other four different local check varieties under drought and low nitrogen stressed environment for yield and other secondary traits associated with stress tolerance.

Materials and Methods

Plant materials and agro-morphological traits

The field experiment was conducted at the research farm of the National Maize Research Program (NMRP), Rampur, Chitwan, Nepal from September 2011 to June 2012. The plant materials used in the research were twelve inbred lines (Table 1), their twenty line × tester hybrids and four check varieties obtained from NMRP, Rampur. The Inbreds were selected from different heterotic groups, ten inbred lines were selected to be female lines and two inbred lines were selected as testers to produce twenty hybrids in line/tester mating design (Table 2). The inbred lines were designated to be female and tester on the basis of performance from the previous research conducted by NMRP. These genotypes were planted following split-split plot design with three replications. Drought was main plot factor, nitrogen was sub-plot factor and genotypes were sub-sub-plot factor. Each replication consisted of four blocks and three sub-blocks with twelve genotypes were randomized on each sub-block. Each genotype was planted in three-meter-long row in a plot that consisted of two such rows with 12 plants in a row. The space between two rows was 0.75 m and space between plants was 0.25 m. The irrigated and drought plot was separated by a trench of 1 m deep and plastic sheet was used to avoid flow of irrigated water to the drought imposed plots. The irrigated plots were irrigated weekly and drought plots were rainfed. For high N plots, 120 kg/ha nitrogen and for low N plots, 30 kg/ha nitrogen was used with 3 split dose with 50% basal dose before planting.

Inbred line	Entry	Country of Origin	Parentage/Source
RML-4	1	CIMMYT, Mexico	-
EML-6	4	CIMMYT, Mexico	-
RML-8	7	CIMMYT, Mexico	-
RML-19	10	CIMMYT, Mexico	-
RML-32	13	CIMMYT, Mexico	CA00320
RML-55	16	CIMMYT, Mexico	-
RL-84	19	NMRP, Nepal	Upahar
RL-114	22	NMRP, Nepal	-
RL-153	25	NMRP, Nepal	-
RL-180	28	NMRP, Nepal	Pool 21
RML-17	31	CIMMYT, Mexico	CML 287
RL-111	32	CIMMYT, Mexico	-
RML: Rampur maize line; RL: Rampur line; CIMMYT: NMRP: National maize research program: CML: CIMMYT maize line.			

Table 1: Details of the inbred lines and testers taken for crossing. (Source: NMRP, Rampur).

Inbred Lines (Females)	Tester lines (Males)

	RML-17	Entry	RL-111	Entry
RML-4	RML-4 / RML-17	2	RML-4 / RL-111	3
RML-6	RML-6 / RML-17	5	RML-6 / RL-111	6
RML-8	RML-8 / RML-17	8	RML-8 / RL-111	9
RML-19	RML-19 / RML-17	11	RML-19 / RL-111	12
RML-32	RML-32 / RML-17	14	RML-32 / RL-111	15
RML-55	RML-55 / RML-17	17	RML-55 / RL-111	18
RL-84	RL-84 / RML-17	20	RL-84 / RL-111	21
RL-114	RL-114 / RML-17	23	RL-114 / RL-111	24
RL-153	RL-153/ RML-17	26	RL-1153 / RL-111	27
RL-180	RL-180 / RML-17	31	RL-180 / RL-111	30
RML=Rampur maize line; RL=Rampur line.				

Table 2: A crossing scheme for line x tester design for line tester hybrids.

Days to anthesis was recorded when more than 50% of the plants of the plot had tasseled with extruded anthers. Days to silking was recorded when more than 50% of the plants in the plot had silk emergence from the cob. Days to leaf senescence was visually recorded when more than 50% of the plants in the field had more than 50% leaf senescence. Anthesis silking interval was calculated as the difference between days to silking and the days to anthesis using the following mathematical formula:

Anthesis silking interval (ASI)=Days to silking - Days to anthesis.

A self-calibrating Minolta Chlorophyll Meter (SPAD-502) was used to measure the amount of total chlorophyll present in leaves. The canopy temperature was measured by using a hand held infrared thermometer. The infrared thermometer was placed to a height and reading was taken pointing to the canopy of the maize genotypes. The canopy temperature depression was calculated by using the following formula:

Canopy Temperature Depression (CTD)=Ambient temperature-Canopy temperature

Leaf rolling score was scored visually from 0 (no rolling) to 5 (tightly rolled). It was measured when leaves were still more upright and measured three times. Leaf senescence score was visually scored on a scale from 0 to 10 during the latter part of grain filling. It was scored three times at 7-10 days interval. Thousand kernel weight was measured by weighing thousand kernels and total grain yield was measured by weighing all the grains from the plot.

Drought and low nitrogen stress tolerance indices in different maize genotypes were estimated by calculating tolerance (TOL), stress susceptibility index (SSI), yield index (YI) and stress tolerance index (STI) by using the following equations [13].

$$TOL = Yp - Ys$$

$$SSI= [1-Ys/Yp]/[1-(\bar{Y}s)/(\bar{Y}p)]$$

$$STI= (Yp-Ys)/(Yp)2$$

where,

Yp=yield of individual genotypes without stress,

Ys=yield of individual genotypes under stressed condition,

¯Ys=average yield of all genotypes with stress and

¯Yp=average yield of all genotypes under stressed condition.

Data analysis

The quantitative data and yield attributing traits were recorded from five plants and secondary traits were taken from the 50% plants of the plot. Average five plants from each of the three replications were taken into consideration for data analysis. The data was analyzed for ANOVA using PROC MIXED syntax of SAS software version 9.4 [14]. The correlation among the morpho-physiological traits was calculated. UPGMA Clustering and Principal component analysis was done using MINITAB 14.

Results

Anthesis silking interval

There was highly significant ($P \leq 0.001$) difference in the anthesis silking interval for genotypes and moisture by genotypes interaction while the other condition and their interactions were not different as shown by ANOVA (Table 3). The mean anthesis silking interval were 0.55, 0.45, 1.2 and 1.36 for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed condition respectively (Table 4). In non-stressed, nitrogen stressed and both moisture and nitrogen stressed condition, the inbred RL-84 and hybrid RML-32/RML-17 had the lowest and the highest ASI respectively. Likewise, in drought stressed condition, RL-114 had the lowest and RML-32/RML-17 had the highest ASI. The ASI had negative correlation with grain yield (r=-0.146) (Table 5).

Source	**df**	**ASI**	**LROLL**	**LSEN**	**SPAD**	**CTD**
Replication(R)	2	1.00	0.18	7.28	3.04	19.27
Moisture (D)	1	65.33	143.23**	176.33*	206.71	420.32*
Error a (RXD)	2	5.38	1.31	9.02	27.06	18.04
Nitrogen (N)	1	0.08	0.15	1.33	2178.68***	42.55*
Moisture x Nitrogen (DXN)	1	1.81	0.12	3.00*	194.39***	2.35
Error b (RXDXN)	4	3.41	0.18	0.30	2.12	5.19
Genotypes (G)	35	25.27***	1.50***	9.67***	120.16***	2.60***
Moisture x Genotypes (DXG)	35	5.09***	0.23***	1.41	5.73	0.34
Nitrogen x Genotypes (NXG)	35	1.61	0.07	0.74	3.06	0.42
Moisture x Nitrogen x Genotypes (DXNXG)	35	1.80	0.05	0.80	5.42	0.35
Error (rxdxnxg)	280	2.45	0.08	0.98	4.77	0.40
Mean		0.89	2.03	2.37	47.73	6.44
R-square		0.65	0.89	0.7	0.84	0.85
CV (%)		75.29	14.62	41.84	4.57	9.89
Significance level: * significant at p=0.05; ** significant at p=0.01; ***: significant at p=0.001. ASI=anthesis silking interval; LROLL=leaf rolling score; LSEN=leaf senescence score; SPAD=spad reading and CTD=canopy temperature depression.						

Table 3: Mean squares from analysis of variance on anthesis silking interval, leaf rolling score, leaf senescence score, SPAD reading and canopy temperature depression of maize genotypes affected by drought and low nitrogen stress.

Genotypes	**ASI (days)**		**LROLL**		**LSEN**		**SPAD**		**CTD (˚C)**	
	D1N1	D2N2	D1N1	D2N2	D1N1	D2N2	D1N1	D2N2	D1N1	D2N2
RML-4	1.00	-2.33	2.58	4.00	2.33	5.67	47.54	41.89	6.47	4.73
RML-6	-2.00	-2.00	1.00	2.42	1.00	1.67	54.32	48.66	7.91	5.42
RML-8	-0.33	1.00	1.17	2.67	1.00	3.00	55.34	48.36	7.71	5.45
RML-19	2.67	1.67	1.67	2.75	1.67	3.67	51.80	45.04	7.31	4.82
RML-32	0.67	0.67	1.42	3.00	1.67	3.00	53.65	48.07	7.56	6.21
RML-55	0.67	2.33	1.25	2.50	1.00	2.33	54.03	47.62	7.82	4.76
RL-84	0.67	1.67	1.58	2.67	2.33	3.00	48.19	43.48	7.32	5.02
RL-114	-0.67	1.00	1.42	2.92	1.00	3.67	54.58	48.12	8.22	5.73
RL-153	1.33	2.67	1.25	2.25	1.00	2.33	52.71	47.22	8.07	4.79
RL-180	2.67	0.67	1.25	2.42	1.00	3.67	54.32	48.49	8.66	5.70
RML-17	-1.33	1.00	1.08	2.75	1.67	2.33	54.02	46.58	9.06	5.79
RL-111	-2.00	1.67	1.25	2.33	1.00	3.00	54.51	48.44	8.61	5.48
Inbred mean	0.28	0.84	1.41	2.72	1.39	3.11	52.92	46.83	7.89	5.33
RML-4/RML-17	-2.67	-0.33	2.08	3.50	1.00	3.00	47.49	44.15	7.48	5.55
RML-4/RL-111	-2.67	-2.33	1.50	2.67	1.00	1.67	55.76	49.01	8.98	5.62

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RML-6/RML-17	0.67	2.33	1.00	2.50	1.00	1.00	54.33	47.34	8.63	5.56
RML-6/RL-111	3.00	2.00	1.75	2.75	2.33	5.00	45.48	40.51	7.54	4.77
RML-8/RML-17	0.67	2.33	1.00	2.67	1.00	1.67	52.49	46.65	7.82	5.75
RML-8/RL-111	2.33	2.33	1.42	2.17	1.00	2.33	51.70	46.32	8.44	5.27
RML-19/RML-17	4.00	3.33	2.25	2.92	3.00	4.33	49.67	42.46	7.83	4.82
RML-19/RL-111	0.67	1.67	1.17	2.50	1.00	2.33	51.48	44.99	8.20	5.39
RML-32/RML-17	2.33	2.67	1.33	2.25	1.67	2.33	53.47	44.60	8.35	4.78
RML-32/RL-111	3.00	3.00	1.58	2.75	3.00	4.33	44.85	40.51	7.17	4.14
RML-55/RML-17	-2.33	-0.33	1.08	2.67	1.00	3.00	52.72	46.75	8.22	5.52
RML-55/RL-111	-0.33	2.33	1.08	2.08	1.00	1.67	52.10	46.92	7.83	5.23
RL-84/RML-17	2.00	3.00	1.92	2.83	3.00	4.33	45.39	41.50	6.89	4.88
RL-84/RL-111	-1.33	1.00	1.00	2.67	1.00	2.33	54.36	47.22	8.19	5.73
RL-114/RML-17	2.33	2.00	1.00	2.25	1.00	1.67	50.63	46.88	8.09	5.63
RL-114/RL-111	0.00	3.00	1.83	2.33	3.00	4.33	42.76	42.82	6.79	4.12
RL-153/RML-17	1.67	2.00	1.25	2.42	1.67	2.33	52.64	45.79	7.50	5.27
RL-153/RL-111	2.00	3.00	1.33	2.25	1.67	3.00	51.69	43.98	7.69	5.64
RL-180/RML-17	-1.00	1.33	2.33	3.83	3.00	5.00	46.36	40.81	7.16	4.75
RL-180/RL-111	3.00	2.67	1.83	2.67	2.33	3.67	41.35	35.71	6.76	3.92
Hybrid mean	0.87	1.85	1.49	2.63	1.73	2.97	49.84	44.25	7.78	5.12
RML-4/NML-2	-3.00	-2.00	1.42	3.17	1.00	3.67	55.74	47.60	7.70	5.45
900 M Gold	0.33	0.33	1.25	2.00	1.00	1.00	53.37	47.48	8.34	5.86
TLBRS07 F16	1.67	1.67	1.58	2.33	1.00	2.33	54.41	47.75	8.05	5.32
Rampur composite	0.33	2.00	1.50	2.42	3.00	3.67	52.99	47.03	7.28	5.15
Check mean	-0.17	0.50	1.44	2.48	1.50	2.67	54.13	47.47	7.84	5.45
GM	0.54	1.34	1.45	2.64	1.59	2.98	51.41	45.52	7.82	5.23

ASI= anthesis silking interval; LROLL= leaf rolling score; LSEN= leaf senescence score; SPAD= spad reading; CTD= canopy temperature depression; TKW= thousand kernel weight; GY= grain yield; GM= grand mean.; D1N1 = irrigated condition with N@120kg/ha and D2N2 = drought condition with N@30kg/ha

Table 4: Secondary traits of different genotypes of maize under different levels of moisture and nitrogen stress.

Traits	**PH**	**ASI**	**LRS**	**LSS**	**SPAD**	**CTD**	**TKW**	**GY**
PH	1.000							
ASI	-0.006	1.000						
LRS	-0.458**	-0.311**	1.000					
LSS	-0.470**	0.022	0.560**	1.000				
SPAD	0.631**	-0.324**	-0.264**	-0.449**	1.000			
CTD	0.484**	-0.227*	-0.216*	-0.354**	0.531**	1.000		
TKW	0.142	-0.006	0.212*	-0.077	0.150	0.157	1.000	

GY	0.653**	-0.146	-0.490**	-0.576**	0.616**	0.501**	0.438**	1.000

Significance level: *: significant at p= 0.05; **: significant at p= 0.01; ***: significant at p= 0.001. PH= plant height; ASI=anthesis silking interval; LRS= leaf rolling score; LSS= leaf senescence score; SPAD= spad reading; CTD= canopy temperature depression; TKW= thousand kernel weight; GY = grain yield.

Table 5: Pearson's Correlation coefficient of secondary traits under drought and low nitrogen stress condition.

Leaf rolling score

ANOVA showed highly significant (P ≤ 0.01) difference for leaf rolling score between moisture, genotype and moisture by genotype interaction. The mean leaf rolling score were 1.45, 1.46, 2.57 and 2.64 for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment respectively (Table 4). Inbreds RML-4 and RML-17 had the highest leaf rolling score for the entire non-stressed and stressed environment. While, the hybrids RL-153/RL-111, RML-4/RML-17, RML-55/RL-111 and 900 M Gold had lowest leaf rolling score for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment respectively. Leaf rolling score had negative highly significant correlation with grain yield (r=-0.490) and thus there was a decrease in grain yield with increasing leaf rolling score (Figure 1 and Table 5).

Leaf senescence score

ANOVA showed highly significant (P ≤ 0.01) difference for leaf senescence score between genotype and significant (P ≤ 0.05) difference for moisture and moisture by nitrogen interaction (Table 3). The mean leaf senescence scores were 1.59, 1.87, 3.03 and 2.98 for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment respectively (Table 4). Inbreds RML-17 and RL-84 had the highest leaf senescence scores for the non-stressed and low nitrogen stressed environment while inbred RML-4 had the highest leaf senescence score for both, drought stress and combined stress of drought and nitrogen. Similarly, the hybrids RML-8/RL-111, RML-8/RML-17, RL-114/RL-111 and RML-32/RL-111 had the lowest leaf senescence score for the non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment respectively. The mean leaf senescence scores for the inbreds were higher than that of hybrids in all stressed and non-stressed environment. Leaf senescence score had negative highly significant correlation with grain yield (r=-0.576) and thus there was a decrease in grain yield with increasing leaf senescence score (Figure 1 and Table 5).

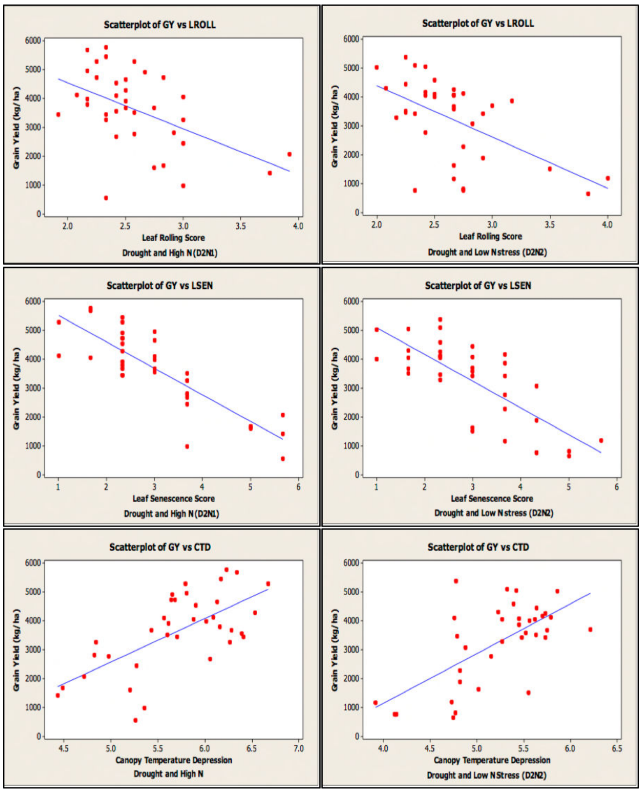

Figure 1: Scatterplot of secondary traits for stressed conditions with grain yield (kg/ha). A) Scatterplot of leaf rolling score with grain yield under drought stress. B) Scatterplot of leaf rolling score with grain yield under drought and nitrogen stress. C) Scatterplot of leaf senescence score with grain yield under drought stress. D) Scatterplot of leaf senescence score with grain yield under drought and nitrogen stress. E) Scatterplot of canopy temperature depression score with grain yield under drought stress. F) Scatterplot of canopy temperature depression with grain yield under drought and nitrogen stress.

SPAD chlorophyll content

There are highly significant (P ≤ 0.001) differences in the chlorophyll content for the nitrogen, genotypes and drought by nitrogen interaction while their interactions were not different as shown by ANOVA (Table 3). The mean chlorophyll content for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment were 51.33, 45.5, 48.61 and 45.46 respectively (Table 4). In non-stressed, drought stressed and both moisture and nitrogen stressed environment, hybrid RML-32/RML-17 had the highest chlorophyll content while the check hybrid RML-4/

NML-2 had highest chlorophyll content for nitrogen stressed environment. Likewise, the inbreds RL-111 and RL-180 had lowest chlorophyll content for both non-stressed and drought stressed environment. RL-180 and RML-111 for the nitrogen stressed and both drought and low nitrogen stressed environment had the lowest chlorophyll content. The mean chlorophyll content for the inbreds was lower than that of hybrids in all stressed and non-stressed environment. SPAD reading had positive highly significant correlation with grain yield (r=0.616) (Table 5).

Canopy temperature depression (CTD)

ANOVA showed highly significant ($p \leq 0.01$) difference for canopy temperature depression between genotype and significant ($p \leq 0.05$) difference for moisture and nitrogen (Table 3). The mean canopy temperature depression was 7.82, 7.04, 5.7 and 5.22 for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment respectively (Table 4). Inbreds RML-4, RL-180, RML-17 and RL-111 had the lowest canopy temperature depression for the non-stressed, low nitrogen stressed drought stressed and combined stress of drought and low nitrogen environment, respectively. Similarly, hybrids RML-19/RL-111, RML-32/RML-17, RML-4/RML-7 and RML-6/RML-17 had the highest canopy temperature depression for non-stressed, low nitrogen stressed, drought stressed and both drought and low nitrogen stressed environment, respectively. Canopy temperature depression had positive highly significant correlation with grain yield (r=0.501) and thus there was a decrease in grain yield with increasing leaf rolling score (Figure 1 and Table 5).

Grain yield (kg/ha)

The ANOVA showed highly significant ($p \leq 0.01$) differences for the grain yield between moisture level, nitrogen level and between genotypes (Table 3). Similarly, interaction between moisture by genotype was significant ($P \leq 0.01$) while the moisture by nitrogen, nitrogen by genotypes and moisture by nitrogen by genotypes were not significant ($P \leq 0.05$). The mean grain yield for the irrigated and drought stressed environments were 5790.3 kg/ha and 3593 kg/ha, respectively. Likewise, the grain yield for the high N dose and N stressed condition was found to be 4956.8 kg/ha and 4427.1 kg/ha, respectively. 900 M Gold (7920.6 kg/ha), RML-4/RL-111 (7202.2 kg/ha) and RML-4/RML-32 (6890.3 kg/ha) had the highest grain yield for all conditions whereas RML-180 (1150.5 kg/ha), RML-17 (1494.7 kg/ha) and RL-114 (1783.7 kg/ha) had the lowest grain yield (Table 6). Under combined stress of both drought and nitrogen, RL-84/RL-111 (5597.91 kg/ha) had the highest yield followed by TLBRS07 F16, RML-4/RML-17 and 900 M Gold which had 5324.8 kg/ha, 5272.87 kg/ha and 5246.4 kg/ha grain yield.

Genotypes	**Grain yield (kg/ha)**			
	Irrigated		**Drought**	
	N@120kg/ha	**N@30kg/ha**	**N@120kg/ha**	**N@30kg/ha**
RML-4	2453.63	2375.17	2157.70	1248.73
RML-6	4466.35	5392.59	2953.62	2366.22
RML-8	4535.37	2311.67	2790.01	1690.69
RML-19	7611.11	8574.43	4944.91	4351.93
RML-32	4754.28	2749.88	3402.67	1572.61
RML-55	2893.13	2571.09	1006.39	845.9
RL-84	3161.60	2533.56	2538.33	1954.36
RL-114	1780.02	2819.82	1745.20	789.57
RL-153	2719.47	1151.56	2875.17	3192.34
RL-180	2295.89	946.88	567.61	791.54
RML-17	2164.85	1662.68	1479.71	671.69
RL-111	2522.56	1906.02	1667.20	1201.02
Inbred Mean	3446.52	2916.28	2344.04	1723.05
RML-4/RML-17	8502.88	8272.83	5512.45	5272.87
RML-4/RL-111	9503.74	9134.61	5825.15	4245.32
RML-6/RML-17	7915.82	8394.73	5116.05	3858.21
RML-6XRL-111	7293.49	5019.50	3660.33	4261.22
RML-8/RML-17	7157.58	7365.71	3835.74	3563.20

RML-8XRL-111	7377.32	5694.00	5168.26	3627.99
RML-19/RML-17	7942.45	6914.57	3841.56	4290.78
RML-19/RL-111	8203.46	8283.38	4861.95	3573.74
RML-32/RML-17	6917.59	6647.96	4234.00	4216.26
RML-32/RL-111	7271.63	7585.38	3958.30	4163.10
RML-55/RML-17	6274.98	4472.43	4082.55	3828.75
RML-55/RL-111	6151.58	5150.70	3596.04	3414.82
RL-84/RML-17	7502.68	6686.75	3716.51	4774.63
RL-84/RL-111	7921.24	6613.40	5699.85	5597.91
RL-114/RML-17	7193.15	7575.12	4264.97	3731.48
RL-114/RL-111	7323.15	7046.32	4308.16	4494.60
RL-153/RML-17	7681.47	6630.66	4746.22	4433.12
RL-153/RL-111	6557.86	5962.24	4146.26	3657.50
RL-180/RML-17	7722.97	4760.82	4479.09	4213.11
RL-180/RL-111	5121.23	4458.85	3584.78	4634.04
Hybrid mean	7029.31	6321.36	4224.64	3996.78
RML-4/NML-2	8674.20	7794.14	4943.31	4022.07
900 M Gold	11619.91	9298.75	5517.38	5246.40
TLBRS07 F16	7015.42	7373.33	6021.01	5324.80
Rampur composite	3949.99	4614.68	3401.83	2879.75
Check mean	6266.85	5831.25	3991.91	3510.13
GM	6074.28	5431.16	3774.64	3364.93
GM= grand mean; RML= Rampur maize line; RL= Rampur line; N= Nitrogen.				

Table 6: Grain yield of different genotypes of maize under different levels of moisture and nitrogen stress.

Dendrogram analysis

The dendrogram for drought and low nitrogen stressed environment based on morpho-physiological characteristics and secondary traits of 36 maize genotypes was constructed (Figure 2). The dendrogram had two major groups and six clusters. Group A and Group B consisted each of 3 clusters. The clusters were obtained on the basis of similarity percentage and related characters. Cluster 2, cluster 3 and cluster 4 consisted of relatively tolerant genotypes than other clusters. The most tolerant genotype (900 M gold) and one of the hybrid RML-32/RML-17 were in the same cluster 4. This cluster 4 consisted of the tolerant genotypes.

Citation: Parajuli S, Ojha BR, Ferrara GO (2018) Quantification of Secondary Traits for Drought and Low Nitrogen Stress Tolerance in Inbreds and Hybrids of Maize (*Zea mays L.*). J Plant Genet Breed 2: 106.

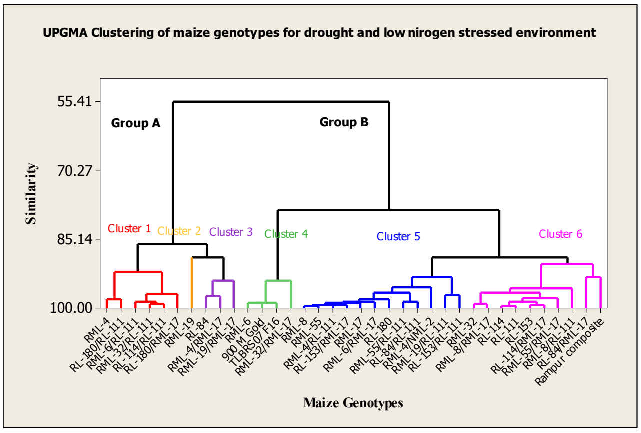

Figure 2: Dendrogram of the maize genotypes under drought and low nitrogen stressed condition.

Principal component analysis for secondary traits

The variation among the genotypes was also studied by Principal component analysis (PCA) for secondary traits and yield for drought and nitrogen stressed condition. The inbreds were grouped together while the hybrids were found to be scattered (Figure 3). The Eigen Analysis of the correlation matrix for drought and low nitrogen stress is shown in Table 7. The data revealed that the four principal components having greater than one Eigen value contributed 94.3% of variation, among 36 genotypes of maize under drought and low nitrogen stressed condition (Table 7). It was found that Principal component 1 (PC1) and Principal component (PC2) contributed 56.7% and 26.7% respectively of the total variation.

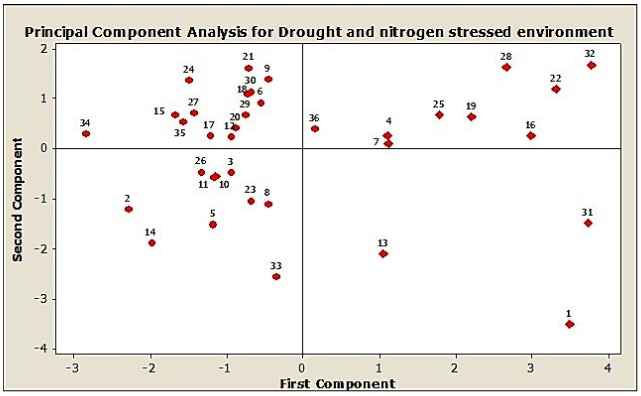

Figure 3: Principal component analysis of the Eigen analysis of the correlation matrix of different genotypes for drought and low nitrogen stressed condition. The number represents different genotypes.

	PC1	PC2	PC3	PC4
Eigen value	3.39	1.60	0.38	0.27
Proportion	0.567	0.267	0.064	0.045
Cumulative	0.567	0.834	0.898	0.943
Eigen vectors				
ASI	0.122	0.698	0.633	-0.033
LRS	0.315	-0.586	0.269	-0.319
LSS	0.469	-0.194	0.270	0.704
SPAD	-0.482	-0.172	0.120	0.588
CTD	-0.431	-0.314	0.655	-0.223
GY	-0.498	0.056	-0.102	0.076

PC1 = principal component 1; PC2 = principal component 2; PC3 = principal component 3; PC4 = principal component 4; ASI=anthesis silking interval; LRS= leaf rolling score; LSS= leaf senescence score; SPAD= spad reading; CTD= canopy temperature depression; GY = grain yield.

Table 7: The first four components of the secondary traits used for principal component analysis of the Eigen analysis of the correlation matrix for drought and low itrogen stressed condition.

Stress indices

The tolerance was higher in hybrids as compared with the inbred lines and the check 900 M gold had the highest tolerance in both drought and low N stressed condition (Table 8). The hybrid RML-4/RL-111 had the highest tolerance in both the drought stress and N stressed conditions. Similarly, the check 900 M gold had the highest SSI and STI in both of the drought and low N stressed conditions. The hybrids RML-4/RML-17 and RML-4/RML-111 has the highest SSI and STI as compared with the other inbred lines and hybrids. Among the inbred lines, RML-55 and RML-8 had the highest SSI for drought and low N stress, respectively while the RML-6 line had highest STI for both drought and low N stressed conditions (Table 8). The mean TOL and STI were higher in hybrids as compared with the inbred lines but the mean SSI for low N stress was found to be higher in inbred lines.

Genotypes	**Yield stability index (YSI)**		**Stress susceptibility index (SSI)**		**Yield index (YI)**		**Stress tolerance index (STI)**	
	Drought	**Nitrogen**	**Drought**	**Nitrogen**	**Drought**	**Nitrogen**	**Drought**	**Nitrogen**
RML-4	0.69	0.77	0.79	1.75	0.47	0.41	0.12	0.17
RML-6	0.53	1.02	1.21	-0.16	0.74	0.88	0.38	0.57
RML-8	0.64	0.53	0.93	3.55	0.62	0.45	0.23	0.29
RML-19	0.59	0.96	1.04	0.28	1.29	1.39	1.04	1.5
RML-32	0.64	0.52	0.91	3.67	0.69	0.49	0.27	0.35
RML-55	0.33	0.85	1.71	1.15	0.26	0.39	0.07	0.13
RL-84	0.77	0.77	0.59	1.76	0.63	0.51	0.19	0.25
RL-114	0.54	1	1.17	-0.01	0.35	0.41	0.08	0.13
RL-153	1.52	0.76	-1.33	1.82	0.84	0.49	0.17	0.24
RL-180	0.41	0.59	1.51	3.14	0.19	0.2	0.03	0.05
RML-17	0.55	0.62	1.15	2.85	0.3	0.26	0.06	0.08
RL-111	0.63	0.72	0.94	2.11	0.4	0.35	0.09	0.13
Inbred Mean	0.65	0.76	0.88	1.83	0.57	0.52	0.23	0.32
RML-4RML-17	0.63	0.94	0.95	0.44	1.5	1.53	1.32	1.88
RML-4RL-111	0.53	0.84	1.19	1.18	1.42	1.51	1.39	2.05
RML-6RML-17	0.54	0.92	1.18	0.64	1.25	1.39	1.07	1.58
RML-6RL-111	0.63	0.82	0.95	1.34	1.1	1.05	0.72	1.01
RML-8RML-17	0.5	0.97	1.28	0.25	1.03	1.24	0.79	1.19
RML-8RL-111	0.66	0.72	0.88	2.09	1.22	1.06	0.84	1.16
RML-19RML-17	0.54	0.92	1.18	0.58	1.13	1.27	0.88	1.31
RML-19RL-111	0.5	0.88	1.27	0.89	1.17	1.34	1.02	1.54
RML-32RML-17	0.61	0.95	1	0.39	1.18	1.23	0.84	1.2
RML-32RL-111	0.54	1.02	1.19	-0.13	1.13	1.33	0.88	1.31
RML-55RML-17	0.72	0.78	0.72	1.66	1.1	0.94	0.62	0.85
RML-55RL-111	0.61	0.86	1.01	1.1	0.98	0.97	0.58	0.83
RL-84RML-17	0.58	0.99	1.06	0.06	1.18	1.3	0.88	1.28
RL-84RL-111	0.76	0.87	0.61	0.95	1.57	1.38	1.2	1.65
RL-114RML-17	0.53	0.96	1.2	0.3	1.11	1.28	0.86	1.29
RL-114RL-111	0.6	0.97	1.02	0.26	1.22	1.31	0.93	1.33
RL-153RML-17	0.63	0.87	0.95	1.01	1.28	1.25	0.96	1.36
RL-153RL-111	0.61	0.88	1	0.95	1.09	1.09	0.72	1.02
RL-180RML-17	0.68	0.72	0.82	2.16	1.21	1.02	0.8	1.09
RL-180RL-111	0.84	1.02	0.41	-0.14	1.14	1.03	0.58	0.78
Hybrid Mean	0.61	0.89	0.99	0.8	1.2	1.23	0.89	1.29

RML-4NML-2	0.53	0.84	1.19	1.18	1.25	1.34	1.08	1.6
900 M Gold	0.5	0.82	1.27	1.33	1.5	1.65	1.65	2.48
TLBRS07 F16	0.77	0.95	0.58	0.37	1.58	1.44	1.19	1.64
Rampur composite	0.72	1	0.72	0.03	0.87	0.85	0.39	0.54
Check Mean	0.63	0.9	0.94	0.73	1.3	1.32	1.08	1.56
GM	0.63	0.85	0.95	1.14	0.99	0.99	0.69	0.99

RML=Rampur maize line; RL=Rampur line.

Table 8: Drought and low nitrogen stress tolerance indices in different maize genotypes.

Discussions

Secondary traits are much more important than grain yields under stressed environments as they are precise for identification of drought and low nitrogen tolerant genotypes and determine the degree to which the crop was stressed [15]. The major secondary traits (ASI, leaf rolling score, leaf senescence score, SPAD reading and canopy temperature depression) were quantified in this study for drought and low N tolerance breeding. The mean ASI for the inbreds was found higher than that of hybrids in all stressed and non-stressed condition. The mean value of ASI for the non-stressed condition was shorter than that of stressed condition. The ASI was found to be shorter under irrigated conditions, but it was relatively longer ASI and significantly reduced under drought stressed condition [16], which is in accordance with our findings. The ASI was negatively correlated with grain yield (r=-0.15) which is in accordance with the findings from Araus et al. [17]. Under drought stress, the silk grows slowly and the ASI increases and the longer ASI are external indicators of low grain filling and barren cobs [18]. So, breeders are working for reducing anthesis-silking interval but increasing various yield components under drought for stress tolerance breeding. The genotypes susceptible to stress shows very large ASI and reduced yield.

The leaf rolling score was significantly negatively correlated (r=0.49) with grain yield. A similar correlation was found in tropical maize source populations. The mean leaf rolling score for the inbreds was higher than that of hybrids in all stressed and non-stressed environment. The leaf rolling score for the stressed environment was higher than that of non-stressed environment. Drought stress causes abscissic acid (ABA) accumulation and passes to the leaf causing leaf rolling [19]. The genotypes which are very susceptible to drought stress shows high leaf rolling score. According to Bolanos [20], grain yield under drought stress shows strong correlation with leaf rolling score. Banziger found that the genotypes with low leaf rolling score showed better recovery after irrigation and were tolerant to drought stress.

The leaf senescence score for the stressed environment was higher than that of non-stressed environment. Increase in leaf senescence score under stressed conditions as explained by Banzinger et al. [3] was due to high potential evapotranspiration and leads to reduced leaf expansion. This causes reduction in radiation interception and ultimately reduction in crop yield. Leaf senescence is accelerated by the accumulation of abscisic acid on the leaf under drought and low nitrogen stress.

The mean value of chlorophyll content for the non-stressed environment (both irrigated and optimum nitrogen) was higher than that of stressed environment. This was verified by Smith [21] they found that mean chlorophyll level (SPAD reading) increased with increasing soil fertility. According to Wilson and Allison [22], the drought affected plants are usually lighter green than their unstressed counterparts with a lower level of leaf nitrogen and chlorophyll concentration. This leads to decrease in chlorophyll content and shows low SPAD reading under stressed condition.

The mean canopy temperature depression for the hybrids was higher than that of inbreds in all stressed and non-stressed environment. The mean value of canopy temperature depression for the non-stressed environment was higher than that of stressed environment. Blum [23] found that the canopy temperature depression variation is a reflection of variations in stomatal conductance. According to Bolanos and Edmeades [24], those genotypes which are able to form osmotically active substances in response to drought stress can take up more water to maintain turgor for longer time under drought. This causes reduction in canopy temperature and such genotypes are better adapted to stress environment. Drought and nitrogen stress both reduces the photosynthesis rate during pre-flowering, flowering and grain filling duration which causes reduction in grain production. The additional reduction in grain production was due to increased energy and nutrient consumption of stress adaptive responses like increased root growth. Abscisic acid produced during extreme drought stress passes to the grain causing abortion of tip grains during grain filling and thus reducing the total grain yield of a genotype. The genotypes with stress adaptive traits shows least reduction in grain yield under stressed condition compared to their unstressed counterpart and they were the most tolerant traits for that particular stress.

The dendrogram was constructed to study similarity of genotypes under drought and low N stress and it had two major groups and six clusters. Group A and Group B consisted each of 3 clusters. The cluster 4 consists of most tolerant genotypes. The most tolerant genotype (900 M gold) and one of the hybrid RML-32/RML-17 were in the same cluster. This gave us idea that this hybrid from cluster 4 could be considered as the best hybrid for higher tolerance against drought and low nitrogen stress condition as predicted by dendrogram and yield data. Dendrogram was constructed to group different maize genotypes under stressed conditions. The secondary traits which contributed more positively to PC1 were leaf senescence score, leaf rolling score and anthesis silking interval whereas grain yield, canopy temperature depression and SPAD reading contributed negatively. This means that populations with high PC1 had high leaf senescence score, leaf rolling score and anthesis silking interval.

The maize genotypes used in the study showed significant response to secondary traits for the stress tolerance. The stress indices tolerance and stress tolerance index showed higher value as compared with stress susceptibility index. Based on TOL and STI, the hybrids RML-4/RML-17, RML-4/RL-111, RML-32/RML-17 and RML-32/RL-111 showed higher level of tolerance to both drought and low N stressed condition as compared with other inbred lines and hybrids. Similar results were seen in transgenic wheat for drought stress tolerance [25]. Significant differences were observed among the genotypes as well as for drought and nitrogen stress condition for almost all measured secondary traits, yield attributing traits and yield. On an average, leaf rolling score for optimum moisture and nitrogen conditions was 1.45 and for drought and nitrogen stressed condition was 2.64. The mean chlorophyll content for optimum and both drought and nitrogen stressed condition was 51.33 and 45.46, respectively. The mean canopy temperature depression for optimum and both drought and nitrogen stressed condition was 7.82 and 5.22 respectively. For drought stressed condition and nitrogen stressed condition, the mean yield was 3593 kg/ha and 4956.8 kg/ha, respectively [26]. The mean of hybrids were higher than the yield of the inbred lines used in this study.

Conclusions

The correlation of the secondary traits was found useful in the selection of stress tolerant genotypes of maize. Among the secondary traits, leaf rolling score and canopy temperature depression were useful in the selection of tolerant genotypes. Those valuable traits were found to be of higher value under the stressed condition. The UPGMA cluster analysis and Principal component analysis for drought and low nitrogen stress were also found significant for selecting tolerant genotypes. Based on quantification of secondary traits and stress indices, the hybrids were found to be more tolerant under both drought and low nitrogen stressed conditions. The hybrids RML-4/RML-17, RML-32/RML-17, RML-8/RML-17, RML-32/RL-111, RML-4/RML-17 and RML-4/RL-111 were found to be more tolerant compared with other hybrids based on secondary trait quantification and stress indices and they had higher yield under stressed conditions. All the secondary traits under study were found to be useful for determining the tolerant genotypes. The correlation of secondary traits provides breeders the flexibility to select the genotypes based on the secondary traits. Therefore, for maize breeders, secondary traits are of great value for selection, evaluation and release of new variety offering higher level of tolerance to environmental stress.

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