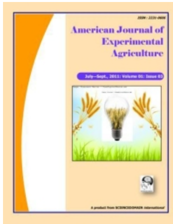

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Effect of Nitrogen Supply and Genotypic Variation for Nitrogen Use Efficiency in Maize

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Authors' contributions

This work was carried out in collaboration between all authors. Author M designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author KR performed the statistical analysis. Author HP managed the analyses of the study and edit the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Effect of nitrogen supply and genotypic variation for different traits related to nitrogen use efficiency (NUE) were studied in order to enhance the understanding of genetic basis of NUE and to find genetic materials for developing low-N tolerant maize genotypes.
Methodologies: Ten genotypes (5 open pollinated varieties and 5 hybrids) were evaluated at four N levels (0; 30; 90; 180 kg $N.ha^{-1}$) in split plot randomized block design with three replications at farmer field in Tulungagung, East Java, Indonesia, from November 2011 to February 2012.
Results: The results showed that genotypes exhibiting contrasted responses to N nutrition. Nitrogen deprivation caused varied reductions of plant height, leaves area, chlorophyll content, stay green, N uptake, total dry matter, grain yield, grain number and a thousand grain weight among genotypes; but did increase days to 50% anthesis, 50% silking, anthesis-silking interval, crop recovery efficiency of applied N (RE_N), physiological efficiency of applied N (PE_N), agronomic efficiency (AE) and NUE significantly (P = .05).

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Heritability estimates (h^2) were high (h^2 > 0.5) for most of measured traits at all N levels and ranged from -0.892 to 0.998. This indicated that it is possible to select genotypes are adapted to low N under both low and high N fertilization. High genotypic variation for grain yield was observed at all N levels, while for RE_N, PE_N and AE were found at high-N and NUE at low-N. Reduction of N level from 180 to 90, 30 and 0 kg N.ha^{-1} caused reduction of 7.8%, 14.4% and 49.4% grain yields respectively. High grain yield were found in Bisi-2, Pioneer-21, NK-33, Bisma and DK-979 at high-N; and less yield reduction caused by N level reduction were found in DK-979, Madura, Bima-3, Bisma and NK-33, whilst high NUE traits were found in NK-33 and Pioneer-21.

Conclusions: NK-33, Pioneer-21, DK-979 and Bisma are expected to be as genetic materials for developing tolerant low-N varieties.

Keywords: Maize; nitrogen use efficiency; genotypic variation; heritability estimate.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

NUE: nitrogen use efficiency; NUp: nitrogen uptake; RE_N: crop recovery efficiency of applied N, PE_N: physiological efficiency of applied N; AE: agronomic efficiency; HI: harvest index; OPVs: open pollinated varieties, TDM: total dry matter.

1. INTRODUCTION

Nitrogen (N) is an essential nutrient and constituent of 3-4% dry matter [1], often becomes a limiting factor for plant growth and development [2-6] because it plays an important role in plant metabolism [7]. The use of N by plant involves several steps, including uptake, assimilation and translocation then when plant is aging, recycling and remobilization [8]. Therefore, nitrogenous fertilizers widely applied by farmers and have contributed to remarkable increase in plant production during the past 50 years [9], especially staple foods such as maize is highly responsive to N and requires large quantity of N [10,11]. Five million tons of N fertilizer used annually to fields of maize production in the industrialized world, and use is on the rise in developing nations [11]. In 2011, the world demand for nitrogen fertilizer was 105.348 million tons and predicted to grow 1.7% annually for 2011-2015 [12].

Nowadays, excessive N application has become a concern because accumulation of a large amount of N in ecosystems leads to significant direct and indirect negative effects on environmental quality, biodiversity decrease and human health [9,13-16], because only about 33% N fertilizer removed by plant and the rest lost by leaching, denitrification, and volatilization [17]. Under tropical conditions, maize crop only absorbed about 40-50% N fertilizer [10,18]. Incomplete capture and poor conversion of N fertilizer causes global warming through emissions of nitrous oxide (N_2O) [8]. Lowering N fertilization and cropping genotypes with better NUE are the ways to overcome the N application problems.

There are several definitions for NUE which have been developed, depending on agronomic, genetic and physiological studies [19]. NUE definition by Moll et al. [20] is the grain yield per unit of N available in soil including fertilizer. Two components of NUE: nitrogen uptake efficiency (NUpE) is the ability of plant to remove N from the soil, and nitrogen utilization efficiency (NUtE), is the ability of plant to use N to produce grain yield [19]. In the agronomic frame work, NUE is yield per N applied, agronomic efficiency (AE) is yield increase per N applied, crop recovery efficiency of applied N (RE_N) is N uptake increase per N applied and physiological efficiency of applied N (PE_N) is yield increase per increase of N uptake from fertilizer [9].

Improving NUE expected to reduce negative impact of excessive N fertilizer application, and it could be increased by production practice system: plant rotation, forage-only production, genotypes, conservation tillage, NH_4–N source and irrigation [17]. In order to develop genotypes that have better nitrogen use efficiency needed a better knowledge of physiological and genetic basis of NUE [7]. N uptake and utilization efficiencies require that those processes associated with absorption, translocation, assimilation, and redistribution of N operate effectively [20]. Genotype x nitrogen interaction is significant for traits associated with NUE [7,21,22]. Several studies found genetic potential in maize genotypes for improvements of NUE and promising genetic material with better N uptake and utilization efficiency and used as parents to develop population adapted to low-N [4,23].

So the present study was conducted with aims to: (i) understand effect of N deprivation to traits related to NUE in maize; (ii) investigate genotypic variation for NUE in maize and identify new sources for breeding maize with greater NUE in order to develop tolerant low-N maize genotypes.

2. MATERIALS AND METHODS

The experiment was conducted on November 2011-February 2012 at farmer field in Tulungagung, East Java, Indonesia with altitude 85 meters above sea level, temperature 28-31ºC, rain fall 1682 mm.year^{-1} and humidity 63-71%. The experiment soil characterized as alluvial hydromorph, pH: 6.2 (H_2O reagent) and 5.24 (KCl reagent); CO: 1.27%; N: 0.13%; P_2O_5: 34.33 ppm, SO_4: 51.45 ppm; Fe: 39.55 ppm; Cu: 12.48 ppm; Zn: 3.26 ppm; K: 0.22 me/100 g; Na: 0.31 me/100 g; Ca: 13.4 me/100 g; Mg: 3.16 me/100 g. Total organic-C, total N and total P were analysed by Kurmis, Kjeldahl and Bray I mehods respectively; whilst other macro-nutrients and micro-nutrients were analyzed using Modified (Wolf) Morgan Extracting Reagent.

Ten maize genotypes from five open pollinated varieties (OPVs) consist of Bisma, Lamuru, Arjuna, Sukmaraga, Madura; and five hybrids consist of Pioneer-21, NK-33, DK-979, Bisi-2, Bima-3 were planted at four levels of N fertilizer: without N fertilizer, low-N (30 kg N.ha^{-1}), medium-N (90 kg N.ha^{-1}), high-N (180 kg N.ha^{-1}) in split plot randomized block design and replicated three times. The genotypes allocated to the main plot, whereas N levels distributed in sub plot. Each genotype was planted in single row, 3 m long, 0.75 m apart and 0.20 m within row. Two seeds were sown per hole and later thinned to one at 2 weeks after sowing (WAS).

Phosphate and potassium fertilizers were applied 100 kg P.ha^{-1} and 75 kg K.ha^{-1} simultaneously with planting, whereas N fertilizer as urea (46%) was added as single application for 30 kg N.ha^{-1} treatment at 2 WAS, and three times for 90 and 180 kg N.ha^{-1} treatment; 1/3 apart at 2 WAS, 1/3 apart at 5 WAS, and 1/3 apart at anthesis.

Five representative plants were used as sample plants to collect the data. At maturity, plants were harvested and separated into leaves, stem and cob, dried at 70ºC for 48 h in oven (MEMMERT type ULM 400 capacity/resolution 220ºC/0.1ºC) to constant weight then were analyzed for total N content by Kjeldahl method.

Total chlorophyll was analyzed at anthesis by Arnon [24]; 0.25 g fresh cob leaves were crushed in 10 ml 80% acetone and extract centrifuged 15 minutes at 2500 rpm. Absorbance of supernatant was recorded at 645 and 663 nm in spectrophotometer (MERCK type PHARO 100 capacity/resolution 320-1100 nm/0.1%/0.001 Abs). Chlorophyll content (expressed as mg/g-1 of each sample) was estimated as follow:

Chlorophyll a ($mg.g^{-1}$) = 12.7 (A663) – 2.69 (A645) x VW
Chlorophyll b ($mg.g^{-1}$) = 22.9 (A645) – 4.86 (A663) x VW
Total Chlorophyll t ($mg.g^{-1}$) = [20.2 (A645) – 8.02 (A663) x VW]/1000

Where: A = absorbance at the given wavelength, W = weight of fresh leaf sample, V = final volume of chlorophyll solution.

The data were recorded on days to 50% anthesis and days to 50% silking of the plants in the plot, and anthesis-silking interval (ASI). At maturity, plant height, total leaves area, total dry matter were determined. Stay green was determined for each plot by visually assessing degree (%) of green leaves at 1 week before harvest. Grain yield, grain number per cob and weight of 1000 grains were determined at harvesting.

The following parameters were calculated to estimate NUE based on agronomic study [9]:

- ✓ Nitrogen uptake = total dry matter above ground X N concentration (g N/plant).
- ✓ Crop recovery efficiency of applied N (RE_N) = (U_N-U_0)/F_N (kg N-uptake/kg N-fertilizer).
- ✓ Physiological efficiency of applied N (PE_N) = (Y_N-Y_0)/(U_N-U_0) (kg grain/kg N-uptake).
- ✓ Agronomic efficiency (AE) = (Y_N-Y_0)/F_N (kg grain/kg N-fertilizer).
- ✓ Nitrogen use efficiency (NUE) = YN/FN (kg grain/kg N-fertilizer).

Where:
F_N: amount of N fertilizer applied ($kg.ha^{-1}$).
Y_N: crop yield with applied N fertilizer ($kg.ha^{-1}$).
Y_0: crop yield in a control treatment with no N fertilizer ($kg.ha^{-1}$).
U_N: total plant N uptake in aboveground biomass at maturity in a plot that received N fertilizer ($kg.ha^{-1}$).
U_0: the total N uptake in aboveground biomass at maturity in a plot that received no N fertilizer.

The data were analyzed by analysis of variance (ANOVA) to estimate the significance of genotypes, N levels and their interactions. Tukey's HSD (honestly significant difference) test was calculated to compare treatment means. Heritabilities were calculated to investigate the genotypic variation by broad-sense heritability derived from the ratio of genotypic variance to phenotypic among genotypes estimated from the analysis of variance, base on expectation of mean squares [25]:

$h^2 = \sigma^2g/ \sigma^2p$
$\sigma^2g = (Mg – Me)/r$
$\sigma^2e = (Me/r)$
$\sigma^2p = (Mg/r)$

Where: h^2 = heritability estimate; Mg = mean square of genotypes; Me = mean square of error; σ^2g = genotypic variance; σ^2p = phenotypic variance; σ^2e = environmental variance.

3. RESULTS AND DISCUSSION

N fertilization, genotypes and N x genotypes interaction had significant effect ($P = 0.01$) on all the traits except harvest index (Table 1). N deprivation caused varied reduction significantly among genotypes on plant height, leaves area, chlorophyll content, stay green, N uptake, total dry matter and yield components (grain yield, grain number per cob and a thousand grain weight); but increased days to 50% anthesis, 50% silking, and anthesis-silking interval on several genotypes. The effect of N deprivation gives preliminary information for understanding plant reaction to N fertilization.

Table 1. Mean squares of ten genotypes planted at four N fertilizer levels for traits related to NUE

S.O.V →	Genotypes	E_a	N levels	N levels x genotypes	E_b
	df				
	9	18	3	39	60
Traits	**MS**				
Plant height (cm)	4520.8**	38.1	13985.5**	296.5**	23.6
Leaves area (m^2)	0.145**	0.001	0.278**	0.019**	0.001
Chlorophyll content($mg.g^{-1}$)	10.1**	0.3	179.8**	2.8**	0.2
Stay green (%)	224.7**	22.0	4761.7**	55.9**	24.3
Days to 50% anthesis	268.5**	0.3	46.9**	1.1**	0.3
Days to 50% silking	320.7**	0.5	51.8**	1.3**	0.4
Anthesis-silking interval (d)	10.1**	0.3	0.3**	0.7**	0.3
Grain yield ($Mg.ha^{-1}$)	23.8**	0.2	77.9**	1.0**	0.2
Grain number per cob	20336.9**	1070.2	145983.7**	1730.3**	2176.7
A thousand grain weight (g)	19504.0**	172.4	11553.3**	389.5**	152.4
Total dry matter ($Mg.ha^{-1}$)	10.5**	0.170	69.91**	0.891**	0.115
Harvest index (HI)	0.018**	0.002	0.0037	0.0027	0.0024
NUp (g)	2.7**	0.1	27.7**	0.6**	0.1
RE_N ($kg.kg^{-1}$)	2.0**	0.1	30.7**	0.6**	0.1
PE_N ($kg.kg^{-1}$)	586.9**	158.2	9709.4**	375.0**	113.3
AE ($kg.kg^{-1}$)	1872.2**	175.6	42942.3**	666.1**	102.0
NUE ($kg.kg^{-1}$)	555.5**	85.8	3726.5**	249.7**	59.4

***, Significant at 0.01 levels of probability; MS, mean squares; E, error, NUp, nitrogen uptake; RE_N, crop recovery efficiency of applied N; PE_N, physiological efficiency of applied N; AE, agronomic efficiency; NUE, nitrogen use efficiency.*

Heritability estimates were high ($h^2 > 0.5$) for most of measured traits at all N levels and ranged from -0.892 to 0.998 (Table 2). Higher heritabilities were observed at high-N for grain yield, a thousand grain weight, N uptake, RE_N, PE_N and AE; and at low-N for plant height, leaves area, stay green, harvest index and NUE. Previous researcher [26] recorded that maize breeding materials heritabilities were similar at both low-N and high-N even though G x Location and error effects were higher under LN.

Table 2. Heritability Estimates for traits at no-N, low-N, medium-N and high-N

Traits	N fertilizer levels (kg $N.ha^{-1}$)			
	0	**30**	**90**	**180**
Plant height (cm)	0.974	0.995	0.966	0.979
Leaves area (m^2)	0.955	0.993	0.967	0.992
Chlorophyll content($mg.g^{-1}$)	0.995	0.995	0.996	0.994
Stay green (%)	0.998	0.992	0.994	0.996
Days to 50% anthesis	0.956	0.854	0.912	0.873
Days to 50% silking	0.997	0.920	0.925	0.964
Anthesis-silking interval (ASI) (d)	-0.892	0.862	0.861	0.672
Grain yield ($Mg.ha^{-1}$)	0.881	0.977	0.969	0.985
Grain number per cob	0.640	0.716	0.814	0.635
A thousand grain weight (g)	0.945	0.968	0.976	0.977
Total dry matter ($Mg.ha^{-1}$)	0.510	0.946	0.975	0.975
Harvest index	0.625	0.807	0.400	0.328
Nup (g)	-0.204	0.839	0.916	0.960
RE_N ($kg.kg^{-1}$)	-	0.839	0.930	0.962
PE_N ($kg.kg^{-1}$)	-	0.689	0.500	0.857
AE ($kg.kg^{-1}$)	-	0.888	0.856	0.952
NUE ($kg.kg^{-1}$)	0.739	0.901	0.741	0.476

**a heritability of zero does mean that phenotypic variance is entirely environmental and non-additive genetic or non genotypic variance.*

Plant height and leaves area were reduced with N reduction on all genotypes except Madura was not responsive to N and had the lowest of plant height and leaves area (Table 3). Without N fertilization, all genotypes exhibited stressed strongly, however they afford to grow optimum at high-N and no different performances except Bima-3 from hybrids and Madura from OPVs which are old varieties. High genotypic variation had been observed for plant height and leaves area at all N levels (Table 2). Genotypic variations for plant height and leaves area were higher at low-N than other N levels. Previous studies [27,28] also indicated that maize leaves number varied with N levels among genotypes.

Chlorophyll content and stay green were significantly affected by N levels among genotypes (Table 3). Chlorophyll content and stay green demonstrated high genotypic variation at all N levels (Table 2), this was due to the difference of N uptake capacity among genotypes associated with chlorophyll content, pre-anthesis N-accumulation and post-anthesis N-remobilization. Nitrogen is a structural element of chlorophyll and protein molecules thereby affects formation of chloroplasts and accumulation of chlorophyll in them [29]. N total and chlorophyll content which are part of physiological traits are indicators that mainly reflect the metabolic activity of individual leaves with regards to N assimilation and recycling, regardless of the level of N fertilization [19]. One of N-efficient maize characteristics is slow leaf senescence through maintenance of post-anthesis N uptake [30]. Improving N uptake, leaf area duration and chlorophyll content could be used to select new varieties exhibiting a “stay-green” phenotype [31]. N reduction led to reduced chlorophyll content and stay green on all genotypes except Madura was constant at all N treatments (Table 3). At low-N, medium-N and high-N, Pioneer-21, NK-33 and DK-979 had higher chlorophyll content and stay green than other genotypes. Without N fertilization, all genotpes were not significantly different and earlier leaf senescence occurred starting 6 weeks after sowing. Similar results were obtained by different authors [21,27,32]. N remobilization generally starts earlier when plants are under low-N compare to high-N fertilization [33] as a consequence of unavailable soil N, and then leaf senescence, which is associated with active remobilization, is also accelerated [34]. Leaf longevity is enhanced by increase in soil N supply [35], and reduced N availability accelerates post flowering leaf senescence than at high-N [36].

Higher genotypic variations for days to 50% anthesis and silking were found at no-N fertilizer treatment than other treatments (Table 2). N reduction from high-N to medium and low-N didn't affect days to 50% anthesis and silking, but they increased significantly at no N fertilization treatment in all genotypes except Madura was earlier mature and had no difference of 50% anthesis and silking at all N levels. ASI tended to increase with reducing N levels, even though it was not significantly different (Table 4). Madura, Sukmaraga, Bima-3, Bisma, Lamuru and Arjuna had shorter ASI than Pioneer-21, NK-33, DK-979 and Bisi-2. Previous researchers [21,32] reported that ASI increased under low-N condition, and recorded that plants were earlier in days to 50% silking than days to 50% anthesis under N stress. ASI could then have a physiological meaning in relation to stress tolerance and increasing ASI bring the consequence there could be a deficit in ovule fertilization. Genotypes that have short ASI would have a more efficient nitrogen metabolism at low N [7]. We observed in the present study that plants had short ASI would lead to high yield.

Total dry matter (TDM) or biomass production decreased significantly among genotypes when N levels reduced from 180 to 30 and 0 kg/ha (Table 4). There were no significant differences of TDM between hybrids and OPVs except Madura, this because most of OPVs were used by authors are leafy maize type which have greater number of leaves and leaf area (Bisma, Lamuru, Sukmaraga) than their conventional counterparts.

N deprivation caused reduction of grain yield, grain number and a thousand grain weight, but harvest index was not affected by N reduction (Fig. 1 and Table 5). Greater genotypic variations were observed on grain yield and a thousand grain weight than grain number and harvest index (Table 2). High genotypic variations for grain yield were observed at all N fertilizations (high-N, medium-N and low-N), but it was low at no N fertilization because all genotypes were strongly stressed. Previous studies indicated that grain yield was not significantly different among upland rice genotypes at low-N [37], and genotype x N interaction is essential due to variation in the adaption of the plant to low-N rather than to variation in the adaption to high-N [7].

At high-N, there was no significant difference of grain yield among genotypes except Madura had the lowest grain yield. However hybrids (Pioneer-21, NK-33, DK-979, Bisi-2 and Bima-3) tended to yield more than OPVs (Arjuna, Sukmaraga, Lamuru, Madura), except Bisma that was found as potential OPVs. At medium-N, NK-33, Bisma, DK-979, Arjuna and Bisi-2 were higher than others, whilst at low-N, Pioneer-21 and NK-33 had higher grain yield than others. In the present study, we also found a fact that newer hybrids (Pioneer-21, NK-33, DK-979 and Bisi-2) had higher grain yield than older hybrid (Bima-3) (Fig. 1). Some researchers reported that evaluation of bread wheat under conditions of low N fertilization, it was found that modern cultivars were more responsive to N in terms of economic fertilizer levels compared with old cultivars [38] and inbred lines of maize were more reduction in yield than hybrid group under N limited conditions [21].

Table 3. Performance of ten genotypes at four N fertilizer levels for plant height, leaves area, chlorophyll content and stay green traits

	Plant height (cm)				Leaves area (m^2)				Chlorophyll content ($mg.g^{-1}$)				Stay green (%)			
Genotypes	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**
Pioneer-21	206.4	234.6	238.2	242.9	0.63	0.73	0.68	0.58	1.54	6.25	8.76	9.03	39.2	68.0	70.5	75.0
NK-33	226.9	252.7	246.8	251.0	0.49	0.60	0.66	0.92	1.69	5.90	7.98	8.90	39.2	68.1	69.6	77.6
DK-979	188.7	221.9	244.0	248.7	0.52	0.74	0.67	0.68	1.68	4.26	7.44	7.25	43.6	64.0	63.2	77.0
Bisi-2	187.1	247.7	249.9	249.6	0.66	0.91	0.84	0.94	0.90	3.18	5.88	6.43	43.8	57.1	64.0	71.9
Bima-3	147.1	192.8	206.6	212.4	0.51	0.74	0.86	0.89	0.79	2.92	6.55	6.57	36.2	63.1	58.0	73.1
Arjuna	187.3	236.3	240.9	243.0	0.65	0.90	0.75	0.77	0.82	3.24	6.48	6.57	40.3	52.0	52.8	68.4
Sukmaraga	203.6	254.6	257.3	256.9	0.58	0.84	0.77	0.92	0.78	3.29	4.72	6.08	40.4	52.8	51.8	65.7
Lamuru	201.2	253.8	255.9	256.8	0.60	0.79	0.80	0.80	1.10	3.61	5.87	6.00	38.1	54.6	54.7	64.8
Bisma	209.3	251.0	248.6	249.1	0.68	0.95	0.80	0.94	1.20	3.15	5.77	6.17	39.6	57.1	70.2	70.1
Madura	191.9	193.4	200.6	195.2	0.46	0.48	0.46	0.48	4.21	4.82	4.38	4.80	42.2	58.5	55.7	58.8
HSD $_{(0.05)}$																
G		9.0				0.04				0.77				6.9		
N		3.3				0.02				0.32				3.4		
GxN		14.7				0.09				1.44				14.9		

Table 4. Performance of ten genotypes at four N fertilizer levels for days to 50% anthesis and silking, anthesis-silking interval (ASI) and total dry matter traits

	Days to 50% anthesis				Days to 50% silking				Anthesis-silking interval (d)				Total dry matter ($Mg.ha^{-1}$)			
Genotypes	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**
Pioneer-21	60.3	57.3	58.0	57.3	64.7	60.7	60.7	59.7	4.33	3.33	2.67	2.33	4.43	7.06	7.90	8.20
NK-33	60.0	57.3	57.3	57.7	64.7	61.0	61.0	61.0	4.67	3.67	3.67	3.33	4.75	6.81	8.28	8.56
DK-979	60.3	57.7	57.3	57.7	64.0	61.3	60.7	61.3	3.67	3.67	3.33	3.67	4.67	6.70	8.09	8.44
Bisi-2	61.3	58.3	58.7	58.7	64.7	62.0	62.3	62.3	3.33	3.67	3.67	3.67	4.53	7.33	8.19	8.28
Bima-3	62.3	61.7	61.7	61.3	64.7	63.3	62.7	62.7	2.33	1.67	1.00	1.33	4.38	6.68	7.46	7.58
Arjuna	59.0	55.3	55.3	55.3	61.7	57.7	57.7	57.7	2.67	2.33	2.33	2.33	4.01	6.73	7.76	7.64
Sukmaraga	61.3	58.3	59.0	58.7	62.7	60.7	60.7	60.7	1.33	2.33	1.67	2.00	4.49	6.97	7.86	8.16
Lamuru	59.3	57.3	56.7	57.0	61.7	59.7	59.7	59.7	2.33	2.33	3.00	2.67	4.28	7.13	7.62	8.21
Bisma	59.3	55.3	55.0	55.3	61.7	59.0	58.7	58.7	2.33	3.67	3.67	3.33	4.63	7.35	8.19	8.31
Madura	44.7	44.3	44.3	43.7	45.7	45.7	45.7	45.0	1.00	1.33	1.33	1.33	3.92	3.98	3.99	4.10
$HSD_{(0.05)}$																
G		0.9				1.0				0.81				0.60		
N		0.4				0.4				0.39				0.23		
GxN		1.8				1.9				1.73				1.03		

In the experimental conditions used by authors, hybrids and OPVs (except Madura) exhibiting similar result for some traits, this may be due to high-N treatment (180 kg N.ha^{-1}) was not the maximum N level anymore, particularly for hybrids which are more responsive to N fertilizer. We noticed during the experiment that N fertilizer used by farmers was higher than 180 kg/ha for hybrids and produced greater grain yield. On the other side, OPVs such as Bisma, Sukmaraga, Lamuru, Arjuna from local populations perhaps have adapted to low-N. Certain maize varieties originating from local populations have a better capacity to absorb and utilize N under low N fertilization conditions whereas others do not [39].

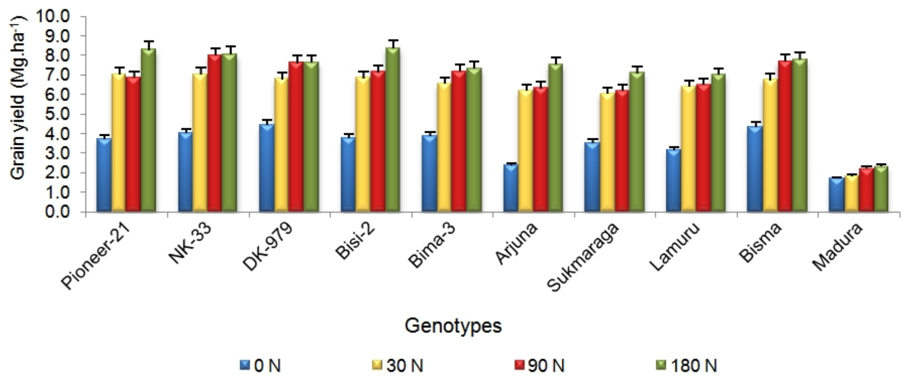

Fig. 1. Grain yield of ten genotypes at four N levels

Reduction of N levels from 180 to 90, 30 and 0 kg N/ha caused varied reductions of grain yield ranged from 0.3 to 68.8% (Fig. 2). Among genotypes had high yield, NK-33, DK-979 and Bisma were less reduced than Pioneer-21 and Bisi-2, whereas Madura had the lowest yield and was not different at all N levels. Some scientists confirmed that yield reduction does not exceed 35–40% [34] and 43% [40] for selection of low-N stress tolerant genotypes. Selection under stress condition is more effective than selection under non-stress conditions for improving grain yield in environments where that specific a biotic stress occurs [41]. Therefore, N reduction from 180 kg N.ha^{-1} to 90 and 30 kg N.ha^{-1} can be used to select genotypes were able to adapt to low-N.

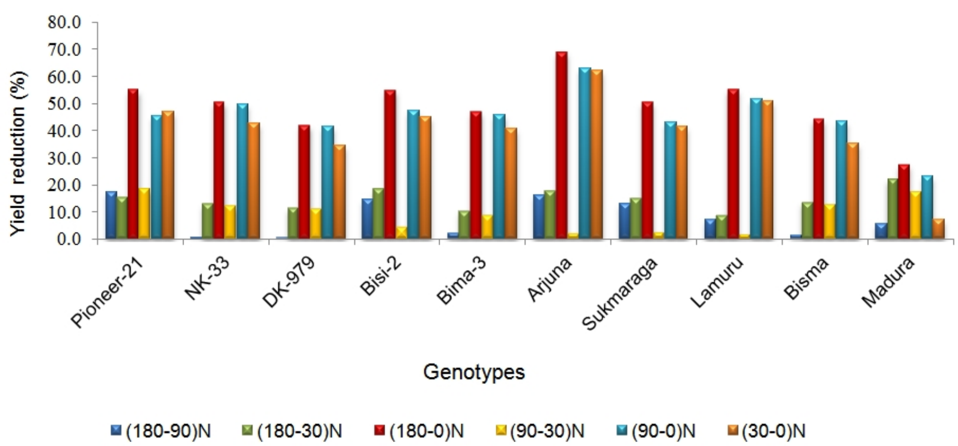

Fig. 2. Grain yield reduction of ten genotypes due to reducing N levels

N deprivation caused a thousand grain weight reduction (Table 5) and high genotypic variation for a thousand grain weight was observed at high-N (Table 2). HI only was significantly affected by genotypes, which are Bima-3 and Madura had lower HI than other genotypes. Previous study indicated that NHI on rice was not affected by N and genotype [6], but other researchers reported that HI on maize and rice was affected significantly by N, genotypes and their interaction [21,22].

Higher genotypic variation for grain number per cob was observed at medium-N than other N treatments. Significant reduction of grain number only occurred at no N treatment. The reduction grain number is due to ovule abortion after fertilization that was affected by N stress [42-44]. Some ovule abortions were observed by authors during the present experiment. After fertilization, the sink demand must be too high compared with the availability of resources, thus leading to embryo abortion in genotype-dependent manner [7].

As it already had been predicted that N uptake was reduced with reducing N level (Table 5). Because of N uptake measurement was taken at maturity, high genotypic variation was observed at high-N (180 kg $N.ha^{-1}$) and gradually decreased with reducing N level (Table 2). Similar result had been reported [7,21] that low variation in whole plant N-uptake under low N-input suggests that there was a limiting factor in nitrogen availability in the soil and in plant capacity to absorb nitrogen. In the present experiment, high N uptake obtained by Pioneer-21, NK-33, and Bisi-2 from hybrids; Bisma and Arjuna from OPVs at high-N. Without N fertilization, N uptakes by NK-33, DK-979 and Lamuru were higher than others, whereas Madura had the lowest N uptake and there were no significant differences at all N levels.

NUE parameters were used based on the agronomic studies (agronomic indices) with respect to some considerations: study emphasizes crop response to N, field experiment and one cropping season [9]. There were genotypes x N interactions for crop recovery efficiency of applied N (RE_N), physiological efficiency of applied N (PE_N), agronomic efficiency (AE) and nitrogen use efficiency (NUE). Reduction N levels caused increasing NUE parameters (Table 6). Similar results were reported by different authors in varied plants, on rice [6,37], wheat [45-48]; maize [4,21,27,49,50], millet [28]. Generally NUE parameters are high under low-N levels and decrease with increasing N level. Decreased NUE at high-N to higher volatilization losses because the plant was unable to assimilate all of N taken up [48].

High genotypic variations were observed at high-N for NUp, RE_N, PE_N and AE, whereas NUE at low-N. At low-N, Bisi-2, Bisma and Sukmaraga showed high RE_N (3.99, 3.32 and 3.00 $kg.kg^{-1}$); whereas high PE_N was obtained by NK-33, Pioneer-21, Lamuru and Bima-3 (74.77, 59.5, 54.7 and 53.3 $kg.kg^{-1}$); high AE was found in Bima-3, Pioneer-21, Lamuru, Bisi-2 and NK-33 (128.0, 109.4, 108.8, 102.1 and 100.1 $kg.kg^{-1}$); and high NUE was obtained by NK-33 and Pioneer-21 (67.2 and 64.5 $kg.kg^{-1}$).

Crop recovery efficiency of applied N (RE_N) indicates the capacity of the crops to absorb N applied, while physiological efficiency (PE_N) reflects the ability of the crops to utilize N absorbed to produce economic yield. Agronomic efficiency is the product of the efficiency of RE_N and PE_N. Increasing RE_N and/or PE_N will lead to an increase of AE. RE_N affected by N application method (amount, timing, placement, N form) as well as factors that determine the size of the crop N sink (genotype, climate, plant density, a-biotic/biotic stresses), whilst PE_N depends on genotypic characteristics, environmental and management factors particularly during reproductive growth. NUE is important for farmers because it integrates the use efficiency of both indigenous and applied N resources [9].

Table 5. Performance of ten genotypes at four N fertilizer levels for grain number per cob, a thousand grain weight, harvest index and N uptake

	Grain number per cob				A thousand grain weight (g)				Harvest index				N uptake (g)			
Genotypes	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**
Pioneer-21	237.2	370.9	386.2	427.0	221.3	269.2	262.1	288.2	0.39	0.43	0.40	0.44	0.90	1.82	3.00	3.93
NK-33	229.9	359.6	340.7	397.0	255.4	270.2	288.0	292.9	0.38	0.43	0.42	0.37	1.03	1.75	2.60	3.21
DK-979	300.7	406.9	411.6	445.0	219.0	235.3	259.1	260.4	0.47	0.39	0.44	0.38	1.09	2.01	2.66	2.82
Bisi-2	249.9	369.2	362.5	417.1	225.1	266.1	282.8	291.3	0.38	0.41	0.40	0.42	0.91	2.88	3.12	3.58
Bima-3	183.4	375.7	395.3	398.9	238.7	269.1	267.1	285.7	0.28	0.38	0.38	0.41	0.85	2.10	2.89	2.95
Arjuna	221.8	358.3	361.6	381.0	193.9	237.7	238.0	285.6	0.41	0.44	0.44	0.40	0.88	2.31	3.39	3.67
Sukmaraga	237.0	352.2	355.9	396.9	224.5	247.0	258.4	264.2	0.38	0.37	0.37	0.37	0.88	2.36	2.98	3.10
Lamuru	175.4	306.1	324.8	373.1	271.7	291.2	301.0	304.1	0.36	0.38	0.39	0.38	1.01	2.17	2.56	2.79
Bisma	259.7	355.8	381.6	405.7	260.6	278.1	299.8	295.8	0.41	0.39	0.42	0.40	0.90	2.54	3.96	3.81
Madura	197.2	228.8	257.4	262.3	145.7	148.5	148.2	151.3	0.24	0.28	0.32	0.34	0.92	1.28	1.07	1.02
HSD $_{(0.05)}$																
G		47.9				18.0				0.07				0.54		
N		31.9				8.4				0.03				0.17		
GxN		141.1				37.4				0.15				0.77		

Table 6. Performance of ten genotypes at four N fertilizer levels for NUE traits

	RE_N ($kg.kg^{-1}$)				PE_N ($kg.kg^{-1}$)				AE ($kg.kg^{-1}$)				NUE ($kg.kg^{-1}$)			
Genotypes	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**
Pioneer-21	-	1.9	1.4	1.0	-	59.5	25.1	24.8	-	109.4	34.5	25.2	69.6	64.5	37.9	34.9
NK-33	-	1.5	1.1	0.7	-	74.8	44.8	25.4	-	100.1	44.0	18.6	66.8	67.2	51.3	38.2
DK-979	-	1.9	1.1	0.6	-	43.1	42.7	27.0	-	77.6	41.4	14.9	69.4	55.7	51.9	41.9
Bisi-2	-	4.0	1.5	0.9	-	26.7	26.4	28.3	-	102.1	37.4	25.4	68.3	39.9	39.1	38.6
Bima-3	-	2.5	1.4	0.7	-	53.3	32.5	41.3	-	128.0	44.1	28.8	47.2	48.7	36.5	42.6
Arjuna	-	2.9	1.7	0.9	-	30.7	21.7	20.8	-	89.2	36.5	19.0	72.7	47.0	34.9	33.3
Sukmaraga	-	3.0	1.4	0.8	-	28.1	20.7	26.7	-	83.9	29.4	19.8	67.4	42.5	34.2	38.1
Lamuru	-	2.4	1.1	0.6	-	54.7	35.9	35.8	-	108.8	37.3	21.4	51.5	50.3	42.0	41.6
Bisma	-	3.3	2.1	1.0	-	23.6	19.3	20.1	-	79.2	39.9	19.7	80.1	43.9	33.0	34.2
Madura	-	0.7	0.1	0.0	-	23.3	33.9	62.9	-	4.3	5.6	3.5	30.5	23.6	34.0	37.7
$HSD_{(0.05)}$																
G		0.56				18.4				13.6				13.6		
N		0.23				7.3				5.6				5.3		
GxN		1.00				32.2				30.6				23.3		

RE_N, crop recovery efficiency of applied N; PE_N, physiological efficiency of applied N; AE, agronomic efficiency; NUE, nitrogen use efficiency

The explanation above is noticeable in the present study, Madura as old variety exhibiting low performance for all measured traits related to NUE due to the capacity to absorb, accumulate and utilize N in plant is lower than others. However, this variety is able to adapt to drought stress so that it can be considered as a genetic material for developing drought stress maize genotypes. Other OPVs indicated moderate NUE traits, this may be due to they are originating from local population have adapted to low-N. Certain maize varieties are originating from local populations have a better capacity to absorb and utilize N under low N fertilization conditions, whereas others do not [39].

4. CONCLUSION

Our study concluded that genetic variation was differently expressed under different N levels. Heritability estimates were high for most of measured traits related to NUE at all N levels. This suggests two important things, the first is there were genetic materials for improving NUE in evaluated maize genotypes; the second is selection for genotypes are tolerant to low N fertilization at low N-input (direct selection) is as effective as at high N-input (indirect selection) [51]. Genetic variation for grain yield and a thousand grain weight were high at all N levels, whereas RE_N, PE_N and AE were higher at high-N and NUE at low-N.

Although most of OPV's and hybrids utilized N in same efficient way for biomass production (TDM), but some OPVs (Sukmaraga, Lamuru and Arjuna) indicated lower yielding ability and hybrids vice versa, except Bima-3; whilst Madura exhibited highest physiological efficiency but lowest for dry matter accumulation, grain yield and agronomical efficiency. This revealed NUE is a complex agronomic trait controlled by a large number of genes which plant growth and N nutrition interact in a complex way and are constantly changing from the vegetative stage to the grain-filling period [19].

Improving NUE had been assessed based on grain yield and NUE parameters. High grain yield were found in Bisi-2, Pioneer-21, NK-33, Bisma and DK-979 at high-N; and less yield reductions caused by N level reduction were found in DK-979, Madura, Bima-3, Bisma and NK-33; whilst high NUE parameters were indicated in NK-33 and Pioneer-21. Therefore, NK-33, DK-979, Pioneer-21 and Bisma are expected to be as genetic materials for developing low-N tolerant genotypes. The use of N-efficient genotypes for crops which require large quantity of N input, such as maize, would be advantageous economically, particularly in the developing countries where many poor nutrient soils found and the high cost of fertilizer are main constraints in cropping system. In addition, the hazard of environmental pollution caused by excessive fertilization would be reduced by cropping the N-efficient maize genotypes.

Furthermore, the study subjected for correlation analysis between NUE traits to find traits closely related to NUE, as "selection criteria" for identifying genotypes with high NUE or low-N tolerant genotypes in maize breeding programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eckert D. Efficient Fertilizer Use Manual-Nitrogen. 1999. Accessed 23 June 2007. Available: www.back-to-basics.net/efu/pdfs/Nitrogen.pdf.
2. Field CJ, Mooney HA. The photosynthesis-nitrogen relationship in wild plants. In: Givinish TJ, editor. The Economy of Plant Form and Function. Cambridge University Press, New York; 1986.
3. Frink CR, Waggoner PE, Ausubel JH. Nitrogen Fertilizer: Restropect and Prospect. Proc. Natl. Acad. Sci. USA. 1999;96(4):1175-1180. PMID:9989997 [PubMed].
4. Worku M, Tuna H, Abera W, Wolda L, Diallo A., Afriyie ST, Guta A. Developing low N tolerant maize varieties for mild atltitude sub humid agro ecology of Ethiopia. Seventh Eastern and Southern Africa Regional Maize Conference. 2001:197-201.
5. Yuan ZY, Li LH, Huang JH, Han XG, Wan SQ. Effect of nitrogen supply on the nitrogen use efficiency of an annual herb, *Helianthus annuus* L. J. Integr. Plant. Biol. 2005;47(5):539-548.
6. Tayefe M, Gerayzade A, Amiri E, Zade AN. Effect of nitrogen fertilizer on nitrogen uptake, nitrogen use efficiency of rice. In: Baby S, Dan Y, Editors, International Proceeding of Chemical, Biological and Environmental Engineering. 2011;24:470-473.
7. Gallais A, Hirel B. An approach to the genetics of nitrogen use efficiency in maize. Journal of Exp. Botany. 2004;55(396):295-306. DOI:10.1093/jxb/erh006.
8. Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. Annals of Botany. 2010;105(7):1141-1157. DOI:10.1093/aob/mcq028.
9. Dobermann AR. Nitrogen use efficiency – state of the art. Agronomy – Faculty Publications. Agronomy and Horticulture Department of Nebraska – Lincoln. 2005. Accessed 16 April 2012. Available: www.digitalcommons.unl.edu/agronomyfacpub/316.
10. Machado AT, Sodek L, Fernandes MS. N-partitioning, nitrate reductase and glutamine synthetase activities in two contrasting varieties of maize. Pesq. Agropec. Bras. 2001;36 (2):249-256.
11. Moose S, Below F, Buckler ES. Gene discovery for maize responses to nitrogen. Research project. University of Illinois at Urbana-Champaign.USA. 2005. Accessed 12 May 2007. Available: nitrogenes.cropsci.illinois.edu/NSF-PG%20Maize%20NUE%20proposal.pdf.
12. FAO. Current world fertilizer trends and outlook to 2015. Food and Agriculture Organization of the United Nations. Rome. 2011. Accessed 24 August 2012. Available: ftp://ftp.fao.org/ag/agp/docs/cwfto15.pdf.
13. Bainbridge D, George M. Problems with nitrogen pollution. Earth Times. San Diego. USA. 1999. Accessed 22 September 2005. Available: www.sdearthtimes.com/et1099/et1099s11.html.
14. Pretty J, Brett C, Gee D, Hine RE, Mason CF, Morison JIL, Raven H, Rayment MD, Bijl GVD. An assessment of the total external costs of UK agriculture. Agric. Syst. 2000;65(2):113-136. PII: S0308-521X(00)00031-7.

15. Schweigert P, van der Ploeg RR. Nitrogen use efficiency in Germany agriculture since 1950: facts and evaluation. Berichte über Landwirtschaft. 2000;80:185-212. Accessed 16 April 2012. Available: www.dr-schweigert.de/N-Bilanz.htm.
16. Townsend AR, et al. Human health effects of a changing global nitrogen cycle. Frontiers Ecol. Environ. 2003;1(5):240-246.
17. Raun WR, Johnson GV. Improving nitrogen use efficiency for cereal production. Agron. J. 1999;91(3):357-367.
18. Peoples MB, Herridge DF, Ladha JK. Biological N fixation: an efficient source of N for sustainable agricultural production. Plant and Soil. 1995;174(1&2):3-28.
19. Hirel B, Chardon F, Durand J. The contribution of molecular physiology to the improvement of nitrogen use efficiency in crops. J. Crop Sci. Biotech. 2007;10(3):123-132.
20. Moll RH, Kamprath EJ, Jackson WA. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 1982;74:562-564.
21. Hefny MM, Aly AA. Yielding ability and nitrogen use efficiency in maize inbred lines and their crosses. International J. Agri. Research. 2008;3(1):27-39.
22. Gueye T, Becker H. Genetic variation in nitrogen efficiency among cultivars of irrigated rice in Senegal. Journal of Agricultural Biotechnology and Sustainable Development. 2011;3(3):35-43.
23. Ajala SO, Menkir A, Kamara AY, Alabi SO, Abdulai MS. Breeding strategies to improve maize for adaptation to low soil nitrogen. In: African Crop Science Conference Proceedings. 2007;8:87-94.
24. Arnon DF. Copper enzymes in isolated chloroplasts, poliphenol oxidase in beta vulgaris. Pl. Physiol. 1949;24(1):1-15.
25. Snedecor GF, Cohran WG. Statistical Methods.7th Edn. The Iowa State University Press, Ames; 1980.
26. Presterl T, Seitz G, Landbeck M, Thiemt EM, Schmidt W, Geiger HH. Improving nitrogen use efficiency in European maize: estimation of quantitative genetic parameters. Crop Sci. 2003,43(4):1259–1265. DOI: 10.2135/cropsci2003.1259.
27. Gungula DT, Togun AO, Kling JG. The influence of N levels on maize leaf number and senescence in Nigeria. World J. Agricultural Sci. 2005;1(1):1-5.
28. Gupta N, Gupta AK, Gaur VS, Kumar A. Relationship of nitrogen use efficiency with the activities of enzymes involved in nitrogen uptake and assimilation of finger millet genotypes grown under different nitrogen inputs. The Scientific World Journal. 2012. 10p. ID 652731. DOI:10.1100/2012/625731.
29. Bojović B, Marković A. Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). Kragujevac J. Sci. 2009;31:69-74.
30. Mi G, Chen F, Zhang F. Physiological and genetic mechanisms for nitrogen-use efficiency in maize. J.Crop Sci. Biotech. 2007;10(2):57-63.
31. Coque M, Martin A, Veyrieras JB, Hirel B, Gallais A. Genetic variation for N remobilization and postsilking N-uptake in a set of maize recombinant inbred lines.3. QTL detection and coincidences. Theoretical Applied Genetic. 2008;17(5):729-747. DOI 10.1007/s00122-008-0815-2.
32. Monneveux P, Zaidi PH, Sanchez C. Population density and low nitrogen affects yield-associated traits in tropical maize. Crop Sci. 2005;45(2):535-545.
33. Masclaux-Daubresse C, Reisdorf-Cren M, Orsel M. Leaf nitrogen remobilisation for plant development and grain filling. Plant Biology. 2008;10(1):23-36. DOI:10.1111/j.1438-8677.2008.00097.x.
34. Gallais A, Coque M. Genetic variation and selection for nitrogen use efficiency in maize: a synthesis. Maydica. 2005;50(3&4):531-537.

35. Racjan I, Tollenaar M. Source-sink ratio and leaf senescence in maize. II. Nitrogen metabolism during grain filling. Field Crops Research.1999;60(3):255-265. DOI:10.1016/S0378-4290(98)00143-9.
36. D'Andrea KE, Otegui ME, Cirilo AG, Eyherabide G. Genotypic variability in morphological and physiological traits among maize inbred lines-nitrogen responses. Crop.Sci. 2006;46(3):1266-1276. DOI:10.2135/cropsci2005.07-0195.
37. Fageria NK, de Morais PO, dos Santos AB. Nitrogen use efficiency in upland rice genotypes. Journal of Plant Nutrition. 2010;33(11):1696-1711. DOI: 10.1080/01904167.2010.496892.
38. Ortiz-Monasterio JI, Sayre KD, Rajaram S, McMahon M. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen regimes. Crop Science. 1997;37(3):898-904. DOI:10.2135/cropsci1997.0011183X003700030033x.
39. Hirel B, Gouis JL, Ney B, Gallais A. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. Nitrogen Nutrition Special Issue. J. of Exp. Botany. 2007;58(9):2369–2387. DOI:10.1093/jxb/erm097.
40. Banziger M, Betran FJ, Lafitte HR. Efficiency of high nitrogen environment for improving maize for low-nitrogen environment. Crop Science. 1997;37(4):1103-1109. DOI:10.2135/cropsci1997.0011183X003700040012x.
41. Banziger M, Diallo AO. Progress in developing drought & stress tolerant maize cultivar for Eastern and Southern Africa. In: Seventh Eastern and Southern Africa Regional Maize Conferrence. 2001;7:189-194.
42. Lemcoff JH, Loomis RS. Nitrogen influences in yield determination in maize. Crop Sci. 1986;26(5):1017-1022. DOI:10.2135/cropsci1986.0011183X002600050036x.
43. Uhart SA, Andrade FH. Nitrogen deficiency in maize. II. Carbon-nitrogen interaction effects on kernel number and grain yield. Crop Science. 1995;35(5):1384-1389. DOI:10.2135/cropsci1995.0011183X003500050020x.
44. Below FE, Cazetta JO, Seebauer JR. Carbon/nitrogen interactions during ear and kernel development of maize. In: Physiology and modelling kernel set in maize. CSSA special publication no.29. 2000. DOI:10.2135/cssaspecpub29.c2.
45. Kanampiu FK, Raun WR, Johnson GV, Anderson MP. Effect of nitrogen rate on plant nitrogen loss in winter wheat varieties. J. Plant Nutr. 1997;20(2&3):389-404. DOI:10.1080/01904169709365259.
46. Campillo R, Jobet C, Undurraga P. Effects of nitrogen on productivity, grain quality, and optimal nitrogen levels in winter wheat cv. Kumpa-inia in andisols of southern Chile.Chilean J. Agricultural Research. 2010;70(1):122-131.
47. Giambalvo D, Ruisi P, Miceli GD, Frenda AS, Amato G. Nitrogen use efficiency and nitrogen fertilizer recovery of durum wheat genotypes as affected by interspecific competition. Agronomy Journal. 2010;102(2):707-715. DOI:10.2134/agronj2009.0380.
48. Dhugga, KS, Waines JG. Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Science 29. 1989(5):1232-1239. DOI:10.2135/cropsci1989.0011183X002900050029x.
49. Bertin P, Gallais A. Physiological and genetic basis of nitrogen use efficiency in maize: I. Agrophysiological results. Maydica. 2000;45(1-4):53–66.
50. De Souza LV, Miranda GV, Galvão JCC, Eckert FR, Mantovani EE, Lima RO, Moreira LJ, Guimarães LJM. Genetic control of grain yield and nitrogen use efficiency in tropical maize. Pesq. agropec. bras, Brasília. 2008;43(11):1517-1523.

51. Gallais A, Coque M, Bertin P. Response to selection of a maize population for adaptation to high or low nitrogen fertilization A. Maydica. 2008;53:21-28.

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