

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

Makhziah^{1*}, Kusurningrum Rochiman², Hery Purnobasuki³

¹Agrotechnology Department, University of Pembangunan Nasional Veteran East Java, Jl. Raya Rungkut Madya, Surabaya (60294), Indonesia

²Animal Nutrition Department, University of Airlangga, Kampus C Mulyorejo, Surabaya (60115), Indonesia

³Biology Department, University of Airlangga, Kampus C, Jl Mulyorejo, Surabaya (60115), Indonesia

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

ABSTRACT

Effect of nitrogen (N) supply and genotypic variation for nitrogen use efficiency (NUE) traits were evaluated in order to enhance understanding of basic genetic of NUE and find genetic material for developing tolerant low-N genotypes in maize breeding. Ten genotypes from open pollinated varieties (OPV) and hybrids were cropped at four N fertilizer rates (0; 30; 90; 180 kg N.ha⁻¹) in split plot randomized block design with three replications at field, between November 2011 and February 2012. The results showed that N deprivation caused varied reduction on plant height, leaves area, chlorophyll content, stay green, grain yield, grain number, a thousand grain weight, N uptake; but increased days to 50% anthesis, 50% silking and anthesis-silking interval (ASI), N uptake efficiency (NUpE), agronomic efficiency (AE), N utilization efficiency (NUE) and NUE significantly ($P < 0.05$). High genotypic variation ($h^2 = 0.80-0.99$) for morphology, physiology, agronomic and NUE traits were observed at all N rates except stay green and NUE at low-N rate, grain number at medium-N rate, NUpE at high-N rate, whereas genotypic variation for harvest index (HI) was low at all N rates. Evaluation genotypes under low-N indicated that hybrids were more efficient to N than OPV.

Keywords: nitrogen use efficiency; genotypic variation; tolerant low-N.

ABBREVIATIONS

NUE: nitrogen use efficiency; **Nup**: nitrogen uptake; **NUpE**: nitrogen uptake efficiency, **NuE**: nitrogen utilization Efficiency; **AE**: agronomic efficiency; **HI**: harvest index, **OPV**: open pollinated varieties.

1. INTRODUCTION

Nitrogen (N) is an essential nutrient and constituent of 3-4% dry matter (Eckert, 1999), often becomes a limiting factor for plant growth and development (Field and Mooney, 1986; Frink et al. 1999; Worku et al., 2001; Yuan et al., 2005; Tayefe et al., 2011) because it plays an important role in plant metabolism (Gallais and Hirel, 2004). The use of N by plant involves several steps, including uptake, assimilation and translocation then when plant is aging, recycling and remobilization (Masclaux et al., 2010b). For that reason, nitrogenous fertilizers widely applied by farmers and have contributed to remarkable increase in plant production during the past 50 years (Dobermann, 2005), especially staple food such as maize is highly responsive to N and requires large quantity of N (Moose et al., 2005; Machado et al., 2001). It's estimated 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 (Moose et al., 2005). In 2011, the world demand for nitrogen fertilizer is 105.348 million tons and is predicted to grow 1.7% annually for 2011-2015 (FAO, 2011).

Nowadays, excessive N application has become a concern because accumulation of a large amounts of N in ecosystems leads to significant direct and indirect negative effects on environmental quality, decrease of biodiversity, human health (Bainbridge and George, 1999; Dobermann, 2005; Pretty et al., 2000; Schweigert and van der Ploeg, 2000; Townsend et al., 2003), because only about 33% N fertilizer removed by plant (nitrogen use efficiency) and the rest lost by leaching, denitrification, and volatilization (Raun and Johnson, 1999). It's only about 40-50% N fertilizer is absorbed by maize crop under tropical conditions (Machado et al., 2001; Peoples et al., 1995). Incomplete capture and poor conversion of N fertilizer causes global warming through emissions of nitrous oxide (N_2O) (Masclaux et al., 2010b). Nitrogen use efficiency (NUE) defined as the grain yield per unit of N available in soil, including fertilizer (Moll et al., 1987). Some parameters of NUE have been developed, NUE also is the grain yield per unit N applied, agronomic efficiency of applied N is yield increase per unit N applied, N uptake efficiency or crop recovery efficiency of applied N is increase in N uptake per unit N applied, N utilization efficiency or physiological efficiency is yield increase per increase in N uptake from fertilizer (Dobermann, 2005).

Improving NUE expected to reduce negative impact of excessive N fertilizer application, and it can be increased by production practice system: plant rotation, forage-only production, genotypes, conservation tillage, NH_4-N Source and irrigation (Raun and Johnson, 1999). In order to develop genotypes that have better nitrogen use efficiency needed a better knowledge of physiological and genetic basis of NUE (Gallais and Hirel, 2004). N uptake and utilization efficiency requires that those processes associated with absorption, translocation, assimilation, and redistribution of N operate effectively (Moll, et al. 1982). Genotype x nitrogen interaction is significant for trait associated with NUE (Gallais and Hirel, 2004). Ajala et al. (2007) stated that several studies found genetic potential in maize genotypes for improvement of NUE and promising genetic material with better N uptake and utilization efficiency and used as parents to develop population adapted to low-N.

So the present study is conducted with aims to: (i) understand effect of N deprivation to traits related with NUE in maize; (ii) investigate genetic variation for NUE in maize and identify new sources for breeding maize with greater NUE.

2. MATERIAL AND METHODS

Experiment was conducted on November 2011-February 2012 at field in Sumber Gempol district, Tulungagung East Java, Indonesia with altitude 85 meters above sea level. The experiment site characterized as alluvial hydromorph contents P_2O_5 : 34.33 ppm, SO_4 : 51.45 ppm; Fe: 39.55 ppm; Cu: 12.48 ppm; Zn: 3.26 ppm; N: 0.13%; CO: 1.27%; K: 0.22 me/100 g; Na: 0.31 me/100 g; Ca: 13.4 me/100 g; Mg: 3.16 me/100 g.

Ten maize genotypes from open pollinated varieties (OPV) consist of Bisma, Lamuru, Arjuna, Sukmaraga, Madura; and hybrids consist of P-21, NK-33, DK-979, Bisi-2 and Bima-3 were planted at four rates of N fertilizer, no 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 rates 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 and 75 kg K/ha simultaneously with planting, while N fertilizer in the form of urea (46%) was added as single application for 30 kg N/ha treatment at 2 WAS, and three times for 90 and 180 kg N/ha treatment; 1/3 apart at 2 WAS, 1/3 apart at 5 WAS, and 1/3 apart at anthesis.

Data were collected from five representative plants used as sample plants. At maturity, plants were harvested and separated into leaves, stem and cob, dried at 70°C for 48 h in oven to constant weight then were analyzed for total N content by Kjeldahl method.

Total chlorophyll was analyzed at anthesis by Arnon method (1949); 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 a spectrophotometer. Chlorophyll content (expressed as mg/g-1 of each sample) was estimated as follow:

Chlorophyll a (mg.g⁻¹) = $12.7 (A_{663}) - 2.69 (A_{645}) \times VW$

Chlorophyll b (mg.g⁻¹) = $22.9 (A_{645}) - 4.86 (A_{663}) \times VW$

Total Chlorophyll t (mg.g⁻¹) = $[20.2 (A_{645}) - 8.02 (A_{663}) \times VW]/1000$

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

Data were recorded on days to 50% anthesis and 50% silking of the plants in the plot, and also interval between them. At maturity, plant height, total leaves area, total dry matter were determined. Stay green was determined for each plot by visually assessing the 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 (Dobermann, 2005):

- Nitrogen uptake = total dry matter above ground X N concentration. (g N/plant)

- Nitrogen uptake efficiency (NUpE) = (UN-U0)/FN (kg N-uptake/kg N-fertilizer)

- Agronomic efficiency (AE) = (YN-Y0)/FN (kg grain/kg N-fertilizer)

- Nitrogen utilization efficiency (NUE) = (YN-Y0)/(UN-U0) (kg grain/kg N-uptake)

- Nitrogen use efficiency (NUE) = YN/FN (kg grain/kg N-fertilizer)

Where:

FN: amount of N fertilizer applied (kg.ha⁻¹).

YN: crop yield with applied N fertilizer (kg.ha⁻¹).

Y0: crop yield in a control treatment with no N fertilizer (kg/ha).

UN: total plant N uptake in aboveground biomass at maturity in a plot that received N fertilizer (kg.ha⁻¹).

140 U0: the total N uptake in aboveground biomass at maturity in a plot that received no N
141 fertilizer.

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143 Statistical analysis used was analysis of variance (ANOVA) to estimate the significance of
144 genotypes, N rates and their interactions. Tukey's HSD (honestly significant difference) test
145 was calculated to compare treatment means. Heritabilities were calculated to investigate the
146 genotypic variation by broad-sense heritability was derived from the ratio of genotypic
147 variance to phenotypic among genotypes estimated from the analysis of variance, base on
148 expectation of mean squares (Snedecor and Cochran, 1980):

149 $h^2 = \sigma^2_g / \sigma^2_p$

150 $\sigma^2_g = (Mg - Me)/r$

151 $\sigma^2_e = Me/r$

152 $\sigma^2_p = Mg/r$

153 Where:

154 h^2 = heritability estimate; Mg = mean square of genotypes; Me = mean square of error; σ^2_g =
155 genotypic variance; σ^2_p = phenotypic variance; σ^2_e = environmental variance.

156 3. RESULTS AND DISCUSSION

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158 N fertilization, genotypes and N x genotypes interaction led to significant effect ($P = 0.01$) on
159 all the traits except harvest index among genotypes (Table 1). N deprivation caused varied
160 reduction significantly on plant height, leaves area, chlorophyll content, stay green, yield
161 components (grain yield, grain number per ear and a thousand grain weight) and N uptake
162 among genotypes; but increased days to 50% tasseling, 50% silking, and anthesis-silking
163 interval on several genotypes, while harvest index was not affected significantly. The effect
164 of N deprivation will gives preliminary information for understanding plant reaction to N
165 fertilization.

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167 High genotypic variation had been observed at all N rates for plant height and leaves area
168 (Table 2). These traits were reduced with N fertilizer rates reduction on all genotypes except
169 Madura had the least of plant height and leaves area and was not responsive to N rates
170 (table 3). Genotypic variation for plant height and leaves area was higher at low-N rates than
171 other N rates. Without N fertilization, all genotypes were stressed strongly, but they were
172 afford to grow optimum at high-N rates and no different performance, except Bima-3 from
173 hybrid and Madura from OPV had less of plant height and leaves area. This result is
174 comparable to that already observed by Gupta et al. (2012) that plant height and leaf area
175 were affected N fertilization and were found differences among the genotypes under
176 different soil conditions. Gungula et al. (2005) also recorded that maize leaves number
177 varied with N rates among genotypes.

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Table 1. Mean squares of ten genotypes planted at four N fertilizer rates for morphology, physiology, agronomy and NUE traits

S.O.V	→	Genotypes	E _a	N rates	N rates 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 (ASI) (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 ear	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	
Harvest index	0.0061	0.0027	0.0064	0.0038	0.0026	
Nup (g)	2.7**	0.1	27.7**	0.6**	0.1	
NupE (kg.kg ⁻¹)	2.0**	0.1	30.7**	0.6**	0.1	
AE (kg.kg ⁻¹)	1872.2**	175.6	42942.3**	666.1**	102.0	
NUtE (kg.kg ⁻¹)	586.9**	158.2	9709.4**	375.0**	113.3	
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

Chlorophyll content and stay green were affected N fertilizer rates significantly among genotypes (Table 3). Chlorophyll content and stay green demonstrated high genotypic variation at all N rates (Table 2), this may due each genotypes has different N uptake capacity that associated with chlorophyll content and N remobilization after anthesis. Nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Bojović and Marković, 2009). One of characteristics of N-efficient maize is slow leaf senescence through maintenance of post-anthesis N uptake (Mi et al, 2007). Improving N-uptake, leaf area duration and chlorophyll content could be used to select new varieties exhibiting a "stay-green" phenotype (Coque et al., 2008). N rates reduction led to reduce chlorophyll content and stay green on all genotypes except Madura that constant at all N treatment (Table 3). At low-N, medium-N and high-N rates, Pioneer-21, NK-33 and DK-979 had higher chlorophyll content and stay green than other genotypes, but at no N fertilization treatment, all of genotypes were not different significantly. Earlier leaf senescence was occurred (6 WAS) had been observed at no N fertilization treatment by authors. Similar results were obtained by different authors (Monneveux et al., 2005; Gungula et al., 2005; Hefny and Aly, 2008). N remobilization generally starts earlier when plants are under a low-N fertilizer regime compare to high-N fertilization (Masclaux et al., 2008a) as a consequence of unavailable soil N-nitrogen, and then leaf senescence, which is associated with active remobilization, is also accelerated (Gallais and Coque, 2005). Leaf longevity is enhanced by increase in soil N supply (Racjan and Tollenaar, 1999), and reduced N availability accelerates post flowering leaf senescence than at high N (D'Andrea et al., 2006).

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Table 2. Heritability Estimates for traits at N fertilizer rates

Traits	N fertilizer rates (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)	0.000	0.862	0.861	0.672
Grain yield (Mg.ha ⁻¹)	0.881	0.977	0.969	0.985
Grain number per ear	0.640	0.716	0.814	0.635
A thousand grain weight (g)	0.945	0.968	0.976	0.977
Harvest index	0.328	0.328	0.033	0.659
Nup (g)	0.000	0.839	0.916	0.960
NupE (kg.kg ⁻¹)	-	0.839	0.930	0.962
AE (kg.kg ⁻¹)	-	0.888	0.856	0.952
NUtE (kg.kg ⁻¹)	-	0.689	0.500	0.857
NUE (kg.kg ⁻¹)	0.739	0.901	0.741	0.476

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

226 Higher genotypic variation had been observed at no N fertilizer treatment for days to 50%
227 anthesis and silking than other treatments (Table 2). N reduction from high-N to medium and
228 low-N didn't affect days to 50% anthesis and silking, but they increased significantly at no N
229 fertilization treatment in all of genotypes except Madura that is early mature and had no
230 different at all of N rates. ASI increased with reducing of N rates, even though statistically it
231 was not different significantly (Table 4). Madura, Sukmaraga, Bima-3, Bisma, Lamuru and
232 Arjuna had shorter ASI than Pioneer-21, NK-33, DK-979 and Bisi-2. Monneveux et al. (2005)
233 reported that ASI increased under low-N condition, while Hefny and Aly (2008) recorded that
234 plants were earlier in days to 50% silking than days to 50% anthesis under N stress. Gallais
235 and Hirel (2004) stated that ASI could then have a physiological meaning in relation to stress
236 tolerance and increasing ASI bring the consequence there could be a deficit in ovule
237 fertilization. Genotypes that have short ASI would have a more efficient nitrogen metabolism
238 at low. In this experiment, we have observed that plants had short ASI would lead high yield.

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

Genotypes	Plant height (cm)				Leaves area (m ²)				Chlorophyll content (mg.g ⁻¹)				Stay green (%)			
					N rates (kg.ha ⁻¹)											
	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		

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Table 4. Performance of ten genotypes at four N fertilizer rates for days to 50% tasseling and silking, tasseling-silking interval and N uptake traits

Genotypes	Days to 50% anthesis				Days to 50% silking				Anthesis-silking interval (d)				N uptake (g)			
									N rates (kg.ha ⁻¹)							
	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	0.90	1.82	3.00	3.93
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	1.03	1.75	2.60	3.21
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	1.09	2.01	2.66	2.82
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	0.91	2.88	3.12	3.58
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	0.85	2.10	2.89	2.95
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	0.88	2.31	3.39	3.67
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	0.88	2.36	2.98	3.10
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	1.01	2.17	2.56	2.79
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	0.90	2.54	3.96	3.81
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	0.92	1.28	1.07	1.02
HSD (0.05)																
G		0.9				1.0				0.81				0.54		
N		0.4				0.4				0.39				0.17		
GxN		1.8				1.9				1.73				0.77		

As it already has been predicted that N uptake was reduced followed reduced N fertilization rates (Table 4). Because of N uptake measurement was taken at maturity, high genotypic variation was observed at high-N rates (180 kg N.ha⁻¹) and gradually decreases with reducing of N rates (Table 2). Similar result had been reported (Gallais and Hirel, 2004; Hefny and Aly, 2008). 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 the capacity of the plant to absorb nitrogen (Gallais and Hirel, 2004). In this experiment, high N uptake was obtained by Pioneer-21, NK-33, and Bisi-2 from hybrids; Bisma and Arjuna from OPV at high N rates, while at no N fertilization, NK-33, DK-979 and Lamuru had higher N uptake than others. Madura had the least N uptake and no different significantly at all N rates.

N deprivation caused reduction of grain yield, grain number and a thousand grain weight, but it was not different significantly on harvest index (Table 5). Greater genotypic variation was observed on grain yield and a thousand grain weight than grain number and harvest index (Table 2). High genotypic variation of grain yield was observed at N fertilization treatments (high, medium low N) but low at no N fertilization because all genotypes were stressed strongly. Fageria et al., (2010) reported that grain yield did not differ significantly among upland rice genotypes at low-N. Gallais and Hirel (2004) declared that 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.

At high-N (180 kg N.ha⁻¹), no significant different grain yield was observed among genotypes except Madura had the least grain yield. Apparently hybrids (Pioneer-21, NK-33, DK-979, Bisi-2 and Bima-3) tended to yield more than OPV (Arjuna, Sukmaraga, Lamuru, Madura), except Bisma that was found as potential OPV has no different yield with hybrids. At medium-N (90 kg N.ha⁻¹), NK-33, Bisma and DK-979 were higher than others and at low-N (30 kg N.ha⁻¹), Pioneer-21 and NK-33 had higher grain yield than others. In this experiment, 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) (Figure 1). Ortiz-Monasterio et al., (1997) confirmed 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 rates compared with old cultivars. Hefny and Aly (2008) also noted that inbred lines were more reduction in yield than hybrid group under N limited conditions.

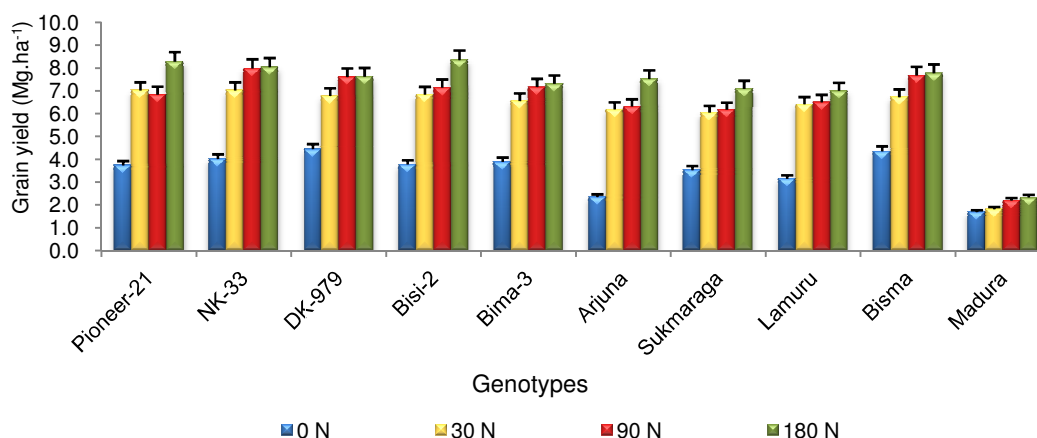


Figure 1. Grain yield of ten genotypes at four N fertilizer rates

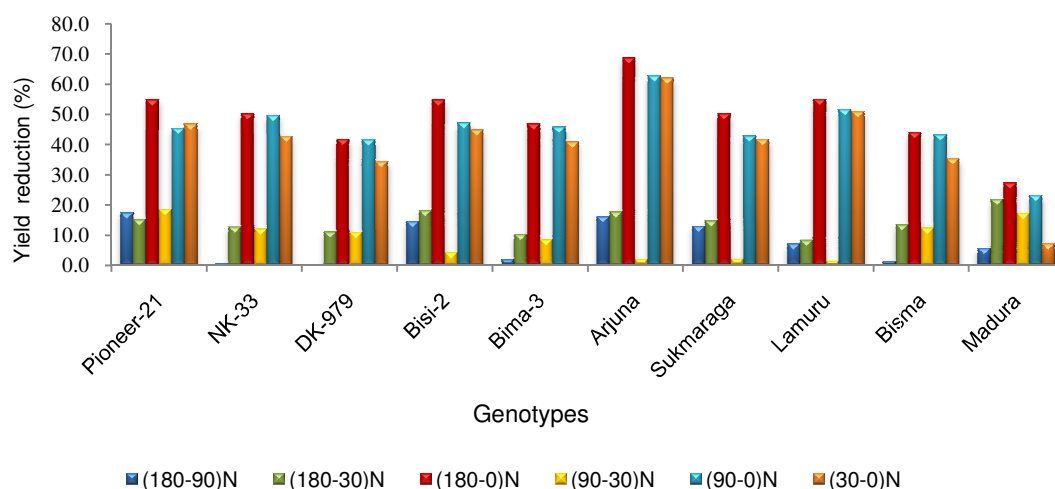


Figure 2. Grain yield reduction of ten genotypes at four N fertilizer rates

Reduction of N rates from 180 to 90, 30 and 0 kg N/ha caused varied reduction of grain yield ranged from 0.3 to 68.8% (Figure 2). According to Gallais and Coque, (2005), yield reduction does not exceed 35–40% for selection of low-N stress tolerant genotypes. Banziger et al. (1997) also stated when a panel of tropical maize lines was studied using the condition that the decrease in yield was not lower than 43%. 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 (Banziger and Diallo, 2001). Therefore, N reduction from 180 kg N/ha to 90 and 30 kg N/ha can be used to select genotypes were able to adapt to low-N. Among genotypes that had high yield at high-N input, NK-33, DK-979 and Bisma were less reduced than Pioneer-21 and Bisi-2 when N fertilizer rate was reduced (low-N), while Madura had the least yield and no different at all N rates.

N deprivation caused a thousand grain weight reduction and high genotypic variation for a thousand grain weight was obtained at high-N but HI was not affected N rates, genotypes and genotype x N interactions significantly. Similar result reported by Tayefe et al., (2011) that NHI was not affected significantly by N and genotype, but it is contrast with result was achieved by Hefny and Aly (2008) that HI was affected by N, genotypes and their interaction.

High genotypic variation for grain number was observed at medium-N and grain number was reduced significantly at no N fertilization treatment. The reduction grain number is due to ovule abortion after fertilization that affected N stress (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995; Below et al. 2000). Some ovule abortion was observed by authors in this 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 (Gallais and Hirel (2004).

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Table 5. Performance of ten genotypes at four N fertilizer rates for grain yield, grain number, a thousand grain weight and harvest index

Genotypes	Grain number per ear				A thousand grain weight (g)				Harvest index			
	N rates (kg.ha ⁻¹)											
	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	0.46
NK-33	229.9	359.6	340.7	397.0	255.4	270.2	288.0	292.9	0.38	0.42	0.43	0.37
DK-979	300.7	406.9	411.6	445.0	219.0	235.3	259.1	260.4	0.47	0.36	0.45	0.40
Bisi-2	249.9	369.2	362.5	417.1	225.1	266.1	282.8	291.3	0.38	0.40	0.42	0.42
Bima-3	183.4	375.7	395.3	398.9	238.7	269.1	267.1	285.7	0.28	0.38	0.37	0.42
Arjuna	221.8	358.3	361.6	381.0	193.9	237.7	238.0	285.6	0.41	0.43	0.44	0.38
Sukmaraga	237.0	352.2	355.9	396.9	224.5	247.0	258.4	264.2	0.38	0.34	0.35	0.38
Lamuru	175.4	306.1	324.8	373.1	271.7	291.2	301.0	304.1	0.36	0.37	0.38	0.39
Bisma	259.7	355.8	381.6	405.7	260.6	278.1	299.8	295.8	0.41	0.38	0.41	0.40
Madura	197.2	228.8	257.4	262.3	145.7	148.5	148.2	151.3	0.30	0.37	0.42	0.46
HSD _(0.05)												
G		47.9				18.0				0.08		
N		31.9				8.4				0.03		
GxN		141.1				37.4				0.15		

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

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

N uptake efficiency (NUpE), Agronomic efficiency (AE), N utilization efficiency (NUE) and N use efficiency (NUE) were significantly different under different N rates, genotypes and their interaction. Reduction N rates caused increasing of NUE parameters (Table 6). Similar results were reported by different authors in varied plants, rice (Tayefe et al., 2011), wheat (Ortiz-Monasterio et al., 1997; Kanampiu et al., 1997; Campillo et al., 2010; Giambalvo et al., 2010); maize (Bertin and Gallais, 2000; Hefny and Aly, 2008; Sousa et al. 2008); millet (Gupta et al., 2012), N efficiency is generally high under low-N rates and it decreases with increasing N rate. Dhugga and Waines (1989) attributed decreased NUE at high-N to higher volatilization losses because the plant was unable to assimilate all of N taken up.

High genotypic variation for NUE was observed at low-N, while for NUpE was and AE was at high-N. At low-N (30 kg N.ha⁻¹), high NUpE was obtained by Bisi-2, Bisma and Sukmaraga (3.99, 3.32 and 3.00 kg.kg⁻¹); high AE was obtained by Bima-3, Pioneer-21, Lamuru, Bisi-2 and NK-33 (127.9, 109.4, 108.8, 102.1 and 100.1 kg.kg⁻¹); high NUpE was obtained by NK-33, Pioneer-21, Lamuru and Bima-3 (74.77, 59.5, 54.7 and 53.3 kg.kg⁻¹); and high NUE was obtained by NK-33 and Pioneer-21 (67.2 and 64.5 kg.kg⁻¹).

4. CONCLUSION

In conclusion, this study showed that the presence of nitrogen x genotype interaction related to NUE will help to enhance understanding of basic genetic of NUE in maize. NUE parameters and genotypic variation are essential in search of promising genetic material for improving NUE in effort to develop low-N tolerant maize genotypes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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 530
 531
 532
 533
 534

535 **Name, affiliation and email of corresponding**
536 **author:**
537 **- Makhziah**
538
539 Agrotechnology Department, University of Pembangunan Nasional Veteran East Java, Jl
540 Raya Rungkut Madya, Surabaya, Indonesia. Email: makhziah@yahoo.com
541 Names, affiliation and emails of all coauthors:
542 **1. Kusriiningrum Rochiman,**
543
544 Animal Nutrition Department, University of Airlangga, Kampus C Mulyorejo, Surabaya
545 (60115), Indonesia. E-mail: -
546 **2. Hery Purnobasuki,**
547
548 Biology Department, University of Airlangga, Kampus C, Jl Mulyorejo, Surabaya (60115),
549 Indonesia. E-mail: herypurba@yahoo.com
550 Suggested reviewers (Name, affiliation, email):
551 **1. Mandayana Tayefe**
552
553 Department of Agriculture, Islamic Azad University, Lahijan Branch, Lahijan , Iran. E-mail:
554 m.tayefe@yahoo.com
555 **2. Anil Kumar**
556
557 Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and
558 Humanities, G.B. Pant University of Agriculture and Technology, Uttarakhand Pantnagar 263
559 145, India. E-mail: anilkumar.mbge@gmail.com
560 **3. Ricardo Campillo**
561
562 Instituto de Investigaciones Agropecuarias INIA, Casilla 58-D, Temuco, Chile. E-mail:
563 rcampill@inia.cl
564 **4. N.K. Fageria**
565
566 National Rice and Bean Research Center of EMBRAPA, Santo Antônio de Goiás, Brazil.
567 E-mail: fageria@cnpaf.embrapa.br
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